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Acute Kidney Injury: Pathogenesis and Novel Therapies

TH-OR001
The Kidney Defends the Urinary System from Infection by Secreting NGAL
Neal A. Paragas, Andong Qiu, Jonathan M. Barasch. Medicine, Columbia
University, NY, NY.
Background: NGAL is a critical component of innate immunity because it binds
catecholate-siderophores which microorganisms require to capture iron. Urinary(u) NGAL
is expressed at mg/L levels after either septic or aseptic diseases of the kidney and it has two
potential functions, epithelial growth and/or bacteriostasis. Here we examined the activity
of uNGAL in the growth of CFT073, a uropathogenic E. coli (UPEC).
Methods: To examine the role of uNGAL in bacteriostasis. (1) We designed a
conditional allele of NGAL. (2) We created a bioluminescent mouse that releases Luciferase
and mCherry when the NGAL locus is activated.
Results: We found NGAL significantly inhibited UPEC growth in vitro, which could be
rescued by the addition of iron. In a mouse pyelonephritic model, the intensity and timing
of urinary CFUs was mirrored by uNGAL, including a decrease in uNGAL coincident
with the resolution of infection. To determine the source of uNGAL, we made a NGAL
reporter mouse NGAL-Luc2/mC to visualize kidney expression in vivo and we found that
UPEC detritus introduced into the bladder induced NGAL-Luc2/mC expression distantly
in the kidneys. Reporter expression was consistent with the bladder and kidney Ngal
expression according to QPCR. By high power in situ hybridization we located Ngal to
alpha intercalated cells. To determine the physiological role of uNGAL, we made a global
NGAL KO and found that UPEC infections had delayed resolution. We verified by knocking
out Ngal in the intercalated cells by HoxB7-cre of the CD. To determine whether UPEC
signaling directly activates kidney epithelia, we developed an in vitro assay using the CDs
of Ngal-Luc2/mC kidneys. These cells express Ngal-Luc2/mC in response to co-culture
with UPECs and expression was reversed by antibiotics. Additional studies suggest that
TLRs are the critical local sensors of infection.
Conclusions: uNGAL is essential for clearance of a UPEC in a model of acute UTI.
The kidney responds to infections localized to the bladder by secreting NGAL. These
findings provide an explanation for the massive secretion of NGAL from the kidney in
both septic and aseptic diseases, demonstrating that the kidney defends the urinary system
via exocrine delivery of NGAL.
Funding: NIDDK Support

TH-OR002
Proximal Tubule Specific Expression of Heme Oxygenase-1 Is Protective
in Acute Kidney Injury Subhashini Bolisetty, 1,2 Abolfazl Zarjou, 1,2
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Birmingham, AL; 2Division of Nephrology, Nephrology Research and Training
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Background: Heme Oxygenase-1 (HO-1), an anti-oxidant enzyme is induced
during oxidative stress and has cytoprotective properties. HO-1 deficient mice are highly
sensitive to acute kidney injury (AKI) secondary to ischemia, rhabdomyolysis and
nephrotoxins. Although HO-1 is cytoprotective, it has been suggested that generalized
HO-1 overexpression might lead to harmful effects due to excessive amounts of its reactionproducts: iron, carbon monoxide, biliverdin and bilirubin.
Methods: Proximal tubules are the target of maximal injury in models of AKI and
are also the site where HO-1 induction is most abundant. Hence, we hypothesized that
targeted overexpression of HO-1 in the proximal tubule segment of the kidney will
confer cytoprotection against AKI. Therefore, we generated proximal tubule specific
HO-1 overexpressing (PEPCK-HO-1) mice using the cre-lox system and tested them in
models of AKI.
Results: Compared to age and sex matched HO-1wildtype littermates, PEPCK-HO-1
mice demonstrated significant renal protection (structural and functional) in two different
intrinsic models of AKI: cisplatin nephrotoxicity and glycerol-induced rhabdomyolysis.
Following rhabdomyolysis, HO-1 wildtype mice demonstrated significantly higher mortality
compared to PEPCK-HO-1 mice, along decreased tubular casts and necrosis and better
preserved kidney function (BUN-control: 145 ± 17; PEPCK-HO-1: 42.8 ± 6; P < 0.001,
creatinine-control: 1.55 ± 0.25; PEPCK-HO-1: 0.3 ± 0.14; P < 0.001). In the cisplatin
model of AKI, PEPCK-HO-1 mice had significantly fewer casts, necrotic tubules and
better-preserved kidney architecture along with preserved renal function (BUN-control:
106.67 ± 13.5; PEPCK-HO-1: 32.5 ± 3.8; P < 0.001, creatinine-control: 0.63 ± 0.1; PEPCKHO-1: 0.28 ± 0.04; P < 0.05).
Conclusions: These studies demonstrate for the first time that proximal-tubule specific
HO-1 overexpression alone is sufficient to protect against AKI and targeting the HO-1
system may serve as a novel therapeutic strategy.
Funding: Other NIH Support - R01 DK059600, R01 DK075332 and O’Brien Center
P30 DK079337, Private Foundation Support

TH-OR003
Endothelial HIF2, but Not HIF1 Modulates Inflammation and Protects
from Renal Ischemia-Reperfusion Injury Hideto Sano, Hanako Kobayashi,
Volker H. Haase. Nephrology and Hypertension, Vanderbilt University Medical
Center, Nashville, TN.
Background: Hypoxia inducible factors (HIF)-1 and -2 are basic helix-loop-helix
transcription factors that regulate cellular responses to hypoxia. Pharmacological activation
of HIF signaling prior to injury protects kidneys from ischemia-reperfusion injury (IRI).
However, it is unclear which HIF homolog, cell type, and which HIF target genes confer
cytoprotection.

Oral Abstract/Thursday

Methods: To address this question, we inactivated HIF-1 (HIF-1eKO), HIF-2 (HIF2eKO) or both (HIF-1/HIF-2eKO) in endothelial cells using VEcadherin-driven Cre
recombinase. KO mice and Cre-negative littermates were subjected to unilateral or bilateral
IRI. Renal injury was assessed by histologically, mRNA and protein analysis, and by analysis
of inflammatory cell infiltration at 2 hours, day 1 and 3 days after reperfusion.
Results: At day 3 following IRI renal injury was exacerbated in HIF-1/HIF-2eKO
mice compared to control as determined by BUN levels (1.4-fold increase), histological
injury score (1.5-fold increase), Kim-1 expression (1.6-fold increase) and inflammatory
cell infiltration (8.5-fold increase). To determine which HIF homolog was cytoprotective,
we subjected HIF-1eKO and HIF-2eKO mice to unilateral IRI. Injury in HIF-2eKO IRI
kidneys was increased, whereas renal injury between HIF-1eKO and littermate controls
was not different. Increased presence of CD45-positive cells in IRI kidneys from HIF1/HIF-2eKO and HIF-2eKO correlated with enhanced VCAM-1 expression, whereas
E-selectin and ICAM-1 did not change significantly. To examine whether endothelial
HIF modulated HIF-mediated ischemic preconditioning, we activated HIF prior to injury
with a prolyl-hydroxylase inhibitor (PHI). Although PHI treatment was cytoprotective
in wild type mice, cytoprotection was not found in HIF-1/HIF-2eKO mice compared to
vehicle-treated KO mice.
Conclusions: Our data suggest that a) endothelial HIF-2 but not HIF-1 is cytoprotective
in renal IRI and that b) endothelial HIF-2 exerts its cytoprotective effect partly by
suppressing IRI-associated inflammation. Furthermore, we provide evidence that endothelial
HIF is required for HIF-mediated ischemic preconditioning.
Funding: NIDDK Support

TH-OR004
Critical Role of Sphingosine Kinase-1 Signaling in A1 Adenosine ReceptorMediated Renal Protection Sang Won Park,1 Mihwa Kim,1 Kevin M. Brown,1
Volker H. Haase,2 H. Thomas Lee.1 1Anesthesiology, Columbia University, New
York, NY; 2Medicine, Vanderbilt University, Nashville, TN.
Background: Acute kidney injury (AKI) is a devastating clinical problem without
effective therapy and renal ischemia reperfusion (IR) injury is a major cause of AKI. We
previously demonstrated that activation of renal A1 adenosine receptors (ARs) attenuated
multiple pathways of cell death including necrosis, apoptosis and inflammation after
renal IR.
Methods: Here, we tested the hypothesis that renal A1AR activation protects against
IR injury by induction of sphingosine kinase-1 (SK1) and sphingosine-1 phosphate (S1P)
synthesis.
Results: In cultured human proximal tubule epithelial (HK-2) cells, a selective A1AR
agonist 2-chlorocyclopentyladenosine (CCPA) significantly induced SK1 (without changing
SK2) mRNA and protein expression and increased the synthesis of S1P. CCPA also induced
SK1 and S1P in mouse kidney cortex. Further supporting a critical role of SK1 in A1ARmediated renal protection, A1AR agonist failed to protect SK1-/- mice (Cr=2.7±0.1mg/dL,
N=6) but protected SK2-/- mice (Cr=1.2±0.2mg/dL, N=6, P<0.01) subjected to 30 min
of renal ischemia and 24 h of reperfusion. In addition, in vivo gene knockdown of S1P1
receptors with small interfering RNA (Cr=2.2±0.2mg/dL, N=6, P<0.01) or a selective S1P1
receptor antagonist W146 (Cr=2.4±0.2mg/dL, N=6, P<0.01) completely abolished the renal
protection provided by CCPA (Cr=1.2±0.2mg/dL, N=5). Finally, mice specifically deficient
in proximal tubule S1P1 receptors (S1P1Rflox/floxPEPCKCre/-, Cr=2.3±0.2mg/dL, N=6) were
not protected against renal IR with CCPA. Mechanistically, A1AR activation increased
HIF-1a nuclear translocation in HK-2 cells and in mouse kidney cortex. Furthermore,
2-methoxyestradiol (a selective HIF-1a inhibitor) blocked A1AR-mediated induction of
SK1 in HK-2 cells and in mouse kidney cortex.
Conclusions: Taken together, our data show that induction of proximal tubule SK1
and subsequent S1P1R activation is critical for A1AR-mediated renal protection. Selective
renal proximal tubular induction of SK1 and S1P1 receptor signaling after IR may provide
a novel therapeutic approach for the prevention and treatment of AKI.
Funding: NIDDK Support

TH-OR005
Activation of Proximal Tubule Sphingosine 1 Phosphate Receptor-1
(S1P1) Ameliorates Cisplatin Induced Nephrotoxicity in Mice Amandeep
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Charlottesville, VA; 3Center for Immunity, Inflammation and Regenerative
Medicine, University of Virginia, Charlottesville, VA.
Background: Sphingosine 1-phosphate (S1P), a sphingolipid that is the natural ligand
for a family of five G-protein coupled receptors (S1P1-5Rs), regulates cell survival and
lymphocyte circulation. The pan S1PR agonist, FTY720 (fingolimod) attenuates ischemiareperfusion injury (IRI) by activating S1P1 on proximal tubule-PT cells directly, an effect
previously thought to be due predominantly to their canonical effects of S1P1 activation
on B and T cells leading to lymphopenia.
Methods: FTY720 reduced cisplatin-induced AKI, therefore in the current study
we sought to determine whether the protective effect was mediated by proximal tubule
(PT) S1P1. We used conditional renal PT S1P1 null (PEPCK-CreS1pr1fl/fl) and control
mice (PEPCK-Cre) to assess renal injury by monitoring plasma creatinine (mg/dl), flow
cytometry to analyze inflammatory cell infiltration and Real Time RT-PCR for changes
pro-inflammatory cytokines.
Results: Compared to control mice (0.79±0.02) cisplatin induced more injury in PT
S1P1 null mice (1.22±0.13, p<0.05) and FTY720 reduced injury in control mice (0.47±0.06,
p<0.01) but not in PT S1P1 null mice (1.32 ±0.22, p=n.s.). There were no differences in

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circulating lymphocytes counts in FTY720 treated and control S1P1 null mice. Control or PTX3 null mice treated with cisplatin have significantly higher levels of pro-inflammatory cytokines (CXCL1, MCP-1, TNF-α and IL-6) compared to vehicle-treated mice. Treatment of control mice with FTY720 significantly attenuates the mRNA levels of aforementioned cytokines. Decrease in cytokine production with FTY720 results in less neutrophils and macrophages infiltration at day 3 in kidneys.

Conclusions: In summary, S1P1 expressed in PTX3 attenuates cisplatin-induced AKI. We conclude that FTY720 administration might represent a novel strategy in the prevention of cisplatin-induced AKI.

Funding: NIDDK Support

TH-OR006

IL-2/anti-IL-2 Antibody Complexes Attenuate Renal Ischemia-Reperfusion Injury through Expansion of Regulatory T Cells

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Background: Regulatory T cells (Tregs) can contribute to suppression of immunologic damage or facilitation of the recovery process following renal ischemia-reperfusion injury (IRI). However, isolation and expansion of rare Tregs are practically hard for clinical application. Recently, complexes (IL-2C) of interleukin-2 (IL-2) and anti-IL-2 antibodies have been shown to control various inflammatory diseases by inducing expansion of Tregs. Therefore, we investigated whether IL-2C can control renal IRI.

Methods: C57BL/6J mice underwent bilateral renal ischemia. IL-2C or vehicles were administered for 3 consecutive days from 5 days before or 1 day after renal IRI. Renal function was assessed with infiltration of inflammatory cells, tissue cytokines and tissue recovery. Anti-CD25 antibodies (PC61) were administered to assess the effect of DC depletion on impacts of IL-2C. We also performed IRI using IL-10 knockout mice to clarify the role of IL-10.

Results: IL-2C induced a 3-5 fold expansion of Tregs in both spleen and kidney. IL-2C treatment before renal IRI significantly improved renal function, attenuated histological renal injury and apoptosis. IL-2C significantly decreased expression of pro-inflammatory cytokines (MCP-1, IL-6) and also reduced infiltration of both neutrophils and macrophages. IL-2C treatment after IRI significantly increased tubular cell proliferation in recovery phase. Depletion of Tregs using PC61 abrogated the beneficial effects of IL-2C. When the IL-10 knockout mice were used, the beneficial effects of IL-2C were abrogated, and these results suggested that IL-10 might mediate the renoprotective effect of IL-2C.

Conclusions: IL-2C attenuated acute renal damage and also facilitated renal recovery in renal IRI, by inducing Tregs with an IL-10-dependent manner. Because IL-2C are easy to manipulate as well as effective for renal IRI, IL-2C is promising for clinical application to renal IRI.

TH-OR007

Pentraxin-3 Suppresses Postischemic Acute Kidney Injury by Inhibiting P Selectin-Mediated Renal Leukocyte Recruitment

Maciej Lech,1 Christoph Rommelke,1 Regina Groebnay,1 Cecilia Garlanda,1 Alberto Mantovani,1 Bernd Uh1,2 Christoph A. Reiche1,1 Fritz Krombach,1 Hans J. Anders,1' "Klinische Biochemie, Medizinische Poliklinik der LMU, Munich, Germany; 2Institut Clinico Humanitas, Rozzano, Italy; 3Walter Brendel Centre of Experimental Medicine, Munich, Germany.

Background: Ischemia-reperfusion injury is a neutrophil-dependent sterile inflammation that is unable to control the causative trigger and therefore induces unnecessary tissue damage. Little is known about the factors that limit postischemic inflammation, e.g. in acute kidney injury, a common medical problem associated with poor survival. The long pentraxin PTX3 regulates multiple aspects of host defense and tissue inflammation therefore we hypothesized that PTX3 would be involved in ischemia-reperfusion injury.

Results: PTX3 induction in postischemic kidneys was largely limited to CD11c+ renal dendritic cells. Lack of PTX3 aggravated postischemic acute renal failure and kidney injury as evidenced by massive tubular necrosis and TNF-α or IL-6 release. Remarkably, neutrophil and macrophage infiltrates were also massively increased in PtX3-deficient mice although intrarenal chemokine CXCL2 and CCL2 expression were independent of the PTX3 genotype. PTX3 rather modulated leukocyte recruitment via interacting with P-selectin as P-selectin inhibition completely abrogated the enhanced leukocyte recruitment and tissue injury in postischemic kidneys of PtX3-deficient mice. In-vivo microscopy revealed increased leukocyte adhesion and transmigration in postischemic microvessels of PtX3-deficient mice. Finally, intravenous injection of recombinant PTX3 shortly after surgery suppressed renal leukocyte recruitment and prevented postischemic kidney injury.

Conclusions: Together, PTX3 is an endogenous suppressor of ischemia reperfusion injury by blocking P-selectin-mediated adhesion of leukocytes to activate endothelia of postischemic tissues, a mechanism that can be mimicked with recombinant PTX3 for therapeutic purposes. Vice versa, PtX3 loss-of-function mutations may predispose to ischemia-reperfusion injuries such as postischemic acute renal failure.

TH-OR008

TRPM2 Channels Mediate Hypoxic Cell Death and Contribute to Ischemic AKI

William Brian weave,1 Weiwei Wang, GuoFeng Gao, Wenyi Zhang, Barbara A. Miller. Departments of Medicine and Pediatrics, Penn State College of Medicine, Hershey, PA.

Background: TRPM2 channels belong to the TRP family of non-selecive cation channels. TRPM2 channels are activated by TNF-α and by oxidant stress, and TRPM2 expression enhances susceptibility to cell death in certain in vitro settings. However, the role of TRPM2 in cell injury or cell death in vivo is unknown. Since TNF-α and oxidant stress have been implicated in the pathogenesis of AKI, we evaluated the role of TRPM2 channels in AKI.

Methods: We created mice with the TRPM2 gene flanked by loxP sites and then bred them with Ell-a-re mice which express Cre recombinase ubiquitously to create a global TRPM2 knockout mouse. The resulting mice developed normally and had normal kidney function and histology. Ischemic AKI was induced in the TRPM2 KO mice and control littermates by clamping both renal arteries for 24 minutes. Renal function was assessed by measurements of BUN and creatinine. Histology and histochemistry of kidney sections were used to measure structural injury, apoptosis and neutrophil infiltration. Gene expression was determined using quantitative RT-PCR.

Results: Ischemia-reperfusion injury in TRPM2 KO mice

<table>
<thead>
<tr>
<th>BUN</th>
<th>Creatinine</th>
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<tr>
<td>WT</td>
<td>TRPM2 KO</td>
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<tr>
<td>0 hrs</td>
<td>19±1</td>
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<td>6 hrs</td>
<td>59±12</td>
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<tr>
<td>24 hrs</td>
<td>118±19</td>
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BUN and creatinine levels at 6, 24 and 48 hrs (not shown) after ischemia were significantly lower in the TRPM2 KO mice compared to littermates. Likewise, tissue injury scores, apoptosis and kidney neutrophil infiltration at both 24 and 72 hrs post ischemia were all significantly lower in TRPM2 KO vs WT mice. Analysis of gene expression showed >2-fold increases in certain pro-survival genes, e.g. akt, peroxiredoxin-2 and BCL2L10, in kidneys of KO vs WT mice. Primary cultures of proximal tubule cells prepared from WT and TRPM2 KO kidneys were subjected to hypoxia by incubation in a GAS/Pak chamber for 24 and 48 hours. At both time points, cell survival in the TRPM2 KO cells exceeded that of the WT cells (<0.001).

Conclusions: These results point to an important role for TRPM2 channels, likely via direct effects in renal epithelial cells, in the pathogenesis of ischemic AKI.

Funding: Pharmaceutical Company Support

TH-OR009

Mice with Absent Heat Shock Protein Induction Are Protected Against Ischemic Renal Injury

Xiaokui Wei1, Shaozing Chen, Melody A. Miller, Dipica Haribhai, Calvin B. Williams, Scott K. Van Why. Pediatrics, Medical College of Wisconsin, Wauwatosa, WI.

Background: Heat Shock Factor 1 (HSF1) regulates inducible heat shock protein (HSP) expression and HSPs protect against renal cell injury in vitro. Whether HSPs provide protection against ischemic renal injury in vivo is unclear.

Methods: To determine whether inducible HSPs protect against ischemic renal injury in vivo, inducible HSP70 and 25 expression and renal injury from bilateral renal ischemia was studied in HSF1 functional knock-out mice (HSF KO) and compared with HSF wild type (WT).

Results: There was no difference between WT and HSF KO in baseline renal HSP70 or 25 levels. WT kidneys had the expected induction of HSP70 and 25 after 45 min of bilateral ischemia and 24 hr reflow, but KO mice had no induction of either HSP. Baseline serum creatinine was also comparable (0.22 mg/dL WT versus 0.19 mg/dL KO). Serum creatinine at 24 hrs reflow in WT was 2.1 mg/dL compared with 0.9 mg/dL in HSF KO (p=0.0002).

Flow cytometry was used to study mononuclear cells isolated from kidneys from both strains. In sham operated kidneys, there was no difference in number of CD4+ and CD8+ T cells in WT compared with HSF KO. However, after 45 min of bilateral ischemia and 1 hr reperfusion, CD4+ and CD8+ cells in WT kidneys increased by 58% (p=0.02) and 75% (p=0.08) respectively from uninjured controls. There was no significant change in either CD4+ or CD8+ cells (p=0.6 and 0.77 respectively) in the HSF KO kidneys after ischemia compared to uninjured controls. In addition, Fox p3+ T regulatory cells decreased by 40% in WT kidneys after ischemia, but did not change in HSF KO (p=0.02 WT versus KO).

Conclusions: This study demonstrates 1) HSP induction is completely absent in HSF1 KO mice kidneys subjected to ischemia, 2) absence of HSP induction, contrary to expectation, is associated with protection against ischemic renal injury in vivo, and 3) HSF KO mice kidneys have altered T-cell infiltration immediately following ischemia. So, inducible HSPs may contribute to early ischemic renal injury by facilitating T cell response.

Funding: NIDDK Support, Private Foundation Support
miR-21 Contributes to Renal Protection Conferred by Delayed Ischemic Preconditioning
Alison J. Kriegel,1 Niall Xu,1,2 Yong Ling,1 Kristie Usa,1 Domagoj Mladinov,1 Yi Fang,2 Xiaojing Ding,2 Mingyu Liang,2 Department of Pathology, Medical College of Wisconsin, Milwaukee, WI; Division of Nephrology, Fudan University Zhongshan Hospital, Shanghai, China.

Background: Delayed ischemic preconditioning (IPC) effectively protects kidneys from ischemia-reperfusion injury. We examined the role of miRNA miR-21 in the renoprotective effects of delayed IPC in the mouse kidney.

Methods: Mice were subjected to 15 min ischemic preconditioning and, 4 days later, 30 min bilateral renal ischemia and 24 h reperfusion. Tail vein injection of locked nucleic acid (LNA)-modified anti-miR (10 mg/kg) was used to knock down miR-21 in vivo. Renal injury was evaluated by the plasma creatinine, histological change, and TUNEL assay. A miR-21 target protein was analyzed by Western blot.

Results: A 15 min renal IPC substantially attenuated I/R injury induced 4 days later in mice, as indicated by decreases in plasma creatinine and histological damage score (P<0.05). Knockdown of miR-21 expression was increased in the delayed IPC + I/R group by 179% ± 17% compared to the Sham + I/R group (P<0.05; n=6/group). Time-course analysis indicated that miR-21 was upregulated at 4 h after IPC compared to the Sham group, and remained significantly higher 4 days after IPC (P<0.05 each time point; P>0.05). Administration of LNA anti-miR-21 at the time of IPC decreased miR-21 levels in the kidney detectable by real-time PCR by 98% (P<0.05; n=5-6/group), and significantly exacerbated functional and histological damage of subsequent I/R injury in the mouse kidney (P<0.05).

Conclusions: These results support a novel mechanism in which upregulation of miR-21 contributes to the protective effect of delayed IPC against subsequent renal I/R injury.

Funding: Other NIH Support - HL085267, DK084405, HL029587, and a CTSl grant, Government Support - Non-U.S.

TH-OR010

TH-OR011

Anti-TGF-β Antibody (ID11) May Attenuate High-Turnover Renal Osteodystrophy by Stimulating Sost Expression

Background: TGF-β plays an important role in normal bone remodeling. Its elevated expression in the bone marrow of patients with high-turnover renal osteodystrophy suggests that this growth factor may also contribute to the pathogenesis of bone disease in the setting of chronic kidney disease. We have previously reported that anti-TGF-β antibody (ID11) prevents the onset of high bone turnover in an adenine-induced rat model of CKD-MBD.

Methods: To further explore the potential efficacy and mechanism of action of ID11 on high-turnover renal osteodystrophy, we administered ID11 to JcK mice, a genetic model of CKD-MBD that had established high-turnover renal osteodystrophy. ID11 was injected IP at doses of 0.5, 2 or 5 mg/kg three times a week for 7 weeks.

Results: Bone histomorphometric analysis indicated that ID11 significantly suppressed the elevated bone turnover in a dose-dependent manner. To explore the possible mechanism of ID11’s action on bone, we examined expression of genes associated with key pathways regulating bone turnover using a TaqMan Low Density Arrays (TLDA). We have previously shown that expression of the Wnt antagonist, SOST, is elevated in early CKD in JcK mice and humans, but this increase is attenuated by rising PTH levels late in disease. In this study, Sost expression was significantly suppressed in JcK relative to wild-type littermate controls at 17 weeks of age (late in CKD). Importantly, ID11 treatment resulted in a significant increase in Sost expression in parallel with reduction in bone turnover despite elevated serum PTH levels.

Conclusions: Our data suggest that antagonism of TGF-β attenuates high-turnover renal osteodystrophy independent of changes in serum PTH levels and the effect of ID11 on bone may be at least partially mediated by stimulation of Sost expression.

TH-OR012

Sclerostin, a Bone osteoparcin Factor, Is Increased in Chronic Kidney Disease
Sclerostin, a Bone osteopenic Factor, Is Increased in Chronic Kidney Disease

Sclerostin is a small peptide secreted by osteocytes. Recent experimental data suggest that sclerostin blocks bone formation and better predicts bone turnover than alkaline phosphatase. We assessed the relationship between serum sclerostin and glomerular filtration rate (GFR) in CKD patients.

Methods: A prospective cohort of 86 patients (43 women, 7 diabetic) was studied. Sclerostin was measured by EIA (Biomedical, Wien). Normal values were 46.4 ± 23.3 pmol/L. Glomerular filtration rate was measured by the gold standard inulin clearance. Serum sclerostin was measured by EIA (Biomedical, Wien). Normal values were 46.4 ± 23.3 pmol/L.

Results: Mean age was 51 ± 19 years, GFR 63 ± 27 mL/min/1.73 m², sclerostin 68.8 ± 56.1 pmol/L, and serum phosphate 0.24 ± 0.04 mEq/L. Sclerostin was strongly and inversely correlated with GFR (r=-0.6, p<0.001), positively with age (r=0.38, p<0.001) and with serum phosphate (r=0.25, p=0.02).

Conclusions: We found a new and strong inverse relationship between sclerostin and GFR in CKD patients (Fig 1). Since sclerostin reduces bone turnover, and accumulates in CKD, this may suggest a role on renal osteodystrophy. As in postsenopausal osteoporosis, for which sclerostin antagonists have been developed, sclerostin might soon become a new player in CKD mineral and bone disease.

Funding: Other NIH Support - HL085267, DK084405, HL029587, and a CTSl grant, Government Support - Non-U.S.

TH-OR013

Decreased Trabecular Tissue Mineralization Is Associated with Fracture in Patients with Kidney Disease
Thomas L. Nickolas, Xiaowei Sherry Liu, Chiyuan Amy Zhang, Donald J. McMahon, Elizabeth Shane. Columbia University.

Background: In renal osteodystrophy (ROD), abnormal Vitamin D metabolism and remodeling cause defects to tissue mineral density (TMD), which affects tissue-level mechanical properties. Abnormal TMD may contribute to high fracture (Fx) risk in CKD without Fx. By digital topological analysis (DTA), Tb bone voxels were classified into one of 3 envelopes: surface (s), central (c) and intervening (middle; m); between surface and central, TMD was calculated for each envelope. Serum iPTH, 25OH and bone formation (BSAP, PINP) and resorption (CTX, Trap5b) markers were measured.

Results: Results: CKD-Fx, CKD-nonFx, and Controls were well matched for age, sex and race (all p>NS). TbTMD was significantly lower in CKD-Fx than Controls. At the DT, s- and m-TMD were lower by 2.6% and 3.0%, respectively (all p<0.05). At the DT, s- and c-TMD were lower by 2.2%, 2.6% and 3.1%, respectively (all p<0.05). TbTMD in patients with CKD-nonFx did not differ from that of either CKD-Fx or Controls. At the DT, each SD decrease in s-, m- and c-TMD was associated with 1.7%, 1.4% and 1.1% increased odds of Fx (p<0.05 for all). Higher levels of iPTH, BSAP, PINP and CTX were significantly and inversely correlated with s-, m- and c-TMD; r values ranged from -0.40 for PINP vs DT TMD (p=0.009) to -0.53 for iPTH vs DR sTMD (p=0.001).

Conclusions: CKD patients with Fx have low TbTMD that is likely related to SHPT and high bone turnover. HRpQCT may provide a noninvasive approach to measurement of TbTMD in CKD; validation against iliac crest bone biopsy is needed.

Funding: NIDDK Support

TH-OR014

The Pathogenesis of the Early CKD-MBD in Stage 2 CKD
Keith A. Huska, Yifu Fang. Pediatrics, Washington University School of Medicine, St. Louis, MO.

Background: CKD induces the CKD-MBD associated with increased mortality by stage 2. Vascular calcification (VC) is a strong risk factor in CKD. The CKD-MBD in stage 2 CKD consists of stimulation of VC, the onset of osteodystrophy, and an increase in FGF23 levels, while Ca, Pi and PTH levels remain normal. The increase in FGF23 serves as a biomarker of the CKD effect on the skeleton. We have linked the skeleton in CKD to stimulation of VC. The pathogenesis of the initial stimulus for osteocytic secretion of FGF23 is unclear. Early changes in phosphate homeostasis, changes in bone remodeling, and PTH are the candidate factors under investigation. Here we examine the actions of either modifying Pi balance through intestinal Pi binding or a skeletal anabolic factor (a neutralizing monoclonal antibody to a Wnt inhibitor, DK1) on the early CKD-MBD.

Methods: Male mice, 3-4 months old, were fed a diet containing 5% sodium chloride and partial kidney ablation at 12 weeks of age to create an early CKD-MBD mouse model. Treatment protocols with Vehicle, CaAc (3% w/w mixed in diet), or the DKK1 mab (30 mg/kg iv IP) were conducted from 14-22 wks or 22-28 wks.

Results: The reduction in serum measured by inulin clearance was 75% of normal (stage 2 CKD). Aortic Ca levels were increased by 22% at week 22.
and progressively increased to 28 wks. The serum levels of BUN, Ca, Pi and PTH were normal, but a transient elevation of PTH at 14 weeks was discovered. MicroCT of the femurs revealed cortical but not trabecular bone loss which was corrected by the DKK1 mab but not by CaAc therapy.

**Conclusions:** We conclude that an early osteodystrophy in CKD may be causatively associated with the stimulation of VC, and that the stimulus to FGF23 secretion is independent and may be Pi or PTH.

**Funding:** NIDDK Support, Pharmaceutical Company Support

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**TH-OR015**

**Effect of Calcium Carbonate Supplement on Phosphate Balance and Homeostasis in Patients with Stage 3 and 4 Chronic Kidney Disease**

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**Background:** In chronic kidney disease (CKD), calcium carbonate (CaCO3) given with meals is used to bind dietary phosphate (Pi) and thus lower plasma Pi. Data on the effect of CaCO3 on Pi balance and homeostasis in CKD are limited.

**Methods:** Three patients, of a projected eight, with stage 3/4 CKD participated in two 3wk balance studies using a randomized crossover design. Patients received 500 mg elemental Ca as CaCO3 or placebo with meals 3x/24h. Diets consisted of 957 mg Ca/24h and 1500 mg Pi/24h. Wk 1 was outpatient equilibration period. Patients were inpatients wk 2-3, and all 24h urine and feces were collected and analyzed for Ca & Pi. Ca & Pi balances were calculated as diet (mg/24h) – fecal (mg/24h) – urine (mg/24h). Fasting blood and urine were collected for biochemistries at the end of each week. Repeated measures ANOVA for were calculated as diet (mg/24h) – fecal (mg/24h) – urine (mg/24h). Fasting blood and urine were collected for biochemistries at the end of each week. Repeated measures ANOVA for Ca and Pi absorption and balance were compared between CaCO3 and placebo.

**Results:** FGF23 levels were decreased by CaAc but not by the DKK1 mab. Vascular Ca levels were decreased by the DKK1 mab but not by CaAc therapy.

**Conclusions:** CaCO3 given with meals decreases FGF23 levels and decreases vascular Ca levels in patients with advanced CKD.

**Funding:** Pharmaceutical Company Support

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**TH-OR016**

**Reduced Fracture Risk with Early Corticosteroid Withdrawal after Kidney Transplant: An Analysis of the USRDS**

Lukas Nikkel,1 Sumit Mohan,2 Chiyuan Amy Zhang,1 Donald J. McMahon,1 Stephanie Boutroy,3 Geoffrey K. Dube,4 Bekir Tanriverdi,1 David J. Cohen,1 Lloyd Ratner,4 Christopher S. Hollenbeck,5 Mary B. Leonard,1 Elizabeth Shane,1 Thomas L. Nickolas,6 1Columbia University; 2Children’s Hospital of Philadelphia; 3Penn State Hershey.

**Background:** Co-administration of calcineurin inhibitors (CNI) with corticosteroid (CS) after kidney transplant (KTx) results in severe bone loss and very high fracture (Fx) risk. Early CS withdrawal (ECSW) has not been associated with bone loss in comparison to CS based immunosuppression (CSBI) after KTx. We hypothesized that Fx rates in KTx recipients managed with ECSW are lower than with CSBI.

**Methods:** Using the United States Renal Data System (USRDS), 77,625 adults undergoing first KTx between January 1, 2000 and December 31, 2006 were identified. CS use was determined from UNOS immunosuppression forms completed at KTx in an intent-to-treat analysis. Fx after KTx was identified from hospitalization discharge ICD9 codes. Continuous characteristics (age, BMI, HLA mismatches) were compared by Students t tests and binary characteristics were compared by chi-square. Time to first Fx was modeled after KTx using Kaplan-Meier and Cox methods.

**Results:** Patients on ECSW differed slightly from those on CS in age (49.9 vs. 48.2 years, p<0.001), gender (62% male vs. 60%, p<0.001), race (69% white vs. 67%, p<0.001), BMI (27.1 vs. 26.6 kg/m2, p<0.001), and live donor (48% vs. 39%; p=0.001). 2395 Fx resulting in hospitalizations were observed during follow-up (307662 person-years); median (IQR) follow-up was 1448 (808-2061) days. There were 5.8 and 8.0 Fx per 1000 patient-year for ECSW and CSBI, respectively. Fx site distribution was similar between groups. Multivariate Cox models demonstrated a 31% (p=0.001) Fx risk reduction for ECSW versus CSBI after adjustment for other risk factors for Fx. By 24-months after KTx, Fx risk was significantly lower in the ECSW group.

**Conclusions:** ECSW is associated with a 31% reduction in serious Fx risk after KTx. Fx risk begins to decrease in ECSW patients by the second year after KTx. There is a need for prospective studies to understand how ECSW affects bone and mechanisms through which Fx risk is decreased.

**Funding:** Private Foundation Support

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**TH-OR017**

**Effect of Steroid Withdrawal on Growth, FGF23/Klotho and IGF-I/IGFBP3 Axis in Pediatric Kidney Transplantation**

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**Background:** Successful renal transplant improves growth in pediatric patients. Steroids immunosuppression has adverse effects on growth, bone metabolism and GH/IGF axis. The Fibroblast Growth Factor 23 (FGF23) is a phosphatonin produced by bone cells, which is active with its co-receptor Klotho. FGF23 reduces osteoblastic activity and matrix mineralization by autocrine/paracrine mechanisms. We hypothesized that steroids increase the FGF23/Klotho axis activity.

**Methods:** A prospective, randomized, multicenter study, steroid withdrawal on growth, FGF23/Klotho and IGF-I/IGFBP3 levels in pediatric kidney recipients (2-15 years) was evaluated. Six days post-Tx patients receiving tacrolimus (TAC) and mycophenolate mofetil (MMF) were assigned into two groups: steroid withdrawal (SW, n=10) and steroid control (SC, n=10). The ethics committee approved the protocol, written informed consent was obtained. Growth and biochemical parameters were measured up to 12 months post Tx. Data were presented as means±SD, student-t test, Mann-Whitney and Wilcoxon tests. Regression analysis for repeated measurements (mixed models) was performed.

**Results:** A significant improvement in height Z-score at 1 year post Tx in SW vs. SC group (-1.02±1.0 vs. -2.25±1.0; p<0.02) was observed. The SW group showed lower FGF23 levels as compared to SC (SW=5.83±3.1 pg/mL vs. SC=18.6±11.5 pg/mL; p<0.003) at 1 year post Tx; that were similar to those of healthy controls. Delta zIGF-I was higher and changes in delta IGF-I/IGFBP3 levels between groups were found. An inverse relationship between FGF-23 levels and growth was evaluated. Multivariate Cox models demonstrated a 31% (p<0.001) Fx risk reduction for ECSW versus CSBI after adjustment for other risk factors for Fx. By 24-months after KTx, Fx risk was significantly lower in the ECSW group.

**Conclusions:** ECSW is associated with a 31% reduction in serious Fx risk after KTx. Fx risk begins to decrease in ECSW patients by the second year after KTx. There is a need for prospective studies to understand how ECSW affects bone and mechanisms through which Fx risk is decreased.

**Funding:** Private Foundation Support
Ezin is essential for the Phosphate Reabsorption in the Renal Proximal Tubule

Ezin is a crosstalk between membrane proteins and actin cytoskeleton, and also known to be related with the membrane fusion of gastric vesicles in stomach. In the kidney, ezrin is mainly expressed in the apical membrane of proximal tubules and interacts with scaffold protein as NHERF1, which is essential for membrane localization of some transporters and receptors. The physiological roles of ezrin in the kidney are not still unraveled. Therefore, we used ezrin knockdown (Ezinkd/kd) mice as a model for the analysis of the physiological roles of ezrin in the kidney.

Methods: Ezrin knockdown mice was constructed as previously reported (Tamura A et al. J Cell Biol. 2005.). Male mice at 4 to 8 week age were kept in the metabolic cages for 7 days and daily urine was collected for the analysis of urinary excretion of phosphate and other substrates. These mice were sacrificed and kidney was collected for cages for 7 days and daily urine was collected for the analysis of urinary excretion of phosphate and other substrates. These mice were sacrificed and kidney was collected for the immunohistochemistry and western blot analysis. Right tieuae were also collected for the bone analysis.

Results: Whereas there is no apparent difference in the morphology of the kidney between adult wild type and Ezinkd/kd mice, Ezinkd/kd mice showed hypophosphatemia and abnormal bone formation caused by significant urinary loss of phosphate but not calcium. These phenotypes were shown even in the prepur Karnataka (4wk). Furthermore, we determined a decreased apical membrane localization of NaPi2a/phosphate cotransporter, NaPi2b, in the proximal tubules by western blot and immunofluorescence analysis.

Conclusions: These results suggest that ezrin is essential for the membrane localization of NaPi2a at the apical membrane in the proximal tubules and functional or genetic disorders of ezrin might be related with the onset of hypophosphatemic rickets.

Two classes of compounds are represented by NTX116 and NTX2128 with IC50s of 0.03 and 3.4 mM respectively. In the BBMV and everted sleeve techniques, NTX116 inhibited sodium-dependent Pi uptake by an IC50 of ~5 μM. Oral dosing rats with 100 mg/kg NTX2128 resulted in a >50% reduction in serum Pi in 3 days.

Using these NaPi2b inhibitors and a novel Pi-t (SLC20A1) inhibitor in the BBMV, everted sleeve, and ligated jejunal techniques, Pi-t was found to contribute less than 10% to total Pi uptake.

Ezrin knockdown mice (Ezinkd/kd) mice showed hypophosphatemia and abnormal bone formation caused by significant urinary loss of phosphate but not calcium. These phenotypes were shown even in the prepurine (4wk). Furthermore, we determined a decreased apical membrane localization of NaPi2a/phosphate cotransporter, NaPi2b, in the proximal tubules by western blot analysis and immunofluorescence analysis.

Conclusions: These results suggest that ezrin is essential for the membrane localization of NaPi2a at the apical membrane in the proximal tubules and functional or genetic disorders of ezrin might be related with the onset of hypophosphatemic rickets.
TH-OR023

Uregulation of the Ca\(^{2+}\) Channel TRPV5 by Uromodulin - A Potential Mechanism for Hypercalcuria with UMOD Mutations

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Background: Uromodulin (UMOD) encodes Tamm-Horsfall protein. UMOD mutations result in tubulo-interstitial nephropathies. A genome-wide association study linked hypercalciuria, which exceeds the degree of Na\(^+\) wasting, suggesting defective transcellular Ca\(^{2+}\) reabsorption in the distal nephron. We studied if UMOD regulates TRPV5, an apical Ca\(^{2+}\) channel in DCT and CNT critical for transcellular Ca\(^{2+}\) reabsorption.

Methods: We transfected TRPV5 and UMOD in HEK293 cells and analyzed TRPV5 current by whole cell patch-clamping.

Results: Coexpression of TRPV5 and UMOD showed increased TRPV5 current density (492±118 vs 294±104 pA/\(\mu\)F for UMOD plus vector; \(p=0.0005\)). TRPV5 upregulated TRPV5 extracellularly: supernatant from UMOD but not from vector-expressing cells - increased TRPV5 current density in TRPV5-transfected cells (856±148 vs 446±129 pA/\(\mu\)F for UMOD vs vector supernatant; \(p=0.0005\)). Biotinylation showed increased surface expression of TRPV5. Half-maximal effective concentration (EC50) for extracellular UMOD protein treatment in TRPV5-expressing cells was approximately 100 ng/ml, which is 100x lower than in human urine, underlying physiological significance.

Coexpression with disease-mutant UMOD resulted in significantly less TRPV5 current compared to wild type UMOD (344±98 vs 852±121 pA/\(\mu\)F for mutant vs wild type UMOD; \(p=0.0005\)). We analyzed if UMOD regulation of TRPV5 is dependent on TRPV5 N-glycosylation by cotransfecting UMOD with glycosylation-defective N358Q-TRPV5 or with wild type TRPV5. We found that UMOD did not increase N358Q-TRPV5 (453±111 and 422±104 for N358Q vector and N358Q-UMOD, respectively; \(n=3\)) while it increased wild type TRPV5 (446±90 and 802±110 for WT vector and WT+UMOD, respectively; \(p=0.0001\)).

Conclusions: We show TRPV5 upregulation by extracellular UMOD at physiological levels. TRPV5 upregulation requires N-glycosylation of TRPV5, and is impaired by UMOD mutations. We suggest that in UMOD mutations reduced UMOD secretion from the TAL decreases TRPV5-mediated Ca\(^{2+}\) reabsorption in the distal nephron contributing to hypercalcuria.

Funding: NIDDK Support, Other NIH Support - Pediatric Scientist Development Program

TH-OR024

Kidney-Specific Calcium-Sensing Receptor (CaSR) Deficient Mice Display PTH-Independent Hypocalciuria and NKCC2 Activation

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Background: The calcium-sensing receptor (CaSR) is a central player in regulating Ca\(^{2+}\) homeostasis. Various studies have shown that rare loss-of-function mutations in the CaSR can lead to decreased urinary calcium (Ca\(^{2+}\)) excretion in the context of relative hyperparathyroidism and hypocalciuria. In addition to playing a central role in regulating PTH release, evidence suggests that CaSR is directly involved in regulating calcium handling independently of PTH. The current study was designed to specifically examine the role of CaSR in the renal tubule.

Methods: We generated a conditional CaSR knockout (KO) mouse with targeted inactivation of exon 3 using the Cre/Lox system. A nephron-specific knockout of CaSR was generated by crossing CaSR flox/flox mice with animals expressing Cre recombinase in the CaSR can lead to decreased urinary calcium (Ca\(^{2+}\)) excretion in the context of relative hyperparathyroidism and hypocalciuria. In addition to playing a central role in regulating PTH release, evidence suggests that CaSR is directly involved in regulating calcium handling independently of PTH. The current study was designed to specifically examine the role of CaSR in the renal tubule.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Funding: Other NIH Support - Program Project

TH-OR025

Claudin -16-Mediated Renal Magnesium Transport Is Transcriptionally Inhibited by 1,25(OH)\(_2\)D via a Calcium Sensitive Receptor-Dependent Mechanism

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Background: The role of the 1,25(OH)\(_2\)D/Vitamin D in renal Mg\(^{2+}\) handling is obscure. The bulk of filtered Mg\(^{2+}\) is reabsorbed in the TAL via the tight junction protein, claudin-16. We have shown that Mg\(^{2+}\) depletion increases and 1,25(OH)\(_2\)D inhibits claudin-16 gene (CLDN16) transcription. We aimed to further explore the molecular mechanisms underlying the effect of 1,25(OH)\(_2\)D/Vitamin D on claudin-16.

Methods: Adult mice received 1.25(OH)\(_2\)D or 1.25(OH)\(_2\)D plus low Mg\(^{2+}\) diet for 3 days, and kidneys were harvested. HEK293 cells were exposed to treatments 1 and 2, for 24 hours. A luciferase reporter vector containing 2.5 kb human CLDN16 (CLDN16) 5’ flanking DNA sequence, was transfected into CaSR-devoid HEK293 cells (HEK293). HEK293 cells transfected with CaSR (HEK-CaSR), CaSR-harboring OK cells (OK), and OK cells transfected with a dominant negative CaSR construct (OK-DN-CaSR).

Results: Treatments 1 and 2, in mice increased CaSR mRNA in kidneys. Treatment 1, increased claudin-16 mRNA and protein in kidneys but had no effect on claudin-2 mRNA. Treatment 2, reversed the expected increase in claudin-16 mRNA and protein in Mg\(^{2+}\) depleted kidneys. HEK293 cells treated with 1, and 2, showed the same pattern of changes in claudin-16 mRNA as in mice, and no influence on mRNA of the Ca\(^{2+}\) channel, TRPM6. Exposure of transfected HEK293 cells to 1,25(OH)\(_2\)D/Vitamin D minimally decreased hCLDN16 promoter activity but markedly inhibited it in HEK-CaSR cells. A 1,25(OH)\(_2\)D/Vitamin D induced inhibition of hCLDN16 promoter activity in OK cells was completely abolished in OK-DN-CaSR cells. Furthermore, 1,25(OH)\(_2\)D/Vitamin D decreased hCLDN16 promoter activity in Mg\(^{2+}\) depleted HEK293 cells.

Conclusions: In conclusion, 1,25(OH)\(_2\)D/Vitamin D inhibits paracellular, claudin mediated renal Mg\(^{2+}\) transport at the transcriptional level via a CaSR dependent mechanism. The 1,25(OH)\(_2\)D/Vitamin D-induced repression of renal Mg\(^{2+}\) transport may serve as an adaptive mechanism to the 1,25(OH)\(_2\)D/Vitamin D-induced increase in intestinal Mg\(^{2+}\) absorption.

Funding: Government Support - Non-U.S.
Activation of the Calcium-Sensing Receptor Induces Tight Junction Formation in MDCK Cells

Cooperating factors are important for the development of stable epithelial tight junctions (TJ), and we hypothesized that CaSR contributes to the assembly of TJ during epithelial cell polarization.

**Methods:** We assessed the level of CaSR expression in Madin-Darby Canine Kidney (MDCK) cells from both type I, i.e. neomycin (1mM), colchicine (100µM), and type II, i.e. R-568 (800nM), CaSR agonists facilitated increases CaSR expression. Exposure of MDCK cells to both type I, i.e. neomycin (1mM), gadolinium (100µM), and type II, i.e. R-568 (800nM), CaSR agonists facilitated TJ formation under normal Ca2+ conditions, as measured by the relocation of Zonula Occludens 1 (ZO-1) and occludin. Moreover, CaSR activation in the absence of Ca2+ initiated TJ assembly, and this effect occurred without inducing the phosphorylation of MAP-activated protein kinase. Co-immunoprecipitation studies further confirmed that CaSR activation increases the interaction between ZO-1 and the Ca2+-dependent protein, α-actinin-1. By contrast, CaSR inhibition by NPS-2143 (800nM) significantly decreased ZO-1/α-actinin-1 interaction and reduced ZO-1 deposits at cell surface following exposure to culture media containing a 200 µM Ca2+ concentration.

**Conclusions:** These results suggest that CaSR plays a role in the regulation of TJ assembly during epithelial cell polarization.

**Funding:** Other NIH Support - NINDK17433

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**TH-OR027**

PINCH-1 and -2 Are Essential for Podocyte Adhesion, Shape Modulation and Maintenance of Glomerular Filtration Barrier Function

**Results:** PINCH-1 and -2 are components of the actin cytoskeleton. The importance of Tln1 in podocyte biology is poorly understood.

**Methods:** We used cells expressing GLEPP1 and nephrin, podocin, src, fyn, β-arrestin2, or paxillin. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin- GFP were fixed and permeabilized with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin-GFP were fixed and permeabilized with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin-GFP were fixed and permeabilized with subsequent western blot analysis was performed. For immunofluorescence, cells expressing GLEPP1 and paxillin-GFP were fixed and permeabilized with subsequent western blot analysis was performed.

**Conclusions:** These results demonstrate essential roles for PINCH proteins in maintenance of podocyte integrity and the glomerular filtration barrier, highlighting the importance of studying the role of PINCH proteins in human glomerular disease.

**Funding:** NIDDK Support

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**TH-OR029**

Loss of Talin 1 in Podocytes Results in Severe Progressive Albuminuria and Kidney Failure

**Background:** Talin 1 (Talin) is a focal adhesion protein, linking beta integrins to the actin cytoskeleton. The importance of Talin 1 in podocyte biology is poorly understood. The actin-based Unipod system, we generated podocyte-specific Talin 1 KO mice. Albuminuria and creatinine were measured by ELISA. Kidney histology was analyzed by H&E, PAS, trichrome, and electronmicroscopy (EM). Apoptosis was assessed by TUNEL. Primary podocytes were isolated by Dynabeads and sieving. Adhesion was assessed by crystal violet staining. Immunofluorescence was performed for phalloidin and actin related proteins.

**Results:** TH-1KO mice were born in the expected mendelian frequency. Compared to wild type controls, KO albuminuria progressed at 3 weeks (p=0.03, vs WT; KO: 7.3±0.4, n=9; p=0.01), progressive albuminuria which started 2 weeks after birth (mg albumin/mg creatinine (WT vs KO): 2 weeks: 21.46±3.29 vs 942.10±69.42; 4 weeks: 16.60±0.97 vs 1357.00±72.08; 6 weeks: 15.68±0.19 vs 1798.00±115.60; 8 weeks: 18.04±3.23 vs 2021.60±469.93, p<0.05); and kidney failure (serum creatinine (mg/dl) vs KO): 2 weeks: 0.25±0.15 vs 0.72±0.33; 3 weeks: 0.21±0.13 vs 0.96±0.25; 8 weeks: 0.24±0.12 vs 1.25±0.35, p<0.001. The majority of TH-1 KO mice were dead by 8 weeks. Histological examination in TH-1 KO mice revealed glomerular capillary loop dilatation as well as glomerulosclerosis and interstitial fibrosis. Furthermore, GLEPP1 enhances the foot process effacement and basement membrane thickening. TUNEL staining of kidney sections demonstrated no significant difference in podocyte apoptosis between KO and WT mice at P14. Isolated podocytes from KO demonstrated modest reduction in adhesion on type II collagen and beta 1 integrin activation (OD: WT: 0.28±0.03, KO: 0.19±0.02, n=6, p=0.01). However, TH-1 KO podocytes had a major reduction in stress fiber formation compared to WT.

**Conclusions:** Loss of Talin 1 specifically in mice podocytes results in severe albuminuria progressing to kidney failure and death by 8 weeks of age, suggesting its fundamental role in regulating the structural integrity of the glomerulus.

**Funding:** NIDDK Support

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**TH-OR030**

Requisite Role for Nephrin Tyrosine Phosphorylation in Podocyte Morphology and Response to Injury

**Methods:** These results suggest that CaSR plays a role in the regulation of TJ assembly during epithelial cell polarization.

**Funding:** Other NIH Support - NINDK17433

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**TH-OR031**

GLEPP1 Reduces Endocytosis of Nephrin

**Results:** GLEPP1 reduces nephrin phosphorylation and recruits adaptor proteins, such as Nck, to the slit diaphragm, where they link nephrin to the actin cytoskeleton. However, the physiological significance of nephrin tyrosine phosphorylation and its role in the development of glomerular disease is not well understood.

**Funding:** Private Foundation Support, Government Support - Non-U.S.

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
increase nephrin tyrosine phosphorylation at its C-terminus. Additionally, GLEPP1 seems to force cytoskeletal changes which might be of importance maintaining the delicate shape of healthy foot processes. Via this mechanism, GLEPP1 may support the integrity of the slit diaphragm.

TH-OR032

Intravitral Multiphoton Imaging of Mouse Podocytes  Mark Fadi Khayat,1 Charbel C. Khouros,1 Tet-Kin Yeo,1 Petr Pyagy,1 Weiming Yu,1 Sheldon Chen,1 Nephrology/Hypertension, Northwestern University, Chicago, IL;2 Physiology, Northwestern University, Chicago, IL.

Background: Multiphoton intravital microscopy (MPIM) is an ideal methodology, due to its minimal phototoxicity, to image podocytes in their native glomerular environment. However, most glomeruli in the mouse lie beyond the maximum imaging depth of MPIM in the kidney, which is 150 to 200 µm. We intend to show that intravitral imaging of murine podocytes is now feasible with the nephron knockout/green fluorescent protein (GFP) knock-in mouse (Nphs1tm1Rkl/J).

Methods: The Nphs1tm1Rkl/J mouse was genetically designed to have the nephron promoter restrict GFP expression to the podocyte in the kidney. Under anesthesia, the mice were perfused with 300-KDa Texas Red dextran via the retro-orbital sinus, using a 28G insulin syringe. The left kidney was then exposed and imaged through a custom-built window on an upright multiphoton microscope.

Results: Optical sections of the renal cortex revealed green fluorescent podocytes, clearly resolvable down to their primary processes, as they lined the glomerular capillaries perfused with Texas Red dextran. In the female Nphs1tm1Rkl/J mouse, the glomerular depth was as shallow as 60 µm. This allowed detailed intravitral microscopy of an intact kidney, avoiding the need for surgical sectioning of the renal cortex and enabling repeat imaging in the same mouse. Multiple time-lapse series of different glomeruli were collected, typically for hours per session, while maintaining viability of the mouse.

Conclusions: To our knowledge, this is the first time that intrinsically fluorescent podocytes have been imaged intravitally in an intact kidney, made feasible by the proximity of the glomeruli to the kidney capsule. Our technique offers the opportunity podocytes have been imaged intravitally in an intact kidney, made feasible by the proximity of the glomeruli to the kidney capsule. Our technique offers the opportunity to add a visual and temporal component to the study of podocytopathies in the multitude of diseases that affect podocytes. This method can be used for the study of mouse models.

Funding: NIDDK Support

TH-OR034

Inhibition of mTOR in Mice Alters Autophagic Flux in Podocytes and Other Renal Cell Types Davide Pietro Cima,1 Tuncer Onay,1 Aarti Palto,2 Javier De Artega,3 Chengjin Li,2 Susan E. Quaggin.1,2 1Faculty of Medicine, University of Toronto, ON, Canada; 2Samuel Lunenfied Research Institute, Mount Sinai Hospital, Toronto, ON, Canada; 3Division of Nephrology, Hospital Privado, Cordoba, Argentina.

Background: Inhibitors of the mammalian target of rapamycin (mTOR inhibitors) belong to a family of drugs with potent immunosuppressive, anti-angiogenic and anti-proliferative properties. Although they are approved for the treatment of a number of renal diseases, the use of mTOR inhibitors has been associated with a significant incidence of de novo or worsening proteinuria.

Methods: Here we explore the mechanism of proteinuria induced by mTOR inhibition through the generation and characterization of mice carrying a podocyte-selective knockout (mTOR pod-KO) or global postnatal knockout of the mTor gene resulting in a loss of both mTORC1 and mTORC2 functions.

Results: Our results show that while mTOR is dispensable in developing podocytes, mTOR pod-KO mice develop nephrotic-range proteinuria by 3 weeks of age, and progress to endstage renal failure by 5 weeks of age. Human podocytes treated with an mTOR inhibitor rapamycin accumulate autophagolysosomes, damaged mitochondria and increased levels of reactive oxygen species. In vivo, podocytes of mTOR pod-KO mice exhibit an accumulation of the autophagosome marker LC3 (rat microtubule-associated protein 1 light chain 3), autophagosomes, autophagosomal vesicles and damaged mitochondria. Global postnatal deletion of mTOR affects all renal cells leading to marked enhancement of renal injury, demonstrating that mTOR function is not only important for podocytes, but also for the tubular epithelium.

Conclusions: Taken together, our results suggest that disruption of the autophagic pathway may play a role in the pathogenesis of proteinuria in patients treated with mTOR inhibitors and that multiple renal cell types may be affected.

Funding: Government Support - Non-U.S.

TH-OR035

Translational Profiling of Podocytes in Focal Segmental Glomerulosclerosis Ivica Giric,1 Giulio Genovese,2 Martin P. Pollak,2 Benjamin D. Humphreys,1 Brigham and Women’s Hospital, Boston, MA;2 BIDMC, Boston, MA.

Background: Identifying new biomarkers and therapeutic targets for podocytopathies such as focal segmental glomerulosclerosis (FSGS) requires a detailed understanding of the earliest events in disease pathogenesis. Transcriptional profiling has already provided important insight into gene expression changes in FSGS. Yet, a key limitation of this approach to define the podocyte transcriptome in FSGS has been the analysis of whole glomerular mRNA rather than podocyte-specific mRNA.

Methods: We have engineered a novel transgenic mouse line that allows extraction of podocyte-specific mRNA from whole kidney by translating ribosome affinity purification (TRAP). In this model, a GFP-tagged ribosomal protein (eGFP-L10a) was expressed under the control of the collagen 1α1 promoter.

Results: Expression of eGFP-L10a was restricted to podocytes in kidney cortex. Expression analysis of TRAP-isolated RNA by whole genome arrays confirmed robust enrichment of podocyte-specific mRNA (podocin: ∼60-fold, nephrin: ∼32-fold, MYH19: ∼47-fold compared to whole cortex), permitting establishment of a podocyte expression fingerprint. We next crossed col1α1-L10a mice with actn4+/- and mutant actn4/K228E mice to analyze expression profiles of podocytes in a genetic form of FSGS. RNA was extracted by TRAP from cortex of biegenic col1α1-L10a mice with actn4+/- and mutant actn4/K228E and K228E. Global postnatal deletion of col1α1-L10a littermate controls at 2 and 6 weeks of age. Regression analysis comparing podocyte expression profiles between genotypes with age as a covariate identified novel differentially regulated genes, including Cxcl11 (∼12-fold up, p < 0.0001) and Gadd45b (∼5-fold up; p < 0.0001) for actn4+/- vs actn4+/- and DMPK (∼5-fold up; p < 0.0001) for actn4+/- vs K228E. We confirmed some of the largest changes in gene expression at the protein level by immunostaining.

Conclusions: In conclusion, we have developed a new approach to define podocyte-specific gene expression in vivo. We used this technique in a model of FSGS and identified new candidate genes that hold promise in elucidating the earliest signaling events in FSGS, and thereby provide potential new therapeutic targets for FSGS.

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TH-OR036

Multiphoton Imaging of the Development of Glomerulosclerosis In Vivo

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Background: The development of podocyte injury/dysfunction and albuminuria in glomerulosclerosis (GS) is still incompletely understood due to technical limitations in studying the glomerular filtration barrier (GFB) in its native environment in vivo. We aimed to directly visualize the early morphological and functional changes in GFB using in vivo multiphoton microscopy (MPM) and the parmycin (PAN) model of focal segmental GS.

Methods: Munich-Wistar-Frömter rats (200 g) were injected 100 mg/kg body weight PAN ip and surgically instrumented for MPM 4-10 days later. The plasma was labeled using albumin-Atto565, and Lucifer yellow (LY) was infused into the carotid artery to visualize GFB elements.

Results: Hypertrophy and cystic dilations of the sub-podocyte space were present in several, but not all podocytes 4 days after PAN. The largest podocyte cysts, as the core structure, formed small, focal adhesions of the glomerular tuft to the parietal Bowman’s capsule (syncytia) 7-10 days after PAN. The thin podocyte wall of these core cysts were highly permeable to albumin indicated by the intense red fluorescence of cyst fluid. Intact, healthy podocytes (normal shape and no albumin permeability) were also observed making contact with parietal cells in other glomerular regions, but never developed syncytia. Numerous new, densely packed small cells that appeared to be healthy and migrating were observed on the outside of the core cysts, forming a continuous cell layer (bridge) between podocytes and parietal cells. Interestingly, most syncytia were positioned next to a collecting duct segment; intense infiltration of immune cells with high albumin uptake was visible in this region of the periglomerular interstitium. Similar results were obtained in 3-5mo old MWF rats, a model of spontaneous FSGS.

Conclusions: In several animal models of GS, high albumin permeability is focal, restricted to the most injured podocytes and to the area of syncytia. The formation of syncytia appears to involve multiple intra- and extraglomerular cell types. Signs of podocyte repair (replacement) were evident, but their mechanism needs further study. Funding: NIDDK Support

TH-OR037

Novel Concepts in Nephrotic Syndrome: Angiopoietin-Like 4 Induced Hypertiglycridemia Results from a Multisystem Effort To Reduce Proteinuria

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Background: Hypertiglycridemia is a cardinal feature of nephrotic syndrome. A recent study (Clement Nature Medicine 2011) shows that podocyte secreted, but not circulating Angiopoietin-like-4 (Angptl4) also noted in minimal change disease, induces proteinuria. Here, we investigated the role of circulating Angptl4 in nephrotic syndrome.

Results: Circulating Angptl4 is known to inhibit endothelium bound lipoprotein lipase, which reduces tissue uptake of triglycerides from circulation, resulting in hypertiglycridemia. We induced the gamma2-NTS and LPS models of proteinuria in Angptl4 +/- and +/- mice. Angptl4 +/- mice did not develop hypertiglycridemia despite significant residual proteinuria, suggesting that Angptl4 is the key determinant of hypertiglycridemia in nephrotic syndrome. Next, we studied albuminuria in adipose tissue specific Angptl4 transgenic rats (NPHS2-Angptl4), we determined that beyond the initial stages, the bulk of the circulating Angptl4 is contributed to by adipose tissue. Immunogold EM showed binding of Angptl4-V5 to glomerular endothelial surface. Cultured rat glomerular endothelial cells were protected from oxidative stress by recombinant neutral pl Angptl4 similar to that present in the circulation (P<0.001). By inducing puromycin nephrosis in podocyte specific Angptl4 transgenic rats (NPHS2-Angptl4), we determined that beyond the initial stages, the bulk of the circulating Angptl4 is contributed to by peripheral tissues, predominantly adipose tissue. In addition, injection of recombinant neutral pl Angptl4 reduces proteinuria in multiple animal models of proteinuria.

Conclusions: Our studies suggest that in later stages of nephrotic syndrome, adipose tissue secretes Angptl4 into the circulation to reduce proteinuria by glomerular endothelial stabilization, but this increase in circulating Angptl4 also results in hypertiglycridemia. Funding: NIDDK Support

TH-OR038

Acute Podocyte VEGF-A Knockdown Disrupts αvβ3 Integrin Signaling in the Glomerulus

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Methods: To study the molecular mechanism involved in the pathogenic effects of podocyte VEGF-A knockdown, we developed a mouse model that silences all VEGF-A isoforms in the podocyte using an inducible shRNA approach, and we generated an immortalized podocyte cell line (VEGFΔβ3) that downregulates VEGF-A upon doxycycline exposure.

Results: Tet-O-siVEGF podocin-rtTA (siVEGF) mice express VEGF shRNA in podocytes in a doxycycline-regulated manner, decreasing VEGF-A mRNA and VEGF-A protein levels in isolated glomeruli to ~20% of non-induced controls and urine VEGF-A to ~30% of control values a week after doxycycline induction. VEGF knockdown in adult siVEGF mice causes acute renal failure and proteinuria, associated with decreased glomerular volume, mesangiolysis and microaneurysms. Glomerular ultrastructure revealed endothelial cell swelling, GBM lamination and podocyte effacement. VEGF knockdown downregulates podocyte fibronectin and glomerular endothelial αvβ3 integrin in vivo, as determined by immunoblotting and dual-labeling immunohistochemistry. Co-immunoprecipitation showed that VEGFR2 interacts with β3 integrin and neuropilin-1 in the kidney and in cultured podocytes. Podocyte VEGF knockdown disrupts αvβ3 integrin activation in glomeruli, as determined by WOW1 Fab immunolabeling, while VEGFR2-αvβ3 integrin interaction and phosphorylation remain intact. VEGF silencing in cultured VEGFΔβ3 podocytes downregulates fibronectin and disrupts αvβ3 integrin activation cell-autonomously.

Conclusions: 1) In vivo podocyte VEGF-A regulates αvβ3 integrin signaling in the glomeruli; 2) decreased autocrine and paracrine VEGFR2 signaling induced by podocyte VEGF knockdown disrupts VEGFR2-αvβ3 integrin crosstalk and damages the three layers of the glomerular filtration barrier, resulting in proteinuria and acute renal failure. The present studies uncover a specific molecular mechanism mediating VEGF-A requirement in the adult glomerulus, i.e. activation of αvβ3 by VEGFR2 signaling. Funding: NIDDK Support

TH-OR039

TGF-β1 Signals through Smad4 to Diversely Regulate Renal Inflammation and Fibrosis by Impairing TGF-beta/Smad3 and Smad7

Transcriptionally Xiaoming Meng, Xiao Ru Huang, Jun Xiao, Wei Qin, Haiyang Chen, Arthur Chi-Kong Chang, Hui Y. Lan. Department of Medicine and Therapeutics, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, NT, Hong Kong, China.

Background: TGF-β1 plays distinct roles in renal inflammation and fibrosis, but signaling mechanisms by which TGF-β1 diversely regulates these processes remain largely unclear. The present study tested the hypothesis that Smad4 may be a key regulator of TGF-β1 signaling in renal disease.

Methods: We first generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte. We next generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte. We next generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte. We first generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte. We next generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte. We first generated the kidney-specifically conditional Smad4 KO mouse by crossing Smad4fl/fl with KspCre mouse in which Smad4 was specifically deleted from podocyte.

Results: Disrupted Smad4 significantly enhanced renal inflammation by a much greater CD45+ leukocytes(23%) & F4/80+ macrophage infiltration (60%↑) and upregulation of IL-1β, TNFα, and MCP-1 in the UUO kidney and in IL-1β-stimulated macrophages (all p<0.05). In contrast, deletion of Smad4 inhibited kidney fibrosis and TGF-β1-induced collagen 1 expression by fibroblasts (p<0.01). Further studies revealed that loss of Smad7 and its responsive promoter activity, thereby inhibiting IkBα expression while enhancing NF-κB activation, were a central mechanism by which disrupted Smad4 promoted renal inflammation. Interestingly, impaired Smad3 transcription such as Smad3 responsive promoter activities and the binding of Smad3 to collagen promoter (COL1A2), but not Smad3 activation, was an underlying mechanism whereby disrupted Smad4 blocked fibrosis in vivo and in vitro.

Conclusions: TGF-β1 acts by stimulating Smad4 to diversely regulate renal inflammation and fibrosis. Transcriptionally impaired Smad7-dependent anti-inflammatory activity and Smad3-mediated fibrogenesis may be key mechanisms by which disrupted Smad4 enhances renal inflammation while inhibiting fibrosis in vivo and in vitro. Funding: Government Support - Non-U.S.
**TH-OR047**

Dot1 Deficiency Facilitates Derivation of Renal Intercalated Cells from AQP2-Positive Cells and Increases Water Excretion

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**Background:** The mammalian kidney has three distinct types of collecting duct cells: principal cells (PC), α-intercalated cells (α-IC) and β-intercalated cells (β-IC), which are responsible for sodium and water balance, acid secretion, and bicarbonate secretion, respectively. The epigenetic players regulating differentiation of these cells remain elusive. Histone H3 K79 methylation Dot1 may regulate this process as it is expressed in PC in the adult kidney.

**Methods:** To test this hypothesis, the Cre-LoxP system was used to develop a mouse model lacking Dot1 in AQP2-expressing cells (Dot1f/l/AQP2Cre). Double immunofluorescence staining and metabolic analysis were performed to identify the cellular and renal phenotype.

**Results:** With AQP2 and V-ATPase subunits B1/B2 as PC and IC markers, respectively, we found that the mutant mice had ~20% fewer PC in cortex, outer medulla and inner medulla vs controls. This change was coupled with a similar increase in α-IC, featured by AE1 expression. Dot1 deletion in PC abolished histone H3 K79 methylation and had no effect on total H3 or methylation of all other H3 residues tested. Unexpectedly, 68-75% of ICs in Dot1 mutant mice also had no detectable H3 K79 methylation. At least some of these IC also expressed other IC markers (V-ATPase subunit A and carbonic anhydrate II) and/or the β-IC marker pgk-1. In contrast, all PC and IC in controls had H3 K79 methylation. The mutants had significantly increased urine volume (by 40%), 18% decreased urine osmolarity, and 12% lower urinary [Na+] vs. controls.

**Conclusions:** We show here that Dot1 is solely and specifically responsible for K79 methylation in mouse kidney, and at least some α-IC and β-IC may be derived from AQP2-expressing progenitor cells or can be derived from mature PC. We have, in conclusion, identified Dot1 as a new regulator of PC and IC differentiation and, thus, water, Na+ and possibly pH regulation.

**Funding:** NIDDK Support, Private Foundation Support

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**TH-OR048**

CTGF Influences Blood Pressure and Albumin Excretion in Mice

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**Background:** CTGF (CCN2) is a pleiotropic growth factor belonging to the 30-40kDa CCN family of proteins that have been shown to exhibit a diverse array of cellular effects. We, and others, have shown that reduced ctgf expression attenuates the response to hypoxia and glucose.

**Methods:** CTGF protein levels are increased in resistance and capacitance vessels in spontaneously hypertensive rats (SHR) and maneuvers that reduce blood pressure also lead to reduced CTGF expression. Given the role of CTGF in extracellular matrix production, it is proposed that ctgf expression will impact on glomerular physiology and function.

**Results:** The potential effect(s) of CTGF on blood pressure, albumin excretion and kidney histopathology was tested in mice models. We studied CTGF knockout and gene-duplicated mice, both generated through gene targeting using standard gene-targeting methodologies.

**Results:** We observed a graded increase in CTGF gene expression with increasing gene copy number. There was no significant difference in expression between mice or mixed embryonic fibroblasts (MEFs) with 3 and 4 copies of ctgf suggesting a possible plateau effect in which expression does not increase above a specific number of copies of ctgf gene. Analysis of blood pressure by automated tailcuff method revealed an increasing plateau effect in which expression does not increase above a specific number of copies of ctgf gene. A significant increase in blood pressure in this line of mice. There was attenuation of basal albumin excretion rate (AER) in CTGF heterozygotes and increased excretion in 3-copy animals compared to 2-copy (wt) mice. In 9 month mice, there was evidence of increased interstitial fibrosis in kidney sections from 3- and 4-copy animals compared with wildtype and 2-copy (wt) mice. In 9 month mice, there was evidence of increased interstitial fibrosis in kidney sections from 3- and 4-copy animals compared with wildtype and 2-copy (wt) mice. In 9 month mice, there was evidence of increased interstitial fibrosis in kidney sections from 3- and 4-copy animals compared with wildtype and 2-copy (wt) mice.

**Conclusions:** These observations suggest that CTGF expression may be a determinant of blood pressure and kidney function in mice.

**Funding:** Other NIH Support - NHLBI, NIDDK, Private Foundation Support

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**TH-OR049**

The Role of Erythropoietin Receptors in Kidney Disease Progression

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**Background:** Erythropoietin (EPO) is a hormone indispensable for red blood cell production and is produced by the kidneys in the fibroblast-like cells in the kidney and kidney cortex. In addition to the role in erythropoiesis, potential protective activity of EPO is reported in various tissues. EPO binds to receptor complex consisting of EPO receptor (EPOR) and β common receptor (CD131) subunit for tissue protective effect, while it binds to homodimeric EPO for maturation of erythrocytes. However molecular mechanism and the contribution of endogenous EPO in tissue protection is unclear.

**Methods:** In the present study, we analyzed the renoprotective function of endogenous EPO utilizing EPO knockout mice.

**Results:** First we analyzed the expression of EPO receptors during kidney injury. The expression of EPO receptors was decreased whereas the expression of CD131 is increased in mouse models of kidney disease. We further demonstrated that the administration of EPO significantly ameliorated renal dysfunction, and restored the expression of EPO in cisplatin nephrotoxicity.

**Conclusions:** We observed increased renal phenotypes of EPO null mutant mice expressing EPO in hematopoietic lineage (EpoR+/−/Gc). In the baseline analysis, kidney function, blood pressure and histological observation in the kidney of EpoR+/−/Gc mice was indistinguishable from those of EpoR+/−/Gc mice, while serum EPO concentration of EpoR+/−/Gc mice was higher than that of EpoR+/−/Gc mice. EPO expression in the kidney, liver, brain and tests was indistinguishable between both genotypes, suggesting that elevated serum EPO concentration in EpoR+/−/Gc mice might be due to lack of endocytosis via EPO. We also induced cisplatin nephrotoxicity to both genotypes, and found that the expression of osteopontin and TGF-β in the kidney of EpoR+/−/Gc mice was higher than that of EpoR+/−/Gc mice.

**Conclusions:** Taken together, endogenous EPO exerts tissue protective function during kidney injury.

**Funding:** Government Support - Non-U.S.

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**TH-OR043**

Rap1b Ameliorates the DN Progression Via Modulating Mitochondrial Homeostasis

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**Background:** We have demonstrated that Rap1b modulates the progression of diabetic nephropathy (DN), and ameliorates glucose-induced mitochondrial dysfunction in renal tubular cells (Ki 2001, JBC 2002, JASN 2008). However, it is unclear whether or how Rap1b can protect the tubular cell from damage induced by hyperglycemia.

**Methods:** 12 DN patients were observed and 24 Rats with STZ-induced DN were used in this study. Rap1b gene was transferred into the kidney of rats with DN, an increased-microbubble-mediated technique (UMT). Immuno-EM was used to evaluate the localization of Rap1b in mitochondria of HK2 cell. Gene and protein expression analyses were carried out by the QPCR, Western blotting and IIF. Mitochondrial functional analysis was carried out by measuring ROS, TMRE, ATP and mitochondrial apoptosis genes.

**Results:** Rap1b expression was decreased in renal proximal tubular cells of DN patients, which was related to the tubular atrophy and decreased tubular functions. Although overexpression of Rap1b in DN Rats by UMT had no effect on blood glucose levels, it significantly attenuated the development of microalbuminuria, inhibited mitochondria mediated renal tubular cells death and ameliorated mitochondrial dysfunctions in proximal tubular cells. In vitro, Rap1b expression was localized to mitochondrial cristae of HK2 cells. Compared to control, 30 mM D-glucocse (HG) induced mitochondrial dysfunction, altered mitochondrial morphology, transmembrane potential, ATP levels, increased expression of mitochondrial fission gene Drp1. While a decreased expression of mitochondrial fusion gene in HK2 cells was observed. In addition, HG enhanced the overproduction of mitochondrial superoxide, decreased antioxidant enzymes and mitochondrial biogenesis genes expression. Overexpression of apoptosis-related gene and protein expression was observed, and these effects were partially reduced with the transfection of Rap1b in HK2 cells.

**Conclusions:** These data indicate that Rap1b is capable of dampening the DN progression via modulating mitochondrial homeostasis.

**Funding:** NIDDK Support, Government Support - Non-U.S.

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**TH-OR044**

Bif-1 Regulates Mitochondrial Dynamics during ATP Depletion-Induced Apoptosis

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**Background:** Originally identified as a Bax-interacting factor, Bif-1 has sequence homology with endodihthionin and is also called endodihthionin B1. Nevertheless, Bif-1 does not play an essential role in membrane trafficking or endocytosis. It has been suggested that Bif-1 may contribute to mitochondrial damage and the consequent release of apoptotic factors during apoptosis. However, it is largely unclear how Bif-1 regulates mitochondria under these conditions.

**Methods:** In this study, we have examined the role of Bif-1 in mitochondrial regulation during ATP-depletion-induced apoptosis.

**Results:** In renal proximal tubular cells, Bif-1 transfected to mitochondria during ATP-depletion. Knockdown of Bif-1 in these cells suppressed apoptosis. Consistently, Bif-1 knockout mouse embryonic fibroblasts (MEFs) were resistant to ATP-depletion-induced apoptosis. Interestingly, Bif-1 knockout did not affect Bax translocation to mitochondria during ATP-depletion, but it prevented cytochrome c release from mitochondria. Mitochondrial fragmentation induced by ATP-depletion was markedly suppressed in Bif-1 knockout mouse kidney cells, suggesting that Bif-1 may participate in changes of mitochondrial dynamics resulting in mitochondrial damage. Mechanistically, Bif-1 was shown to interact with prohibitin-2, which regulates OPA1, a key regulator of mitochondrial inner membrane fusion.

**Funding:** NIDDK Support, Government Support - Non-U.S.

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
Conclusions: Together, the results reveal a novel pathway of mitochondrial regulation by Bif-1 during apoptosis.
Funding: NIDDK Support, Veterans Administration Support

**TH-OR045**

Cathepin-Mediated Depletion of Sirtuin-1 (SIRT1) in Endothelial Progenitor Cells (EPC)  
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Background: Stress-induced premature senescence (SIPS) of endothelial cells has emerged as a notable contributor to global endothelial cell dysfunction (ECD) in diverse diseases. One of the critical cellular abnormalities mechanistically linked to SIPS is lysosomal dysfunction. In the present study, we attempted to integrate the previous findings on endothelial SIPS with the exponentially growing field of SIRT1 effects on aging processes as applied to senescence of EPC.

Methods: Specifically, we examined the impact of a range of relevant cardiovascular risk factors on the expression of SIRT1, SIPS and apoptosis and documented the role of SIRT1 in the changes of EPC viability.

Results: Furthermore, studies showed reciprocal relations between SIRT1 and p62/SQSTM1 expression in stressed EPC, thus demonstrating an attendant abnormality of autophagy. The described effects of stressors could be partially mimicked by inducing lysosomal membrane permeabilization or inhibiting autophagy and effect of stressors could be reversed by a cell-permeable inhibitor of cathepsins. Here we provide evidence, for the first time, that SIRT1 is an important substrate of cysteine cathepsins B, S, and L. An antioxidant/peroxynitrite scavenger, ebselen, shown in the previous studies to protect endothelial cells and EPC from SIPS, prevented stress-induced SIRT1 depletion and subversion of autophagy by mitigating lysosomal dysfunction.

Conclusions: 1) these data advance the concept of "stem cell aging" by establishing the critical role of lysosomal dysfunction in the development of SIPS through the cathepsin-induced depletion of SIRT1, a hitherto hidden mechanism linking cell stress with apoptosis or SIPS; 2) Ebselen potently protects lysosomal membrane integrity, preventing cathepsin-induced cleavage of SIRT1 in murine EPC, as well as blunting SIPS and apoptotic cell death induced by relevant cardiovascular stressors; 3) the proposed mechanism of SIRT1 depletion in stress has all the attributes of being a paradigm of SIPS.

**TH-OR046**

Protein Domains of Kidney Injury Molecule 1 That Mediate Phagocytosis and Induction of Autophagy  
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Background: Autophagy has been shown to protect against acute kidney injury in vitro and in vivo. We have found that Kidney injury molecule-1 (KIM-1) expression induces autophagy, which is enhanced following phagocytosis. KIM-1 is a type I transmembrane phosphatidylserine receptor which is highly upregulated by proximal tubule cells in the injured kidney. Upon recognition, KIM-1 induces the phagocytosis of apoptotic cells, ox-LDL and other ligands. To identify regions of KIM-1 important for its functions and for therapeutic targeting, we examined which domains of KIM-1 are important for phagocytosis and autophagy.

Methods: We utilized KIM-1 constructs which have mutations in the phosphatidylserine binding site or which produce truncated proteins lacking either the extracellular domain or the cytosolic domain. Phagocytosis was studied by the uptake of fluorescently labeled apoptotic cells and oxidized low density lipoprotein. Autophagy was measured by formation of LC3-GFP puncte and LC3-II bands by western blot analysis.

Results: Expression of wild-type KIM-1 induces autophagy in renal proximal tubule cells, which is enhanced following phagocytosis. Mutation of the phosphatidylserine binding domain of the protein (which recognizes apoptotic cells) inhibits phagocytosis and blocks autophagy induction. The KIM-1 extracellular domain together with the transmembrane domain of the protein was sufficient to induce both phagocytosis and autophagy. Thus the cytosolic domain of KIM-1 was not required for phagocytosis or autophagy. Indeed, when the cytosolic domain was expressed alone there was no phagocytosis or upregulation of autophagy. In addition, wild-type KIM-1 and KIM-1 ectodomain were found to localize to the autophagosome following phagocytosis, while KIM-1 cytodomain or phosphatidylserine binding site mutants were absent from the autophagosome.

Conclusions: KIM-1 induces autophagy through its phagocytic function. Targeting of KIM-1 to the autophagosome is also dependent on the phagoctytic function of the protein. Both KIM-1 phagocytosis and the role of KIM-1 in autophagy are independent of the intracellular domain of the molecule.

Funding: NIDDK Support

**TH-OR040**

Signaling Mechanisms for Targeted Inactivation of Epidermal Growth Factor Receptor-Elicited Inhibition of Renal Fibrogenesis  
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Background: Although development and progression of renal fibrosis is associated with enhanced activation of epidermal growth factor receptor (EGFR), the underlying mechanisms by which EGFR mediates renal fibrosis remain elusive.

Methods: We used genetic, pharmacologic, in vivo, and in vitro experiments to study the molecular basis of EGFR-mediated renal fibrogenesis.

Results: In a mouse model of obstructive nephropathy, a sustained EGFR phosphorylation was detected in the kidney of wild-type mice, and these mice developed more severe renal fibrosis than wild-type mice that have reduced EGFR tyrosine kinase activity. Attenuation of renal fibrosis in waved-2 mice was associated with reduced numbers of renal tubular cells arrested at G2/M, inhibition of α-smooth muscle actin (α-SMA) expression, down-regulation of gene expression of multiple profibrogenic cytokines including transforming growth factor-β1 (TGF-β1), and dephosphorylation of Smad3, STAT3 and ERK1/2. This phenotype was fully recapitulated in injured kidney of wild-type mice given gefitinib, a specific EGFR inhibitor. Furthermore, inactivation of either EGFR or STAT3 reduced UUO-induced expression of lipocalin-2, a molecule associated with the pathogenesis of chronic kidney disease. In cultured renal interstitial fibroblasts, inhibition of EGFR or abbreviated TGF-β1 or serum-induced phosphorylation of EGFR, STAT3, ERK1/2 and Smad3 as well as expression of α-SMA and extracellular matrix proteins.

Conclusions: These data suggest that EGFR may mediate renal fibrogenesis via induction of transition of renal epithelial cells to a profibrotic phenotype, overproduction of inflammatory factors, and activation of renal interstitial fibroblasts. Inhibition of EGFR may hold a therapeutic potential for treatment of fibrotic kidney disease.

Funding: NIDDK Support

**TH-OR048**

The UK Randomised Controlled Trial of Immunosuppression for Progressive Membranous Nephropathy  
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Background: Optimal treatment for idiopathic membranous nephropathy (IMN) remains controversial despite numerous controlled trials, not least because the condition has a variable natural history and even severely affected individuals can undergo spontaneous remission. Only a subset of patients (25-30% in most series) develops progressive loss of kidney function and since the available therapies have considerable adverse effects, a prominent school of thought is that aggressive therapy should be reserved for that subgroup.

Methods: In the mid 1990s we embarked on a multicentre randomised controlled trial to compare the three approaches that were popular at that time (and remain so today): six months of alternating cycles of prednisolone and chlorambucil (PC), twelve months of cyclosporine (Cy); or supportive therapy alone (ST) in patients with IMN who had shown a 20% deterioration in excretory renal function.

Results: 108 patients were recruited from centres across the UK (33 randomised to receive PC, 36 Cy, 37 ST, 2 proved ineligible). Recruitment was completed in 2008; analysis commenced when two year follow-up was complete for the last recruited subjects. Primary end-point was a further 20% decline in renal function; secondary end-points were proteinuria and adverse effects.

Regarding renal function, there was a highly statistically significant difference (p=0.004) in favour of PC.

Comparisons: Hazard Ratio (95% CI) and p-value
Cyclosporin vs Supportive care. HR 1.17 (0.7, 1.96), 2p=0.5
Prednisolone/ Chlorambucil vs Supportive care. HR 0.43 (0.25, 0.76), 2p=0.004

This group also showed the greatest fall in proteinuria. Adverse events were common in all 3 groups, significantly higher in PC and Cy compared to ST but not significantly different between PC and Cy.
Conclusions: We conclude that six months’ therapy with prednisolone and chlorambucil is superior to cyclosporine or supportive therapy alone in patients with SMN whose renal function is deteriorating. This effect is maintained to at least 3 years.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

TH-OR049

Oral Calcitrol for Reduction of Proteinuria in Patients with IgA Nephropathy: A Randomized, Controlled Trial Jilian Liu,1 Jicheng Lv,2 Sufang Shi,3 Yuqing Chen,1 Hong Zhang.3

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Background: Vitamin D has shown efficacy in the reduction of proteinuria in patients with chronic kidney disease. This study aimed to determine the effect of calcitriol on urinary protein excretion in patients with IgA nephropathy.

Methods: In this open-labeled, blank-controlled study, 50 IgA nephropathy patients were enrolled. The main criterion for inclusion was urinary protein excretion greater than 0.8 g/d following renin-angiotensin-system (RAS) inhibitor treatment for at least three months. Patients were randomly assigned (1:1) to receive two doses (0.5 µg) of calcitriol per week or no treatment, for 48 weeks. The primary endpoint of urinary protein excretion was measured from the baseline to 24 hours post-treatment. Concentrations of monocyte chemoattractant protein 1 (MCP-1) and transforming growth factor-β (TGF-β) in serum and urine were also measured.

Results: Measurement of the primary endpoint showed changes in the urinary protein excretion of +21% (from 1.29 to 1.58 g/24 h; 95% CI -9 to 52) in the control group and -19% (from 1.60 to 1.30 g/24 h; 95% CI -42 to 4) in the calcitriol-treated group (p=0.03). A 15% decrease in proteinuria was measured in controls (7 of 24, 29.2%) and the calcitriol-treated treatment group (17 of 26, 65.4%; p=0.02). No significant differences were observed in the decline in the estimated glomerular filtration rate (eGFR) and changes in blood pressure. Incidence of recorded adverse events was similar between the two groups.

Conclusions: Addition of calcitriol to RAS inhibitor had no safety concerns. And it was effective in reducing proteinuria in patients with IgA nephropathy.

Funding: Government Support - Non-U.S.

TH-OR050

The Effect on the Microcirculation of Ergocalciferol (Vitamin D2) Versus Placebo in Chronic Kidney Disease 3-4 and Vitamin D Deficiency: A Pilot, Double Blind, Randomised Controlled Trial Gavin Dreyer,1 Arthur Tucker,2 Martin J. Raftery,3 Magdi Yaqoob.1

1Renal Medicine, China; 2Nephrology, Shanghai Shuguang Hospital, China; 3Nephrology, Affiliated Hospital, Jiangshu University of Chinese Medicine, China

Background: Observational studies have demonstrated reduced cardiovascular disease (CVD) in patients with kidney disease who receive vitamin D therapy. The exact mechanism leading to these observations is unclear and there are very few prospective studies of vitamin D in CKD. We conducted a pilot randomized trial testing the hypothesis that vitamin D therapy improves microcirculatory function, a known surrogate marker for future cardiovascular dysfunction and disease.

Methods: 38 non-diabetic, non-transplant patients with CKD 3-4 and vitamin D deficiency (25 OH vitamin D < 50 nmol/L) were enrolled. 20 received ergocalciferol at 50,000 iu weekly for 1 month followed by 50,000 iu monthly for 5 months. 18 patients received a matching placebo. The primary endpoint was microcirculatory function after 6 months therapy assessed by % change in skin vessel flux measured by laser doppler flowmetry after iontophoresis of acetylcholine (ACh).

Results: Treatment groups were similar at baseline with respect to age, sex, eGFR, blood pressure, proteinuria, medication and tobacco use, Hb, CRP and 25 OH vitamin D levels. After 6 months, 25 OH vitamin D levels were 91.4 nmol/L in the treatment vs 26.2 nmol/L in the placebo group (p<0.001). Percentage change in skin vessel flux after iontophoresis of ACh was similar at baseline in both groups (ergocalciferol -826.0% +/- 170.0%, placebo -785.9% +/- 121.3%, p=0.85) but was significantly higher in ergocalciferol treated patients at 6 months (ergocalciferol 1130% +/- 182.3%, placebo 540.0% +/- 112.6%, p=0.012). eGFR, blood pressure, proteinuria, HB and CRP did not change with treatment.

Conclusions: Improved microcirculatory function after treatment with vitamin D compounds may be contributing to the reduced burden of CVD in previous observational studies. Multi-centre, randomised trials which compare different vitamin D compounds and include measurement of both microcirculatory and hard cardiovascular endpoints are now required to optimise therapy in this patient group.
**TH-OR053**

**Trajectories of Kidney Function before Initiation of Chronic Dialysis**

**Methods:** We used data from the VA, Medicare and USRDS to model pre-dialysis eGFR trajectories among 7,322 patients who initiated chronic dialysis in 2001-2003. We identified four distinct trajectories of eGFR during the two year period before dialysis initiation: 63% of patients experienced relatively slow loss of eGFR from levels that were already severely reduced two years before initiation (Group 1); 25% declined from moderately reduced levels of eGFR two years before initiation (Group 2); 9% had normal levels of eGFR until two years before initiation (Group 3), and 3% had normal levels of eGFR until six months or less before initiation (Group 4).

**Results:** More rapid loss of eGFR was associated with a greater likelihood of hospitalization and inpatient acute kidney injury and a lower likelihood of outpatient nephrology care and vascular access placement during the two year period before initiation. Those with more rapid loss of eGFR were more likely to initiate dialysis in the hospital, at a higher level of eGFR and in the setting of an acute kidney injury. Median survival ranged from 1.0 year (25th to 75th percentile, 0.3 to 4.4 years) for Group 4, to 3.2 years (25th to 75th percentile, 1.3 to 6.2 years) for Group 1. Differences in survival persisted in analyses adjusted for patient characteristics and care practices.

**Conclusions:** Patterns of eGFR decline before initiation of chronic dialysis are heterogeneous and are strongly associated with pre-dialysis care and survival after initiation.

**Funding:** Other NIH Support - NIA, Other U.S. Government Support, Veterans Administration Support

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**TH-OR054**

**A Breath Test for Chronic Kidney Disease and Disease Progression**

**Background:** A novel approach that overcomes many of the conventional diagnostic techniques relies on patterns of volatile biomarkers in the exhaled breath. Some of the volatile organic compounds among the plasma chronic kidney disease (CKD) biomarkers, or their metabolic products, are transmitted to the alveolar exhaled breath through exchange via the lung, at the very onset of the disease. Accurate determination of kidney function is essential in the treatment of CKD in order to identify patients with early renal impairment and to follow the course. We report a novel method to identify CKD and progression that is based on breath testing by using a custom-designed, nanoscale artificial nose (NA-NOSE).

**Methods:** Alveolar exhaled breath samples were collected from 62 volunteers and were analyzed using a custom-designed array of nanosensors that is based on organically functionalized gold nanoparticles, combined with support vector machine (SVM) analysis, to detect statistically significant differences between the sub-populations. Sensitivity and specificity with reference to CKD patient classification according to eGFR, were determined using cross-validation. Chemical composition of the breath samples was studied using gas chromatography linked with mass spectrometry (GC-MS).

**Results:** Excellent distinction was achieved with the nanosensor array between: (i) early stage CKD and healthy states, and (ii) stage-4 and stage-5 CKD states, with an accuracy of 79% and 85%, respectively. Several substances in the breath were identified and could be associated with CKD related biochemical processes or with the accumulation of toxins through kidney function loss.

**Conclusions:** This study focuses on testing the feasibility of a novel method in nanomedicine for identifying early stage of CKD and monitoring disease progression from exhaled breath of patients. The biomarker-based NA-NOSE breath test could form the basis of a future cost-effective, fast and early diagnostic test for CKD and progression. The nanosensor array could distinguish with high accuracy between the exhaled breath of (i) early and advanced CKD stages.

**Funding:** Technion Institute, Government Support - Non-U.S.
TH-OR055
A New Equation To Estimate GFR from Standardized Creatinine and Cystatin C
Lesley Stevens Inker,1 Christopher H. Schmid,1 Hocine Tighiouart,1 John H. Eckfeldt,2 Harold I. Feldman,3 Tom H. Greene,4 Jane Manzi,5 John W. Kusek,6 Josef Coresh,6 Andrew S. Levey,1 Tufts Medical Center; 2University of Minnesota; 3University of Pennsylvania; 4John Hopkins University; 5NIDDK; 6Cleveland Clinic.

Background: GFR estimates based on serum creatinine (cr) are routinely used, however, are imprecise due to variation among people in non-GFR determinants of creatinine. Cystatin C (cys) is a potential alternative filtration marker.

Methods: We developed estimating equations based on cys alone (eGFRcys) and in combination with cr (eGFRcr-cys) in a cross-sectional analysis using separate databases for equation development (13 studies of 5352 people) and validation (5 studies with 1119 people). GFR was measured (mGFR) using urinary clearance of iothalamate in the development dataset, and clearance of other markers in the validation dataset. Cr and cys assays were traceable to high-level reference materials.

Results: Mean mGFR (SD) was 68.39 and 70.41 ml/min/1.73 m2 in the development and validation dataset, respectively. eGFRcr-cys performed better than eGFRcr or eGFRcys, with similar bias, improved precision, and greater accuracy (table). Compared to eGFRcr, eGFR-cr-cys improves classification of subjects with mGFR ≥ or < 60 ml/min/1.73 m2 [net reclassification improvement (NRI) (95% CI) 4.93% (2.19%-7.67%)]. In the subgroup of eGFR of 45-59 ml/min/1.73 m2, NRI was 32.7% (15.4%-50.1%).

Conclusions: The combined Cr-Cys equation is more accurate than equations with either marker alone, and can be used as a confirmatory test for people decreased eGFRcr.

Funding: NIDDK Support

TH-OR056
Assessing Improvement of eGFR Risk Classification in Meta-Analysis: An Example of CKD-EPI and MDRD Study Equations (for the CKD-PC Collaborators)
Kunihiro Matsushita, Yingying Sang, Mark Woodward, B. Khan Mahmoodi, Brad C. Astor, Andrew S. Levey, Josef Coresh. Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Background: Risk prediction models are assessed by calibration, discrimination, and reclassification. Net reclassification improvement (NRI) is a popular reclassification statistic in clinical epidemiology. However, methods to assess NRIs in a meta-analysis are not well described. Using an example of the CKD-EPI and the MDRD Study equations, we demonstrate a method to meta-analyze NRIs for eGFR category based on different equations.

Methods: In a sample of 16 of 46 cohorts joining the CKD Prognosis Consortium (CKD-P C), NRI was calculated for reclassification across the following six eGFR categories (<60, 60-89, 45-59, 30-29, <30 ml/min/1.73 m2) for CKD-EPI vs. MDRD Study equations. Standard errors of NRIs were calculated in analogy to McNemar’s test for paired proportions as √[(proportion of reclassification at high risk / low eGFR category in those who developed events + proportion of reclassification to low risk / high eGFR category in those who did not develop events / proportion of reclassification to low risk category in those who did not develop events / proportion of reclassification to high risk category in those who did not develop events)](9 of no events). NRIs were meta-analyzed in a random effects model applying (OR - StATA) SCr method was assessed by analyzing capillary blood samples of 282 KEEP participants and comparing POCT results with those of an Olympus 5400 spectrophotometry instrument, which measures i-STAT cys in venous blood. Correlation between i-STAT and MDRD SCr was calculated.

Results: Mean age SD of the 282 participants was 53.4±15.1 and 76% were women. Mean SCr±SD by the i-STAT method was 0.56±0.2 mg/dl and by the IDMS method 0.73±0.2 mg/dl. Mean eGFR±SD estimated with the MDRD and CKD-EPI equations from the IDMS SCr were 102±25 and 97±19 ml/min/1.73 m2, respectively. Correlation for i-STAT and IDMS SCr was high (r=0.86, p<0.0001) and improved when a correction factor (1.0±0.022) was applied to the i-STAT SCr.

Conclusions: i-STAT SCr measured in capillary blood is as accurate as IDMS SCr when a correction factor is applied. Further linear regression analysis will be done to use i-STAT SCr for GFR estimation with equations.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-OR057
Comparison of Serum Creatinine Measured with a Point-of-Care Testing Device (i-STAT) and with IDMS Methodology among Mexico's Kidney Disease: Data from the CARDIA (Coronary Artery Risk Development in Young Adults) Study
Carmen A. Peralta,1 Eric Vittinghoff,2 Michael Shlipak,1,2 David Siscovick,1 David R. Jacobs,7 Holly J. Kramer,4 Michael Steffes,5 Paul Muntner,3 Kirsten Bibbins-Domingo,1 UCSF, SF, CA; 2SPVAMC, SF, CA; A Washington, Seattle, WA; 3Loyola, Maywood, IL; 4U Minnesota, Minneapolis, MN; 5U Alabama Birmingham, Birmingham, AL; 6U Minnesota Public Health, Minneapolis, MN.

Background: Blacks have been reported to have faster rates of kidney function decline in middle-aged or older adults with or without chronic kidney disease (CKD). Whether race differences in kidney function decline are detectable in young adults without CKD is not well studied.

Methods: CARDIA is a longitudinal cohort of young blacks and whites (age 18-30 at enrollment) with over 20 year follow-up. We included participants at years 10, 15 and 20 with at least two measurements of cystatin C (N=3658). Rapid kidney function decline was defined as annual eGFRcys decline ≥2% per year. We evaluated race differences in rapid decline by study period (period 1: year 10-15 (age 28-40) and period 2: years 15-20 (age 33-45)) using Poisson regression. We adjusted for age, sex, diabetes, albuminuria, and time-averaged cumulative exposure to systolic blood pressure ≥120mmHg throughout follow up.

Results: At baseline, mean age was 35±4, eGFRcys 112±26/ml/min/1.73 m2 for blacks and 105±21/ml/min/1.73 m2 for whites. Blacks were more likely to have rapid eGFRcys decline during both periods, but the magnitude of the differences varied by period. After age adjustment, during period 1, 14.3% of Blacks had rapid decline vs. 1.8% of whites. During period 2, 26.4% of Blacks and 8.7% of whites had rapid decline. Multivariate adjustment did not attenuate race differences: blacks were nearly 8 times as likely to have rapid decline in kidney function as whites during the first period (prevalence ratio for black:whites=7.6 (5.1-11.3)) and nearly three times as likely during period 2 (prevalence ratios 2.7 (2.3-3.2)).

Conclusions: Among young adults without chronic kidney disease, Blacks were more likely to have rapid decline in eGFRcys than whites. Future studies are needed to elucidate the mechanisms predisposing young blacks to rapid kidney function decline.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support
APOL1 Variants Are Associated with Subclinical Albuminuria and Lower Glomerular Filtration Rate in the Black Young Adults: Findings from the Coronary Artery Risk Development in Young Adults (CARDIA) Study

Kirsten Biddles-Domingo,1 Eric Vittinghoff,2 Myriam Fornage,2 George W. Nelson,1 Cheryl Ann Winkler, Jeffrey B. Kopp.1 Department of Medicine and Epidemiology and Biostatistics, University of California, San Francisco, CA; 2University of Texas Health Science Center at Houston, Houston, TX; 3National Institutes of Health, Bethesda, MD.

Background: Genetic variants in APOL1, encoding apolipoprotein L1, are strongly associated with focal segmental glomerulosclerosis, HIV-associated nephropathy, and hypertensive kidney disease, but the association with subclinical kidney disease has not been examined.

Methods: The Coronary Risk Factors in Young Adults (CARDIA) is an on-going longitudinal study that enrolled 5115 participants age 18-30 years in 1985-86. We genotyped APOL1 kidney risk mutations in all participants with samples at Year 10 and explored the association of 2 APOL1 mutations with repeated measures of albuminuria (urine albumin/creatinine ratio >30 mg/g) and cystatin C-corrected glomerular filtration rate (eGFR) from Years 10, 15, and 20 among 1764 black participants. We also assessed effects of metabolic syndrome.

Results: The APOL1 risk genotype (2 APOL1 risk alleles as homozygotes or compound heterozygotes) was present in 12.6% of black participants. After adjustment for visit year, age, and sex, albuminuria (nearly all of which was microalbuminuria) was associated with the APOL1 risk genotype (adjusted prevalence 17.2% vs 6.0%, p<0.0005). The APOL1 risk genotype was associated with 5.5% lower eGFR at each exam year (p=0.003) and also predicted eGFR<60 ml/min (adjusted prevalence 3.3% vs 1.0%, p=0.02). The average eGFR was significantly higher for DDLT recipients (β=0.53, 95% CI: 0.47, 0.60) compared to white patients, which reverses after controlling for age. A survival advantage for heterozygotes was present in 12.6% of black participants.

Conclusions: The increased prevalence of albuminuria and reduced eGFR may indicate subclinical kidney disease associated with APOL1 risk alleles.

Funding: NIDDK Support, Other NIH Support - NHLBI

Black Patients Experience Lower Mortality Compared to White Patients in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD

P. Kovesdy,1,2 Evan H. Lott,3 Jun Ling Lu,4 Sandra M. Malakauskas,1 Jennie Z. Ma,1,2 Mark D. Okusa,3 Kamyar Kalantari-Zadeh,4 Salem VA Medical Center; 1VA Informatics and Computing Infrastructure; 1Salem Research Institute; 1Harbor UCLA.

Background: Blacks with end stage renal disease experience significantly lower mortality compared to whites. A similar paradoxical association in non-dialysis dependent CKD is unknown.

Methods: We compared 54,154 black and 468,233 white patients in a nationally representative cohort of US veterans with non-dialysis dependent CKD stages 1-5 in 2005-2006. Crude mortality rates (Model 1) were compared with the Kaplan Meier method and in Cox models. In order to explain observed differences in mortality, models were adjusted for age (Model 2), sociodemographics (Model 3), comorbidities (Model 4), and laboratory variables (Model 5).

Results: Blacks were younger, more likely to be unmarried and uninsured and to have diabetes and hypertension, but less likely to have coronary artery disease. Over a median follow-up of 4.7 years, 14,848 blacks (64.3 deaths/1000 patient-years (95% CI: 63.4, 64.3-64.5)) and 143,107 whites died (75.2 (71.8-72.5)). Black race was associated with lower crude mortality (hazard ratio, 95% CI: 0.89, 0.88-0.90), which reversed after adjustment for age (1.09, 1.07-1.11). Further adjustments resulted in black race being again associated with lower mortality (Figure; all p values <0.001). Similar trends were seen in all stages of CKD.

Conclusions: Blacks with non-dialysis dependent CKD experience lower mortality compared to white patients, which reverses after controlling for age. A survival advantage of blacks re-emerges upon controlling for differences in various characteristics. General population studies are needed to clarify the reasons for the selection of black patients with CKD possessing such different characteristics.

Funding: NIDDK Support, Veterans Administration Support

National Trends in Prevalence of Chronic Kidney Disease in Kidney & Liver Transplant Recipients

Anca Tisea,1 Yihuang Huang,1 Yahakn B. Shahnian,1 Vanessa Grubbs,2 Neil R. Powe,2 Nilka Rios Burrows,3 Desmond Williams,3 Rajiv Saran.1 1’U of MI; 2UCSF; 3CDC.

Background: High prevalence of CKD has been recognized as a major complication of solid organ transplantation. We postulated that prevalence of CKD in organ transplant recipients may be on the rise, as average age of both transplant donors and recipients has increased.

Methods: Adult solid organ (kidney (KT) and liver (LT)) transplant recipients (age≥20) enrolled between 1999 and 2008 were identified in the US Scientific Registry of Transplant Recipients (SRTR). Patients with a death event or missing eGFR prior to 1-year post-transplant were excluded. GFR was estimated by the MDRD equation. Logistic regression was used to model the probability of eGFR<60 ml/min/1.73m2 or ESRD at 1-year post-transplant, adjusted for transplant year, recipient and donor demographics by donor type (deceased (DD) or living (LD) donor).

Results: In adjusted models, the odds of CKD at 1-year post KT were significantly lower for both DDKT recipients and LDKT recipients (OR=0.96, and OR=0.93 respectively, Figure Panel [a], bars). Correspondingly, there was a significantly higher mean eGFR (lines) for both donor types (p<0.05). Similar results were seen in CKD prevalence for LT recipients (Panel [b]). In adjusted models, the odds of CKD at 1-year post LT were 3.4% lower per year (p=0.01) for DDLT recipients, but remained relatively unchanged for LDLT recipients (p=0.47). The average eGFR was significantly higher for DDLT recipients (β=0.53, p=0.05).

Conclusions: Prevalence of post-transplant CKD among KT and LT recipients has steadily decreased in the US over the last decade, despite aging donors and recipients. Explanations for such overall trends may include changes in immunosuppression practices or other practices, and require further study.

Funding: Other U.S. Government Support
TH-OR062

Pregnancy in a Prospective Cohort of Women with CKD 3-5: Maternal Outcomes

Sajeda Youssouf, Matt Hall, Liz Lightstone, Nigel J. Brunkull, Sue Carr; 1John Walls Renal Unit, Leicester General Hospital, Leicester, United Kingdom; 2Renal Section, Dept of Medicine, Imperial College Healthcare NHS Trust, London, United Kingdom.

Background: CKD is associated with an increased risk of adverse fetal outcomes at all levels of renal dysfunction. For some women, pregnancy is associated with an accelerated decline in renal function. This retrospective analysis of data collected prospectively since 2003 sought to identify maternal outcomes in women with excretery renal dysfunction at conception.

Methods: We analysed prospective data from 3 specialist renal-obstetric services in the United Kingdom and identified those with excretery renal dysfunction (CKD 3 to 5) as women with a pre-conception estimated GFR <60ml/min or a serum creatinine >110µmol/l (>1.25mg/dl) prior to 12 weeks gestation.

Results: 42 pregnancies in 39 women with CKD stage 3 to 5 were identified, of whom sufficient data was available in 30 women. Mean maternal age at conception was 32±4.5 years. Mean eGFR prior to conception was 46 ± 10 ml/min, with 4 (12.5%) having eGFR <30ml/min and serum creatinine (154 vs 115 µmol/l, p=0.059) identified more advanced renal dysfunction in those going on to require RRT.

Conclusions: Women with CKD 3 to 5 are at risk of loss of renal function as a result of pregnancy, particularly if eGFR ≤30 ml/min.

Funding: Clinical Revenue Support

TH-OR063

Serum Uric Acid Predicts Incident Stage 3 Chronic Kidney Disease in a Middle Aged Population with Excess Obesity and Diabetes: The Strong Middle Heart Family Study

Jason G. Umana,1,2 Hong Wang,1 Nawar M. Shara,1,2 MedStar Health Research Institute;1 Georgetown-Howard Universities Center for Clinical and Translational Science.

Background: Hyperuricemia has been associated with prevalent and incident CKD, with hypertension, and with the metabolic syndrome in observational studies of unselected and low risk populations. Studies in higher risk populations are limited by concerns that serum uric acid (UA) may more sensitively detect subtle decrements in GFR than creatinine-based methods. We sought to determine if elevated UA predicted incident stage 3 CKD (CKD3) in a population with high prevalence of obesity, diabetes and at high risk for CKD and CVD.

Methods: The genetic epidemiologic Strong Heart Family Study (SHFS) included 3665 American Indians (60% female) with median age 39(14-93)y. At the baseline examination (2001-03), 22.8% of participants had DM, 33.4% had HTN, 57.3% were obese with BMI ≥30, 18% (648) had albuminuria (ACR ≥30mg/g), and 6% (217) had CKD3-5 (MDRD eGFR ≤60ml/min/1.73m² or ESRD). After excluding those with albuminuria or decreased eGFR at baseline, we assessed the association of serum UA and incident CKD3 in the remaining 2898 participants by conditional logistic regression, accounting for the extended family structures in the study population.

Results: Over a median 5.2y follow up, there were 170 incident cases of CKD3. The 1st, 2nd, 3rd, and 4th quartiles of serum UA spanned values from 0.5-0.4, 4.1±0.5, 5.1±6.0, and 6.1±11.1 mg/dl, respectively; mean serum UA in the 4th quartile was 7.04±0.84 mg/dl. The multivariate-adjusted (for age, sex, LDL-C, HDL-C, HTN, DM, and smoking) odds ratios for incident CKD3 (95% CI) across increasing quartiles of serum UA were: 1 (referent), 0.98(0.48-1.99), 1.4(0.71-2.99), and 2.65(1.7-6.00).

Conclusions: In this high risk group with an excess of obesity and DM, clinically-elevated values of serum UA, occurring only in the 4th quartile of our population, were significantly associated with incident CKD3. This extends observations from other populations with lesser burdens of disease.

Funding: Other NIH Support - NHLBI, NCCR

TH-OR064

Association of Tenofovir Exposure with Kidney Disease Risk in HIV-Infection

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Background: Despite the widespread use of highly active antiretroviral therapy (ART) therapy, HIV disease remains associated with increased risk of kidney disease. Whether or not tenofovir use is associated with higher risk of kidney disease is controversial.

Methods: We designed a cohort of 10,841 HIV-infected patients who initiated antiretroviral therapy in the Veterans Health Administration from 1997-2007. Using proportional hazards survival regression and marginal structural models, we evaluated association of cumulative exposure to tenofovir with kidney outcomes: first occurrence of (1) proteinuria, (2) rapid decline in kidney function (≥3ml/min/1.73m² annual decline), and (3) estimated glomerular filtration rate (eGFR) <60ml/min/1.73m². Time-dependent models adjusted for antiretroviral drugs, demographics, baseline comorbid conditions, and current measurements.

Results: During follow-up, 3,400 proteinuria, 3,078 rapid decline, and 1,712 CKD events occurred. After multivariable adjustment, each year of exposure to tenofovir was associated with increased risk of all 3 endpoints. Other ARVs showed weaker or inconsistent associations with kidney disease risk. Among those who discontinued tenofovir use, the risk of kidney disease events did not appear to decrease during follow-up.

Conclusions: Tenofovir exposure was independently associated with increased risk for kidney disease events, and did not appear to be reversible. The balance between tenofovir’s efficacy and these probable adverse effects requires further study.

Association of Cumulative Tenofovir Exposure with Risk of Kidney Disease Outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Hazard Ratio (95% CI) per year of Tenofovir</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (n=3480 events)</td>
<td>1.23 (1.24-1.25)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rapid decline (n=1078 events)</td>
<td>1.61 (1.55-1.71)</td>
<td>0.0033</td>
</tr>
<tr>
<td>CKD (n=1712 events)</td>
<td>1.23 (1.12-1.35)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Funding: NIDDK, Support, Other NIH Support - 1R03AG043871 - NIA NIH HH, K24AI069994 - NIAID NIH HH, Veterans Administration Support

TH-OR065

Hemoglobin A1C Levels and Mortality in the ESRD Population: Findings from the DOPPS

Sylvia Paz B. Ramirez,1 Jyothi R. Thumma,1 Francesca Tentori,1 Brenda W. Gillespie,2 Masaaki Inaba,2 Robert G. Nelson,3 Ronald L. Proins,1 Bruce M. Robinson,1 Abor Research, MI;2 Univ of MI, MI;3 Osaka City Univ, Japan; *NIDDK, AZ

Background: Lowering hemoglobin A1C (A1C) levels to <7% can lower the risk of developing the microvascular complications of diabetes, but the value of maintaining this A1C goal in diabetic patients (pts) who have already progressed to kidney failure is less certain. In this study, we present analyses evaluating the relationship between glycemic control based on mean A1C levels and mortality in international DOPPS data.

Methods: S.437 hemodialysis pts from 12 countries (DOPPS 3 & 4, 2006-2010) were identified who had diabetes at study entry. Associations between average A1C over 8 months (mo) after study entry and subsequent mortality was assessed using multivariable Cox regression models adjusting for age, sex, body mass index, vintage, comorbid conditions and serum albumin.

Results: Pts had 1 to 3 reported A1C values over 8 mo. 5% and 95% %ile A1C levels were 4.9 and 9.2%. The relationship between average A1C levels and mortality risk was U-shaped, with the lowest risk associated with A1C levels of 7-8%. All-cause mortality risk appears to be greatest at A1C levels ≥9% and <5%, however a trend toward higher mortality risk was seen below A1C levels of 7%.

Association of A1C Levels With All-Cause Mortality

<table>
<thead>
<tr>
<th>Hazard Ratio (95% CI)</th>
<th>% of diabetes medication:</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.05</td>
<td>7%</td>
</tr>
<tr>
<td>1.12</td>
<td>8%</td>
</tr>
<tr>
<td>1.14</td>
<td>9%</td>
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<tr>
<td>1.15</td>
<td>10%</td>
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<tr>
<td>2.00</td>
<td>5%</td>
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</tbody>
</table>

Among patients with A1C <7%, mortality was higher among patients taking diabetes medications (p=0.01 for interaction).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

16A
Conclusions: These analyses support the importance of measuring A1C levels in the ESRD population. Non-albuminuric levels may be higher than A1C levels as compared to the general population since mortality risk appears to be lowest at A1C levels of between 7-8%. Use of diabetes medications is common among patients with A1C <7%; avoiding excessively low blood glucose levels by tapering these medications may be a readily modifiable practice to improve outcomes.

Funding: Pharmaceutical Company Support

TH-OR066
Intensive Glucose Lowering and End Stage Kidney Disease Vläde Perkovic,1 Hidio Jan Lambers Heerspink,2 John P. Chalmers,3 Mark Woodward,1 Min Jun,1 Alan Cass,1 Mark E. Cooper,4 Michel Marre,5 Carl Erik Mogensen,6 Carl Erik Mogensen,6 Sophia Liu
1Department of Nephrology, Fuxing Hospital, Capital Medical University, Beijing, China.

Background: Blood glucose levels have been linked to the risk of kidney disease, but the effects of intensive glucose control on major kidney outcomes among people with diabetes are not known.

Methods: This analysis from ADVANCE compared the effects of an intensive glucose lowering target (HbA1c < 6.5%) using a gliclazide MR based regimen to a standard target (HbA1c 7%) on a hemoglobin A1c of 6.5%. The outcomes assessed were end-stage kidney disease (ESKD), defined as maintenance dialysis or transplantation), renal death, confirmed death of diabetes, and sustained doubling of creatinine to above 200 μmol/L, and sustained doubling of creatinine to the same level (below 7%) on major renal events. The outcomes assessed were end-stage kidney disease (ESKD), defined as maintenance dialysis or transplantation), renal death, confirmed death of diabetes, and sustained doubling of creatinine to above 200 μmol/L, and sustained doubling of creatinine to the same level (below 7%) on major renal events.

Results: A total of 107 patients with type 2 diabetes were randomly recruited in this study. These patients were further divided into three groups according to their urinal albumin to creatinine ratio. Group A (n=44) consisted of type 2 diabetic patients without diabetes nephropathy (serum creatinine<106 μmol/L and normoalbuminuric). Group B consisted of patients (n=30) with microalbuminuria (A1C 20-200 μg/mg). At the same time 102 healthy controls were selected for the healthy controls. Plasma level of PAI-1 was measured by ELISA and PAI-1 polymorphism was measured DNA sequences.

Results: (1) The plasma PAI-1 level in type 2 diabetes is higher than those in healthy controls. (2) Plasma PAI-1 level was correlated positively with glucose, triglyceride, High density lipoprotein and uric acid. (3) The genotype 4G/4G distribution frequency in group C were 42.4%, which were significantly higher than those normal controls (28.7%). (4) The PAI-1 level are (68.33±15.95)ng/L, (64.57±19.39)ng/L and (58.00±18.34)ng/L in genotype 4G/4G, 4G/5G and 5G/5G. (5) Multiple logistic analysis showed that the plasma PAI-1 were the important risk factors of type 2 diabetic nephropathy. (6) No clear effect on doubling of creatinine (HR 1.15, CI 0.82-1.63, p=0.42) or sustained doubling of creatinine to the same level (below 7%) on major renal events. The outcomes assessed were end-stage kidney disease (ESKD), defined as maintenance dialysis or transplantation), renal death, confirmed death of diabetes, and sustained doubling of creatinine to above 200 μmol/L, and sustained doubling of creatinine to the same level (below 7%) on major renal events.

Conclusions: Plasma PAI-1 level goes up in 2 diabetes. High PAI-1 level is an important risk factor for type 2 diabetic. (2) Individuals with 4G/4G genotype have higher plasma PAI-1 levels. (3) PAI-1 4G/4G polymorphism is associated with the development and progression of predominant proteinuria diabetes nephropathy.

TH-OR068
Low Plasma Adiponectin Levels Predict Increase of Urinary Albumin/ Creatinine Ratio in Type 2 Diabetes Patients Ina Maria Kacso,1 Alina Lenghel,1 Remus Aurel Orasan,2 Rodica Rahaian,3 Mirela Gherman.1 1University of Medicine and Pharmacy, Cluj Napoca, Romania; 2Emergency County Hospital, Cluj Napoca, Romania; 3Nefromed Dialysis Centers, Cluj Napoca, Romania.

Background: Experimental studies have shown that adiponectin has anti-inflammatory and anti-proliferative effects. The purpose of the study was to assess the value of plasma adiponectin as a predictor for progressive diabetic kidney disease in type 2 diabetes (T2D) patients.

Methods: In a one-year prospective follow-up study we included microalbuminuric type 2 diabetes patients. Exclusion criteria were acute infection/inflammation, uncomplicated hypertension or history of coronary heart disease, stroke, atherosclerosis obliterans. The main outcome measure was change in urinary albumin/creatinine ratio (UACR) between one year and baseline (AUCR). Results: Fifty-six patients (66% males) completed the study. Mean UACR was in the microalbuminuric range (81.58±26.42 μg/g creatinine) and GFR close to normal (mean GFR=81.63±4.006 mmol/min). At baseline, simple regression disclosed significant correlations between UACR on one hand and plasma adiponectin (r=0.54, p=0.00002) and GFR (r=0.29, p=0.03) on the other hand, findings confirmed by multiple regression analysis (p=0.0007). Baseline plasma adiponectin was significantly correlated to body mass index (r=-0.28, p=0.04), waist circumference (r=-0.27, p=0.05), HDL cholesterol (r=0.35, p=0.01), LDL cholesterol (r=-0.27, p<0.04). A UACR in simple regression significantly to baseline adiponectin values (r=0.38, p=0.004); in multiple regression baseline plasma adiponectin remained the only predictor of A UACR (p=0.001). If patients are divided according to A UACR in nonprogressors (A UACR<0) and progressors (A UACR>0), logistic regression shows that baseline GFR (OR=1.04, C95%: 1.00-1.09, p=0.048) and plasma adiponectin (OR=1.16, C95%: 1.02-1.32, p=0.02) are the only factors that predict whether the patient will be a progressor or not.

Conclusions: In microalbuminuric T2D patients lower plasma adiponectin levels seem to be predictive of increasing UACR.

Funding: Government Support - Non-U.S.

TH-OR069
The Majority of Type 2 Diabetic Patients with Renal Impairment Have Non-Albuminuric Renal Disease – The Swedish National Diabetes Register (NDR) Hamir Afghahi,1 Mervece Miftaraj,2 Ann-Marie Svensson,3 Henrik Hellström.1,2 Born Elisson Eriksen.1,4 4Nephrology, Kårgårdsavdelningen, Skövde, Västra Götaland, Sweden; 2Center of Registers in Region Västra Götaland, Gothenburg, Västra Götaland, Sweden; 3Medicine, Sahlgrenska University Hospital, Gothenburg, Västra Götaland, Sweden; 4Nephrology, Sahlgrenska University Hospital, Gothenburg, Västra Götaland, Sweden.

Background: Albuminuria and renal impairment are two manifestations of renal disease but are not entirely linked in patients with type 2 diabetes (T2D). The aim of this cross-sectional study was to study prevalence and clinical characteristics associated with non-albuminuric renal impairment in T2D in a nation-wide population-based diabetes register.

Methods: 99446 patients with T2D and serum creatinine reported to the Swedish National Diabetes Register in 2009 were included. Renal impairment was defined as eGFR < 60 ml/min/1.73 m² (MDRD) or albuminuria as AER > 20 μg/min. A registry linkage was performed between the NDR and the Swedish Prescribed Drug Register to evaluate ongoing anti-diabetic, lipid-lowering, anti-hypertensive and aspirin medication.

Results: 17% of all patients had renal impairment and 62% of these were non-albuminuric. Patients with non-albuminuric renal impairment were more often women, non-smokers and more seldom had a history of CVD and heart failure and had lower HbA1c, triglycerides, BMI and systolic blood pressure compared to patients with albuminuric renal impairment. 27% of the patients with non-albuminuric renal impairment had no ongoing treatment with any RAAS-blocking agent. These patients had lower BMI and systolic blood pressure and were older, more often women and smokers and fewer patients had a history of CVD and heart failure compared to patients with non-albuminuric renal impairment and ongoing RAAS-blockade.

Conclusions: The majority of patients with type 2 diabetes and renal impairment were non-albuminuric. Non-albuminuric renal impairment can be explained by the use of RAAS-blockers but since only 75% of these patients were treated with RAAS-blockade this also supports the concept of different underlying pathophysiology mechanisms.

TH-OR070
Effect of Fenofibrate on Cardiovascular Events According to Changes in Plasma Creatinine Levels during the Pre-Randomization Period: The Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study Jean-Claude Ansagnier,1 Karine Le Malicot,1 Ru-Dee Ting,2 Anthony C. Keech.3 1Institut Rivages Fournier SA, Dus, France; 2NHMRC-CTU University of Sydney, Australia.

Background: Assessment of renal function (eGFR and albuminuria) is essential in risk stratification for cardiovascular disease (CVD) in subjects with type 2 diabetes mellitus (T2DM). FIELD was a double-blind, placebo-controlled, randomized study in
Complications of Diabetes Mellitus: Clinical Studies
Oral Abstract/Thursday

TH-OR071
Chronic Kidney Disease and Diabetes Mellitus: Can We Do More To Modify Cardiovascular Risk after Coronary Bypass Graft Surgery? Sean Gallagher, Matt Lovell, Dan A. Jones, Andrew Wragg, Akhil Kapur, Rakesh Uppal, Magdi Yaqoob.

Background: Chronic kidney disease stage 3 (CKD3) and diabetes mellitus (DM)/in combination is anecdotally associated worse outcomes following coronary artery bypass graft surgery (CABG). Long term mortality data upon patients with both CKD3 and DM following CABG is currently lacking.

Methods: We analysed prospective data upon 2713 consecutive patients undergoing CABG at a tertiary cardiac centre between 2003 and 2007. Patients were divided into four groups for analysis: NoCKD/NoDM, NoCKD/DM, CKD/NoDM and CKD/DM. All-cause mortality was determined via Office of National Statistics data.

Results: There were 1286 No CKD/NoDM patients, 553 No CKD/DM patients, 611 CKD/NoDM patients and 263 CKD/DM patients. In hospital mortality (0.78% vs 0.54%, p=0.037) and 5 year mortality (94.3±9.8%, p=0.685) were not different between NoCKD/ NoDM and NoCKD/DM groups despite more LV dysfunction (41.4% vs 35.8%, p=0.037), and more previous MIs (54.0±9.0%, p=0.001) in the NoCKD/DM group. Comparing CKD/NoDM with CKD/DM; there was no difference in hospital mortality (2.45±1.90%, p=0.806) but significant difference in 5 year mortality (86.2±78.0%, p=0.0261).

Conclusions: The absolute risk reduction (ARR) in CABG events was 1.4% (relative reduction 11% p=0.035). Plasma creatinine changes in the upper tertile (+16%) were associated with the highest 5-year risk on placebo (17.7%) and the largest ARR with fenofibrate (3.5% p=0.005). ARR was 0.6% and 0.4% in the middle and lower tertile (58%), respectively. These results persist after adjustment for age, gender, prior CVD, use of ACE inhibitors, angiotensin receptor blockers and diuretics. Conversely, lower eGFR by tertiles or by chronic kidney disease stage is associated with higher risk of CVD.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-OR072
Sevelamer Carbonate Improves Metabolism and Reduces Risk Factors for Progressive Nephropathy in Type 2 Diabetes with Stage 2-4 CKD by Sequestering Oral Advanced Glycation End Products Helen Vlassaras,1 Jamie Urbahn,1 James B. Post, Fabrizio Grosjean,2 Gary E. Striker.1 ‘Medicine and Geriatrics, Mount Sinai School of Medicine, New York, NY; 2Epidemiology, University of Sydney, Australia; 3Medicine, University of Pavia, Italy.

Background: Increased inflammation and oxidative stress (Infl/OS) in stable diabetes mellitus (DM) are partly due to advanced glycation end products from food, and restricting AG can mitigate these risk factors in DM. High levels of circulating AG are associated with the development of CVD events in this population.

Methods: The aim of this study, in contrast, relative advanced glycation products (AGEs) and TNF α were decreased and TNF α a trend to decrease. Peripheral blood mononuclear cell AGE-receptor 1 and SIRT1 levels were decreased and TNF α were decreased by SC. These changes did not occur with CC. SC has a significant impact on CVD events in CKD when compared with diabetes alone. A U-shaped curve has been seen with calcium carbonate. A larger randomised trial is indicated to confirm these results.

Funding: Pharmaceutical Company Support

TH-OR073
Advanced Diabetic Nephropathy with Nephrotic Range Proteinuria: Long-Term Efficacy of Subcutaneous Adrenocorticotropic Hormone (ACTH) Therapy on Proteinuria and Urinary Vascular Endothelial Growth Factor (VEGF) Levels James A. Turulin,1 Claude Mabry Galphin,2 Brad H. Rovin.2 1Internal Medicine/Nephrology, University of Tennessee College Medicine, Chattanooga, TN; 2Renal Division, Ohio State University, Columbus, OH; Clinical Research, Southeast Renal Research Institute, Chattanooga, TN.

Background: Activation of melanocortin receptor-1 (MC1R) in podocytes and endothelium can lower proteinuria. We have shown that 6 months of ACTH gel reduces proteinuria in over 50% of patients with nephric diabetis nephropathy. Because ACTH increases expression of vascular endothelial growth factor (VEGF), we investigated whether the reduction in proteinuria with ACTH gel involves alteration of VEGF expression.

Methods: A total of 14 patients with diabetic nephropathy and 3.0 gm proteinuria/24 hrs on ACE inhibitor alone or 2.0 gm/24 hrs on combination ACE/ARB were enrolled. All patients had eGFr>20 mls/min and HbA1c ≤ 9%. Patients were randomized to ACTH gel (16U or 32U) SQ daily for 6 months. Using a Luminex or ELISA assay, urinary VEGF and monocyte chemoattractant protein concentrations were measured at baseline and after 6 months of ACTH gel. All urinary samples were normalized to Cr.

Results: Table-1 Data

Funding: NIHDKK Support, Pharmaceutical Company Support

TH-OR074

Background: Due to altered red blood cell survival and erythropoietin therapy glycated hemoglobin (HbA1c) may not accurately reflect long-term glycemic control in patients with diabetes and chronic kidney disease (CKD). Glycated albumin (GA) and fructosamine are potential alternative markers since their production is not affected by anemia or ESA. The exact relationship between glucose and the different indices of glycemia in advanced CKD have yet to be established. The aim of this study was to determine the correlation of glucose with the different indices of glycemia in advanced CKD.

Methods: A total of 20 diabetic patients with nephrotic range proteinuria in over 50% of patients with nephrotic diabetic nephropathy. Because ACTH may represent a novel therapy for advanced diabetic nephropathy, and may act in part by restoring appropriate expression of VEGF.

Conclusions: ACTH gel reduces proteinuria in diabetic nephropathy for up to 6 months after withdrawal of therapy. ACTH gel increased urinary VEGF 5-fold with a non-significant trend toward increased MCP-1. ACTH gel may represent a novel therapy for advanced diabetic nephropathy, and may act in part by restoring appropriate expression of VEGF.

Funding: NIHDKK Support, Pharmaceutical Company Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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than one proteomic technique identified a wider range of candidates. Further evaluation is usually with up-regulation of factors in kidney parenchyma. We identified markers that approaches brought disease-gene identification in close proximity.

duplex collecting system identified suggestive linkage for loci on chromosome 3 and 4. candidate genes was performed and revealed novel variants implicated in CAKUT of end-stage renal disease in children. Structural anomalies belonging to the CAKUT spectrum include renal agenesis, multicystic dysplastic kidney, and duplex collecting system. Not much is known about the origin of CAKT. Often, renal abnormalities are found in close relatives of CAKT cases, showing a genetic contribution to CAKT. Many studies demonstrate common molecular pathology in CAKT, and glycodelin; LC/MSE identified increased immunoglobulins, kininogen and IBP1.

significant down-regulation of alpha-1-microglubulin (AMBP) and gelsolin. Reduced considerations to be important. The aim of this study is to identify new genetic factors involved in CAKT aetiopathogenesis.

Identification of Novel Genetic Factors for Congenital Anomalies of the Kidney and Urinary Tract (CAKUT) (M. Renkema,1,2 Ernie M.H.F. Feitz,4 Helen McNeill,5 Barbara Franke,2 Nine V. Knoers.1,2)

In renal malformations, SELDI-TOF detected a down-regulated protein of 40,890Da, or appeared to originate in the distal pelvis, but only partially propagated in the proximal small contractions that appeared near synchronously along the full length of the renal pelvis. Immunofluorescence of kidney sections from Crim1KST264/− mice display renal anomalies, focal glomerular cysts and progressive interstitial fibrosis. A portion of Crim1KST264/KST264 mice develop hydronephrosis of unknown aetiology. This study further characterized the underlying renal abnormalities that differentiate CAKT and Crim1KST264/− mice from controls.

We tested the hypothesis that lack of Ang II production in angiotensinogen (AGT)-null mice impairs papillary collecting duct elongation thus contributing to the hypoplastic renal medulla phenotype observed in these mice. Conclusions: Papillae were dissected from AGT+/−/− and −/− mouse metanephi on postnatal day 3 and grown in 3-dimentional collagen matrix gels located on air-fluid interface in the presence of media (control, n=6 genotypes) or Ang II (10 M, n=6 genotype) for 24 hours. Images were acquired at time of dissection (“0” hours) and after 24 hours of culture. A significant change in papillary length, determined by Slidebook 4.0 software, at 24 hours relatively to time “0” was compared between the groups. We next examined the role of Ang II AT1R in papillary collecting duct growth, cell proliferation and apoptosis using P3 papillae from Hoxb7-GFP+ mouse grown ex vivo for 24 hours in the presence of the specific AT1R antagonist, candesartan. In papillae from control mice, we observed a significant reduction in papillary length after 24 hours of culture (87±4.2 vs. 100±0%, p<0.01). In contrast, papillary length in group (media, n=5) did not differ from baseline (101±4.3 vs. 100±0%, p=0.6. The number of proliferating phospho-histone H3 (pH3)-positive collecting duct cells, visualized with anti-phospho-histone H3 antibody, was lower whereas the number of pH3-negative cells undergoing apoptosis was higher in candesartan- vs. media-treated papillae (pH3: 12±1.4 vs. 21±1.2, p<0.01; Casp 3: 3.8±0.5 vs. 17±0.2, p<0.01).

Conclusions: In summary, Ang II, acting via the AT1R, promotes papillary growth by stimulating collecting duct cell proliferation and survival. We conclude that defects in collecting duct elongation may be causally linked to medullary hypoplasia observed in AGT- and AT1R-null mice.

MRI Reveals Postnatal Functional Obstruction and Abnormal Peristalsis in Crim1KST264/KST264 Mice (Lorine J. Wilkinson,1 Nyoman Dana Kurniawan,2 Yu Leng Phua,1 Joan Li,1 Melissa H. Little,1 Richard J. Lang.1)

MRI Reveals Postnatal Functional Obstruction and Abnormal Peristalsis in Crim1KST264/KST264 Mice (Lorine J. Wilkinson,1 Nyoman Dana Kurniawan,2 Yu Leng Phua,1 Joan Li,1 Melissa H. Little,1 Richard J. Lang.1)

Developmental Biology and Inherited Kidney Diseases: From Genes to Systems Biology

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Developmental Biology and Inherited Kidney Diseases: From Genes to Systems Biology
Analysis Identifies Two Novel Nephropathy Loci

Roel Sterken, Natalia High

Resolution Genome Scans Combined with Principal Component Analysis (PCA) to identify engaging mRNA and their targets. PAR-CLIP on mature cells identified 860 high confidence mRNA clusters (showing a unique photo-crosslinking signature); a lower estimate of the true number of mRNA targets in podocytes due to the scale of the experiment. The clusters mapped to 634 genes (1 to 5 mRNA clusters per target gene). 89% of clusters matched exons. Of these, 70% targets in podocytes (parentheses denote # of members), sf-miR-30a(6) ranked higher in immature. Higher expression of miR-29a(4), miR-18a(1) and several additional mRNA genomic clusters was found in podocytes compared to other epithelia (panel A).

Figure (A) mRNA profile heat map (top 70%) with hierarchical clustering of samples (cell lines). mRNA are grouped according to genomic arrangement and the clusters of a particular gene are represented by the order in which the exons are present in that gene. The clusters and analyses are represented by the following:

Conclusions: We provide mRNA profiles from human podocytes based on RNA deep sequencing. These profiles, complemented with biochemically confirmed transcription-wide in-vivo mRNA target PAR-CLIP data, can serve to direct further research on miRNA involvement in podocyte development and disease.

Funding: Other NIH Support - The project described was supported by Grant Award Number U1DR024143 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research.

TH-OR080

High Resolution Genome Scans Combined with Principal Component Analysis Identifies Two Novel Nephropathy Loci

Roel Sterken, Natalia High

Resolution Genome Scans Combined with Principal Component Analysis to identify engaging mRNA and their targets. PAR-CLIP on mature cells identified 860 high confidence mRNA clusters (showing a unique photo-crosslinking signature); a lower estimate of the true number of mRNA targets in podocytes due to the scale of the experiment. The clusters mapped to 634 genes (1 to 5 mRNA clusters per target gene). 89% of clusters matched exons. Of these, 70% targets in podocytes (parentheses denote # of members), sf-miR-30a(6) ranked higher in immature. Higher expression of miR-29a(4), miR-18a(1) and several additional mRNA genomic clusters was found in podocytes compared to other epithelia (panel A).

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Funding: Other NIH Support - The project described was supported by Grant Award Number U1DR024143 from the National Center for Research Resources (NCRR), a component of the National Institutes of Health (NIH) and NIH Roadmap for Medical Research.

TH-OR082

Systems Approach Identifies HIPK2 as a Critical Regulator of Kidney Tubulointerstitial Fibrosis

Yuanmeng Jin, Peter Y. Chuang, Vivette D. D’Agati, John C. He.

Background: Tubulointerstitial fibrosis is a common process that leads to the progression of kidney disease. We describe an integrated computational/experimental approach to identify upstream protein kinases that regulate gene expression changes in kidneys of HIV-1 transgenic mice (Tg26), which exhibits significant tubulointerstitial injury. Using this approach, we identify the homeo-domain interacting protein kinase 2 (HIPK2), a previously unrecognized protein kinase for kidney disease, is critically involved in the regulation of the gene network in HIV AN kidney. We find that HIPK2 expresses mostly in kidneys of HIV-1 transgenic mice (Tg26), which exhibits significant tubulointerstitial injury. We confirm that HIPK2 mediates HIV-induced apoptosis of renal tubular epithelial cells (RTEC). HIPK2 also mediates TGF-beta and HIV-induced expression of EMT markers. Here, we further determined the role of HIPK2 in vivo by crossing HIPK2-/- with Tg26.

Methods: HIPK2-/- mice on a mixed C57B6/129svj background were first backcrossed onto the FVB/N background for 6 generations and then cross with Tg26 to generate mice that are HIPK2 deficient and express the HIV-1 transgene. HIPK2-/-;Tg26 mice were compared to HIPK2+/+;Tg26 at 8 weeks of age. Renal function, proteinuria, and kidney histology were assessed in these mice. The activation of signaling pathways were also studied in kidneys of these mice.

Results: Compared to HIPK2+/+;Tg26 group, the HIPK2-/-;Tg26 mice had a significant decrease in serum urea nitrogen, urine protein/creatinine ratio, collagen deposition and tubulo-interstitial injury and fibrosis scores. Knockout of HIPK2 also significantly attenuated the activation of TGF-beta/smad, Wnt/beta-catenin, Nox1, and p38 pathway in kidneys of Tg26 mice.

Conclusions: HIPK2 is a critical regulator of kidney fibrosis in the HIVAN model and a potential target for anti-fibrosis therapy.

Funding: NIDDK Support
TH-OR083

Geographic Differences in Genetic Susceptibility to IgA Nephropathy: GWAS Replication Study and Geospatial Risk Analysis


Background: In a recent GWAS, we localized 5 common susceptibility loci for IgAN.

The goals of this study are to replicate these findings in independent cohorts and to model the genetic risk for IgAN in the worldwide populations.

Methods: We tested for association, assessed heterogeneity, and calculated a genetic risk score for IgAN in independent cohorts of 5 nationalities (French, Italian, German, Hungarian & AA, 1,120 cases & 1,665 controls) in addition to the original GWAS cohorts (Asians and Europeans; combined N=8,687). After validation, the risk model was applied to 1,042 HGDP individuals (57 populations); spatial interpolation of the risk trend surface was used to construct topographical IgAN risk maps across 6 continents.

Results: 4 of 5 loci demonstrate significant replication and no heterogeneity (OR=0.70-0.88, P=8x10-4-10^{-2}). Heterogeneity is observed only for the MHC locus of TAP1/2-PSMB9/9 (I2=75%, p<0.05). This locus replicates in Italians & Germans (OR=0.60-0.67, P=5x10^{-3}-10^{-2}), but not in French, Hungarians, or AAs. In the combined analysis, all 5 loci remain highly significant with minor alleles conferring protection (OR=0.66-0.81, P=5x10^{-3}-10^{-2}) in both A1c and A1E in independent GWAS cohorts & in HGDP individuals. Taken together, these findings suggest that the genetic risk for IgAN is widespread in the human population.

Conclusions: These data suggest that the five IgAN risk loci contribute to the known geographic variation in disease prevalence.

Funding: NIDDK Support

TH-OR084

Mitochondrial Mistargeting Causes Autosomal Dominant Renal Fanconi Syndrome

Markus Reindl, Enrico Klotzjuk, Horia Stanescu, Detlef Böerger, Carsten Broeker, Dominika Peindl, Kathrin Renner, Karin Eberhart, Joerg Reinders, Katja Dettmer, Robert Klera, Richard Warth, University of Regensburg, Regensburg, Germany; 2University College London, United Kingdom.

Background: Renal Fanconi syndromes are characterized by a generalized renal proximal tubular dysfunction. For this study the causative genetic factor as well as the underlying molecular pathology in an extended family with autosomal dominantly inherited renal Fanconi syndrome without kidney failure was unraveled.

Methods: Whole genome multiplex parametric linkage analysis was performed resulting in a significant LOD score (> 3) for a single locus. All genes in the linked area were sequenced resulting in the identification of a heterozygous mutation in a gene, which we call Fanconin that leads to a de-novo formation of a mitochondrial targeting motif. To assess the functional impact a stable permanently transfected inducible renal proximal tubular cell model was generated. Immunohistochemical analysis showed appropriate intracellular localization of Fanconin.

Results: Underline represents presenting author.

Conclusions: This novel mitochondrial chaperone can be a causative factor for Fanconi syndrome.

Funding: NIDDK Support

TH-OR085

Glycemic Control and Mortality in Hemodialysis Patients with Diabetes Mellitus: A Six Year Cohort Study

Miklos Z. Molnar, Joni L. Ricks, Csaba P. Kovacs, Anuja P. Shah, Allen R. Nissenson, Mark E. Williams, Kamya Kalantar-Zadeh, Harold Simmons Center, Torrance, CA; 2Salem VA Medical Center, Salem, VA; 3DaVita, Inc, Denver, CO; 4Joslin Diabetes Center, Harvard Medical School, Boston, MA.

Background: Observational studies examining the association of hemoglobin A1c (A1c) with outcomes in diabetic patients (pts) on maintenance hemodialysis (MHD) have used different methodologies & reached somewhat contrasting conclusions.

Methods: We examined mortality-predictability of A1c & random serum glucose over time in a cohort of diabetic MHD pts treated in DaVita dialysis clinics from July 2001 through June 2006 with follow-up through June 2007.

Results: We identified 54757 diabetic MHD pts with A1c data (age, 63±13 years, 51% men, 30% African Americans). Adjusted all-cause death hazard ratio (HR) and 95% confidence interval for baseline A1c increments of 0.8-0.9%, 9.0-9.9% and ≥10%, compared to 7.0-7.9% (reference), were 1.06(1.01-1.12), 1.05(0.99-1.12), & 1.19(1.12-1.28); and for time-averaged A1c were 1.11(1.05-1.16), 1.36(1.27-1.45), & 1.59(1.46-1.72), respectively.

Conclusions: Poor glycemic control (A1c ≥ 8%) or serum glucose ≥ 200mg/dl appears associated with high death in MHD pts. Very low glycemic levels also add mortality risk. Clinical trials are needed to better define the target A1c levels in long-standing diabetic pts on MHD.

Funding: NIDDK Support

TH-OR086

Analysis of Cholesterol Homeostasis Characterizes Dialysis Patients as “Cholesterol Absorbers”

Kyril S. Rogachev, Tobias Pindorf, Oliver Weingärtner, Julius Popp, Dietrich Lutjohann, Gunnar H. Heine.

1Nephrology & Hypertension, Saarland University Hospital, Homburg, Germany; 2Clinical Chemistry/Pharmacology, University of Bonn, Bonn, Germany; 3Psychiatry, University of Bonn, Bonn, Germany; 4Cardiology, Angiology, Intensive Care, Saarland University Hospital, Homburg, Germany.

Background: Recent clinical trials on cholesterol-lowering in chronic kidney disease patients yielded conflicting results, which might result from different treatment strategies used. Serum cholesterol levels are determined by both endogenous synthesis and intestinal absorption, which are differentially influenced by various classes of cholesterol-lowering agents. Assessment of cholesterol homeostasis has thus been proposed for guidance of lipid-lowering therapy. We analysed established surrogate markers of cholesterol homeostasis in patients with chronic kidney disease.

Methods: In 113 hemodialysis patients, we measured lathosterol and desmosterol as markers of cholesterol synthesis, and cholestanol, sitostanol and campesterol as markers of cholesterol absorption via gas chromatography. 229 healthy subjects served as controls.

Results: Hemodialysis patients displayed a striking shift towards cholesterol absorption compared to synthesis (p<0.001). High absorption markers and concomitantly low synthesis markers indicated poor outcome among dialysis patients in univariate Kaplan-Meier analysis, and in multivariate Cox regression analysis after adjustment for potential confounders.

Conclusions: Our analysis of cholesterol homeostasis characterises hemodialysis patients as “cholesterol absorbers”. These findings supplement data from a recent randomised controlled trial on dual cholesterol-lowering therapy in chronic kidney disease.
Declining Interdialytic Weight Gain (IDWG) in Chronic Hemodialysis Patients (HD) — An Ominous Sign? Results of an International Study

ADRIAN MORALES-GUINZBURG,1 Cristina MARELLI,1 Adam TASHMAN,2 Michael EITTER,1 Daniela MARCELLI,1 Gero D. VON GERSDORFF,1 Mathias SCHALLER,1 Yuedong WANG,1 Nathan W. LEVIN,1 Peter KOTANKO,2 Len A. USVAVY,1 1FMC Latin America, Buenos Aires, Argentina; 2University of California — Santa Barbara, Santa Barbara, CA; 3FMC Asia Pacific, Hong Kong, Hong Kong; 4FMC Europe, Bad Homburg, Germany; 5RRI, NY, NY; 6University of Cologne Medical Center, Germany.

Background: Chronic HD patients (pts) frequently present with high IDWG, presumably due to fluid over intake. It has been reported that in pts from US, contrary to widespread believe, declining IDWG precedes death (Kotanko 2009). We extend this observation to diverse HD populations from 3 continents.

Methods: HD databases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and KfH in Germany were queried. IDWG was calculated as % of post-HD weight. IDWG dynamics were analyzed by estimating the mean IDWG level before death and its 1st derivative using quintic splines.

Results: Chronic HD pts from 23 countries were studied (Europe 17 [N=12333; age 71.7, 59% males]; South-East Asia 4 [N=1484; age 68; 53% males]; Argentina [N=10517; age 63.1; 58% males]; USA [N=3473; age 69.9; 56% males]). IDWG [mean (SD)] before death was 2.3 (2.6); 3.8 (3.2); 2.9 (3.1); 2.9 (3.1); same order as above. Irrespective of the region, IDWG dropped in males between 0.23% in South-East Asia and 0.68% in Europe in the 2 years preceding death (left).

The rate of IDWG change was identical in Europe, Argentina, and US, and less in South-East Asia (right). The results were materially identical in females and pts from KfH (not shown).

Conclusions: This international study corroborates that opposite to common believe, a decline in IDWG may be an ominous sign. Insights into pre-death biology may aid the development of alert systems to facilitate timely interventions.

Declining Interdialytic Weight Gain (IDWG) in Chronic Hemodialysis Patients (HD) — An Ominous Sign? Results of an International Study

<table>
<thead>
<tr>
<th>Hazard Ratio (95% CI)</th>
<th>Model A (not adjusted for nutritional indicators)</th>
<th>Model B (adjusted for nutritional indicators)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevalence</td>
<td>15.9%</td>
<td>10.2%</td>
</tr>
<tr>
<td>Prevalence</td>
<td>24.2%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Prevalence</td>
<td>13.8%</td>
<td>10.9%</td>
</tr>
<tr>
<td>Serum potassium (mEq/l)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>4.4 – 4.9</td>
<td>5.7 – 6.0</td>
</tr>
<tr>
<td>&gt;4</td>
<td>5.5 – 5.9</td>
<td>6.0 – 6.6</td>
</tr>
</tbody>
</table>

| Prevalence            | 15.9%                                         | 10.2%                                         |
| Prevalence            | 24.2%                                         | 21.4%                                         |
| Prevalence            | 13.8%                                         | 10.9%                                         |

| Serum potassium (mEq/l) |                                               |                                               |
| ≤4                    | 4.4 – 4.9                                     | 5.7 – 6.0                                     |
| >4                    | 5.5 – 5.9                                     | 6.0 – 6.6                                     |

Conclusions: By current standards useful to inform reimbursement decisions in publicly funded health care systems, frequent home HD appears attractive compared with conventional HD. However, the attractiveness varies based on the mix of comparator dialysis modalities. The modality mix provided by a health system and used in patients who would be offered frequent home HD is a critical factor when considering establishing a program.

Funding: Government Support - Non-U.S.

TH-OR087

Declining Interdialytic Weight Gain (IDWG) in Chronic Hemodialysis Patients (HD) — An Ominous Sign? Results of an International Study

ADRIAN MORALES-GUINZBURG,1 Cristina MARELLI,1 Adam TASHMAN,2 Michael EITTER,1 Daniela MARCELLI,1 Gero D. VON GERSDORFF,1 Mathias SCHALLER,1 Yuedong WANG,1 Nathan W. LEVIN,1 Peter KOTANKO,2 Len A. USVAVY,1 1FMC Latin America, Buenos Aires, Argentina; 2University of California — Santa Barbara, Santa Barbara, CA; 3FMC Asia Pacific, Hong Kong, Hong Kong; 4FMC Europe, Bad Homburg, Germany; 5RRI, NY, NY; 6University of Cologne Medical Center, Germany.

Background: Chronic HD patients (pts) frequently present with high IDWG, presumably due to fluid over intake. It has been reported that in pts from US, contrary to widespread believe, declining IDWG precedes death (Kotanko 2009). We extend this observation to diverse HD populations from 3 continents.

Methods: HD databases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and KfH in Germany were queried. IDWG was calculated as % of post-HD weight. IDWG dynamics were analyzed by estimating the mean IDWG level before death and its 1st derivative using quintic splines.

Results: Chronic HD pts from 23 countries were studied (Europe 17 [N=12333; age 71.7, 59% males]; South-East Asia 4 [N=1484; age 68; 53% males]; Argentina [N=10517; age 63.1; 58% males]; USA [N=3473; age 69.9; 56% males]). IDWG [mean (SD)] before death was 2.3 (2.6); 3.8 (3.2); 2.9 (3.1); 2.9 (3.1); same order as above. Irrespective of the region, IDWG dropped in males between 0.23% in South-East Asia and 0.68% in Europe in the 2 years preceding death (left).

The rate of IDWG change was identical in Europe, Argentina, and US, and less in South-East Asia (right). The results were materially identical in females and pts from KfH (not shown).

Conclusions: This international study corroborates that opposite to common believe, a decline in IDWG may be an ominous sign. Insights into pre-death biology may aid the development of alert systems to facilitate timely interventions.

TH-OR088

Associations of Serum Potassium with All-Cause Mortality: Results from the Dialysis Outcomes and Practices Patterns Study (DOPPS)

Kain Saran,1 Jinzhao Zhang,1 Anaconda Sen,1 Hal Morgenstern, Francesca Tenorri, Antonio Alberto Lopes,1 David C. Mendelssohn,1 Vittorio E. Andreucci,1 Hideki Kawanishi,2 Bruce M. Robinson.1,2 1University of Michigan; 2Arbor Research Collaborative for Health; 3Bahia da Universidade Federal da Bahia; 4Humber River Regional Hospital; 5Universita Federico II; 6Tischira General Hospital.

Background: Severe hyperkalemia, defined here as serum potassium (SK) > 6 mEq/l, has been observed in approximately 10% of maintenance hemodialysis (HD) patients. We investigated the relation between SK and mortality in a large international HD cohort study.

Methods: We analyzed data on 37,967 in-center hemodialysis (HD) patients from 12 countries in DOPPS phases 1-3 (1996-2008). Patient demographics, laboratory values and dialysis treatment data were obtained at study enrollment. Cox regression was used to estimate the hazard ratio (HR) and 95% CI for the effect of SK on all-cause mortality, adjusting for several potential confounders, excluding and including 4 nutritional markers. SK was categorized and treated as 5 indicator variables in the model (<4, 4-4.49, 4.5-4.99, 5-5.49, ≥5.5 mEq/l).

Results: When adjusting for patient demographics, 13 summary comorbid conditions, socioeconomic status, dialysis treatment and adherence indicators, but not nutritional markers, there was a U-shaped association between SK and mortality (Figure, Model A). When also adjusting for BMI, albumin, creatinine and normalized PCR, a positive monotonic association was observed (Model B).

Conclusions: The association between hyperkalemia and mortality seems to be confounded by poor nutritional status. Therefore, SK < 4.0 mEq/l should alert physicians to the potential presence of poor nutritional status in HD patients. Levels approaching 6 mEq/l or higher, however, warrant appropriate therapeutic maneuvers.

TH-OR090

Anti-Inflammatory & Anti-Oxidative Nutrition in Hypoaalbuminemic Dialysis Patients (AIONID) Double-Blind Randomized Placebo-Controlled Trial

Kamyar Kalantar-Zadeh,1 Martin Lee, Ramanath B. Dukkipati, Jennie Jing, Youngmee Kim, Anne Cole Voss, Deborah A. Benner, Miklos Z. Molnar, Iain C. Macdougall, John Tayek, Keith C. Norris, Csaba P. Kovesda, Joel D. Kopple.1 FMC Europe, Bad Homburg, Germany; 2FMC Latin America, Buenos Aires, Argentina; 3FMC Asia Pacific, Hong Kong, Hong Kong; 4FMC Europe, Bad Homburg, Germany; 5RRI, NY, NY; 6University of Cologne Medical Center, Germany; 7LABioMed/Harbor-UCLA.

Background: There is interest in providing frequent home nocturnal hemodialysis (NHD) to patients on dialysis, and decision makers require information on its cost-effectiveness. Methods: We did a cost-utility analysis of frequent home NHD compared with conventional hemodialysis (4 hours thrice weekly, including in-centre, satellite, and home HD) taking the perspective of the health payer over a lifetime horizon. Data on transition probability of health states, quality of life, costs, and other inputs were from a recent RCT. Costs, including training costs, were obtained using micro-costing, trial, and administrative data (CANS). We determined the incremental cost per quality adjusted life year (QALY) gained, and robustness was assessed using scenario, sensitivity, and probabilistic sensitivity analyses.

Results: Compared with conventional HD (61% in-centre, 14% satellite, and 25% home as in the trial), frequent home NHD led to incremental costs of $290 and additional 0.38 QALYS, with a cost/QALY gained of $5740. In sensitivity analyses, when the annual probability of failing home NHD and commencing conventional HD was assumed to be ≤6%, home NHD was dominant (greater benefit at lower costs), however if ≥21%, the cost/QALY gained was $753/QALY. The cost/QALY gained increased to $29k if average training time for NHD increased from 3.65 weeks to 6 weeks. Results were sensitive to quality of life estimates. In scenario analyses where the comparator modality was changed, frequent home NHD was dominant compared to in-centre HD, and was associated with a cost/QALY gained of $35k, $203k, and $481k when compared with satellite HD, home HD, and peritoneal dialysis respectively.

Conclusions: Based on current standards useful to inform reimbursement decisions in publically funded health care systems, frequent home HD appears attractive compared with conventional HD. However, the attractiveness varies based on the mix of comparator dialysis modalities. The modality mix provided by a health system and used in patients who would be offered frequent home HD is a critical factor when considering establishing a program.

Funding: Government Support - Non-U.S.
Results: Out of 84 pts (age: 59±12 yrs, vintage: 33 mo), 74 completed the entire 16 wks.

Group (n) A (n=19) B (n=22) C (n=22) D (n=21) ANOVA p
Assignments ONS+PTX ONS+placebo PTX+placebo placebo-placebo
Age (yrs) 59.2±13.6 84.6±10.3 57.7±13.9 63.9±9.1 0.09
Gender (% men) 53 36 34 41 0.77
Pre-trial albumin (g/dL) 3.60±0.45 3.60±0.20 0.51
Change in albumin 2.15±1.23 2.10±1.30 0.51
Change in albumin 0.10±0.24 0.10±0.21
Paired t-test p 0.001 0.004 0.008 0.58 0.57

Paired t-test showed significant alb increase of +0.14 to +0.21 g/dL in any intervention other than combined placebos. However, the intention-to-treat analysis of changes in albumin using predetermined equation (post-albumin - pre-albumin) showed that only ONS was associated with a significant change: ONS, PTX or both were associated with change in alb of +0.17 (p=0.02), +0.11 (p=0.12) & +0.16 g/dL (p=0.10), respectively. No serious adverse events were observed.

Conclusions: Daily intake of CKD-specific high-protein ONS with anti-inflammatory/anti-oxidative ingredients for 16 wks was well tolerated and associated with improved albumin level of about +0.17 mg/dL per ITT (p=0.02).

Funding: NIDDK Support

TH-OR091

More Efficient Removal of Serum Bilirubin by a Novel Artificial Liver Support System: A Pilot Study

Dehua Gong, Dongdong Zhu, Daxi Ji, Zhi-Hong Liu. Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.

Background: In this study, we established a novel artificial liver support (ALS) system based on fractionated plasma separation and adsorption system integrated with continuous venous-venous hemofiltration (FSA-CVVH), and compared its efficacy with the traditional system of plasma separation and adsorption (PSA).

Methods: Sixteen patients with hyperbilirubinemia due to acute liver failure were included. For PSA, plasma was separated and perfused through an adsorber. For FSA-CVVH, albumin-rich plasma was separated using a fraction plasma separator, followed by a concentration through ultrafiltration using a hemofilter. The concentrated plasma was then perfused through an adsorber and returned to the patients with a simultaneous infusion of the same amount of replacement fluid via a predilution route into blood. The duration for each session of ALS was 8 hours.

Results: The comparisons of total bilirubin clearance (TB, ml/min) by PSA versus FSA-CVVH at the time points of 0.5, 2, 4, and 6 hour were as follow: 22.3 ± 2.2 vs. 28.7 ± 3.1, 12.2 ± 4.4 vs. 21.9 ± 9.1 (P < 0.05), 9.0 ± 2.8 vs. 16.1 ± 4.3 (P < 0.01), 9.5 ± 3.9 vs. 11.2 ± 3.7, 8.3 ± 3.0 vs. 9.3 ± 4.1, respectively. The average reduction rates (%) of TB, bile acid, urea and creatinine were as follow: 29.1 ± 27.2% for single session of PSA versus FSA-CVVH were 46.1 ± 8.3%, 54.4 ± 5.2% (P < 0.05), 36.5 ± 5.2% vs. 47.6 ± 14.7%, 14.9 ± 16.8% vs. 31.4 ± 10.6% (P < 0.01), and -17.1 ± 14.7 vs. 5.3 ± 6.2% (P < 0.05), respectively. On the other hand, there was a significant decline of albumin level (10.3 ± 5.9%, P < 0.01), prolongation of prothrombin time (11.2 ± 2.7%), P < 0.01 and activated partial thromboplastin time (26.6 ± 30.4%, P < 0.05) observed after PSA but not FSA-CVVH.

Conclusions: In conclusion, compared with PSA system, the novel FSA-CVVH system provides a more efficient removal of both albumin-bound toxins, like bilirubin, and water-soluble toxins, like urea and creatinine, with less deleterious effects on serum albumin level and blood coagulation.

TH-OR092

Comparing Erythropoiesis Stimulating Agent Dose and Responsiveness between Peritoneal and Hemodialysis Patients

Uyen Duong, 1 Kamyar Kalantar-Zadeh, 1,2 Miklos Z. Molnar, 1 John J. Sim, 1 Ramanath B. Dukkipati, 1 Parta Hatamizadeh, 1 Deborah A. Benner, 1 Joel D. Kopple, 1 Kamyar Kalantar-Zadeh, 1,4 Harold Simmons Center, Torrance, CA; 1Salem VA Medical Center, Salem, VA; 1Kaiser Permanente, Los Angeles, CA; 1Harbor UCLA, Torrance, CA; 1DaVita Inc., Denver, CO.

Background: It is not clear which potentially modifiable laboratory measures are associated with greater survival in maintenance hemodialysis (MHD) patients (pts). Method: In a large cohort of 140146 MHD pts who underwent MHD treatment for at least 3 months in all legacy DaVita dialysis clinics between July 2001 & June 2006, changes in values across consecutive 20 calendar quarters for laboratory measures that may represent “malnutrition inflammation complex syndrome” (MICS) were calculated. Values were standardized with mean of 0 and standard deviation of 1, and hazard ratios for the increase or decrease (as commensurate) in 1 standard deviation were estimated.

Results: Pts were 61.5±15.5 years old & included 45% women, 32% African Americans, 14% Hispanics & 44% diabetics. Cox models were case-mix adjusted (sex, age, race/ethnicity, diabetes, dialysis vintage, Kt/V, BMI) & MICS adjusted (serum creatinine, albumin, calcium, nPCR, ferritin, bicarbonate, TIBC, TSAT, phosphorus, lymphocyte percentage & blood hemoglobin): The three indicators of protein energy wasting (lower serum levels of albumin, creatinine & lymphocyte percentage) showed the strongest association with death risk, followed by lower serum bicarbonate & higher levels of serum phosphorus & calcium.

Conclusions: Measures of nutritional/inflammatory status are by far the strongest predictors of survival in MHD patients. Interventions to improve nutritional/inflammatory status may improve longevity. This hypothesis needs testing in randomized trials.

Funding: NIDDK Support

TH-OR093

Superiority of the Survival-Predictability of Laboratory Measures of the Nutritional Status in Hemodialysis Patients: A Comparative Effectiveness Study

Jessica Miller, 1 Csaba P. Kovesdy, 1 Miklos Z. Molnar, 1 John J. Sim, 1 Ramanath B. Dukkipati, 1 Parta Hatamizadeh, 1 Deborah A. Benner, 1 Joel D. Kopple, 1 Kamyar Kalantar-Zadeh, 1,4 Harold Simmons Center, Torrance, CA; 1Salem VA Medical Center, Salem, VA; 1Kaiser Permanente, Los Angeles, CA; 1Harbor UCLA, Torrance, CA; 1DaVita Inc., Denver, CO.

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Funding: NIDDK Support
TH-OR094
Hepcidin Isoforms – Biomarkers for Optimal Management of Renal Anemia in Hemodialysis Patients Satoshi Yamazaki,1 Yusuke Sasaki,2 Masahiro Hagiwara,1 Shunichi Furutaka,1 Minoru Murakami,1 Yasushi Shimokama,3 Masaya Ikeda,1 1Dept. of Nephrology, Saku Central Hospital, 2Minamisaku-Gun, Nagano, Japan; 3Product Research Dept., Chugai- pharmaceutical Co., Ltd., Kamakura-shi, Kanagawa, Japan.

Background: It has been known that serum levels of hepcidin-25 (Hep-25) and its isoforms, hepcidin-20 and -22, are significantly higher in hemodialysis (HD) patients than in healthy subjects, however, the significance of these isoforms has not been well elucidated. In the present study, we analyzed the relationship between serum levels of hepcidin isoforms and clinical parameters in HD patients to clarify the possibility of hepcidin isoforms as biomarkers for anemia treatment.

Methods: We enrolled 100 HD patients, provided written informed consent in Saku Central Hospital. Patients with infection, severe inflammatory disorders or hematologic disorders were excluded. Anemia and other complications were treated according to national and K/DOQI guidelines. Erythropoiesis stimulating agents (ESA) was used to maintain a target hemoglobin (Hb) level of 10-11 g/dL. Clinical parameters including iron parameters and serum hepcidin isoforms were measured. Patients characteristics were analyzed in 4 groups divided by median of serum levels of Hep-25 (High, Low) and the ratio of active hepcidin (Hep-25/total hepcidin isoforms levels) (High, Low).

Results: Patients with high Hep-25 levels and low active hepcidin ratio, named HL group, showed the lowest Hb and highest mean cellular hemoglobin (MCH) than any other groups. HL group also exhibited high transferrin saturation (TSAT) and serum ferritin levels. Instead, patients with low Hep-25 levels and low active hepcidin ratio (LL group) showed the highest Hb and lowest MCV than any other groups. LL group exhibited low TSAT and serum ferritin levels. There was no difference in ESA dosage between groups.

Conclusions: Present results indicate that patients with high Hep-25 levels and low active hepcidin ratio characterized enough iron storage and supply but low hematopoietic activity. It is recommended that these patients should be treated ESA rather than iron supplementation. Moreover, iron status and hematopoietic activity might affect proteolysis of hepcidin isoforms.

TH-OR095

Background: There are limited data on the population epidemiology of acute kidney injury (AKI).

Methods: To determine the incidence rate of dialysis-requiring AKI from 1998-2008, we analyzed data from the US Census and the Nationwide Inpatient Sample (NIS), a nationally representative sample of hospitalizations, accommodating for NIS’s sampling scheme. Cases were identified using validated ICD9 codes (Waikar 06). To explore reasons for temporal trends, we used multivariate logistic regression, with dialysis-requiring AKI as the primary outcome, year as main predictor, and additional covariates such as demographic variables and prespecified AKI risk factors.

Results: From 1998-2008, the incidence rate of dialysis-requiring AKI increased from 201 to 496 cases/million person-yrs, averaging 9% per yr (OR 1.09, 95% CI 1.08-1.10). Since 2005, the population incidence of dialysis-requiring AKI has exceeded that of renal replacement therapy (RRT)-requiring end-stage renal disease (ESRD).

Conclusions: Process-of-care factors frequently influenced the decision: Nurse availability (27%); vascular access issues (25%); dialysis need at night time (17%) or holiday and weekends (17%). Physician’s perception of futility correlated well with their choice to withhold dialysis, whereas prediction of renal recovery was associated with greater likelihood that the patient did not need dialysis.

Conclusions: Decisions to initiate dialysis are widely variable and uncertainty is common. Perception of patient prognosis and process-of-care factors influence the decisions.

Funding: NIDDK Support

TH-OR097
A Multi-Center Pilot Study To Assess the Safety and Efficacy of Selective Cytopheretic Device (SCD) Therapy in Patients with Acute Kidney Injury (AKI) James A. Tumlin,1 Lakhmir S. Chawla,2 Ashita J. Tolwani,3 John J. Dillon,3 Ravindra L. Mehta,3 Kevin W. Finkel,4 J. Ricardo Da Silva,5 Alexander S. Yevzlin,6 David Humes,7 SERRI; George Washington University; 8University of Alabama; 9Mayo Clinic; 10UCSD; 11University of Texas; 12Cytheris, Inc.; 13University of Wisconsin; 14University of Michigan.

Background: Dialysis requiring AKI in critically ill patients is associated with hospital mortality rates between 30-60%. Currently, there are no proven therapies that can improve renal function or reduce overall mortality.

Methods: To address this problem, we conducted a multicenter, single arm, pilot study of the Selective Cytopheretic Device (SCD), a novel cartridge that is able to selectively bind and deactivate activated neutrophils from the circulation. Enrolled patients received up to 7 days of SCD therapy in conjunction with citrate-based continuous renal replacement therapy (CRRT). The primary endpoints of the study were safety of the SCD and mortality at 28 and 60 days.

Results: A total of 35 patients were enrolled by 6 centers in the United States between May 2010 and January 2011. The mean age of patients enrolled was 56.9 years with 60.0% (21/35) male. The mean Sequential Organ Failure Assessment (SOFA) score at baseline was 11.3 and 68.6% (31/35) were ventilated. The overall mortality rate was 28.6% (10/35) in hospital, 25.7% (9/35) at 28 days and 31.4% (11/35) at 60 days. The rate of renal recovery in survivors was 73.1% (19/26) by day 28 and 100% (24/24) by day 60. During the study, a total of 28 serious adverse events were reported of which 7.1% were determined to be by investigators to be related to the SCD device. Elastase, IL-6, and sICAM, markers associated with inflammation, decreased substantially over the course of therapy.

Conclusions: This trial provides preliminary data of the safety and efficacy of the SCD treatment. The SCD compares favorably to the 50-60% mortality reported in previous AKI trials. The addition of the SCD to conventional citrate-based CRRT was well tolerated and led to a reduction in serum markers of activated neutrophils.

Funding: Pharmaceutical Company Support
TH-OR098
High Cut-Off Hemodialysis for the Management of Myeloma Kidney: An International Study
Colin A. Hutchison, Anne Bevins.
1The Binding Site Group Ltd, Birmingham, United Kingdom; 2Renal Unit, University Hospital Birmingham, United Kingdom.
Background: The early reduction of serum free light chains (FLC) improves clinical outcomes for patients with acute kidney injury secondary to multiple myeloma. We studied an international cohort of patients treated FLC removal by high cut-off (HCO) hemodialysis to determine treatment patterns and clinical outcomes associated with its use in this setting.
Methods: Data was collected from 54 patients, from 18 centers in 10 countries, using electronic case report forms. Rates of renal recovery were determined in relationship to baseline variables, treatment patterns and degree of FLC reduction achieved.
Results: Patients were predominantly Caucasian, median age 65 years (range 43-81), with a median GFR of 8 (1-27). Baseline serum biochemistry was: creatinine 633.5µmol (168-2263), calcium 2.5mmol (0.91-3.83); albumin 34g/L (14-46) and iCa 4.95mg/L (0.57-5.7). Myeloma kidney was the primary diagnosis in 81% of the population who had a renal biopsy. Monoclonal κ and γ, FLC levels were: κ 5070µg/L (range 2520-20200) and γ 4200µg/L (range 300-13300), respectively at presentation. 68.75% of patients were treated with the Theratite dialyzer; another 31.25% used the C0100H8. 78% received bortezomib and 34% received thalidomide as part of their initial treatment. In total there were 626 HCO dialysis sessions, with a median of 3 (3-3) treatments per patient. HDF was used in 3 patients. Median FLC reduction was 72.96% (15.09-99.62%) by day 12 and 93.03% (40-2399.96%) by the last dialysis treatment. There was no difference in the percentage FLC reduction achieved between bortezomib and thalidomide treatment groups (p=0.140). Dialysis independence occurred in 55.6% of patients, median time 32 days (10-249). Independence of dialysis was greater in patients who had a reduction in serum FLCs by day 12 (p=0.038). No significant adverse events related to the study device were reported.
Conclusions: Targeting early FLC reductions in patients with AKI secondary to multiple myeloma should become standard of care. This study adds further weight to the potential benefit of FLC removal by HCO-HD in this setting.

TH-OR099
Exposure to Potentially Toxic Hydro- and Halocarbons Released from Dialyzer and Tubing Sets During Hemodialysis
Hyon Ji Julie Lee, Madeleine V. Pahl, Nostrala D. Vaziri, Donald R. Blace.
1Nephrology and Hypertension, University of California, Irvine, CA; 2Chemistry, University of California, Irvine, CA.
Background: While much is known about the effect of ESRD and dialysis on the composition of solutes in the plasma, little is known about their impact on composition of gaseous compounds in the exhaled breath. This study was designed to explore the effect of uremia and hemodialysis (HD) on the composition of exhaled breath.
Methods: Exhaled breath samples were collected from 10 ESRD patients immediately before, during and after dialysis. Ten age-matched healthy subjects served as controls. To determine the potential introduction of gaseous compounds from the components of dialysis, gasses were eluded from dialyzers, tubing sets, dialysate and water supplies. A five column/detector gas chromatography (GC) system was employed to measure different hydrocarbon, halocarbon, oxygenate, and alkyl nitrile sulfur containing compounds.
Results: The concentration of 11 hydrocarbons and 4 halocarbons in the patients' breath rose rapidly after the onset of the HD. All of the 15 compounds appearing in the breath of the patients, little is known about their impact on composition of gaseous compounds in the exhaled breath. This study was designed to explore the effect of uremia and hemodialysis (HD) on the composition of exhaled breath.

Conclusions: The present study documented release of several potentially toxic hydrocarbons and halocarbons to the circulation from the dialyzer and tubing sets during HD. The long-term exposure to these compounds may contribute to the morbidity and mortality in ESRD population and this issue should be considered in manufacturing of new generation of dialyzers and dialysis tubing sets.

TH-OR100
2Na-MRI Monitoring of Sodium Removal in Dialysis Patients
Anke Dahlmann, Peter Linz, Florian Eicher, Kathrin Dörfler, Matthias Hammam, Christoph Kopp, Alexander Cavallaro, Dominik N. Muller, Kai-Uwe Eckardt, Friedrich C. Luft, Michael Uder, Jens Titze.
1Nephrology & Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany; 2Interdisciplinary Centre for Clinical Research, University of Erlangen-Nuremberg, Erlangen, Germany; 3Radiology, University of Erlangen-Nuremberg, Erlangen, Germany; 4Experimental & Clinical Research Centre, Charité & MDC, Berlin, Germany; 5Experimental Medicine I, University of Erlangen-Nuremberg, Erlangen, Germany.
Background: Sodium and water are removed by hemodialysis. While water removal can easily be determined by ultrafiltration rates and weight loss, no valid method for the assessment of tissue Na+ content is available. 2Na magnetic resonance could offer spectroscopic and imaging (MRI) options.
Methods: We used 2Na magnetic resonance spectroscopy and imaging at 3Tesla (T) to quantify Na+ content in skeletal muscle of the lower leg. 15 dialysis patients (9 men, 6 women) were measured before and after regular dialysis with 2Na MRI. Blood pressure was inversely correlated with ultrafiltration rate (r=-0.00007x+0.90452, R2= 0.47), but not with blood pressure.
Conclusions: Non-invasive measurement of tissue Na+ content with 2Na MRI provides a novel clinical tool to assess Na+ removal in patients with end-stage renal disease during hemodialysis treatment. The method could allow efficacy monitoring for Na+ removal and permit a better understanding of the relationship between body Na+ accumulation, blood pressure, and cardiovascular disease in patients with end-stage renal disease.

TH-OR101
Increasing Protein-Bound Solute Clearances Independent of Urea Clearance
1Medicine, Stanford, Palo Alto, CA; 2Medicine, AECOM, New York, NY.
Background: The toxicity of bound solutes could be evaluated better if we could adjust the clearance of such solutes independent of unbound solutes. This study evaluated a method to increase the clearance of bound solutes independent of unbound solutes during nocturnal dialysis. We tested the hypothesis that bound solute clearances could be increased by raising the dialysate flow and dialyzer size above the low levels which are sufficient to achieve target Kt/Vurea values during the extended hours of nocturnal treatment.
Methods: Nine patients on thrice-weekly in-center nocturnal dialysis underwent two experimental sessions week one apart. The sessions were designed to provide the same urea clearance but widely different bound solute clearances. The Low KoA-Qd session employed a smaller dialyzer, dialysate flow of 300 ml/min, and blood flow of 350 ml/min. The High KoA-Qd session employed a larger dialyzer and dialysate flow of 800 ml/min while restricting blood flow to 270 ml/min. The bound solutes p-cresol sulfate (PCS) and indoxyl sulfate (IS) were measured by HPLC and the unbound solute urea was measured enzymatically.
Results: Results showed (mean±sd; p<0.05 High KoA-Qd vs Low KoA-Qd; p<0.05 PCS and IS clearance vs urea).

Urea
Clearance (ml/min) 193±16 294±20
Kt/Vurea 1.9±0.3 1.9±0.4
Removed in dialysate (g) 43±6 41±11

PCS
Clearance (ml/min) 14.6±7.0 29.7±9.0
Removed in dialysate (mg) 207±86 375±200

IS
Clearance (ml/min) 14.5±5.4 26.8±3.8
Removed in dialysate (mg) 153±74 201±137

Urea clearance and removal were similar during both treatments. As expected, the clearances of the bound solutes PCS and IS were much lower than the clearance of urea. But the High KoA-Qd session nearly doubled the clearances of PCS and IS without changing the urea clearance. Higher clearances during the High KoA-Qd session resulted in the removal of most of the IS in the dialysate.

Conclusions: Increasing dialyzer size and dialysate flow during nocturnal dialysis increases the clearance of bound solutes and could provide a means to further test the contribution of such solutes to morbidity in ESRD.

Funding: Other NIH Support - NIH Nephrology Research Training Grant T32DK73757
Associations of Dialysate Bicarbonate (DB) with Mortality: Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS) Francesco Tentori,1 Jin Yao Zhang,2 Anaenda Sen,3 Hal Morgenstern,4 Bruce M. Robinson,1,2 Hugh C. Rayner,1 Rachel B. Fissell,1 Raymond C. Vanholder,5 Tadashi Tomo,6 Rachelle K. Porter,1,2 The Research Collaborative for Health,1 University of Michigan,2 Birmingham Heartlands Hospital,3 Cleveland Clinic Foundation,4 University Hospital Gent,5 Oita University.

Background: Severe pre-dialysis metabolic alkalosis has been associated with elevated mortality risk in patients on maintenance hemodialysis (HD) [Bonner AJKD 2004]. We hypothesized that use of high dialysate bicarbonate (DB), which could result in severe post-dialysis metabolic alkalosis, may adversely affect clinical outcomes.

Methods: This study included 16,899 patients on thrice-weekly in-center HD using DB as the predominant dialysate buffer (20 to 45 mEq/L) from 12 countries in DOPPS 2 (2002-2004) and 3 (2005-2008). Cox regression was used to estimate the effect of DB on all-cause mortality, adjusting for potential confounders including predialysis serum bicarbonate. DB was analyzed as a continuous variable and as a categorical variable (<33, 33-36.9, ≥37 mEq/L).

Results: DB varied across facilities and countries. The overall median facility mean DB was 35 mEq/L (inter-quartile range=33.2, 36.5), with the lowest median level in Japan (30) and the highest level in the US (37). About 65% of facilities prescribed the same DB dose to more than 90% of its patients. DB was positively associated with all-cause mortality in the total sample and in facilities using a single DB for most patients (Table). The adjusted HR for DB did not vary appreciably by level of pre-dialysis serum bicarbonate, serum or dialysate potassium, serum or dialysate calcium.

Conclusions: High DB was associated with elevated mortality risk in the DOPPS. Changes in DB can easily be implemented in dialysis treatment and may have a beneficial impact on clinical outcomes.

Table: Adjusted HR (95% CI) for all-cause mortality, by category of dialysate bicarbonate or continuous DB and patient sample

<table>
<thead>
<tr>
<th>Dialysate bicarbonate (mEq/L)</th>
<th>All patients (N=16,899)</th>
<th>Facilities with more than 90% patients on the same dialysate bicarbonate prescription (N=8,345)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(mEq/L)</td>
<td>%</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Per 10 mEq/L (continuous)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;33</td>
<td>12.1</td>
<td>1.03 (1.01-1.05)</td>
</tr>
<tr>
<td>33-36.9</td>
<td>51.0</td>
<td>1.09 (1.07-1.12)</td>
</tr>
<tr>
<td>≥37</td>
<td>25.0</td>
<td>1.04 (1.01-1.06)</td>
</tr>
</tbody>
</table>

*Adjusted for severe uncorrectable anemia, age, sex, blood pressure, diabetes, duration of HD, serum albumin, dialysis vintage, predialysis bicarbonate level, serum potassium, serum calcium, and calcium x phosphorus product.

**Participants from patients from the initial cross-sectional and facilities with at least 5 patients and ≥60% of patients prescribed the same dialysate bicarbonate.

Funding: Pharmaceutical Company Support

Reserve Conservation in Hemodialysis: Two Simple, Practical, Eco-Sensitive, Cost Efficient Initiatives Alwijn Tiptop, John W.M. Agar. Renal Unit, Geelong Hospital, Melbourne, Victoria, Australia.

Background: Conventional hemodialysis (HD) is a rapacious (abuser of water and power, yet most HD services ignore its environmental impact. While HD session duration, frequency and equipment varies, our measured mean mains water use (4-5 hr x 3/wk HD) is 500L/min: 66% reject rate). Further, the metered power-draw from our paired Fresenius 4508B + Aquauno RO home systems is ∼0.1 ± 0.1 nmole, p<0.001, N=4). On a high Na diet, radio-telemetric BP showed a mean systolic BP elevation of 5 ± 2 mmHg during the day and a 10 ± 2 mmHg elevation during night in KAP-mAGT animals comparing to littermate control mice; no significant difference in BP was observed when fed a normal salt diet. Plasma renin concentration was reduced to 80% of control animal values by proximal tubule mAGT overexpression in animals given a high Na diet, but was not different between mouse lines during normal Na intake (n=7).

Astonishingly, plasma AT1A concentration was not altered by overexpression of proximal tubule mAGT (n=7). Renal renin mRNA tended to decrease in targeted mice (79 ± 7% of controls, N=7, p=0.08), while renal NHE3 and ENaC expression, documenting Cre in SM throughout the vasculature including small arterioles. The mechanisms responsible for the hypertensive effects of mAGT overexpression are unresolved, but studies suggest that further attention should be focused on alterations in vascular renin-angiotensin system.

Results: The KAP-mAGT animals showed kidney-specific KAP-AGT mRNA expression, normal immunostaining-detected AGT only in proximal tubule. Urinary uncleaved AGT was markedly increased in KAP-mAGT mice (5.3 ± 2.1 nmole vs. wild-type controls (0.1 ± 0.1 nmole, p<0.001, N=4). On a high Na diet, radiotelemetric BP showed a mean systolic BP elevation of 5 ± 2 mmmHg during the day and a 10 ± 2 mmHg elevation during night in KAP-mAGT animals comparing to littermate control mice; no significant difference in BP was observed when fed a normal salt diet. Plasma renin concentration was reduced to 80% of control animal values by proximal tubule mAGT overexpression in animals given a high Na diet, but was not different between mouse lines during normal Na intake (n=7).

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Conclusions: In summary, proximal tubule overexpression of mAGT leads to hypertension and salt-sensitivity without recruitment of the systemic renin-angiotensin system. The mechanisms responsible for the hypertensive effects of mAGT overexpression are unresolved, but studies suggest that further attention should be focused on alterations in NHE3 and ENaC.

Funding: NIDDK Support, Other NIH Support - NHLBI

Overexpression of Mouse Angiotensinogen in Renal Proximal Tubule Causes Salt-Sensitive Hypertension in Mice Donald E. Kohnan, Deborah Stuart, Jean-Marc Lelouel, Jian Ying. University of Utah Health Sciences Center, Salt Lake City, UT.

Background: The role of proximal tubule angiotensinogen (AGT) in modulating blood pressure has previously been examined using mice co-expressing proximal tubule human AGT and human renin. These animals are hypertensive, however the question remains whether alterations in “endogenous” mouse proximal tubule AGT, interacting with endogenous renin, affects blood pressure (BP).

Methods: Mouse AGT cDNA was knocked-in to the endogenous kidney androgen promoter (KAP) gene using an internal ribosomal entry site-based strategy.

Results: The KAP-mAGT animals showed kidney-specific KAP-AGT mRNA expression, normal immunostaining-detected AGT only in proximal tubule. Urinary uncleaved AGT was markedly increased in KAP-mAGT mice (5.3 ± 2.1 nmole vs. wild-type controls (0.1 ± 0.1 nmole, p<0.001, N=4). On a high Na diet, radiotelemetric BP showed a mean systolic BP elevation of 5 ± 2 mmHg during the day and a 10 ± 2 mmHg elevation during night in KAP-mAGT animals comparing to littermate control mice; no significant difference in BP was observed when fed a normal salt diet. Plasma renin concentration was reduced to 80% of control animal values by proximal tubule mAGT overexpression in animals given a high Na diet, but was not different between mouse lines during normal Na intake (n=7).

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Conclusions: In summary, proximal tubule overexpression of mAGT leads to hypertension and salt-sensitivity without recruitment of the systemic renin-angiotensin system. The mechanisms responsible for the hypertensive effects of mAGT overexpression are unresolved, but studies suggest that further attention should be focused on alterations in NHE3 and ENaC.

Funding: NIDDK Support, Other NIH Support - NHLBI

Angiotensin II Type 1A Receptors in Vascular Smooth Muscle Cells Contribute to Blood Pressure Control, Salt Sensitivity and Hypertension Matthew A. Sparks, Johannes Stegbauer, Susan B. Gurley, Thomas M. Coffman. Nephrology, Duke Hospital.

Background: Ang II acting through AT1 receptors promotes hypertension. However, AT1 receptors are expressed in many tissues relevant to BP control. In previous studies, we found that AT1 receptors in proximal tubule play a key role in determining level of BP. Here, we examine the impact of vascular actions of AT1 receptors in chronic BP homeostasis.

Methods: We generated a mouse with a conditional allele of the Agtr1a gene encoding the major AT1 receptor isofrom, AT1A. To eliminate AT1A from VSMCs, we used transgenic Cre mice driven by SM22 promoters. In a previous study, we found that a heterologous SM22-Cre transgene induced deletion of AT1A from large conduit arteries, but not resistance vessels. In these mice, BP and hypertensive responses were unaffected. Therefore, we now utilize a transgenic line with Cre knocked-in to the SM22 locus (KIS2M-Cre). Knock-in KIS2M-Cre mice were crossed with the mTmG reporter to map Cre expression, documenting Cre in SM throughout the vasculature including small arterioles. We then generated mice homozygous for the conditional Agtr1a allele also bearing KIS2M-
Cret(SMKOs). Compared to controls, acute constrictor responses to ang II were decreased by 25% in SMKO (p<0.05), consistent with partial deletion of AT1A from resistance arteries and arterioles. Resting BP measured by telemetry was reduced in SMKO (105.6±2.3 mmHg vs. 114.3±1.8 mmHg; P<0.01). While BPs in the control mice were not affected by varying sodium, MAP fell by 5.0±0.8 mmHg in SMKO (p<0.01) mice during low salt and increased in the SMKO mice by 7.5±0.3 mmHg (P<0.01) on high salt. Similarly, hypertension induced by chronic infusion of ang II was attenuated in SMKO (MAP:121.7±2.8 mmHg vs. 145.3±3.5 mmHg; P=0.004) and this was associated with diminished aortic media thickening(63±3.8± vs. 82.2±8 mm; P=0.006) and reduced cardiac hypertrophy(3.5±0.2 vs. 7.4±0.6 heart/body mg/g; P<0.01; SMKO).  

**Conclusions:** Thus, a mutation causing partial attenuation of Ang II-induced vasoconstriction reduces BP, enhances sodium sensitivity and confers substantial resistance to hypertension indicating a major role for vascular AT1A receptors in BP control and hypertension pathogenesis.

_Funding:_ NIDDK Support, Veterans Administration Support

**TH-OR107** Responses of the Renal Afferent Arterioles to Increased Pressure and Angiotensin II: Role of P47phox  

Enyin Lai, Christopher S. Wilcox, Anton Wellstein, Zaiming Luo, Kathryn Sandberg, William J. Welch.  

_Nephrology and Hypertension, Georgetown University, Washington, DC._

_**Background:** The tone of the renal afferent arteriole regulates renal vascular resistance (RVR) and transmission of arterial pressures into the kidneys. We reported that mice isolated perfused afferent arterioles challenged with either angiotensin II (Ang II; 10−5 M) or an increasing perfusion pressure generated reactive oxygen species (ROS: from PEG-SOD, a peroxynitrite scavenger; E.DHET and DETA-NONOate) and that the contractile responses to both were blunted by 10−5 M tempol. We detected the mRNA for p47phox, Nox-2 and -4 and in a gene array from individual microdissected afferent arterioles. Since Ang II infusion downregulated Nox-2 and only Nox-2 requires p47phox, we tested the hypothesis that Nox-2/p47phox is the source of the ROS that enhanced afferent arteriol tone and thereby RVR with Ang II infusion. Ang II increased MAP and RVR in p47phox+/+ mice (77±3 to 91±3 mmHg; P<0.05 and 7.5±0.4 to 10.5±0.8 mmHg/mL/min/100 g; P<0.05) but did not change these variables in -/- mice (74±3 to 76±1 mmHg; NS and 8.9±0.5 to 9.7±0.7 mmHg/mL/min/100 g; NS). Afferent arterioles dissected from mouse kidneys and the isolated perfused afferent arterioles from p47phox+/+ or -/- mice (18.8±0.9 vs 9.1±0.5 μm; NS). Compared to isolated perfused afferent arterioles from p47phox+/+ mice, those from -/- mice had a lesser myogenic response (MR; increase in wall tension with perfusion pressure from 40 to 135 mmHg; 3.1±0.4 vs 1.4±0.2 dynes/cm² mmHg; P<0.05) and a lesser change in luminal diameter with 10−5 M Ang II (4.6±0.4 vs -2.9±0.3 μm; P<0.03). These were accompanied by lesser increases in E.DHET. Two weeks Ang II infusion (200 ng/kg/min) enhanced the reduction in luminal diameter with 10−5 M Ang II in afferent arterioles from p47phox+/+ mice (-5.9±0.4 μm; P<0.05) but not from -/- mice (2.5±0.5 μm; NS). We concluded that ROS derived from Nox-2/p47phox enhanced Ang II hypertension by augmenting the increase in renal vascular resistance and the afferent arteriolar myogenic and Ang II contractile responses.

_Funding:_ NIDDK Support, Other NIH Support - P01HL-068868, R01-DK049870

**TH-OR108** Intrarenal Dopamine Counteracts Angiotensin II-Mediated Progressive Renal Injury  

Ming-Zhi Zhang,1 Suwan Wang,1 Xiaofeng Fan,1 Shilin Yang,2 Raymond C. Harris.1  

_Vanderbilt University, Nashville, TN; 3Nashville, TN._

_**Background:** Angiotensin II (Ang II) is a mediator of progressive renal injury. Previous studies by us and others have indicated that dopamine may modulate actions of Ang II in the kidney. The current studies examined the role of altering intrarenal dopamine on Ang II-mediated renal fibrosis. We also used a model of increased intrarenal dopamine, COMT KO mice, which have increased dopamine metabolizing enzymes, COMT and MAO. After 8 weeks of Ang II infusion, there were no significant differences in blood pressure between either wild type and COMT or AKD KO mice. Altogether, wild type, AKD mice had increased, and COMT mice had decreased albuminuria and tubulointerstitial injury. In response to Ang II, there was increased expression of both glomerular and tubulointerstitial injury markers (HIF1α, CTGF, FSP1, KIM-1, collagen I podocyte VEGF) in AKD KO mice and decreased expression in COMT KO mice. There was also differential macrophage infiltration in the two models. We have recently reported that Ang II-mediated tubulointerstitial fibrosis is mediated by src-dependent EGFR activation. In AKD KO mice, Ang II infusion further increased expression of p-src and pTyr845-EGFR, while their expression was markedly attenuated in COMT KO mice. These results demonstrate a role for intrarenal dopamine to buffer the detrimental effects of angiotensin II upon the kidney.

_Funding:_ NIDDK Support, Veterans Administration Support

**TH-OR109** Impaired Pressure Natriuresis Is Associated with Interstitial Inflammation in Salt Sensitive Hypertension  

Marta Franco Guevara,1 Tapia Edilia,1 Ursino Pacheco,1 Jose Santamaria,1 Yasmir Quiroz,2 Richard J. Johnson,1 Bernardo Rodriguez-Iturbe.2  

_Nephrology, Instituto Nacional de Cardiologia I.Ch., Mexico City; Mexico; 3Renal Service, Hospital Universitario, Zarla, Maracay, Venezuela; 2Nephrology, University of Colorado, Denver, CO._

_**Background:** Impairment of pressure natriuresis is a physiopathological characteristic of salt-sensitive hypertension. The shift to the right of the pressure natriuresis is likely a consequence of changes present in tubulointerstitial areas of the kidney, among them, accumulation of immunocompetent cells and increase in renal angiotensin II (ATII) activity.

_**Methods:** We studied the pressure natriuresis relationship in relation to severity of interstitial inflammation in salt sensitive hypertension (SSHT). SSHT was induced in 250-270g male Wistar rats by administration of L-NAMe for 3 weeks; after a 2 week washout period a high (4%) salt diet was given (SSHT group, n=6). One group received metofilit mycophenolate (MMF) (20mg/kg/day) during the L-NAMe administration for reduction of tubulointerstitial inflammation and amelioration of salt-induced hypertension (MMF group, n=8) (AJP2001:281:F38-F47). Controls were untreated rats under high (C-HSD, n=5) and normal (0.4%) salt diet (C-NSD, n=9). Pressure natriuresis was evaluated after 6 weeks using an aortic clamp to modify renal artery pressure (RAP) with measurements at 90, 110, 130 and 150 (hypertensive rats) mmHg of RAP.

_**Results:**

| CEDS-CD86 | MMF | C-HSD | C-NSD |
| 84.4±6.2 | 50.2±2.3 | 21.6±1.2 | 17.6±1.1 |
| 0.2±0.8 vs. 0.3±0.1 | °0.005 vs. the rest |

_**Conclusions:** Impairment of pressure natriuresis in salt sensitive hypertension is associated with tubulointerstitial infiltration of immune cells and ATII expressing cells and corrected by suppression of tubulointerstitial inflammation.

_Funding:_ Government Support - Non-U.S.

**TH-OR110** Intra-Renal Delivery of Autologous Endothelial Progenitor Cells (EPC) Improves Renal Function after Revascularization in Swine Renal Artery Stenosis (RAS)  

Xiang-Yang Zhu,1 Alfonso Eirin,1 Behzad Ebrahimii,1 Zihun Li,1 Amir Lerman,2 Stephen C. Textor,1 Lilach O. Lerman.1  

_Divisions of Nephrology and Hypertension, Mayo Clinic; 2Cardiovascular Diseases, Mayo Clinic, Rochester, MN._

_**Background:** Percutaneous transluminal renal angioplasty (PTRA) alone fails to restore the renal function and microvascular network in a swine model of RAS. This study assessed if intrarenal replenishment with EPC in conjunction to PTRA would improve renal function in swine RAS.

_**Methods:** Pigs with 10 weeks of RAS were studied 4 weeks after PTRA (+stenting) or sham, with or without adjunct intrarenal delivery of autologous EPC expanded from peripheral blood, and controls (n=7 each). Stenotic kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were evaluated in vivo with multidetector computed tomography, and microvascular remodeling and fibrosis ex vivo with micro-CT and trichrome staining respectively.

_**Results:** PTRA normalized blood pressure and GFR in all revascularized pigs (p<0.05 vs. RAS, °p<0.005 vs. Normal). EPC were retained (12%) and engrafted in injected kidneys. Stenotic kidney renal blood flow (RBF) and glomerular filtration rate (GFR) were normalized only in PTRA+EPC-treated pigs (Figure). Similar to our prior report, EPC+RBF+GFR activation. In AADK KO mice, Ang II infusion further increased expression of p-src and pTyr845-EGFR, while their expression was markedly attenuated in COMT KO mice. These results demonstrate a role for intrarenal dopamine to buffer the detrimental effects of angiotensin II upon the kidney.

_Funding:_ NIDDK Support, Veterans Administration Support

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

_Underline represents presenting author._
Increased Expression and Activity of the Sodium Chloride Cotransporter Mediates Salt Sensitivity in Mice

**Xinwan Wang, Laureano D. Asico, Ines Armando, Pedro A. Jose. Center for Molecular Physiology Research, Children’s National Medical Center, Washington, DC.**

**Background:** We have reported that C57BL6 and SJL mice from Jackson Laboratory have different responses to a chronic salt load: blood pressure increases in C57BL6 but not in SJL mice. Our purpose for the current study is to determine if the salt sensitivity of C57BL6 mice is related to increased expression of sodium transporters in renal distal convoluted tubule.

**Methods:** We examined the protein abundance of the sodium chloride cotransporter (NCC) which is predominantly expressed in the distal tubule by semiquantitative immunoblotting and measured blood pressures, urinary sodium excretion and aldosterone levels in the two mouse strains (10 weeks old, male) on normal (0.8%, NS) and high NaCl (6%, HS) diets (n=5/group).

**Results:** The blood pressures (telemetry) were similar at baseline and increased in C57BL6 and SJL mice after 1 and 3 weeks of HS. The urinary sodium excretions (6%, HS) diets (n=5/group).

**Conclusions:** We conclude that increased renal NCC expression and activity, which modifies the clinical outcomes in the AASK trial participants.

**Funding:** Other NIH Support, Private Foundation Support

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**TH-OR111**

**Increased Expression and Activity of the Sodium Chloride Cotransporter Mediates Salt Sensitivity in Mice**

**Xinwan Wang, Laureano D. Asico, Ines Armando, Pedro A. Jose. Center for Molecular Physiology Research, Children’s National Medical Center, Washington, DC.**

**Background:** We have reported that C57BL6 and SJL mice from Jackson Laboratory have different responses to a chronic salt load: blood pressure increases in C57BL6 but not in SJL mice. Our purpose for the current study is to determine if the salt sensitivity of C57BL6 mice is related to increased expression of sodium transporters in renal distal convoluted tubule.

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**Conclusions:** We conclude that increased renal NCC expression and activity, which modifies the clinical outcomes in the AASK trial participants.

**Funding:** Other NIH Support, Private Foundation Support

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**TH-OR112**

**The Glutathione S-Transferase µ-1 Null Allele GSTM1(0) Is Associated with an Accelerated Progression of Hypertensive Kidney Disease in the African American Study of Kidney Disease (AASK)**

**Jamison W. Chang,1 Virginia; 2Georgetown University.**

**Background:** We previously identified Gstm1 as a candidate gene influencing susceptibility to renal vascular injury in a mouse model with lesions that closely resemble renal vascular pathology in human hypertensive nephrosclerosis (HN). There is also evidence that humans who are homozygous for the null allele of Gstm1(0) have increased risks of vascular disease. We evaluated whether the null allele of GSTM1 modifies the clinical course of African Americans with CKD due to hypertension in the African American Study of Kidney Disease (AASK).

**Methods:** 722 AASK trial participants with DNA samples were genotyped and classified into three groups based on the number of null alleles: homozygous null (0/0), heterozygous (0/1), and homozygous active (1/1). Differences in the time to 50% or 25ml/min/1.73m² decline in glomerular filtration rate (GFR), time to dialysis, or the composite events of time to GFR event or dialysis or the time to GFR event, or dialysis or death were compared between genotype groups. The effect of the GSTM1 genotype was explored using Cox regression.

**Results:** The genotype groups differed significantly in the time to a GFR event or dialysis (p<0.04) and in time to a GFR event, dialysis, or death (p=0.02, figure 1). The hazard ratios for the time to GFR event or dialysis in those with 2 (0/0) or 1 (0/1) null alleles relative to those with none (1/1) were 1.96 (95% CI, 1.12 to 3.44, p=0.01) and 1.72 (95% CI, 1.02 to 2.89, p=0.04), respectively. For the time to GFR event, dialysis, or death the hazard ratios (in same order) were 2.15 (95% CI, 1.26 to 3.69, p<0.005) and 1.73 (95% CI, 1.05 to 2.87 and p=0.03).

**Conclusions:** The GSTM1 null allele is associated with a more rapid progression to important clinical outcomes in the AASK trial participants.

**Funding:** Other NIH Support, Private Foundation Support

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**TH-OR113**

**Blood Pressure Components and Decline in Kidney Function in Older Adults**

**Dena E. Birkh,1 Ronit Katz,2 Michel B. Chonchol,3 Michael Shlipak,4 Mark J. Sarnak,5 Linda F. Fried,6 Anne B. Newman,4 David Siscovick,3 Carmen A. Peralta.4**

**Background:** Progressive decline in kidney function (KF) is associated with mortality in older adults. Although hypertension is known to contribute to KF decline in the general population, the relative contributions of elevated systolic (SBP), diastolic (DBP), and pulse pressure (PP) to KF decline in community-dwelling older adults are not known.

**Methods:** We used linear and logistic regression to examine the separate and combined associations of SBP, DBP, and PP at baseline with KF decline among 4365 older adults in the Cardiovascular Health Study. We used cystatin C to estimate glomerular filtration rate (GFR) at three occasions over seven years of follow-up. Rapid decline in KF was defined as loss of ≥23ml/min/year.

**Results:** Average age was 72.2 and mean (SD) SBP, DBP, and PP were 153(21), 71(11), and 65(18) mm Hg, respectively. SBP and PP were significantly associated with KF decline. In adjusted linear models, each 10 mm Hg increment in SBP was associated with a more modest decline of 0.10 ml/min/year (-0.19, -0.08, p < 0.001); each 10 mm Hg increment in DBP was associated with a more rapid decline of 0.13 ml/min/year (-0.19, -0.08, p < 0.001), and an added decline of 0.13 ml/min/year (-0.21, -0.09, p < 0.001). Each 10 mm Hg increment in DBP was associated with a more rapid decline of 0.10 ml/min/year (-0.20, 0.01, p < 0.05). In adjusted logistic models, SBP had the strongest association with each at the time of GFR event, with each 10 mm Hg increase conferring a 14% increased hazard of rapid decline (95% CI 1.04, 1.07, p < 0.01). In models combining BP components, only SBP was independently associated with rapid decline. Findings were similar regardless of BP medication use.

**Conclusions:** Our findings suggest that elevated BP, particularly SBP, contributes substantially to declining KF in older adults.

**Funding:** NIDDK Support, Other NIH Support
Female residents of Iowa City who delivered between 1976 and 1982 were included in the study. A cohort of 2,102 female residents and their offspring were追踪ed over a median follow-up of 1.4 years (IQR: 0.6, 3.0). African American (AA) race and urine protein to creatinine ratio (uP/C) > 2 were associated with postpartum hypertension (eBP). Age, older glomerular renal function (GFR) and proteinuria showed a significant increase in risk of RT or death. Results of Multivariate Cause-specific Relative Hazards Models, N=205

<table>
<thead>
<tr>
<th>Factor</th>
<th>HR for BP Control, Estimate (95%CI)*</th>
<th>HR for RT/death, Estimate (95%CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, per year</td>
<td>0.96(0.92,1.02)</td>
<td>1.01(0.95,1.07)</td>
</tr>
<tr>
<td>uP/C &gt; 2</td>
<td>1.31(1.02,1.67)</td>
<td>2.50(1.95,3.21)</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>1.54(1.02,2.33)</td>
<td>9.51(4.20,19.77)</td>
</tr>
<tr>
<td>Glomerular CKD</td>
<td>0.89(0.47,1.69)</td>
<td>2.10(1.00,4.39)</td>
</tr>
<tr>
<td>uP/C &gt; 4</td>
<td>0.79(0.50,1.27)</td>
<td>7.06(2.70,18.62)</td>
</tr>
<tr>
<td>&gt; 0.2</td>
<td>0.72(0.45,1.16)</td>
<td>1.26(0.26,6.12)</td>
</tr>
<tr>
<td>&gt; 0.2</td>
<td>0.17(0.05,0.61)</td>
<td>4.54(0.95,21.61)</td>
</tr>
</tbody>
</table>

* HR=Hazard ratio; adjusted for variables show plus BP med use.

Conclusions: In pediatric CKD, eBP develops regardless of GFR and demographics. In those with eBP, achieving controlled BP is more difficult in AA patients and those with uP/C > 2.

Funding: Other NIH Support - National Institute of Neurological Disorders and Stroke (U01-DK-66143), National Institute of Child Health and Human Development (U01-DK-66174), National Heart, Lung, and Blood Institute (U01-DK-66116)

TH-OR116
Factors Contributing to the Development and Control of Elevated Blood Pressure in Pediatric CKD Patients

Amy Kogon,1,2 Christopher B. Pierce,2 Tammy M. Brady,2 Mark Mitsnefes,2 Bradley A. Warady,2 Susan L. Furth,2 Joseph T. Flynn,1,2 1Seattle Children's Hospital; 2CKiD Study Group.

Background: Few data exist regarding longitudinal blood pressure (BP) status changes in pediatric chronic kidney disease (CKD). Using Chronic Kidney Disease in Children (CKiD) cohort study data, we assessed factors associated with the rates at which normotensive patients develop elevated BP (eBP) and patients with eBP develop controlled BP.

Methods: Development of eBP: Children with no history of eBP, measured BP >90th %ile and no BP medication use at baseline, were followed prospectively with annual visits for the outcome of a measured BP >90th %ile. Analysis was performed using Cox proportional hazards models. Control of eBP: Children with measured BP >90th %ile were followed prospectively for the outcome of measured BP >90th %ile at two consecutive visits. To account for the competing events of progression to replacement therapy (RT) or death, cause-specific relative hazards were reported.

Results: Of 124 children (21% of CKiD cohort) with normal baseline BP and median follow-up of 1.5 years (IQR: 0.9, 3.0), 30% were followed prospectively for the development of eBP within 2.5 years, regardless of glomerular filtration rate (GFR, race, gender, and body size). 205 children (35% of cohort) had baseline eBP; 85 developed controlled BP and 43 progressed to RT with median follow-up of 1.4 years (IQR: 0.6, 3.0). African American (AA) race and urine protein to creatinine ratio (p/C) > 2 were associated with persistently eBP. Older age, glomerular CKD and GFR <45 showed an increased risk of RT or death.

Conclusions: In pediatric CKD, eBP develops regardless of GFR and demographics. In those with eBP, achieving controlled BP is more difficult in AA patients and those with p/C > 2.

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Conclusions: These studies demonstrate for the first time that the post-stenotic ARAS kidney releases inflammatory biomarkers and retains circulating EPC. These cytokine likely accelerate kidney injury and provide signals to EPC that may participate in reparative processes within the affected kidneys. These observations identify novel therapeutic targets to attenuate tissue injury and promote repair in the stenotic kidney.

Background: A time-depending effect of ADDs genes was detected. We observed an epistatic interaction on PNat (p<0.01) between ADD1 and genes related to tubular transporters (WNK1, MDR1, NEDD4L) and MYLK gene, encoding myosin light chain kinase which is a calcium/calmodulin dependent enzyme.

**Results:** We found an epistatic interaction on PNat (p<0.01) between ADD1 and genes related to tubular transporters (WNK1, MDR1, NEDD4L) and MYLK gene, encoding myosin light chain kinase which is a calcium/calmodulin dependent enzyme.

**Methods:** We performed a Na load test (iv. infusion of 2L of 0.9% NaCl in 2h) in patients displaying increased numbers, but impaired function of CACs. We performed modified Boyden chamber. GL-3 was visualized by immunofluorescence and electron microscopy of CACs to further investigate causes of this functional impairment and identified an excessive accumulation of GL-3 in Fabry disease patients.

**Results:** Fabry patients showed a hyperactive, but impaired endothelial function and signs of cardiac hypertrophy, which normalized after enzyme replacement therapy. Fabry patients displayed increased numbers, but impaired function of CACs. We performed immunofluorescence and electron microscopy of CACs to further investigate causes of this functional impairment and identified an excessive accumulation of GL-3 in Fabry disease patients.

**Conclusions:** Cardiovascular disease in Fabry patients may be in part related to CAC dysfunction. Enzyme replacement therapy improves CAC function and may attenuate tissue injury and promote repair in the stenotic kidney.

**Background:** Recent experimental findings demonstrate vascular endothelial growth factor (VEGF-C) mediated water-free storage of salt in the interstitium, which prevents a salt-sensitive blood pressure state. It is unknown whether this mechanism plays a role in salt homeostasis and regulation of blood pressure in humans. Therefore, we investigated circulating VEGF-C levels and blood pressure during different well-controlled salt intakes in healthy subjects and in chronic kidney disease (CKD) patients.

**Methods:** In two cross-over studies, non-diabetic proteinuric CKD patients (n=32), and healthy subjects (n=31) were treated with consecutively a high sodium diet (HS, aim 200 mmol Na+/d) and a low sodium diet (LS, aim 50 mmol Na+/d) in random order, during two 6-week (CKD) and two 1-week (healthy subjects) periods.

**Results:** We found that VEGF-C levels are higher during HS than during LS in CKD patients (median (IQR) 1228 (1024-1471) and 1004 (857-1177) pg/mL, resp; p<0.034) as well as in healthy subjects (881 (758-1023) and 773 (748-921) pg/mL, resp; p=0.070). In CKD patients HS was associated with higher NT-proBNP levels (HS: median (IQR) 91 (60-137) and LS: 62 (41-93) pg/mL, resp; p=0.005) and body weight (HS: mean (SD) 91 (3) and LS: 89 (3) kg, resp; p=0.013), consistent with ECV expansion, and with higher mean arterial pressure (HS: mean (SD) 105 (15) and LS: 101 (11) mmHg, resp; p<0.001), indicating salt-sensitivity. In healthy subjects blood pressure was not affected by dietary salt (HS: 87 (7) and LS: 86 (7) mmHg, resp; p=0.251), despite a rise in ECV (HS: mean (SD) 20.8 (0.5) and LS: 19.8 (0.5), resp; p=0.023).

**Conclusions:** Our findings support a role for VEGF-C mediated salt homeostasis in humans. Considering the salt-sensitivity of blood pressure, this buffering mechanism appears to be inefficient in proteinuric CKD patients. Future studies need to state the clinical and therapeutical relevance of this VEGF-C regulatory mechanism in humans.

**References:**


**Funding:** Other NIH Support - HL085307, DK73608, DK77013, HL77131 and UL1-RR024150, and by Mayo Clinic Center for Individualized Medicine.

**TH-OR119**

**Vascular Endothelial Growth Factor C Levels Are Modulated by Dietary Salt Intake in Humans**

**Aim:** To investigate the effect of dietary salt intake on circulating VEGF-C levels and blood pressure in healthy subjects and in chronic kidney disease (CKD) patients.

**Methods:** In two cross-over studies, non-diabetic proteinuric CKD patients (n=32), and healthy subjects (n=31) were treated with consecutively a high sodium diet (HS, aim 200 mmol Na+/d) and a low sodium diet (LS, aim 50 mmol Na+/d) in random order, during two 6-week (CKD) and two 1-week (healthy subjects) periods.

**Results:** We found that VEGF-C levels are higher during HS than during LS in CKD patients (median (IQR) 1228 (1024-1471) and 1004 (857-1177) pg/mL, resp; p<0.034) as well as in healthy subjects (881 (758-1023) and 773 (748-921) pg/mL, resp; p=0.070). In CKD patients HS was associated with higher NT-proBNP levels (HS: median (IQR) 91 (60-137) and LS: 62 (41-93) pg/mL, resp; p=0.005) and body weight (HS: mean (SD) 91 (3) and LS: 89 (3) kg, resp; p=0.013), consistent with ECV expansion, and with higher mean arterial pressure (HS: mean (SD) 105 (15) and LS: 101 (11) mmHg, resp; p<0.001), indicating salt-sensitivity. In healthy subjects blood pressure was not affected by dietary salt (HS: 87 (7) and LS: 86 (7) mmHg, resp; p=0.251), despite a rise in ECV (HS: mean (SD) 20.8 (0.5) and LS: 19.8 (0.5), resp; p=0.023).

**Conclusions:** Our findings support a role for VEGF-C mediated salt homeostasis in humans. Considering the salt-sensitivity of blood pressure, this buffering mechanism appears to be inefficient in proteinuric CKD patients. Future studies need to state the clinical and therapeutical relevance of this VEGF-C regulatory mechanism in humans.

**References:**


**Funding:** Other NIH Support - HL085307, DK73608, DK77013, HL77131 and UL1-RR024150, and by Mayo Clinic Center for Individualized Medicine.

**TH-OR120**

**Cardiovascular Disease in Fabry Patients Is Related to Dysfunctional Circulating Angiogenic Cells**

**Aim:** To investigate the impact of this disease on the biology of circulating angiogenic cells (CACS) and endothelial function in patients with Fabry disease and healthy controls.

**Methods:** 26 patients with untreated Fabry disease, 16 patients after enzyme replacement therapy (ERT) and 26 healthy controls were investigated. Endothelial function was assessed by the EndoPat device, left ventricular hypertrophy by echocardiography. Circulating angiogenic cells were analyzed by fluorescence associated cell sorting (FACS) analysis (CD34+/CD133+/KDR+). The migratory capacity of CACS was assessed by a modified Boyden chamber. GL-3 was visualized by immunofluorescence and electron microscopy. Alpha-Gal A was knocked out in CACS by a specific siRNA.

**Results:** Fabry patients showed a hyperactive, but impaired endothelial function and signs of cardiac hypertrophy, which normalized after enzyme replacement therapy. Fabry patients displayed increased numbers, but impaired function of CACs. We performed immunofluorescence and electron microscopy of CACs to further investigate causes of this functional impairment and identified an excessive accumulation of GL-3 in Fabry disease CACS. Enzyme replacement therapy attenuated CAC dysfunction in patients via a reduction in GL-3 accumulation in vitro and in vivo. SiRNA-mediated knockdown of alpha-Gal A in healthy CACS also led to an impairment of migratory capacity.

**Conclusions:** Cardiovascular disease in Fabry patients may be in part related to CAC dysfunction. Enzyme replacement therapy improves CAC function and may attenuate development of cardiovascular disease in the long-term.

**Funding:** Government Support - Non-U.S.

**TH-OR121**

**Gitelman Syndrome: Hypertension in Adulthood Is a Common Sequela, and Female Patients Have Higher Potassium Requirements**

**Aim:** To investigate the prevalence of hypertension in Gitelman Syndrome (GS) and the potassium requirements in female patients.

**Methods:** We performed a retrospective study of all patients with genetically proven Gitelman syndrome known to our specialist adult nephrology service. Differences between group medians were tested for significance by Mann-Whitney U-testing.

**Results:** 20 female patients were identified, with a median age of 41 years (range 10-73). Hypertension was present in 13/20 (65%) patients. The median 24-hour urinary potassium excretion was significantly lower in female patients than in male patients (p<0.05). The median 24-hour urinary potassium excretion was significantly lower in female patients than in male patients (p<0.05). The median 24-hour urinary potassium excretion was significantly lower in female patients than in male patients (p<0.05).

**Conclusions:** Hypertension is a common sequela of Gitelman Syndrome, and female patients have lower potassium requirements than male patients.

**Funding:** Government Support - Non-U.S.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**

**30A**
Results: 57 patients (21 male and 16 female) with median age 39±14 y were studied. All were hyper-remnemic at diagnosis. 35% (n=13) patients were hypertensive (defined as BP > 130/80 despite amiloride or anti-hypertensive agents). Of these, 13, 11 male (p=0.017) with median age 53 y, vs two female, 45 y (p=0.55). One patient was already hypertensive by age 21 y. Overall, currently normotensive patients were significantly younger than median 37.5 y (p=0.03), and also younger at the time of genetic diagnosis: 35.5 vs 47 y (p=0.04).

Conclusions: Despite obese salt-wasting in GS, secondary hypertension has developed in only one of our cohort, and may be an expected feature of the aging GS population. It appears to be more prevalent in male patients. It may be related to chronic hyperreninemia and/or hyperfiltration. The higher female requirement for potassium supplementation we observed may be related to the effects of oestrogens on expression or function of the NCCT. Mutations in exon 26 (C-terminal) may affect targeting of NCCT and hence explain the apparently more severe phenotype observed in these patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-OR124

An ADPKD Cyst Epithelial Cell Secreted Factor Activates STAT3 and Promotes M2-Like Polarization in Macrophages

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Background: Alternatively-activated macrophages (M2 MΦ) are known to play a pro-fibrotic role in chronic kidney disease (CKD). Since MΦ have been demonstrated within the stroma of autosomal dominant polycystic kidney disease (ADPKD), we assessed whether MΦ are present and whether ADPKD cells affect their polarization.

Methods: Immunohistochemical characterization of macrophages in human ADPKD tissue was performed using HAM56 and anti-CD163 antibodies. Primary human ADPKD Φ membranes underlying cysts. Many of these MΦ inducibly express and M2-like macrophage polarization. M2 MΦ induces Arg1 expression and M2-like macrophage polarization. M2 MΦ are present and whether ADPKD cells affect their polarization.

Results: HAM56 identified MΦ within ADPKD stroma, especially adjacent to basement membranes underlying cysts. Many of these MΦ were stained for the M2 marker CD163. Since murine proximal tubule epithelial cells have been shown to influence MΦ polarization in vitro, we tested whether ADPKD epithelial cells could affect polarization of BMDM. Strikingly, ADPKD MΦ co-culture caused a >200 fold induction of the M2 MΦ marker Arg1 in both BMDM and RAW cells, while the inflammatory MΦ marker Nos2 was not induced. ADPKD cell conditioned media (ADPKD CM) also induced Arg1 expression (75 fold), indicating the presence of an ADPKD cell-derived, soluble, M2-polarizing factor. Specifically, M2 MΦ activation, which is essential for Arg1 induction and M2 polarization induced by the cytokines IL4 and IL13, was not detected in RAW cells after ADPKD CM treatment. However, ADPKD CM included a rapid activation of STAT3 in RAW cells, and the presence of a STAT3 inhibitor completely blocked the ADPKD CM Arg1 induction.

Conclusions: ADPKD cyst cells produce a soluble factor that activates STAT3 and induces Arg1 expression and M2-like macrophage polarization. M2 MΦ in the ADPKD environment are able to promote disease progression. Thus, understanding the mechanisms by which polarization occurs could allow the development of targeted therapies to diminish M2 polarization and disease progression in ADPKD.

Funding: NIDDK Support

TH-OR125

Irel-Xbp1 Unfolded Protein Response (UPR) Pathway Is a Genetic and Molecular Regulator of Cystogenesis through a Polycystin-1 (PC1) Dependent Mechanism

Eunyung Song, Vivien M. Keles,1 Amanda Scala,1 Ming Ma,1 Rachel Gallagher,1 Laurie H. Glumacher,2 Stefan Somlo.1 Internal Medicine/Nephrology, Yale School of Medicine, New Haven, CT.1 Immunology Infectious Diseases and Medicine, Harvard Medical School, Boston, MA.

Background: This work examined the relationship between up-regulation of the unfolded protein response (UPR), a housekeeping pathway involved in counteracting ER stress and cyst formation due to the absence of the Autosomal Dominant Polycystic Liver Disease (ADPLD) gene orthologs, Prkcsh and Sec63, both of which are in ADPKD.

Methods: We used mouse models of Prkcsh, Sec63, and Xbp1b based on conditional inactivation in the kidney by Ksp-Cre, as well as kidney tubule cell lines isolated from these animals.

Results: Inactivation of Prkcsh did not result in up-regulation of any of the three branches of the UPR pathway. Inactivation of Sec63, on the other hand, resulted in selective activation of the Irel-Xbp1 pathway, as evidenced by Xbp1 splicing, protein expression and up-regulation of Xbp1 transcriptional targets. The other two branches of the UPR pathway, Atf6 and Perk, remained at basal levels. Double knockout (DKO) animals with conditional inactivation of Sec63 and Xbp1b in distal nephron segments (Xbp1floxflox; Xbp1bcre/cre; Ksp-Cre) showed a marked exacerbation of the cystic phenotype compared to single knockout (SKO; Sec63floxflox; Ksp-Cre) mice, Xbp1floxflox; Ksp-Cre mice had no cysts. DKO mice had a >2.5 fold increase in kidney weight (KW), kidney-to-body weight ratio (KW/BW), cystic index (CI) and BUN compared to SKO animals. Introduction of a 3-copy PrkcshIBAC transgene in DKO mice resulted in a significant reduction in the KW, KW/BW ratio, CI and BUN compared to the DKO animals alone. Furthermore, expression of the Xbp1bIBAC transgene in a conditional target ER chaperone Bip was decreased in the cystic epithelia of DKO vs. SKO animals. This correlated with a decrease in the expression of PC1 and PC2 in the DKO vs. SKO backgrounds.

Conclusions: Taken together our data demonstrate that the most conserved branch of the UPR pathway, Irel-Xbp1, can act as a modifier of cyst formation by impacting the biogenesis of PC1/PC2.

Funding: NIDDK Support
Dependent manner, in Pkd1 mutant (Pkd1−/−) to remove the dead cells from kidney tissues, to prevent cyst formation. We thus developed a novel strategy by targeting pathways that can induce only cystic renal epithelia apoptosis, leading to the growth of cystic renal lesions have been designed to “normalize” the activity of a specific signaling molecule. Recent efforts in our laboratory have focused on developing a novel inhibitor of apoptosis proteins 1 and 2 (cIAP1 and cIAP2), to form a pro-survival signaling protein complex that includes receptor associated protein kinase 1 (RIPK1), and the cellular C-tail death domain of TNF-α receptors (2). We observed that in Pkd1−/− mice, the kinase activity of FADD, determining the apoptosis of the epithelial-to-mesenchymal regulator Snai1 and of the developmental signaling molecules such as Gli1 and Hh ligands, leading to the apoptosis of cystic renal epithelial cells. This complex specifically induces cell death in an epithelial manner, indicating increased Jak2 activity in ADPKD cells. Through a chemical inhibition of Jak2 and JAK3, we were able to induce apoptosis in ADPKD cells, and to reduce the growth of cystic renal lesions in a PKD model (Pkd1 inactivation at P7). The kidney weight, the kidney body weight ratio, serum creatinine, cyst volume were measured. Proliferation index was assessed by using Ki67 and BrdU incorporation.

Results: A sustained activation of the transcription factor STAT3 was observed in ischemic injured and uninjured mouse polycystic kidneys and in human ADPKD kidneys. Furthermore, Jak2 inhibitors reduced STAT3 activation in human ADPKD cells in a dose-dependent manner, indicating increased Jak2 activity in ADPKD cells. Through a chemical library screen, we identified a novel anti-parasitic compound pyrvinium as a inhibitor of STAT3 function. Treatment with pyrvinium decreased STAT3 activation and cell proliferation in human ADPKD cells and reduced renal cyst formation in an adult and a neonatal PKD mouse models. Moreover, we demonstrated that another STAT3 inhibitor, S3I-201, reduced cyst formation and growth in a neonatal PKD mouse model (Pkd1−/− at P7). The kidney weight, the kidney body weight ratio, serum creatinine, cyst volume were measured. Proliferation index was assessed by using Ki67 and BrdU incorporation.

Conclusions: Blocking STAT3 signaling with pyrvinium or similar drugs may be an attractive therapy for human ADPKD.

Funding: NIDDK Support, Private Foundation Support

TH-OR127

Smac-Mimetic Prevents Renal Cyst Formation By Inducing TNF-α-Dependent Cystic Epithelial Cell Death
Lucy X. Fan, Xia Zhou, Wei Liu, William E. Sweeney, Ellis D. Avner, Xiaogang Li

Background: Past efforts to pharmacologically interfere with the development and growth of cystic renal lesions have been designed to “normalize” the activity of a specific signaling molecule. Recent efforts in our laboratory have focused on developing a novel strategy by targeting pathways that can induce only cystic renal epithelia apoptosis, leading to the removal of the dead cells from kidney tissues, to prevent cyst formation.

Results: In this study, we present for the first time evidence that second mitochondria-derived activator of caspase (Smac) or a Smac mimetic induces cell death in a TNF-α-dependent manner, in Pkd1 mutant (Pkd1−/−) renal epithelial cells without any effect on Pkd1 wild type (Pkd1+/+) or Pkd1 heterozygous (Pkd1+/-) renal epithelia. Following activation by TNF-α, the C-tail death domain of TNF-α receptor 1 (TNFR1) recruits a multiprotein complex that includes receptor associated protein kinase 1 (RIPK1), and the cellular C-tail death domain of TNF-α receptors (2). We observed that in Pkd1−/− mice, the kinase activity of FADD, determining the apoptosis of the epithelial-to-mesenchymal regulator Snai1 and of the developmental signaling molecules such as Gli1 and Hh ligands, leading to the apoptosis of cystic renal epithelial cells. This complex specifically induces cell death in an epithelial manner, indicating increased Jak2 activity in ADPKD cells. Through a chemical inhibition of Jak2 and JAK3, we were able to induce apoptosis in ADPKD cells, and to reduce the growth of cystic renal lesions in a PKD model (Pkd1 inactivation at P7). The kidney weight, the kidney body weight ratio, serum creatinine, cyst volume were measured. Proliferation index was assessed by using Ki67 and BrdU incorporation.

Conclusions: Blocking STAT3 signaling with pyrvinium or similar drugs may be an attractive therapy for human ADPKD.

Funding: NIDDK Support, Private Foundation Support

TH-OR128

Heptacope Nuclear Factor Factor 1 beta (Hnf1b/MODY5) Controls Nephron Morphogenesis
Filippo Massa, Evelyn Fischer, Serge Garbay, Marco Pontoglio

Background: The deficiency for Hepatocyte Nuclear Factor 1 beta (Hnf1b), a gene encoding for a homeobox-containing transcription factor, is a major cause for developmental renal malformation characterized by the occurrence of cysts and hypo-dysplasia. The current knowledge about its molecular and cellular mechanisms is still incomplete.

Methods: A homozygous germ-line deletion of Hnf1b in mouse leads to embryonic lethality shortly after implantation (E6.5) due to defective differentiation of extraembryonic tissues. To circumvent this early lethality we inactivated this gene specifically in the embryonic pronephric and mesonephric renal epithelial and mesenchymal compartment with a set of Cre recombinases (Mox2Cre, RARβCre and Six3-GFP::Cre).

Results: Similarly to what was already shown (Okamane et al.,2010), we found that a total of 12 mutations (2xMox2-Cre driven) Hnf1b inactivation led to a drastic defect of renal morphogenesis. Branching of the ureteric Bud (UB) was distorted and the induction of renal vesicles was impaired.

Our results also demonstrated that Hnf1b-deficient embryos displayed Wolffian duct malformations, characterized by the emergence of multiple bulges (thus UBs). To further identify the potential role played by Hnf1b in developing nephrons, we used a Cre recombinase specifically expressed in Metanephric Mesenchyme (MM) and not in the UB (Six5-GFP::Cre). Contrary to the Mox2-Cre driven deletion, this inactivation did not prevent the induction of renal vesicles. However, renorn formation precursors gave rise to abrogated glomerular structures and did not produce renal tubules.

Interestingly, with a highly chimeric inactivation of Hnf1b in the MM (RARβ-Cre), we were able to identify a further crucial role of Hnf1b in proximal tubular morphogenesis. In fact, a partial loss of Hnf1b led to defective opening of the segment, and mutant animals suffered from defective reabsorption of glucose.

Conclusions: Our results demonstrate that HNF1 beta plays essential roles in the first morphogenetic events that shape nephrons and, subsequently, in proximal tubular elongation.

These results provide a novel perspective on the function of a gene that is frequently mutated in children or fetuses with cystic/dysplastic kidneys.

Funding: Government Support - Non-U.S.
to sites of DNA damage. Third, we show that ATM is activated in turn by the 'TIP60 complex'. And we demonstrate colocalization to TIP60-positive nuclear foci for other proteins of NPHP-RC genes. In 4 different families with NPHP-RC we identify mutations in

**TH-OR131**

**Disrupted Retrograde Ciliary Transport Causes Sensenbrenner Syndrome with Nephropathies**


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**Background:** Intraglomerular transport (IFT) occurs along microtubules in the cilium, an antenna-like organelle present on the apical surface of almost every vertebrate cell (including renal cells). The IFT-B and IFT-A protein complexes regulate upward- and downward ciliary transport, respectively, in association with motor proteins such as kinesins and dyneins. The IFT-A complex consists of 6 proteins of which 3 (IFT122, IFT121 and IFT43) are associated with Sensenbrenner syndrome, an autosomal recessive disorder characterized by skeletal- and ectodermal defects.

**Methods:** Exome-sequencing; SNP array analysis; Sanger sequencing; Immunocytochemistry.

**Results:** We used exome-sequencing to identify the genetic defect in a Norwegian family with Sensenbrenner syndrome accompanied by nephropathies-like nephropathy. We identified multiple genic variants, however, only the compound heterozygous mutations in IFT144 in 2 patients were in perfect linkage with disease. IFT144 is encoded by the gene RPP14 and consists of 466 amino acids. The IFT144 protein is present in the base and the tip of the cilium. IFT144 is absent in cilia from fibroblasts from one of our patients.

**Conclusions:** We conclude that WDR19 is a novel SNS gene and that defective tip-to-base (retrograde) ciliary transport causes Sensenbrenner syndrome. **Funding:** Private Foundation Support, Government Support - Non-U.S.

**TH-OR132**

**Proteomic Analysis of Class IV Lupus Nephritis: Global Versus Segmental**


**Background:** There have been several attempts to standardize definitions and increase reproducibility in classifying lupus nephritis. The last classification added subcategories (global (IVG) and segmental (IVS)). Whether this subdivision has an impact on clinical practice or patient’s outcome is controversial.

**Methods:** A retrospective review with 2DE gel electrophoresis and MALDI-TOF/ MS analysis of renal biopsies.

**Results:** Renal biopsies from patients with IVG, IVS, ANCA, normal were used to extract proteins. Proteins were then separated by 2DE gel electrophoresis and the expression levels of the protein spots were determined. Principal Component Analysis (PCA) Plot was applied for the four groups using the dataset of statistically differentially expressed protein spots. Global and segmental groups cross over each other and clearly separate to sites of DNA damage, 40 patients had active disease, 22 were in partial remission and 26 in complete remission. DQA1 05:01; DQB1 02:01 tissue type and poor long term outcome, suggesting that an interaction between anti-PLA2R levels, HLA genes and PLA2R polymorphism is unknown. We measured anti-PLA2R antibodies in 88 IMN patients typed for DQA1: DQB1 and PLAR2 SNP and describe the results in the context of disease activity and outcome.

**Conclusions:** Antibodies to PLAR2 have been identified in 70% cases of idiopathic membranous nephropathy (IMN), which has a strong association with HLA DQ/A1. The interaction between anti-PLA2R levels, HLA genes and PLAR2 polymorphism is unknown. **Funding:** Private Foundation Support

**TH-OR133**

**C3 Glomerulopathy Is a Disease of the Alternative Pathway of Complement**

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**Background:** C3 Glomerulopathies (C3GN) is a recently described glomerulonephritis (GN) with extensive C3 deposits. The purpose of this study was to describe the clinicopathologic findings and study the alternate pathway of complement (AP) in cases of C3GN.

**Methods:** The diagnosis of C3GN was based on a proliferative GN on light microscopy (LM) with extensive C3 deposits and absence of immunoglobulins (Igs’), kappa and lambda light chains on immunofluorescence microscopy (IF).

**Results:** Ten cases of C3GN were identified. There were 6 male and 4 female patients. The age range was from 4 to 73 years (mean 44.4 yrs). The serum creatinine ranged from 0.5 to 3.1 mg/dL (mean 1.52 mg/dL). Urinary protein ranged from 0.3 to 9 gms (mean 2.7 gms/day). UA showed hematuria and proteinuria in all cases. All cases had low C3 and normal C4. Ten cases showed a mesangiocapillary GN with extensive C3 deposition in the mesangium and capillary walls on IF. IF was negative for IgG, kappa and lambda light chains. EM often showed large confluent hazy electron dense deposits in the mesangium, with subendothelial, intramembranous and occasional subepithelial deposits. Based on the presence of subepithelial deposits, 4 of the 10 cases were previously diagnosed as post-infectious GN. Three cases were previously labeled MPGN type I. Evidence of AP activation was demonstrated in all cases. Specific abnormalities of the AP were as follows: Autoantibodies to C3 convertase (2 cases), antibodies to factor H (1 case), hereditary deficiency of factor H (1 case), heterozygous risk alleles for p.His402 (1 case), c.C2867T p.T956M factor H missense mutation (2 cases), heterogeneous risk alleles for factor H p.Va6 and p.His402 (1 case), mutation in factor 1 gene and polymorphisms in factors B and H (1 case), frameshift c.2171delC, p.Thr724Hisstop725 variant mutation in factor H resulting a truncated factor H (1 case), and deletion of CHFR1-CHFR1 (1 case).

**Conclusions:** This study shows that C3GN can result from a diverse set of abnormalities affecting the AP. Treatment needs to be tailored based on the underlying etiology of AP dysfunction. Finally, C3GN should be considered in differential diagnosis of post-infectious GN.

**Funding:** NIDDK Support

**TH-OR134**

**Association of Anti-PLA2R with Disease Activity and Outcome in Idiopathic Membranous Nephropathy**

*Durga A.K. Kanisicierla,*1 *Paul E. Brenchley,*1 *Michael Venning,*1 *Kay V. Poulton,*1 *Edward A. McKenzie,*1 *Jennet O. Gummadova,*1 *Colin D. Short,*1 *Renal Medicine, Manchester Royal Infirmary, United Kingdom; FLS, Manchester University, United Kingdom.

**Background:** Anti-PLA2R antibodies have been identified in 70% cases of idiopathic membranous nephropathy (IMN), which has a strong association with HLA DQ/A1. The interaction between anti-PLA2R levels, HLA genes and PLAR2 polymorphism is unknown. **Funding:** Private Foundation Support

**TH-OR135**

**Response of Anti-PLA2R to Adrenocorticotropic Hormone (ACTH) Gel in Membranous Nephropathy**

*Laurence H. Beck,*1 *Cattran, C.C.,*2 *B. Appel,*3 *G. Cattran,*4 *G. B. Appel,*4 *J. Salant.*1 *Boston University; Mayo Clinic; Columbia University; Toronto General Hospital.

**Background:** Anti-PLA2R antibodies to the phospholipase A2 receptor (PLAR2) define most cases of primary membranous nephropathy (MN); their presence correlates with clinical disease activity. We analyzed anti-PLA2R in patients treated with long-acting ACTH gel
after discontinuing treatment with ACTH gel. Two other patients also showed an increase in anti-PLA2R while 2 others, after receiving rituximab for perceived failure of ACTH gel, fully cleared to treatment. All 12 patients experienced a reduction in anti-PLA2R (17-100%) by 6 mo and achieved clinical remission and may have already entered immunologic remission prior to treatment. All 12 patients experienced a reduction in anti-PLA2R (17-100%) by 6 mo and achieved clinical remission and may have already entered immunologic remission prior to treatment.

Conclusions: Measurement of anti-PLA2R provides useful information relating immunological and clinical disease activity in MN patients treated with new agents such as ACTH gel. This study suggests that ACTH gel may work in part by suppressing autoantibody production; the duration and degree of this response need further study.

Funding: NIDDK Support, Pharmaceutical Company Support, Private Foundation Support

TH-OR138

Novel Noninvasive Biomarkers for Disease Activity of IgA Nephropathy (IgAN) Yusuke Suzuki,1 Keiichi Matsuzaki,1 Hitoshi Suzuki,1 Keiko Okazaki1,1 Hiroyuki Yanagawa1,1 Norio Ieiri1,1 Mitsuhito Sato1,2 Toshinobu Sato1,2 Yoshio Taguma1,1 Jan Novak1,3 Osamu Hotta1,4 Yusuhiko Tomino5,6,7 Juntendo University Faculty of Medicine, Tokyo, Japan; 2Sendai Shakaihoken Hospital, Sendai, Japan; 3University of Alabama at Birmingham.

Background: The primary abnormal manifestation in IgAN is recurring bouts of hematuria with or without proteinuria. Although immunohistochemical analysis remains the gold standard not only for diagnosis but also for evaluating the activity of IgAN, sensitive and reasonably specific tests are emerging to guide therapeutic strategy applicable to all stages of IgAN. Present study examined serum levels of galactose-deficient IgA1 (GdIgA1) and IgA-IgG immune complex (IgA-IgG IC) as noninvasive markers for the disease activity.

Methods: We enrolled patients (n=48) with IgAN who showed complete or partial clinical remission after tonsillectomy and steroid-pulse therapy (TSP) and were followed up for 3-5 years. Baseline clinical data were evaluated just before TSP; serum levels of GdIgA1 and IgA-IgGIC and IgA-IgG immune hematuria and proteinuria were assessed just before TSP, 1 year, and 3-5 years after TSP. GdIgA1 and IgA-IgG IC were measured by ELISA.

Results: Cross-sectional analysis revealed that the degree of hematuria and proteinuria were significantly associated with levels of GdIgA1 (p for trend<0.004) and IgA-IgG IC (p for trend<0.001), respectively. Longitudinal analysis showed that from the group of 91.7% patients with heavy hematuria (≥3+) just before TSP, 64.6% patients showed complete disappearance of hematuria (group A), but the remaining patients did not (group B). The median follow-up was 5-year follow-up. Although the levels of GdIgA1 and IgA-IgG IC in the two groups just before TSP were similar (A vs. B: 122.2 vs 107.7 units p=0.36, and 0.77 vs 0.85 OD p=0.43), decrease of GdIgA1 levels in group A was significantly higher than that in group B (27.4 vs 13.6%, p=0.02).

Conclusions: Disease activity of IgAN assessed by hematuria and proteinuria correlated with changes of serum levels of GdIgA1 and IgA-IgG IC, respectively. These new noninvasive disease activity markers can be used to guide the therapeutic approaches.

TH-OR139

APOL1 Is Expressed in Human Kidney with Dynamic Cellular Localization Patterns in Health and Disease Sethu M. Madhavan, John F. O’Toole, Martha Konieczkowski, Leslie A. Bruggeman, John R. Sedor. Med/Health System, Case Western Reserve University, Cleveland, OH.

Background: Genetic variants in APOL1, which encodes apolipoprotein L1 (APOL1), associate with the non-diabetic kidney diseases in patients of African ancestry. APOL1 is a component of HDL, particles and mediates trypanolytic activity. Renal disease-related APOL1 variants have been positively selected in African populations by conferring resistance to trypanosomiasis. Mechanisms by which APOL1 variants promote nephropathy are unknown.

Methods: APOL1 localization was examined using immunohistochemistry in normal human kidney sections and in FSGS, HIVAN, diabetic and hypertensive-associated nephropathy and minimal change disease (MCD) biopsies. Biopsies were genotyped for APOL1 variants. APOL1 expression was also examined in human podocytes, endothelial and proximal tubular cells.

Results: APOL1 was only present in podocytes in normal glomeruli. Podocyte staining of APOL1 was decreased in diseased glomeruli, although podocyte markers, GLEPP1 and synaptopodin, were maintained. Only in MCD glomeruli, APOL1 expression was induced in endothelia concomitant with the loss of podocyte APOL1. APOL1 localized to proximal tubular cells in a vesicular pattern and was detected in the arteriolar endothelium of normal and diseased kidney sections. Unexpectedly, in biopsies but not normal kidney, medium artery and arterioles contained a subset of α-smooth muscle actin-positive cells that stained for APOL1. RT-PCR demonstrated APOL1 transcripts in podocytes and normal glomeruli. Biopsies from APOL1 variants have been positively selected in African populations by conferring resistance to trypanosomiasis. Mechanisms by which APOL1 variants promote nephropathy are unknown.

Conclusions: APOL1 localization was examined using immunohistochemistry in normal human kidney sections and in FSGS, HIVAN, diabetic and hypertensive-associated nephropathy and minimal change disease (MCD) biopsies. Biopsies were genotyped for APOL1 variants. APOL1 expression was also examined in human podocytes, endothelial and proximal tubular cells.

Funding: NIDDK Support

TH-OR137

Natural History of Serum Free Light Chains Prior to the Clinical Presentation of AL Amyloidosis Joseph D. Hebreo,1 Kevin C. Abbott,1 Brendan M. Weiss,1 Stephen W. Olson.1 1Department of Medicine, Nephrology Service, Walter Reed Army Medical Center, Washington, DC.

Background: AL amyloidosis is a rare plasma cell dyscrasia with significant morbidity and mortality. Recent research suggests that disease progression to AL amyloidosis is linked to abnormalities in normal kidney. The cell compartments in which it localizes change with disease, and APOL1 expression was also examined in human podocytes, endothelial and proximal tubular cells.

Results: A greater percent of cases versus controls had a single abnormal serum free light chain (SFLC) ratio and a LLC rate of rise greater than 1 mg/L per year was 100% sensitive and specific for future disease (100% vs. 0%, p<0.001).

Conclusions: An abnormal SFLC ratio and a LLC rate of rise greater than 1 mg/L per year was 100% sensitive and specific for future disease (100% vs. 0%, p<0.001).

Funding: NIDDK Support

TH-OR136

Clinical Pathologic Correlations in 190 Multiple Myeloma Patients with Kidney Biopsy Samih H. Nasr,1 Anthony M. Valeri,1 Sanjeev Sethi,1 Mary E. Fidler,1 Lynn D. Cornell,1 Nelson Leung1. 1Pathology, Mayo Clinic, Rochester, MN; 2Nephrology, Columbia University, New York, NY; 3Nephrology, Mayo Clinic, Rochester, MN.

Background: Renal involvement is common in multiple myeloma (MM). In this largest study to date, we examined kidney biopsy (KBx) findings in MM patients (pt) and correlated them with clinical data at KBx and outcome.

Methods: The characteristics of 190 Mayo Clinic pts with MM who underwent KBx between 1997-2011 are provided. MM was diagnosed before or at time of KBx.

Results: On KBx, paraprotein-related lesions (PRL) were seen in 73% of pts, non-PRL in 25%, and no pathology in 2%. PRL were myeloma cast nephropathy (MCN)(33%) of pts, monoclonal immunoglobulin deposition disease (MIDD)(22%), amyloid (21%), fibrillary GN (FGN)(1%), immunotactoid GN (0.5%) and light chain proximal tubulopathy (0.5%). Two forms of PRL were seen in 6% (MCN and MIDD in 5 pts, MCN and amyloid in 4, MIDD and amyloid in 2, and MCN and FGN in 1). Direct MM infiltration was seen in 1%. Non-PRL included ATN (9%), HTN arteriolosclerosis (6%), diabetic nephropathy (5%), FSGS (3%), APIGN (3%), and interstitial nephritis (2%). 1/3 of MIDD pts were <50 yrs vs. 9% of MCN and 9% of amyloid pts (p=0.035). Serum complete Ig was more common in MCN than amyloid or MIDD. The size of urine paraprotein and % of plasma cells in bone marrow were higher in MCN than amyloid or MIDD. Markedly abnormal FLC ratio was more common in MCN than amyloid. 37% of MCN pts required dialysis at KBx vs. 22% of MIDD and 9% of amyloid pts. 50% albuminuria on UPEP was highest in amyloid-MIDD-SMCN. After a mean follow-up of 30 mos, 52% died. Median pt survival was 45 mos for MCN, 61 for amyloid and 84 for MIDD (p=0.193). Independent predictors of death were reaching ESRD, no treatment with stem cell transplant, and hypercalcemia. Median renal survival was 36 mos for MCN vs. 113 yrs for amyloid vs. 74 for MIDD (p=0.003). The median progression to progression to ESRD was diastasis at KBx.

Conclusions: The current spectrum of renal lesions in MM pts is more heterogeneous than previously reported. KBx is essential to establish the individual diagnoses and provide important prognostic and therapeutic information.

Funding: NIDDK Support

TH-OR135

New Insights into Pathogenesis and Biomarkers of Glomerular Disease Oral Abstract/Thursday
There is an Increased Risk of Peritonitis in the 21 Days Prior to Death in Peritoneal Dialysis Patients: A Case-Crossover Study Neil Bouvall1,2, Andrew Aytoun1,3, Caroline M. Hawley1,2, Sami V. Badve1, Fiona Brown1, Brian E.R. Livingston1, Stephen P. McDonald1, Kym M. Bannister1, Kathryn J. Wiggins1, David W. Johnson1.
1Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry, Adelaide, SA, Australia; 2University of Western Australia, Perth, WA, Australia.

Background: Peritoneal dialysis (PD)-related peritonitis may be associated with increased risks of both infectious and non-infectious death as a result of inflammation, declining health and other factors. However, the evidence for this is primarily descriptive.

Our aim was to examine the relationship between mortality and peritonitis in PD patients, utilizing a novel method of analysis, where patients serve as their own controls.

Methods: All patients on the ANZDATA Registry receiving PD for >7 months between 1/5/2004 and 31/12/2009 and who died on PD or within 30 days of transferring to HD were included. We used a case-crossover design so patients had to be on PD for >7 months to allow a comparator time period. Conditional logistic regression was used to compare the risk of peritonitis in the ‘case’ window 21 days immediately before death, and a ‘control’ window of 21 days fomonths before death. This method eliminated the influence of patient-level confounders.

Results: 1316 PD patient were included with a mean age at death of 70.5±11.7 years, 44.2% female, and a mean time on dialysis of 3.2±2.2 years. 1446 peritonitis episodes were documented, with 43.1% experiencing ≥1 episode. 5.9% of deaths were documented as being due to peritonitis, with 207 (15.7%) experiencing an episode in the 21 days before death. There was a significant 8.0 fold increased odds ratio (95%CI: 5.3-12.1) of an episode of peritonitis in the 21 days before death compared to a 21-day window 6months prior. Sensitivity analysis showed no significant difference in the odds of having peritonitis in a 21 day window 60days prior to death compared to 120, 190 and 250days.

Conclusions: We have established that there is a significantly increased risk of peritonitis in the 21 days prior to death. An examination of the definition of peritonitis-related mortality is required.
mechanisms of Smad2 and Smad3 in PD-associated peritoneal fibrosis was studied in primary cultures of peritoneal mesothelial cells (PMCs) that lack Smad3 or have conditional KO for Smad2.

**Results:** We found that TGF-β1-Smad2/3 was markedly activated in PD patients with severe peritoneal fibrosis and EMT. In a mouse model of PD, mice lacking Smad3 were protected against peritoneal PET dysfunctions and peritoneal fibrosis including EMT such as increased collagen matrix and SMA-positive myofibroblast accumulation (Col1α1 86.6%±sMA-44.9% all p<0.05), but diseased E-cadherin expression. Surprisingly, in contrast to Smad3 KO mice, mice with conditional Smad2 KO from the peritoneal tissues substantially enhanced peritoneal fibrosis (Col1α1 79.9%±p<0.01) and EMT. Similarly, deletion of Smad3 from PMCs prevented, but disruption of Smad2 enhanced TGF-β1-induced collagen matrix expression and EMT in vitro. Further study revealed that increased peritoneal fibrosis in conditional Smad2 KO mice and PMCs was associated with enhanced Smad2 and Smad3 signaling.

**Conclusions:** The present study demonstrates that Smad3, but not Smad2, plays an essential role in PD-related peritoneal fibrosis and EMT. Enhanced Smad3 signaling may be a mechanism by which disrupted Smad2 promotes peritoneal fibrosis and EMT in vitro.

**Funding:** Government Support - Non-U.S.

**TH-OR145**

**Effect of Treatment Time Per Session and Dialyzer Phosphate Concentrate on Predialysis Serum Phosphorus Concentration during Short Daily Hemodialysis** J. Ken Leypoldt, Baris U. Agar, Alp Akonur, Mary E. Gellens, Bruce F. Cullen. Renal (Medical Products), Baxter Healthcare Corporation, McGaw Park, IL

**Background:** Short daily hemodialysis (SDHD) has the potential to reduce predialysis serum phosphorus (P) concentrations; however, not all previous SDHD studies have demonstrated this clinical benefit. In this study we use P kinetic modeling to determine the effect of treatment time per session (T) and dialyzer phosphorus clearance (K) on predialysis serum P concentration during SDHD.

**Methods:** We recently demonstrated that a pseudo-one compartment kinetic model, including P mobilization from other body compartments into extracellular fluids, can be used to describe P kinetics during conventional (4 h) and short (2 h) HD treatments (Hemodial Int 2011, in press). This kinetic model is advantageous since it characterizes individual patient differences in P removal by a single parameter, the P mobilization clearance (Km, determined range: 45-208 ml/min). We used this model, combined with a P mass balance relationship, to perform computer simulations of predialysis serum P concentrations during 6-times per week SDHD.

**Results:** Results from representative simulations are tabulated for patients at steady state with a P central distribution volume of 10 L (estimate of extracellular fluid volume); it was assumed that net P intake was increased by 20% during SDHD. K values were varied over the range reported in previous clinical studies.

**Simulated Predialysis Serum P Concentrations (mg/dL)**

| KM (mg/dL) | CHD SDHD SDHD CHD SDHD SDHD |
|-------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| K (mg/min) | CHD SDHD SDHD CHD SDHD SDHD |
| T (min) | 120 | 120 | 180 | 120 | 120 | 180 |
| 8.34 | 36.8 | 78.5 | 2.52 |
| 8.19 | 36.8 | 78.5 | 2.52 |
| 8.19 | 36.8 | 78.5 | 2.52 |
| 8.19 | 36.8 | 78.5 | 2.52 |
| 8.19 | 36.8 | 78.5 | 2.52 |
| 8.19 | 36.8 | 78.5 | 2.52 |

**Conclusions:** Both increasing T and maintaining a high K during SDHD are important to provide significant reductions in predialysis serum P concentrations.

**Funding:** Pharmaceutical Company Support

**TH-OR146**

**Histone Acetyltransferase Activity Is Involved in the Pathogenesis of Experimental Peritoneal Fibrosis** Akihiro Sagara,1 Norihiko Sakai,1 Yasuyuki Shinozaki,1 Shinni Kitajima,1 Tadaoshi Toyama,1 Akinori Hara,1 Kiyoki Kitagawa,1 Miho Shimizu,1 Kenji Furuichi,1 Shuichi Kaneko,1 Takashi Wada,2 1Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa University, Kanazawa, Ishikawa, Japan; 2Division of Nephrology, Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Faculty of Medicine, Kanazawa, Ishikawa, Japan

**Background:** Peritoneal fibrosis is a serious complication in long-term peritoneal dialysis. However, the precise pathogenic mechanisms of peritoneal fibrosis remain to be determined. Circulating mesenchymal progenitor cells CD45/COLI double positive cells, have been reported to participate in peritoneal fibrosis. In addition, histone acetyltransferase (HAT) has also been demonstrated to be involved in the pathogenesis of fibrotic conditions. Therefore, we examined the participation of HAT activity in experimental mouse model of peritoneal fibrosis.

**Methods:** To address this, peritoneal fibrosis was induced by the injection of 0.1% chlorhexidine gluconate (CG) into the abdominal cavity in mice.

**Results:** Chlorhexidine-induced increases in peritoneal thickness and hydroxyproline content were significantly attenuated in LPA-deficient mice (LPA, KO) compared with wild-types (WT). Peritoneal accumulation of myofibroblasts and connective tissue growth factor (CTGF) expression induced by CG were also significantly reduced in LPA, KO. To determine whether LPA and CG, mediated the accumulation of peritoneal fibroblasts as a source of myofibroblasts, we determined the effect of LPA, selective antagonist AM095 on CG challenge of mice whose fibroblasts could be identified by fibroblast-specific expression of GFP. AM095 dramatically attenuated CG-induced increases of GFP-positive fibroblasts. We also found that LPA directly induced CTGF gene expression by mouse peritoneal mesothelial cells. To determine the molecular pathway through which LPA induced CTGF expression, we performed a series of experiments genetically deleting, knocking down or chemically inhibiting potential components of this pathway. We found that this pathway involves LPA signaling, RhoA activation, actin polymerization, and the transcription factors MRTF-A, MRTF-B and SRF.

**Conclusions:** Our results suggest that LPA and CG, drive PD by inducing fibroblast accumulation and MRTF-SRF-dependent pro-fibrotic gene expression.

**Funding:** Other NIH Support - NIH/NHLBI, Government Support - Non-U.S.
Conclusions: We found that PD patients who had access to ACE/ARBs at least 60% of the time during the second year of PD were approximately 30% less likely to transition off PD for any reason (other than transplant) compared to patients who had no or less access to ACE/ARB. Our data suggest that ACE/ARB therapy may be associated with longer time on therapy for PD patients, though the exact mechanism for this has not been determined.

Funding: Pharmaceutical Company Support

TH-OR149

Abstract Withdrawn

TH-OR150

Effect of BMP-7 and Tamoxifen in Animal Model of Peritoneal Fibrosis Developed in Uremic Rats Filipe Miranda Silva, 1 Dayana G. Viloslada, 1 Humberto Dellê, 1 Erik Halesik, 1 Mari C. Sogayar, 2 Irene L. Noronha. 1

Background: Progressive increase in the thickness of peritoneal membrane and peritoneal sclerosis are considered serious complications of long-term peritoneal dialysis. There is still no recognized treatment to reduce these fibrotic changes. In this context, the use of drugs with anti-fibrotic properties such as bone morphogenic protein-7 (BMP-7) and tamoxifen (TAM) may be of relevance. The aim of this study was to analyze the effect of BMP-7 and TAM in an experimental model of peritoneal fibrosis induced by chlorhexidine gluconate (CG), developed in uremic rats.

Methods: End stage chronic kidney disease (CKD) was induced in male Wistar rats using diet containing 0.75% adenine for 30 days. At day 15, rats with CKD, characterized by hyperfiltration (168±13mmHg), high BUN (64±23mg/dL) and high serum creatinine (0.70±0.4mg/dL) levels, were subjected to intraperitoneal injections of 0.1% CG daily for 15 days to induce peritoneal fibrosis (PF). The animals were divided into 4 groups (n=5 per group): CKD, CKD+rats receiving only vehicle; CKD+PF, peritoneal fibrosis induced in CKD rats; CKD+PF+BMP7, CKD rats with PF treated with BMP-7 (30mg/kg every 3 days intraperitoneally), and CKD+PF+TAM, CKD rats with PF treated with TAM (10mg/kg/day) by gavage.

Results: Treatment with BMP-7 and TAM was effective in reducing the thickness of the peritoneal membrane and the number of macrophages and T-lymphocytes. Reduction of TNF-α and Collagen III mRNA levels was also observed.

Funding: Government Support - Non-U.S.

TH-OR152

Development of Circulating Anti-HLA Antibodies Is Associated with Acute Rejection after Conversion: Interim Report of CTOT-02 Sacha A. De Serres,1 Indira Guleria, 1 Nader Najaffar, 2 David N. Iké, 3 Flavio G. Vincenzi, 2 William E. Harmon, 1 Mohamed H. Sayegh, 1 Anil K. Chandraker, 1 Brigham and Women's Hospital and Children's Hospital Boston, Boston, MA; 2UCSF, San Francisco, CA; 3Rho Federal Systems Division, Chapel Hill, NC.

Background: The clinical characteristics and the impact of development of anti-HLA allotubodies (Abs) in renal transplant recipients is not well defined. This report looks at possible associations between Ab development, clinical characteristics, allograft histology at time of Ab development, and acute rejection following Ab conversion.

Methods: Over 750 subjects have been enrolled in the screening phase of the NIH CTOT-02/CTPT-02 study, a multi-center prospective trial where unsensitized kidney transplant recipients are screened for development of de novo anti-HLA Abs up to 48 months post transplant. Subjects were divided into those who developed anti-HLA antibodies (Ab+) and those who did not (Ab-) as detected by Luminex. Ab+ subjects were offered treatment with anti CD20 therapy.

Results: 92 (15%) subjects developed Abs, at a fairly constant rate throughout the study period. 26, 51 and 15 subjects developed class I, II or both I & II Abs respectively. Mean time of Abs development was 18±10 months post transplant. Compared to Ab- subjects, Ab+ subjects were younger (36±18 vs. 43±17; p<0.01) and had lower serum creatinine (SCr) (1.5±1.8 vs. 1.4±0.6; p=0.65). The proportion of subjects who developed acute rejection (AR) was higher in the Ab+ group (14 vs. 3%; p<0.01); most of the AR (77%) were noted after Ab development, at a mean time of 4.8±4.8mo post enrollment. There was no evidence of an association between Ab development and gender, donor type or DGF status. Scr at time of Ab conversion in Ab+ subjects was similar to the last SCr at follow-up in Ab- subjects (1.5±1.8 vs. 1.4±0.6; p=0.65). The proportion of subjects who developed acute rejection (AR) was higher in the Ab+ group (14 vs. 3%; p<0.01); most of the AR (77%) were noted after Ab development, at a mean time of 4.8±4.8mo post conversion. Moreover, biopsies in 7/18 Ab+ subjects prior to treatment showed evidence of acute rejection.

Conclusions: This interim analysis of CTOT-02 reveals unexpected differences in the baseline characteristics and a high proportion of subclinical and clinical rejection associated with anti-HLA Abs.

Funding: Other NIH Support - This work was supported by the Cooperative Clinical Trials in Organ Transplantation (CTOT)

TH-OR153

The Joint Economic Impact of Acute Rejection and Glomerular Filtration Rate in Contemporary Kidney Transplantation Adrian Gheorghian, 1 Mark Schnitzer, 1 Gilbert Litinlien, 1 Anupama Kalsekar, 1 Krista L. Lentine. 1 Saint Louis University; 2Bristol-Myers Squibb.

Background: The economic implications of acute rejection (AR) in contemporary kidney transplant are not defined. We assessed the combined impact of 1st year AR and estimated glomerular filtration rate (eGFR) on 2nd and 3rd yr Medicare costs.

Methods: Data for Medicare-insured kidney transplant recipients in 2000-2007 (n=32,520) who survived with graft function to 12 mo were drawn from the United States Renal Data System. AR events were ascertained from OPTN reports. AR was classified as Antibody-Treated AR (Ab-AR) or other management (non-Ab-AR). The primary cost measure was payments for all healthcare services made by Medicare during intervals of the 2nd and 3rd yrs post-transplant. Multivariate linear regression was used to quantify: 1) the marginal cost impact of first-year AR and eGFR on subsequent costs, and 2) total predicted costs. Covariates included recipient, donor and transplant factors in the UNOS Kidney Allocation Review Committee survival model.

Results: Ab-AR and non-Ab-AR within the 1st yr were associated with significant incremental costs in 2nd yr costs of $5,755 and $5,019, respectively, after adjusting including eGFR and baseline factors (Table). AR was also significantly associated with 3rd yr costs. Table 2nd & 3rd costs were higher in those with AR compared to no AR. However, markedly stronger variation in costs was seen across eGFR levels (Figure). For example, among those with non-Ab-AR, adjusted total 2nd & 3rd costs were $22,747 with eGFR >60 but $43,881 with eGFR ≤30 ml/min/1.73m2. Cost impacts of AR and eGFR were similar but of lower magnitude after additional adjustment for death and graft failure.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Table. Associations of AR within the 1st Yr and 12-month eGFR with Costs in Years 2 and 3 after Transplant by Multivariate Regression.

<table>
<thead>
<tr>
<th>P-values: <strong>F</strong> P &lt; 0.05; <em>P</em> = 0.0001</th>
<th>2nd Year Costs</th>
<th>3rd Year Costs</th>
<th>2nd Year Costs,*</th>
<th>3rd Year Costs,*</th>
<th>Adjusted for death</th>
<th>Adjusted for death</th>
<th>$ per yr</th>
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<th>$ per yr</th>
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<td>Non-Ab-treated in Yr1</td>
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<td>3,483.01</td>
<td>4,501.2</td>
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</tbody>
</table>

Figure. Predicted Costs in Yr 2 after Transplant according to 1st Yr AR and eGFR level

Conclusions: AR is a significant predictor of post-transplant costs. However, the cost impact of AR is markedly higher among affected patients with reduced compared to preserved eGFR.

Funding: Pharmaceutical Company Support

TH-OR154

Efficacy and Safety of Early Cyclosporine Conversion to Sirolimus with Continued MMF: 5-Year Results of the Post-CONCEPT Study Yvon Lebranchu,1 Matthias Buchler,1 Eric Thervet,2 Isabelle Etienne,3 Pierre-Francois Westeel,4 Jean-Philippe Rerolle,5 Sandrine Girardot-Seguin,6 Bruno Molin.7  
1CH Bretonneau; 2CHU Necker; 3CH Bois Guillaume; 4CH Sud; 5Civil Hospital; 6Roche, Neuilly-sur-Seine; 7Civil Hospital.

Background: CNI induces long-term nephrotoxicity and is associated with moderate renal dysfunction. The de novo introduction of sirolimus (SRL) has demonstrated a positive impact on renal function. However, the adverse effects of early SRL introduction could limit this approach. In the CONCEPT study, the delayed conversion to SRL demonstrated an improved renal function with a similar tolerance, at one year. 60 months follow up study investigates the long term impact of this strategy.

Methods: In this prospective trial, renal function by simplified MDRD formula and renal dysfunction were assessed from 12 to 60 months post-transplantation in the two groups (group A: CsA-MMF; group B: CsA+MMF).

Results: Among the 162 patients (89.5%) entered in the follow up in M12, 139 patients were evaluated at M60; 67 in group A, 72 in group B. eGFR was significantly higher in group A, 58.31 vs 49.89 ml/min/1.73m², respectively (p=0.0012). This difference was observed particularly in population “on treatment” (group A, n= 39) 65.05 vs 51.73 ml/min/1.73m² (p<0.001).

In ITT analysis, 7 deaths (3 in SRL group and 4 in CsA group) related to immunosuppressive treatment, 2 graft losses in group A and 4 BPAR (2 in each group) were observed between M12 and M60.

Concerning the lipid profile and proteinuria, the two groups were not different at M60.

The occurrence of cancers was lower in patients treated by MMF+SR (n= 4) than MMF+CSA (n=12).

Conclusions: The significant improvement in renal function observed at 12 months in patients receiving MMF + SRL was maintained at 60 months compared to MMF + CSA and the occurrence of cancers was lower in patients treated by MMF + SRL.

Funding: Pharmaceutical Company Support

TH-OR155

A Multicentre RCT of Early Switch to Everolimus Plus Steroids or Everolimus Plus CsA Versus CsA, MPA and Steroids in De Novo Kidney Transplant Recipients: 12 Month Analysis Steven J. Chadban,1 Graeme Russ,2 John Kanellis,1 Helen L. Pilmore,1 Yu Scun Kim,2 Si-Yen Tan,2 Nicol Kurstjens,2 Josette M. Eris.1  
1Royal Prince Alfred Hospital; 2Royal Adelaide Hospital; 3Monash Medical Centre; 4Auckland City Hospital; 5Convergence Hospital; 6University Malaya Medical Centre; 7Novartis Australia.

Background: To determine whether addition of everolimus (EVR) to cyclosporine (CsA), mycophenolic acid (MPA) and steroids at 2 weeks post-kidney transplantation can enable elimination of either CsA and MPA or steroids and MPA without compromising efficacy and safety.

Methods: SOCirates is a 36-month (M), prospective, multi-centre, randomised, open-label study. Kidney transplant recipients (KTR) received CsA, MPA, prednisone and basiliximab and were randomized commencing week 2 to add EVR and withdraw CsA+MPA (CsA-WD); add EVR and withdraw steroids & MPA (steroid-WD); or remain on CsA, MPA & steroid (control). Primary outcome was non-inferiority at M12 eGFR (Nankivil formula). Key secondary endpoints were a composite of biopsy proven acute rejection (BPAR), graft loss, death, loss to follow-up and each of these individually.

Results: 126 KTR were randomized to CsA-WD n=49, steroid-WD n=30, or control n=47. The steroid-WD arm was prematurely terminated due to a higher treatment discontinuation rate attributed to acute rejection, unsatisfactory effect, or other reasons. At M12, CsA-WD was non-inferior to control for eGFR 72.3 ±71.3 ml/min/1.73m²(p=0.006).

Change in mean eGFR from week 2 to M12 was numerically greater for CsA-WD vs control (+17.5 vs +5.7ml/min/1.73m²).

Conclusions: Early switch to everolimus enabled withdrawal of CsA and MPA without compromising M12 eGFR. This regimen was associated with improved eGFR from week 2 to M12 but a higher rate of acute rejection. Continuation of MPA may be preferred when switching from a CNI to everolimus strategy.

Funding: Pharmaceutical Company Support

TH-OR156

Endothelial Dysfunction in Renal Transplant Recipients with Functioning Arteriovenous Fistula Yasar Caliskan,1 Mehmet Besiroglu,1 Halil Yazici,1 Ibrahim Altan,2 Ahmet Gurdal,2 Tefik Eced,1 Aydin Turkmen,1 Mehmet Sever.1  
1Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; 2Department of Cardiology, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey.

Background: Endothelial dysfunction is a common precursor and denominator of atherosclerosis and atherosclerotic cardiovascular diseases (CVD) are still the major cause of death among renal transplant recipients. We investigated the effects of functioning arteriovenous fistula (AVF) on endothelial functions in renal transplant recipients.

Methods: A total of 82 renal transplant recipients who were undertaken hemodialysis or peritoneal dialysis before transplantation enrolled in this study. Patients were divided into three subgroups [patients with functioning AVF (n=46), patients whose fistulas were spontaneous stopped or closed (n=25) and peritoneal dialysis patients who had never been constructed AVF]. Serum creatinine, uric acid, albumin, calcium, phosphorus, albumin, PTH, triglyceride, total, LDL- and HDL- cholesterol levels were measured. Endothelial function was measured non-invasively with high resolution ultrasound equipment as the percentage of post-ischemic flow-mediated dilation (FMD) of the brachial artery of the non-dominant or non- fistula arm.

Results: There were no significant differences regarding age, gender, smoking status, time on dialysis, immunosuppressive treatment, blood pressures, serum creatinine, uric acid, albumin, hs-CRP, calcium, phosphorus, albumin, intact PTH, triglyceride, total, LDL- and HDL- cholesterol levels among the study groups. Pulmonary artery pressure and ejection fraction were also similar among the three groups. FMD value of patients with functioning AVF (7.9±6.1%) were significantly lower then patients with non-functioning AVF (12.2±5.8%) and patients never been constructed AVF (10.4±5.8%) (p=0.017). In correlation analysis, FMD was not significantly correlated with any laboratory parameters.

Conclusions: Endothelial dysfunction which is a common precursor of cardiovascular events is more prominent in renal transplant recipients with functioning AVF.

Funding: Government Support - Non-U.S.
TH-OR157

Short Course, High Dose Erythropoietin in Deceased Cardiac Death Kidney Transplant Recipients Zeynep Avdin, Marko J. Mallat, Ton J. Rabelink, Johan W. De Fijter. Nephrology, Leiden University Medical Center, Leiden, Netherlands.

Background: Ischemia-reperfusion injury is a problem in kidneys derived from DCD donors. In preclinical models with ischemic insults, exogenously administered Erythropoietin proved to be renoprotective. Methods: All deceased DCD transplanted recipients were included in this single center prospective, randomized, double blind, placebo-controlled study. Erythropoietin was administered to the recipient as an intravenous bolus of 3.3 x 10^9 IU, 3-4 hours before the transplantation, as well as 24 and 48 hours after reperfusion. Primary end point was the composite of delayed graft function/primary non function. Secondary end points included duration of DGF, acute rejection, measured creatinine clearance (mGFR) and survival at 1 year after transplantation.

For safety purposes any arterial or venous thrombosis was recorded. Results: A total of 92 patients were included in the study. DGF occurred in 39/45 recipients in the EPO treated group (86.7%) as compared with 41/47 patients in the placebo group (87.2%) (P=1.00).

The incidence of PNF was 6.7% in the EPO group (3 patients) and 2.1% in the placebo group (1 patient).

If DGF developed, the median duration was 10 days in the EPO group and 9 days in the placebo group (NS).

Acute rejections occurred in 20.5% in the EPO group and in 26.1% in the placebo group (P=0.62).

The measured creatinine clearance showed no difference at 6 weeks (44±19 min and 46±18 min/m in EPO and placebo group respectively), but was significantly better in the EPO group 1 year after transplantation (68±23 vs 57±25 ml/min) (P=0.05).

One-year patient and graft survival were respectively 96.93% and 96.96% in the EPO and placebo group.

Thromboembolic events occurred in 14 patients in the total group, the majority consisted of thrombosis of the vascular access (4 in EPO group, 1 in placebo group).

Conclusions: High-dose Erythropoietin did not reduce the incidence or duration of DGF. However, treatment with EPO resulted in a significantly better recovery of mGFR, but also more thrombotic events.

TH-OR158

Replacement of Vitamin D by Cholecalciferol or Cholecalciferol Plus Doxercalciferol Lowers Parathyroid Hormone Levels without Raising 25-OH Vit D or FGF23 or Adverse Effects on Kidney Function 4. These results have a low side effect profile in stable long-term KTR's with HPTH. 2. Repletion of Vitamin D can be accomplished within 6 months and results in an average decrease in PTH of 30%.

3. Repletion of Vitamin D with cholecalciferol or active vitamin D (doxercalciferol) does not cause an increase in 1,25 Vit D or FGF23 or adverse effects on kidney function 4. These results have a low side effect profile in stable long-term KTR's with HPTH. 2. Repletion of vitamin D can be accomplished within 6 months and results in an average decrease in PTH of 30%.

Methods: To study the role of macrolactones in recovery from acute kidney injury characterized, predominantly by epithelial apoptosis, we utilized a transgenic mouse expressing the human diptheria toxin receptor (DTR) selectively in kidney proximal tubule.

Results: DT administration induced acute kidney dysfunction in transgenic (TG) mice but not wild-type (WT) mice following DT administration, with increased creatinine and BUN and renal proximal tubule dilatation, cell sloughing and cast formation. Marked renal proximal tubular cell apoptosis was detected by TUNEL-staining. In the TG mice, macrophage/monocyte depletion by clodronate either prior to, or after induction of injury prevented development of histologic injury, increased and prolonged KIM-1 expression, increased apoptosis and delayed cell proliferation. Similar results were seen with macrophage depletion in γ-TGD11c-DTR double transgenic mice. Following ischemia-reperfusion, there was an early increase in macrolactones with "M1" markers, which were reduced by DT, there was increased increased in M1 macrolactones but significant increases in M2 (wound healing) macrolactones; expression of these M2 markers was markedly inhibited by clodronate or in the double transgenic mice. Of interest, prior spleenectomy did not alter the recovery in this model. Furthermore, unlike ischemia/reperfusion injury, there was no increase in renal accumulation of labeled macrolactone in DT-induced injury above that seen in kidneys from untreated animals, but there was an increase in cells double labeled with F4/80 and Ki67, a marker of proliferation.

Conclusions: In this model of AKI characterized by renal tubular apoptosis, M2 macrolactones increase in response to injury and serve as a crucial component mediating epithelial regeneration. There appears to be an important role for local macrophage proliferation in the reparative process. Funding: NIDDK Support, Veterans Administration Support

FR-OR160


Background: During renal ischemia, shifts in the metabolic supply to demand ratio – particularly for oxygen – result in severe tissue hypoxia. Cellular responses to hypoxia are regulated by enzymes that sense cellular oxygen levels and coordinate transcriptional responses to hypoxia or ischemia. Central among these enzymes are three oxygen-sensing prolyl hydroxylases (PHD1-3). Limited oxygen availability results in inhibition of PHDs with subsequent stabilization of hypoxia-inducible factors (HIF’s). Activation of HIF results in a transcriptionally regulated response that reprograms cellular metabolism towards hypoxia adaptation. Thus, we hypothesize that genetic deletion or pharmacologic inhibition of PHDs mediates kidney protection from ischemia.

Methods: Gene targeted mice for PHD1, 2 and 3 (PHD1−/−, PHD2−/−, PHD3−/−) were studied in an ischemic model of acute kidney injury (AKI) by using a hanging weight system. Renal function was determined by FITC-labeled inulin clearance, serum creatinine, renal cytokine levels, renal myeloperoxidase (MPO ELISA), histology and TUNEL staining.

Results: To pursue our hypothesis, we first treated wild type mice with a PHD inhibitor (glyceryl trinitrate, glyceryl trinitrate) before renal ischemia. Mice with DMOG treatment showed attenuated kidney injury following renal ischemia compared to vehicle treated mice. The decrease in GFR following ischemia was attenuated and the increase in serum creatinine reduced. Furthermore, inflammatory cytokines (TNF-alpha, IL-6) and neutrophil by DT administration led to significantly greater functional and histologic injury, increased apoptosis and delayed cell proliferation. Similar results were seen in gene-targeted mice for PHD1, 2 or 3 showed a selective phenotype in Phdl−/− mice with remarkable protection from ischemic AKI. The GFR was tremendously improved, serum creatinine significant lower and histological damage attenuated.

Conclusions: In summary, our results in ischemic kidney show that in Phdl−/− mice with improved renal function following AKI due to ischemia. Thus, PHD inhibitors could prove to be a novel therapeutic agent in the treatment of AKI in the near future.

FR-OR161

Chitinase 3-Like 1 Regulates the Renal Response to Ischemic Injury and Predicts Delayed Allograft Function Insa Marie Schmidt,1 Isaac E. Hall,1 Gilbert W. Moeckel,2 Chirag R. Parikh,1 Lloyd G. Cantley. 1Department of Internal Medicine, Section of Nephrology, Yale University School of Medicine, New Haven, CT; 2Department of Pathology, Yale University School of Medicine, New Haven, CT.

Background: Acute kidney injury can trigger a series of responses that promote tubular repair. Defining those pathways may provide novel targets for therapeutic intervention to prevent the repair process.

Methods: Using proteomic analysis, western analysis and quantitative PCR, we found that Chitinase 3-like 1 (Chi3l1, named Brp-39 in mouse, YKL-40 in humans), is highly upregulated in the kidney and urine during the repair phase after ischemia/reperfusion (IR) injury. The functional importance of this pathway was analyzed using Brp-39 null mice, cultured epithelial cells and urine from patients after kidney transplant.

Results: Mice lacking Chi3l1 demonstrate significantly worse outcomes following AKI compared to control animals, with more severe tubular injury, increased apoptosis, delayed epithelial tubular proliferation, a persistent reduction of kidney function and decreased survival. Stimulation of mouse tubular epithelial cells with Brp-39 induces activation of Akt and Erk, with a reduction of H2O2-induced apoptosis by 50%. Inhibition of Brp-39 stimulated PI3-K/Akt activation prevented the anti-apoptotic effect, suggesting that this pathway is critical for the protective effects of Brp-39. In recipients of deceased-donor kidney transplants who exhibited delayed graft function (DGF, indicative of severe ischemic injury, n=26), urinary YKL-40 levels at 0 (immediately post-op) and 1 day after
FR-OR162

PTEN Loss Defines the Tubule Phenotype of Failed Differentiation Associated with Kidney Fibrosis in Rodent Models and Human Disease Ronpei Lan,1 Hui Geng,2 Aaron Polichnowski,2 Prajjal Kanti Singha,1 Pothana FR-OR163

Exosome-horizontal Transfer of IGF-1 Receptor to Cisplatin-Damaged FR-OR165

IQGAP1 Is Critical for Recovery from AKI by Enhancing Tubular Cytoskeleton Regeneration Li-Wen Lai,2 Kim-Chong Yong,1 Yeong-Hau Howard Lien.1,2,3 (Pharmacology & Toxicology, University of Arizona, Tucson, AZ, ‘Medicine, University of Arizona, Tucson, AZ, ‘Arizona Kidney Disease and Hypertension Center, Tucson, AZ.)

Background: We studied how the “failed differentiation” tubule phenotype develops in cultured cells, mice with tubule specific TGFβ induction, rat models of ischemic and maleate AKI, and human chronic kidney disease.

Results: Tubule specific induction of TGFβ in vivo led to loss of tumor suppressor PTEN and increased Jun N-terminal kinase (JNK) signaling in proximal tubules (PT), and to fibrosis. In cultured PT cells, high TGFβ depleted PTEN, raised JNK signaling and inhibited heterodimerization. Conversely, TGFβ antagonism increased PTEN, decreased JNK signaling and promoted differentiation. Selective Cro-Lox PTEN deletion suppressed PTEN differentiation despite TGFβ status, triggered JNK and JNK signaling and induced growth arrest. PTEN declined in a subpopulation of PT during repair of AKI in vivo. These cells lacking PTEN were growth arrested, but showed increased JNK2 and JNK signaling, failed to re-differentiate, expressed viminatin and keratin, produced PDGF-B and CTGF (subsequently were surrounded by cells expressing these transcripts), whereas JNK/PPTEN recovery was associated with PT re-differentiation and normal repair. Pharmacologic TGFβ antagonism promoted PTEN recovery, PT differentiation, and normal repair. Similar improvement of low PTEN and tubulo-interstitial pathology were induced by contralateral nephrectomy done 2 weeks after unilateral ischemia-reperfusion, confirming the reversibility of this dysfunctional phenotype. Vimentin and keratin expressing tubules with low PTEN and increased phospho-c-Jun were associated with fibrosis also in human chronic kidney disease of diverse etiology.

Conclusions: The low PTEN, vimentin-keratin positive tubule phenotype that expresses dysfunctional JNK-Jun signaling and fibrogenic peptides is associated with fibrosis in diverse animal models and human disease. We propose that it plays a central pathogenic role in triggering fibrotic events in the renal interstitium, and is potentially treatable.

Funding: NIDDK Support

FR-OR166


Background: Ischemia-reperfusion (IR) injury is characterised by the production of pro-inflammatory cytokines, such as IL-6, TNF and MCP-1, that drive macrophage polarisation resulting in tissue damage. We have reported that colony stimulating factor (CSF)-1 stimulates macrophage differentiation towards a reparative phenotype leading to accelerated kidney repair following IR (Alikhan et al. Am J Pathol 2011). Identifying
further cytokines and growth factors involved in macrophage polarization may lead to the development of therapies aimed at limiting the rapid of the inflammatory response and promotion of tissue remodeling.

Methods: Flow cytometry was used to assess macrophage phenotype during the inflammatory and tissue remodeling phases of IR injury (n=5/group) with/without the addition of CSF-1. The production of IFN-γ, TNF-α, IL-6, IL-10, IL-12p70 and MCP-1 was assessed using cytokine bead arrays. In conjunction, macrophages (CD45+CD11b+CD11c+) from damaged kidneys were FACS sorted and subjected to microarray gene expression profiling using IlluminaTM Mouse gene expression arrays. Candidate therapeutic target genes were identified using the Ingenuity Pathway Analysis software.

Results: Total kidney cellularity decreased over the time course of injury but with a concomitant rise in CD45+ cells. Ly6Chigh inflammatory monocytes comprised the majority of infiltrating cells. This infiltration was followed by the upregulation of MHC class II and F4/80. Gene profiling arrays showed the increased expression of the M2 associated glucocorticoid receptor, IL-10 and IGF-1 genes at day 5 and repair associated PDGF, GM-CSF and apoptosis signaling pathways by day 7 in CSF-1 treated mice.

Conclusions: Macrophages play a central role in the endogenous repair process following IR injury as evident by the phenotypic switch from a pro-inflammatory to an anti-inflammatory cell type. Further gene profiling of the cells involved in CSF-1 enhanced repair has highlighted potential therapeutic targets that may prevent or retard the progression of renal disease.

FR-OR167 Hedgehog Signaling Pathway Is Activated during Kidney Repair

Hyeong Cheon Park, Hoon Young Choi, Geum-Ock Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Acute Kidney Injury (AKI) is a frequent cause of long term renal failure or death (1). Unilateral ureteral obstruction (UUO) in mice has been widely used as a model of severe AKI. Hedgehog (Hh) is a core signaling pathway implicated in fundamental processes during embryonic kidney development (2) but its role in the adult kidney has not been explored.

Experiments performed in our laboratory show that persistence of hedgehog signaling in the adult kidney leads to acquisition of mesenchymal hallmarks by tubular epithelial cells, similarly to what is observed after AKI. This similarity prompted us to test the participation of Hh pathway in kidney repair.

Methods: Using Gli1lacZ transgenic mice (3) as an in vivo reporter of Hh activity, we found that Hh signaling is latent in adult fully developed kidneys and is significantly upregulated after UUO. Increased Gli1 activity was more evident at the cortico-medullary junction, where it is mostly concentrated in interstitial cell populations, but was also sparsely detected in cells of various tubular segments.

To test if pharmacological modulation of Hh activity would affect kidney repair, we inhibited Hh signaling at different time points after injury using the specific Smoothened antagonist cyclopamine. Hh inhibition resulted in net improvement of kidney morphology, significant reduction of collagen deposition and diminished apoptosis, indicating a central role of Hh signaling in kidney repair. Interestingly, the favorable effects of Hh inhibition on the injured kidney were dependent on the time of the drug administration, suggesting that Hh might have different functions and signal to different cell populations in different phases of the repair process.

Conclusions: We conclude that activation of the Hh signaling pathway has a key role in kidney repair and its modulation might be useful for future therapy of AKI.


Funding: Other NIH Support - P30DK079328-04, NIH UT Southwestern O’Brien Kidney Research Core Center, Private Foundation Support

FR-OR168 Microparticles from Kidney Derived-Mesenchymal Stem Cells Act as Carriers of Proangiogenic Signals and Contribute to Renoprotection in Acute Kidney Injury

Hyung Cheon Park, Hoon Young Choi, Geum-Ock Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Background: Microparticles (MP) shed from bone marrow mesenchymal stem cells (MSCs) confer protective effects against acute tubular injury via transfer of messenger RNA and microRNA. We recently demonstrated that in vitro expanded kidney derived-MSC (KMSC, Kidney International 2008; 74:879-889) protected peritubular capillary endothelial cells in acute ischemic reperfusion injury (IRI).

Methods: KMSC were cultured in hypoxic chamber in serum deprived MEM with hydrogen peroxide (200 μM) for 24 hours. MP were isolated from supernatants by differential ultrafiltration (2,000x g, 10 min, 100,000x g, 1hr) for electron microscopy and flowcytometric characterization and MP RNA was extracted using ExoMir kit (BIOO Scientific). Isolated MP were cultured with human umbilical vein endothelial cells (HUVEC) on growth factor reduced Matrigel to assess their effect on endothelial tube formation. Microparticles were subjected to rt-PCR, Western blots and immunoisotchemistry in human monocytes.

Results: 1Brigham and Women’s Hospital, Harvard Medical School; 2Warwick Medical School, United Kingdom.

Background: Klotho-FGF23 functions as a receptor for the phosphatase, FGF23 at the kidney. FGF23 levels rise in CKD despite progressive vascular calcification (VC). We postulate functional vascular Klotho expression as a therapeutic target for VC in CKD.

Methods: In vitro model: Human aortic smooth muscle cells (HA-SMCs) +/- Klotho siRNA. Calcification: Alizarin red and Araesano III detection.

Results: We show for the first time Klotho expression in human arteries and HA-SMCs. Analysis of human arteries from CKD and healthy controls revealed Klotho as a state of vascular diseases. FGF23 inhibited Klotho and FGF23/1 deficiency was driven by uremic serum, calcification medium (CM: 2.7 mM CaCl2 +/- 2mM β-glycerophosphate) and TNF-α (20ng/ml), in vitro. Klotho knockdown potentiated development of accelerated calcification. These cells exhibited osteogenic transformation (increased Cbfa1 and ALP), and concomitant loss of myocardin-SRF regulated contractile phenotype. In addition, in vitro studies revealed that vascular cells are a Klotho-dependent target for FGF23. FGF23 (5ng/ml) induced cellular activation of ERK, AKT and proliferative effects, which were abrogated by Klotho knockdown. We next showed that calcitriol or calcidiol reversed loss of Klotho and FGF23/1 in HA-SMCs. Human arterial organ cultures from CKD patients confirmed these findings, with upregulation of Klotho and FGF21 mRNA after calcitriol and paracalcitrol treatment. Furthermore, calcitriol or FGF23 pre-treatment alone followed by combined treatment with CM did not modulate development of HA-SMC calcification. However, pre-treatment with calcitriol and FGF23 together, significantly inhibited the development of calcification. These effects mitigated by Klotho knockdown.

Conclusions: Chronic metabolic stress factors found in CKD promote vascular Klotho deficiency. Functional studies reveal a bifunctional role for vascular Klotho, first as an inhibitor of VC, and second as a co-factor for FGF23 signaling. Furthermore, VDR activators can restore Klotho expression and unmask FGF23 vascular-protective effects.

Funding: Private Foundation Support

FR-OR170 FGF23 Inhibits Innate Immune Responses to Vitamin D in Human Monocytes

Justine Baccetti, Thomas S. Lisse, Jessica L. Sea, Ren Chuen, Katherine Wesseling-Perry, Isidro B. Salusky, Martin Hewison. David Geffen School at UCLA, Los Angeles, CA.

Background: Vitamin D is a potent stimulator of innate immunity. This facet of vitamin D physiology is highly dependent on efficient synthesis of 1,25-dihydroxyvitamin D (1,25D), by monocytes, the 1-alpha hydroxylase CYP27B1 being induced by immune activators. FGF23 suppresses renal CYP27B1 but its role in non-classical actions of vitamin D is less clear. We hypothesized that FGF23 inhibits vitamin D-induced antibacterial activity by targeting monocyte CYP27B1 similar to its actions in the kidney.

Methods: We performed rt-PCR, Western blots and immunohistochemistry in human monocytes obtained from healthy donor peripheral blood mononuclear cells (PBMCm) and from peritoneal dialysate effluent (PDM). We performed rt-PCR, Western blots and immunohistochemistry in human monocytes obtained from healthy donor peripheral blood mononuclear cells (PBMCm) and from peritoneal dialysate effluent (PDM).

Results: In untreated PBMCm, initial rtPCR studies confirmed the presence of transcription of CYP27B1, with klotho and FGF23 being more strongly expressed than FGR2, 3 or 4; there was a positive relationship between FGR1 and klotho expression, as well as between FGR1-4 and CYP27B1. Immunohistochemistry showed that klotho and FGR1 proteins colocalize, this effect being enhanced following treatment with FGF23. Confocal microscopy showed that receptor with FGF23 activated both FGR1 and Akt pathways, known to be downstream of FGRs. Treatment of PBMCm with FGF23 (100 ng/ml) demonstrated a downregulation of Klotho, FGR1 and CYP27B1 expression, as determined by nPCR. This effect was also observed in PBMCm pre-treated with IL-15 to stimulate CYP27B1 transcription. These data show support the hypothesis of decreased CYP27B1 expression, FGF23-treated PBMCm showed lower levels of antibacterial LL37, and other 1,25D targets such as CYP24A1. Similar observations were also made in PDM, which showed stronger expression and colocalization of klotho and FGR1 at baseline.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

41A
Chronic Phosphate Restriction Fails To Prevent Fibroblast Growth Factor 23 (FGF23) Elevation in Chronic Kidney Disease Mouse Model Jaron R. Stubbs, Shiqin Zhang, Ryan Gillihan. Kidney Institute, University of Kansas Medical Center, Kansas City, KS.

Background: FGF23 is a phosphaturic and vitamin D regulatory hormone that is markedly elevated in end-stage renal disease (ESRD). While elevations in FGF23 are associated with morbidity and mortality in ESRD, the mechanism responsible for this elevation is unclear.

Methods: We tested the hypothesis that phosphate retention in chronic kidney disease (CKD) is the primary stimulus for FGF23 elevations by studying the effects of dietary phosphate restriction on FGF23 levels in Col4a3 null mice, a mineralometabolic mouse model with a mineral metabolism profile that mimics that found in humans with CKD.

Results: We observed a significant increase in serum FGF23 in Col4a3 null mice at 8 weeks-of-age (1160.8 ± 401.7 pg/ml vs 183.5 ± 11.3 pg/ml for WT controls; p<0.05), with a marked elevation by 12 weeks (8228.3 ± 2314.0 pg/ml; p=NS). Col4a3 null mice also demonstrated FGF23 levels similar to those of null mice on the control diet at 8 weeks-of-age (1160.8 ± 401.7 pg/ml vs 183.5 ± 11.3 pg/ml for WT controls; p<0.05), with a 3-fold increase in renal Cyp24 gene expression in Col4a3 null mice (p=0.07) compared to WT, suggesting the activation of vitamin D degradation pathways. Phosphate restriction improved 1,25(OH)2D levels in advanced CKD despite persistent FGF23 elevations, suggesting a secondary pathogenic role in development of sHPT.

Conclusion: Our genetic mouse model shows that lack of parathyroid KL does not cause bone abnormalities in mineral metabolism, likely due to adaptations in CaSR and VDR expression. Reduced KL, as found in CKD patients, is therefore unlikely to play a primary pathogenic role in development of sHPT.

Possible Involvement of PTH in the Secreted Frizzled Related Proteins (sFRPs) Regulation and Wnt Signalling Pathway Natalia Carrillo-Lopez, Maria Arias, Pablo Roman-Garcia, Sara Panizo, Manuel Naves, Jorge B. Cannata-Andia. Bone and Mineral Research Unit, Hospital Universitario Central de Asturias. Instituto Reina Sofia de Investigación. REDinREN del ISCIII. Universidad de Oviedo, Oviedo, Asturias, Spain.

Background: The aim of this study was to evaluate in vivo and in vitro the effect of different degrees of secondary hyperparathyroidism and different PTH concentrations on bone turnover-related and Wnt pathway signaling-related gene expression.

Methods: After inducing chronic renal failure (CRF) by 7/8 nephrectomy in 36 rats, one group was fed normal phosphorus (P) diet (NPD) (0.6% P) and another was fed high P diet (HPD) (0.9%). Rats were sacrificed at 8, 16 and 20 weeks. Blood samples were collected and the left tibia was removed to assess gene expression. In the in vitro study, UMR106 cells were exposed to vehicle or different concentrations of PTH (1-34). After 24 hours, cells were collected to analyze gene expression.

Results: After 20 weeks, CRF rats fed HPD showed a significant increase in serum PTH and P levels, together with a significant decrease in serum calcium. Moreover, the bone gene expression of bone turnover markers together with Wnt inhibitors, such as sFRP1, sFRP2, sFRP4 and DKK1, was significantly increased. In vitro, cells exposed to PTH were able to significantly increase FGF23, osteocalcin, OPG, Cbfa1 and cathepsin K gene expression. Like in the in vivo experiments, PTH were also able to significantly increase sFRPs, 1 and 4 gene expression in a concentration dependent manner.

Conclusions: Bone gene expression of bone turnover markers and Wnt-related gene expression measured by qRT-PCR in the in vitro and in vivo studies. RELI: Relative Units referred to Reference group (rats with normal renal function and NPD diet) (a vivo study) and Vehicle group (a in vitro study). In vivo study: *p<0.05 compared to time-matched NPD group and *p<0.05 compared to Reference group. In vivo study: +p<0.05 compared to Vehicle group.

FR-OR174
Conditional Knockout of Dicer in the Parathyroid Shows That miRNAs Are Necessary for the Response of the Parathyroid to Short-and-Long-Term Hypocalcemia
Vitali Shilo, Choft Chai, Justin Silver, Tally Naveh-Many, Nephrology, Hadassah Hebrew University Medical Center, Jerusalem, Israel.

Background: MicroRNAs (miRNAs) are short non-coding RNA molecules that affect protein levels by sequence-specific repression of translation and mRNA degradation. The final step in miRNA maturation is mediated by Dicer, a RNase III-like enzyme expressed in all cell types that is essential for life.

Methods: We have disrupted miRNA maturation specifically in the parathyroid using parathyroid specific dicer knock-out (PT-Dicer-/-) mice to study the role of miRNAs in parathyroid physiology and the development of SHPT.

Results: The PT-Dicer-/- mice develop normally and are fertile with no marked differences in serum PTH and calcium levels compared to control littermates. However, a short-term decrease in serum Ca2+ by EGTA administration at 40 min did not increase serum PTH in the PT-Dicer-/- mice but led to the expected 3-fold increase in serum PTH in the control littermates. To induce secondary hyperparathyroidism (SHPT) mice were fed a calcium-depleted diet for 3 weeks. PT-Dicer-/- mice showed only a moderate 2-fold increase in serum PTH compared to the 10-fold increase in control mice. Moreover, chronic hypocalcemia also failed to increase PTH miRNA levels in the PT-Dicer-/- mice.

Conclusions: These results show that parathyroid miRNAs are necessary for the response of the parathyroid to both acute and chronic hypocalcemia. The impaired response of the PT-Dicer-/- mice to the challenge of hypocalcemia may be due to an effect of miRNAs on calcium sensing, PTH secretion, gene expression and/or parathyroid cell proliferation.

FR-OR175
Transgenic Approach Reveals Strong Protective Effects of Vitamin D Signaling on Podocytes
Youli Wang, Dilip K. Deb, Yan Chun Li.

Department of Medicine, University of Chicago, Chicago, IL.

Background: Podocytes play a key role in maintaining the integrity of the glomerular filtration barrier, and podocyte injury is a major cause for renal dysfunction in diabetic nephropathy. Clinical and animal studies have demonstrated potent anti-proteinuric activity for vitamin D and its analogs, suggesting podocytes as important renoto-protective target of vitamin D signaling.

Methods: To test this hypothesis, we targeted Flag-tagged human (h) VDR to podocytes in DBA/2 mice using the 2.5 kb podocin gene promoter. The transgenic (Tg) mice were analyzed using the model of streptozotocin (STZ)-induced diabetic nephropathy.

Results: Podocyte-specific expression of hVDR was confirmed by Western blotting and immunostaining in IVDR-Tg mice. Diabetic hIVDR-Tg mice exhibited less albuminuria compared to wild-type (WT) counterparts. While treatment with a low dose vitamin D analog doxercalciferol (Dex, 30 ng/kg bw, p.o. daily) did not have any effects on diabetes progression of diabetic nephropathy in WT mice, this treatment almost completely prevented albuminuria and markedly reduced glomerular fibrosis in diabetic hIVDR-Tg mice. Dex and 3-beta hydroxy vitamin D-like ligands exhibited attenuation of podocyte apoptosis in the IVDR-Tg mice. Dex treatment also prevented the elevation of renal renin and fibronectin and preserved the expression of nephrin in the IVDR-Tg mice. Moreover, when the hIVDR transgene was bred into VDR-null mice to generate VDR-/-;hIVDR-Tg (KO) mice that express hIVDR only in podocytes, the IVDR transgene was able to partially rescue the severe renal damages seen in VDR KO mice in the STZ-diabetic model, manifested by marked reduction in albuminuria and podocyte loss. In podocyte culture exposure to high glucose (30 mM) induced apoptosis with up-regulation of p53, Bad, Bak and p-Erk, and vitamin D treatment blocked this pro-apoptotic pathway and podocyte apoptosis.

Conclusions: Taken together, these data provide strong evidence that vitamin D signaling plays a critical role in the protection of podocytes from diabetic injury.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-OR176
Cardiac Remodeling and Vitamin D Status in Chronic Kidney Disease: A CRIC Study
Bonnie Ky, Justine Shults, Martin Kane, Myles S. Wolf, Harold L. Feldman, MD.1 Children's Hospital of Philadelphia; 1University of Pennsylvania; 1University of Miami.

Background: Animal studies link abnormal vitamin D metabolism and left ventricular (LV) hypertrophy. The objective was to determine associations between vitamin D levels and LV remodeling in CKD.

Methods: We evaluated 1283 CRIC study participants with vitamin D levels and echocardiography at Year 1 [52% male, 53% Caucasian, 40% African American; median age 62 (54-67) yrs, and eGFR 45 (35-55) ml/min/1.73m2]. Serum echocardiography at Year 1 [25(OH)D level <20 ng/mL, a level <20 ng/mL was associated with an odds ratio of LVMI of 1.36 (95% CI 1.01, 1.84, p<0.05) in adjusted models. Lower 1.25(OH)D levels were associated with greater LVMI in both unadjusted (p<0.001) and adjusted analyses (p<0.03). Vitamin D levels were not associated with volumes.

Conclusions: These data demonstrate a significant association between lower 25(OH)D and 1,25(OH)2D levels and higher LVMI in CKD.

Figure: LVMI According to 25(OH)D Levels

Funding: NIDDK Support
Methods: In a cohort of familial cases with IHH and suspected autosomal recessive inheritance, we performed an extended candidate gene approach to identify the causative genetic defect.

Results: We detected homozygous or compound-heterozygous mutations in CYP24A1 encoding Vitamin D-24-hydroxylase responsible for the inactivation of 1,25(OH)2-VitD3. CYP24A1 mutations were not only identified in IHH patients given regular 500 IU VitD daily, but also in a second cohort of patients from German Democratic Republic who presented during infancy with suspected VitD intoxication after receiving a bolus prophylaxis of 600,000 IU VitD2 in the late 1980s. The functional analysis of mutant CYP24A1 in a mammalian overexpression system revealed a lack of 24-hydroxylated VitD metabolites after incubation with radioactively labeled 1,25(OH)2-VitD3 indicating a complete loss-of-function.

Conclusions: In conclusion, we not only highlight the role of CYP24A1 mutations as causative for IHH but identify a genetic risk factor for the development of a serious adverse effect of generally advocated VitD prophylaxis.

FR-OR179
Risks of ESRD and Death Following Coronary Revascularization in Individuals with CKD

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Background: Surgical (CABG) and percutaneous (PCI) intervention revascularization are frequently deferred in individuals with CKD out of concern that they will precipitate ESRD, but reliable estimates of the absolute and relative risks of death and ESRD following PCI and CABG are unavailable.

Methods: Individuals with CKD undergoing PCI (8,620) or CABG (4,547) were identified using the 5% Medicare sample. Long-term outcomes were tracked, and ESRD was identified via linkage to the USRDS. Multivariable Cox regression was used to assess the relative hazards (HR) of death and ESRD.

Results: PCI patients were older, less likely to have heart failure, arrhythmias, anemia, COPD, diabetes, or cerebrovascular disease, and more likely to have a myocardial infarction (P<0.001 for all comparisons). The overall HR for death was lower with CABG than PCI (HR 0.89, P<0.001). Early mortality was higher after CABG, but the HR increasingly favored CABG over time (Figure 1). Results were similar when death was censored for ESRD. Conversely, the risk of ESRD was higher throughout follow-up following CABG (HR 1.18, P=0.04). The absolute incidence of ESRD at 3 years was markedly lower (PCI-ESRD, 2.7% vs. CABG-3.6%) than the incidence of death (PCI-20.1%, CABG-15.6%). As result, the adjusted HR of the combined outcome of ESRD or death was higher during the first 3 months (HR 1.30, P=0.01) and lower from 6-36 months (HR 0.66, P<0.001).

Conclusions: Among individuals with CKD undergoing coronary revascularization, the incidence of death is much higher than ESRD. The risk of ESRD is lower throughout follow-up with PCI, but the long-term risk of death or combined death and ESRD is lower with CABG. Our data suggest that overall clinical outcomes are better with CABG than PCI in patients with CKD.

FR-OR180
The Impact of Low Vitamin D Levels on Mortality Is Influenced by Urinary Albumin Excretion Rates in Chronic Kidney Disease

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Background: Reduced 25(OH)-Vitamin D (Vit D) levels and the presence of albuminuria are independent predictors of mortality in chronic kidney disease. The combined impact of these two potentially modifiable risk factors on mortality has to our knowledge not been explored in a population-based cohort.

Methods: We tested this hypothesis in cohort of 13,455 subjects age ≥20 without diabetes or pre-diabetes, and representative of the U.S. population from the Third National Health and Nutrition Examination Survey (1988-1994). Vital status through to 2006 was obtained from linkage with the National Death Index. The interaction of Vit D and albumin/creatinine ratio with mortality was tested. Vit D levels in quintiles [Q1=1–6.2, Q2=6.2–21.5, Q3=21.5–2.7, Q4=27.1–3.4, Q5=34 ng/ml] and urinary albumin/creatinine ratio categorized as ≥30, 30-300 and >300 mg/gm were modeled with all-cause and cardiovascular mortality using weighted Cox regression with Q5 Vit D and albumin <30 as a single referent.

Adjustment was made for demographic factors, cardiovascular conditions, hypertension, body mass index, physical inactivity, smoking, CRP, and eGFR.

Results: The p-value for interaction was 0.02. The relative mortality risks were significantly greater for subjects in the lowest quintile of Vit D, and for subjects with the highest albumin excretion rates. Subjects who fell into both categories experienced the greatest mortality risks. The patterns of association were similar for CV mortality (data not shown).

Conclusions: Vit D deficiency and albuminuria were synergistically associated with higher mortality risk. Combined strategies to correct these related abnormalities could therefore yield greater benefit than therapy focused on either alone.

FR-OR181
Mortality in Individuals with Acute Coronary Syndrome and CKD: An Analysis of the Myocardial Infarction National Audit Project (MINAP) Database

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Background: CKD is a recognised risk factor for cardiovascular mortality. Our aim was to assess the mortality risk following acute coronary syndrome (ACS) across all stages of CKD using the UK’s MINAP database.

Methods: Data from January 2007 to April 2011 on 94,165 individuals with NSTEMI and 54,824 individuals with STEMI were included in multivariate Cox regression models to investigate the association of eGFR (PCI/CABG) with mortality and in multivariate logistic regression models to study the association between intervention and eGFR.

Results: eGFR is strongly associated with mortality after adjustment for confounders following ACS (p<0.001). After NSTEMI, an increase in hazard ratio (HR) for mortality from 1.23 (1.11,1.36) to 3.38 (2.97,3.84) was demonstrated in those that did not receive an intervention as eGFR declined from CKD3a to CKD5 compared to those with CKD1. In those who received an intervention, an increase in HR from 1.68 (1.29, 2.18) to 7.38 (4.50,11.03) was seen across the same eGFR categories.

After STEMI, an increase in HR for mortality from 1.17 (0.99,1.38) to 4.01 (3.21,15.02) in the non-intervention group and an increase in HR from 1.22 (0.99,1.49) to 8.73 (3.24,7.30) in the intervention group was demonstrated as eGFR declined.

In intervention all stages of CKD demonstrated a survival benefit compared with no intervention (p<0.001). After an NSTEMI, the odds ratio (OR) of receiving an intervention decreased from 0.87 (0.82,0.94) to 0.34 (0.28,0.42) as eGFR declined from CKD3a to CKD5 and from 0.66 (0.61,0.72) to 0.26 (0.20,0.34) in the STEMI population.

Conclusions: eGFR is strongly associated with increased mortality after ACS. The greater increment in risk in individuals with NSTEMI who underwent intervention, likely reflects the increased severity of their cardiac disease. In all stages of CKD, intervention demonstrated a survival benefit. However individuals with increasingly severe renal dysfunction were less likely to receive an intervention compared to those with normal kidney function.

FR-OR182
Fracture Risk Is Not Increased in Patients with Chronic Kidney Disease

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Background: In patients with end-stage kidney disease, fractures occur four-times more frequently than in the general population. The incidence of fractures in those with chronic kidney disease (CKD) not receiving renal replacement treatment has not been well-studied.

Methods: We did a population-based longitudinal study with participants aged ≥18 years identified from a province-wide laboratory database from Alberta, Canada between 2002 and 2008. 1,815,957 patients had at least one outpatient serum creatinine measurement and did not require renal replacement therapy at baseline. The eGFR was estimated using the CKD-EPI equation, and categorized as ≥90, 60-89, 45-59, 30-44, 15-29 mL/min/1.73 m². The cohort was linked to administrative data to define demographics, comorbidities and outcomes. Outcomes of non-traumatic incident hip, wrist or clinical vertebral fractures were identified during the follow-up period to March 31, 2009. Poisson regression was used to determine unadjusted and adjusted rates of each fracture outcome by level of eGFR.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
FR-OR183
Effect of IL-1β Inhibition with Canakinumab Compared to Triamcinolone Acetonide on Pain Intensity and New Flares in Gouty Arthritis Patients with Chronic Kidney Disease Stage 2-5

Background: Many patients with gouty arthritis (GA) have pre-existing comorbidities. Chronic kidney disease (CKD) is one of the most common comorbidities and significantly limits treatment options with standard therapy. Canakinumab, a fully human monoclonal anti-IL-1β antibody, is a potential new therapy for treating GA pain and delaying new flares. We present 24-week data for a subgroup of patients with renal insufficiency.

Methods: In 2 multicenter, double-blind, active controlled studies (β-RELIEVED, N=226; β-RELIEVED-II, N=226), patients, ≥18 ≤65 years meeting ACR 1997 criteria for acute GA and contraindicated, intolerant or unresponsive to NSAIDS and/or colchicine, received canakinumab 150 mg sc or triamcinolone acetonide (TA) 40 mg im 3 times. The co-primary endpoints were pain intensity at 72h post dose on a 0-100 mm visual analog scale (VAS) in the most affected joint and time to first new GA flare.

Results: A total of 380 (83.7%) patients had renal impairment (baseline eGFR <90mL/min/1.73 m²); corresponding to CKD stages 2-5): 188 (83.6%) canakinumab group vs 52.6% with TA. Serious AEs (canakinumab: n=15, 8%; TA: n=23, 13%) were not considered to be related to treatment by the investigator. n=6, 3.1%) were not considered to be related to treatment by the investigator. vs 47.4%, OR 0.38, 95% CI 0.25-0.59, p<0.0001). 66.5% of patients had adverse events and 192 (83.8%) TA group. Mean VAS scores for canakinumab and TA were 73.9mm and 75.8mm respectively (p<0.001 versus placebo in all cases). Similarly, canakinumab 150 mg sc or triamcinolone acetonide (TA) 40 mg im 3 times. The co-primary endpoints were pain intensity at 72h post dose on a 0-100 mm visual analog scale (VAS) in the most affected joint and time to first new GA flare.

Conclusions: Lower levels of kidney function are associated with an increased risk of non-traumatic fractures, an association that is primarily related to confounding by age.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
FR-OR187

Proteinuria Predicts a Decline in Glomerular Filtration Rate Particularly in African-Americans and Amercianer Chronic Kidney Disease

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Background: Proteinuria is associated with progressive kidney disease. CKiD cohort cross-sectional data revealed a log-linear relationship between proteinuria and glomerular filtration rate (GFR). Here we evaluate a relationship between urine protein-to-creatinine ratio (Up/c) level and prospective changes in GFR.

Methods: GFR was measured in the first two visits and then annually by iohexol blood-disappearance or estimated using the CKiD equation; proteinuria was assayed by first morning baseline Up/c. We used a linear mixed model with random intercept and slope to account for individual variability in baseline GFR and % decline.

Results: 564 CKiD subjects with median age 11 yrs, GFR 44 ml/min/1.73 m², and baseline Up/c 0.47 were studied: 62% males, 72% African Americans (AA), 22% glomerular (G) cause CKD, 54% on ACE/ARB. For both non-G and G CKD, GFR % decline increased with rising baseline Up/c; linearly in non-G pts and biphasic in G CKD pts. Those with baseline Up/c ≥3.4 had GFR decline larger than expected under a linear relationship.

Conclusions: Up/c level predicts magnitude of future decline in GFR. In pts with G CKD, baseline Up/c is associated with a biphasic rate of decline in GFR, which is accelerated when Up/c ≥3.4. The effects of Up/c are more pronounced in AA.

Funding: NIDDK Support

FR-OR188

Determinants of Progression of Microalbuminuria in Non-Diabetic Persons from the General Population

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Background: Low-grade albuminuria is a risk factor for chronic kidney disease and cardiovascular disease. Knowledge about predictors and consequences of change in albuminuria over time in non-diabetic populations is incomplete. Our aim was to assess predictors for increase in urinary albumin-creatinine ratio (ACR) in a non-diabetic population.

Methods: From the population based Tromsø study, non-diabetic persons with morning ACR in the upper tertile at least two out of three consecutive days at baseline (1994/1995), and who were attending follow-up (2006/2007), were divided into two groups; upper tertile (“increasers”) vs. the two lower tertiles (“non-increasers”) of ACR change. Logistic regression was used to assess predictors of being an “increaser”.

Results: Among the 6528 non-diabetic baseline participants, 2001 had ACR in the upper tertile in at least two specimens. Between baseline and follow-up, 715 participants died. At follow-up 662 persons participated and were divided into 221 “increasers” (ACR change ≥0.45 mg/mmol) and 441 “non-increasers” (ACR change <0.45 mg/mmol). Baseline predictors of being an “increaser”, were age (OR for 5 years 1.26; 95% CI 1.10 – 1.46; P=0.001), gender (OR for men 1.92; 95% CI 1.23 – 2.99; P=0.004), waist circumference (OR for per cm 1.12; 95% CI 1.01 – 1.24; P=0.03) and cystatin C (OR per 0.1 mg/L 1.15; 95% CI 1.04 – 1.28; P=0.006). Increase in systolic blood pressure (OR per 10 mm Hg 1.17; 95% CI 1.08 – 1.27; P<0.001). Increase in HbA1C (OR per % 1.38; 95% CI 1.04 – 1.84), and increase in cystatin C (OR per 0.1 mg/L 1.16; 1.07 – 1.26; P<0.001) also were significant predictors. Smoking cessation, initiation of antihypertensive medication or physical activity were not significant predictors.

Conclusions: In non-diabetic persons with elevated ACR, gender, age, obesity, increasing glucose and blood pressure predicted further increase in ACR. However, most of the increase was not explained by cardiovascular risk factors. Increasing albuminuria seemed to parallel changes in renal function more closely than changes in cardiovascular risk factors.

Funding: Government Support - Non-U.S.

FR-OR198

Comparative Effectiveness of Modifiable Risk Factors To Predict Mortality in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD

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Background: The effect of change in kidney function, in particular the degree of change, and risk of death is not well understood. We aimed to investigate the association between change in eGFR over a one year period and risk of mortality in a community based population.

Methods: We included 843,417 adults who had at least 2 outpatient eGFR measurements in a one-year period, separated by at least two weeks, from a laboratory registry from Alberta, Canada. The percent change in eGFR was calculated using the first and last eGFR within the period. Change in eGFR was categorized as: ≤-50%, >-50% to ≤-25%, >-25% to ≤-10%, >-10% to 24%, >24% to 49%, >49% to 50%, and ≥50% to reflect both decline and increase in eGFR. Cox hazard models, adjusting for baseline covariates, kidney function and proteinuria, were used to estimate the HR of all-cause mortality (follow-up to March 31, 2009) associated with categories of percent change in eGFR. Stable eGFR (change ≤10%) was the reference.

Results: Among the participants (mean age 55.6 years, 42% male), 61.2% had stable, 19.9% had an increase and 18.9% had a decline in eGFR. Compared to participants with stable eGFR, those with the largest decline in eGFR (change ≤-50%) had a greater than threefold increased risk of death.

Participants with moderate declines also exhibited a higher mortality risk, as did participants with the greatest increase in eGFR.

Conclusions: Our results demonstrate an independent and graded association between change in kidney function and mortality, with both a decline and increase in kidney function associated with increased risk. These results suggest that overall variation in eGFR over time, rather than decline alone, may be an important prognostic marker.

Funding: NIDDK Support

FR-OR199

Effective Strategies to Reduce CKD Mortality in the United States

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Background: CKD results in the development of multiple metabolic disorders which individually are associated with increased mortality. The head-to-head relative importance of these abnormalities remains unclear.

Methods: We determined the hierarchy of multiple modifiable risk factors (blood pressure, urine albumin/creatinine ratio (UACR), serum albumin, bicarbonate, calcium, alkaline phosphatase, hemoglobin, white blood cell count (WBC) and cholesterol) in predicting all-cause mortality in a nationally representative cohort of 657,614 US veterans with non-dialysis dependent CKD stage 1-5 in 2005-06. The comparative effectiveness of the modifiable risk factors was examined by constructing classification and regression models.
trees (CART) from time-dependent Cox models adjusted for non-modifiable risk factors of mortality (age, gender, race, marital and insurance status, geographic location and comorbidities).

Results: Patients were 73.9±9.8 years old, 97% were males and 71% were white. 189,365 patients died over a median follow-up of 4.7 years. Of the modifiable risk factors included in the CART model low serum albumin, low hemoglobin, high alkaline phosphatase, high UACR, high bicarbonate and high WBC predicted mortality in decreasing order of significance (Figure).

Conclusions: In a comparative effectiveness model in CKD patients markers of protein-energy wasting, anemia and increased bone turnover appeared to be the most important predictors of mortality, followed by albuminuria. Since these risk factors are readily modifiable, therapeutic interventions targeting them may hold the largest promise of significant benefit towards lowering mortality in CKD.

Funding: NIDDK Support, Veterans Administration Support

FR-OR191

Comparative Effectiveness of Incident Oral Antidiabetic Drugs on Kidney Function: A National Veterans Cohort Study

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Background: Diabetes is a major cause of chronic kidney disease (CKD). Oral antidiabetic drugs (OADs) are the mainstay of therapy for most patients with type 2 diabetes, however, data comparing their effect on kidney function decline are sparse.

Methods: We used national Veterans Administration (VA) databases to assemble a retrospective cohort of 93577 diabetic veterans who filled an incident OAD prescription between 10/1/2001 and 9/30/2008, and had an estimated glomerular filtration rate (eGFR) ≥60 ml/min. Exposure groups were incident users of metformin (n=61104), sulfonylurea (n=30550), or rosiglitazone (n=1923). The primary composite outcome was persistent decline in eGFR from baseline of ≥25% (GFR event) or diagnosis of end-stage renal disease (ESRD). The secondary outcome was an eGFR event, ESRD or death. Sensitivity analyses included 1) using a more stringent definition of GFR event which required reaching an eGFR of 60 ml/min/1.73m², 2) controlling for baseline eGFR ≥60 ml/min/1.73m², 3) using a more stringent definition of eGFR event which required reaching an eGFR of 60 ml/min/1.73m², and 4) controlling for baseline eGFR ≥60 ml/min/1.73m² had nearly 3-fold odds of LVH (2.8; 1.9-4.1) relative to subjects with eGFR ≥60. This reduction in kidney function was also significantly associated with abnormal LV geometry (2.1; 1.6-2.9) and diastolic dysfunction (1.4; 1.1-1.9), but not systolic dysfunction (1.0; 0.6-1.7); an eGFR of 50-44 was also significantly associated with LVH and abnormal LV geometry compared with eGFR>60.

Conclusions: In this large CKD cohort, reduced kidney function appears to have substantial associations with abnormal cardiac structure, weaker associations with diastolic dysfunction, and no independent association with systolic dysfunction.

Funding: NIDDK Support

FR-OR192

Association of Kidney Function with Abnormal Cardiac Structure and Function by Echocardiography in the Chronic Renal Insufficiency Cohort (CRIC) Study

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Background: Heart failure (HF) is a common consequence of chronic kidney disease (CKD) and portends high risk for mortality. The prevalences of abnormal cardiac structure and function preceding clinical HF in a community-based population with CKD is not known.

Methods: We evaluated cross-sectionally the association of kidney function with echocardiographically determined stages of cardiac structural and functional abnormalities among 3496 participants in CRIC without a diagnosis of HF. Kidney function was defined by glomerular filtration rate estimated from cystatin C (eGFRcys) (>60 ml/min/1.73m², 45-59, 30-44, or <30).

Results: The prevalence of left ventricular hypertrophy (LVH) was 27%, 39%, 44%, and 54% for eGFRcys categories ≥60, 45-59, 30-44, and <30 respectively. In fully adjusted multivariate analyses, subjects with eGFRcys levels of <30 had increased risk of 1.21 (95% CI 1.13, 1.28). This association was still significant after additional adjustment for age, sex, race, smoking status, body mass index, systolic blood pressure, estimated glomerular filtration rate, high UACR, high bicarbonate and high WBC predicted mortality in decreasing order of significance (Figure).

Conclusions: In a comparative effectiveness model in CKD patients markers of protein-energy wasting, anemia and increased bone turnover appeared to be the most important predictors of mortality, followed by albuminuria. Since these risk factors are readily modifiable, therapeutic interventions targeting them may hold the largest promise of significant benefit towards lowering mortality in CKD.

Funding: NIDDK Support, Veterans Administration Support

FR-OR193

Chronic Kidney Disease (CKD) Progression and Death among Hispanics and Non-Hispanics in the Chronic Renal Insufficiency Cohort (CRIC) Study

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Background: Although Hispanics have higher crude rates of CKD progression, little is known about the earlier stages of chronic kidney disease (CKD) in Hispanics.

Methods: We conducted a prospective longitudinal analysis of CRIC participants. Race/ethnicity was self-reported and classified in three exclusive categories: Hispanic, non-Hispanic White (NHW), and non-Hispanic Black (NHB). Cox proportional hazards models were used to determine the association between race/ethnicity, 50% eGFR loss or incident dialysis/transplantation (renal outcome), and death.

Results: Among 3785 participants, 13% were Hispanics, 43% NHW, and 44% NHB. Over 3 years of follow up, Hispanics had significantly higher rates of the renal outcome compared with both NHW and NHB (p<0.05) but similar death rates.

<table>
<thead>
<tr>
<th>Hispanic</th>
<th>NHW</th>
<th>NHB</th>
<th>HR (Hispanic v. NHW)</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incident dialysis/transplantation</td>
<td>0.7</td>
<td>2.7</td>
<td>6.2</td>
<td>2.49**</td>
<td>0.94</td>
</tr>
<tr>
<td>Death</td>
<td>2.3</td>
<td>1.8</td>
<td>2.3</td>
<td>0.84</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*p<0.05

Conclusions: Although Hispanics have higher crude rates of CKD progression, Hispanic ethnicity is independently associated with similar or lower rates of this outcome relative to non-Hispanic race/ethnicity in CRIC. Disparities in renal outcomes for Hispanics appear to be explained by factors other than race/ethnicity.

Funding: NIDDK Support

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Underline represents presenting author.

47A
FR-OR194

Chronic Kidney Disease and End Stage Renal Disease Predict Higher Mortality in Primary Upper Gastrointestinal Bleed Patients

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Background: The outcome from upper GI bleeding (UGIB) in CKD and ESRD patients is difficult to discern from the older literature of small patient numbers prior to advanced endoscopy, proton pump inhibitors or H. pylori treatment. We sought a large national database to quantify the role of CKD and ESRD as independent predictors of mortality, for patients hospitalized with a principal diagnosis of primary UGIB.

Methods: We used the Healthcare Cost and Utilization Project’s Nationwide Inpatient Sample (NIS) 2007, the largest US all-payer database publicly available: an administrative database of 8 million admissions in 1,000 hospitals approximating a 20% stratified sample of all US hospitals. Patients over 18 years, discharged with primary diagnoses of UGIB and CKD or ESRD were identified by ICD-9-CM codes. The outcome variables included frequency and in-hospital mortality in CKD and ESRD patients as compared to non-CKD patients. We also analyzed the effect of other risk factors and co-morbid conditions on outcomes of primary UGIB and all-cause mortality using multiple regression modeling.

Results: In the NIS dataset, out of a total of 398,213 admissions with a diagnosis of primary UGIB, 35,985 were for CKD patients, 14,983 for ESRD patients and the remaining 347,245 for patients without renal problems. Primary UGIB hospitalizations in CKD and ESRD patients were 30% and 84% higher respectively, compared to no-renal disease group, after controlling for potentially confounding covariates and adjusting for interaction between the independent variables (OR 1.30, 95% CI 1.17-1.46, P< 0.001 and OR 1.84, 95% CI 1.61-2.09, P<0.001). The CKD patients had 47% higher adjusted all-cause in-hospital mortality when compared to the no-renal disease group (OR 1.47, 95% CI 1.21-1.78, P< 0.001). The ESRD patients with UGIB three times higher adjusted all cause mortality as compared to the no-renal disease group (OR 3.02, 95% CI 2.23-4.1, P<0.001).

Conclusions: Patients with CKD or ESRD admitted with primary UGIB have up to three times higher all-cause in-hospital mortality. The clinicians need to be exceptionally vigilant in the care of these renal patients.

FR-OR195

A Varying Patient Safety Profile across Racial Groups with Chronic Kidney Disease

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Background: Chronic kidney disease (CKD) is a high risk condition for a variety of adverse safety events yet little is known about differential rates of safety events among racial groups with CKD. We sought to examine the incidence of an array of disease-specific adverse safety events in black versus non-black patients with CKD.

Methods: A retrospective observational study of a national VA cohort of veterans with CKD and race/ethnic designation, and at least 1 hospitalization during federal fiscal year 2005 (n= 70,154). Primary measures included hospital discharge coding for Agency for Healthcare Research and Quality Patient Safety Indicators (AHRQ PSI), laboratory records for detection of hyperkalemia and hypoglycemia, and pharmacy records to determine dosing of over prescribed medications with potential safety hazards in CKD (atenolol, glubuline, allopurinol, dipiridamol).

Results: The majority of participants had at least one adverse safety event during the study period (57%, n=40,003). Black veterans (16% of study cohort) were more likely than non-black veterans to experience 1 safety event (33% versus 32%, respectively), and multiple safety events (32% versus 23%, respectively, both <0.001). After adjustment, black veterans were 21% and 50% more likely to have at least one episode of hyperkalemia or hypoglycemia respectively, than non-black veterans, but were 22% less likely to experience a medication error (P<0.001). There was no association between the occurrence of AHRQ PSI and race after adjustment.

Conclusions: Black veterans with CKD are more likely to experience a broad array of safety events than non-blacks with CKD with a preponderance of electrolyte disturbances rather than medication errors or AHRQ PSI. The differential safety phenotype in blacks versus non-blacks may have implications for preventive strategies to improve patient safety in an integrated health system.

FR-OR196

Antibiotics in Patients with Severe Chronic Kidney Disease: An Epidemic of Dosing Errors

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Background: Antibiotics are frequently misprescribed in patients with chronic kidney disease (CKD) and are implicated in over one third of preventable adverse drug events in these patients. To improve the safety of CKD patients, in January 2006, outpatient laboratories in Ontario, Canada began reporting estimated glomerular filtration rate (eGFR).

We sought to describe the rate of ambulatory antibiotic dosing errors in Southwestern Ontario and examine the impact of eGFR reporting on these errors.

Methods: We linked health administrative data for ambulatory residents in Southwestern Ontario from January 2003 to April 2010. Patients had severe CKD (eGFR < 30 ml/min/1.73m2) and were 66 years of age or older. We conducted a population-based intervention analysis with time-series modeling on the monthly rate of dosing errors.

Results: Of the total 1464 prescriptions filled for study antibiotics throughout the study period, 970 (66%) were dosed in excess of guidelines. Nitrofurantoin, which is contraindicated in patients with creatinine clearance < 60 ml/min, was prescribed 169 times. The initiation of eGFR reporting was not associated with a decline in the rate of antibiotic dosing errors (P<0.85, Figure 1). Prior to eGFR reporting the average rate was 636 per 1000 antibiotic prescriptions; after eGFR reporting, the rate was 680 per 1000 antibiotic prescriptions.

Conclusions: Our findings demonstrate that ambulatory antibiotic dosing errors are exceedingly common among severe CKD patients. Moreover, eGFR reporting has not impacted the rate of these errors at the population level. Further interventions to reduce medication errors in CKD patients may therefore be warranted.
Biochemical Data and Growth in Children with Chronic Kidney Disease: A Report from the Chronic Kidney Disease in Children (CKID) Cohort Study

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Background: Growth failure is common among children with chronic kidney disease (CKD) and is associated with declining glomerular filtration rate (GFR). However, its relationship to a variety of biochemical parameters is poorly characterized.

Methods: We examined the cross-sectional association of baseline growth parameters (height [HT] and weight [Wt]) age-specific standard deviation scores (SDS) with hemoglobin [Hb] and serum protein (CRP), and beta arrestin-GFP and then chlorin-GFP, conferring the role of clathrin-coated pits in V2R internalization. Cell fractionation and western blot analysis showed that internalized VP-V2R moves from light vesicles to heavier vesicles along with beta-arrrestin. We also observed a similar pattern of distribution of the Gs alpha subunit (Gs) protein, despite being a Gs protein, and membrane dye, which did not seem to interact directly with the receptor by immunoprecipitation. In contrast, immunoprecipitated c-myc tagged V2R expressed in HEK cells co-IP’d with beta-arrrestin.

Conclusions: This result suggests that cell surface mobility of the V2R is reduced by internalization, and that V2R is then immobilized in specific membrane domains along with proteins that may or may not interact directly with the receptor, but that are involved in its trafficking and/or signaling after VP binding and subsequent internalization.

Funding: NIDDK Support

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FR-OR199

High NaCl-Induced Nuclear Accumulation of the Osmoprotective Transcription Factor NFAT5 Depends on Binding to Osmotic Response Elements (OREs) Yuiichiro Izumi,1 Morgan Gallazzini,2 Maurice B. Burg,3 Joan D. Ferraris. 1NLH/B/SC, National Institutes of Health, Bethesda, MD; 2Centre de Recherche Croissance et Signalisation, Institut National de la Sante et de la Recherche Medicale, Paris, France.

Background: Nuclear Factor of Activated T-Cells 5 (NFAT5), also called TonEBP/OREBP, induces transcription of osmoprotective genes in response to hyper tonicity. High NaCl causes translocation of NFAT5 from cytoplasm to nucleus. Threonine 298 is a known DNA contact site in NFAT5.

Methods: The effects of mutation of threonine 298 on binding of NFAT5 to its cognate DNA element and its nuclear localization were examined. We measured binding of NFAT5-V5 to OREs by Electrophoretic Mobility Shift Assay (EMSA), using an IR- cognate DNA element and its nuclear localization were examined. We measured binding of wild type NFAT5-V5, but not of NFAT5-T298D-V5. That result was confirmed by confocal microscopy of HeLa cells in which high NaCl showed that GSK3α showed normal development. Baseline urine output in 3αCDnull mice showed normal development. The 3αCDnull mice showed normal development. Baseline urine output in 3αCDnull mice showed normal development. The 3αCDnull mice showed normal development. Baseline urine output in 3αCDnull mice showed normal development. The 3αCDnull mice showed normal development. Baseline urine output in 3αCDnull mice showed normal development. The 3αCDnull mice showed normal development. 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Conclusions: These studies show for the first time that GSK3 is critical for renal development and that 3α and 3β isoforms compensate for each other. Also, GSK3α may play a more important role in renal urine concentration than GSK3β because: 1) at basal levels GSK3α deletion caused higher urinary concentrating defect and polyuria compared to GSK3β; and 2) GSK3α, rather than GSK3β, is a critical target for Li.

Funding: NIDDK Support

FR-OR203
Pharmacological Blockade of P2Y1 Receptor Ameliorates Lithium-Induced Polyuria in Rats Bellumlakonda K. Kishore,1 Ioana L. Pop,1 Noel G. Carlson,1 Yue Zhang,1 1Medicine, Nephrology, VMMC & Univ of Utah; 2Neurovirology & GRECC, VMMC & Univ of Utah, Salt Lake City, UT.

Background: Previously, we showed the potential involvement of ATP/UTP/LDP-activated P2Y, or P2Y2, receptors in a rat model of lithium (Li)-induced polyuria, and observed that genetic deletion of P2Y2 receptor results in significant resistance in Li-induced polyuria. In contrast, P2Y1 receptor (P2Y1R) is an ADP receptor expressed predominantly in blood platelets, and in the brain (microglia and astrocytes). Stimulation of P2Y1-R; R also inhibits adenylyl cyclase. We discovered that P2Y1-R mRNA and protein are expressed in rat kidney, and pharmacological blockade of P2Y1-R with clopidogrel bisulfate (Plavix®) results in increased urinary concentration in rats, independent of P2Y2 receptor (companion abstract). Here we document that pharmacological blockade of P2Y1-R with Plavix® significantly ameliorates Li-induced polyuria in rats.

Methods: Groups of rats were fed normal (ND) or Li-added (LD; 40 mmol/kg food) diets with free access to drinking water and with/without added Plavix® (20 mg/kg/day in water) for 14 days, and then euthanized. Water intake and urine output were monitored and kidney tissue was analyzed.

Results: Co-administration of Plavix® resulted in significant improvement in: 1) Li-induced polyuria (P < 0.01) and polydipsia (P < 0.001), and decreased urinary concentration (P < 0.05); and 2) effectively reversed Li-induced increased solute-free water excretion (P < 0.01). These changes in whole body water metabolism were matched with a significant improvement in Li-induced stimulated PFK/PK in APQ2 water channel in the inner medulla (2-fold difference; P < 0.04). Co-administration of Plavix® also significantly augmented Li-induced urinary AVP levels (1.7-fold vs. Li alone group; P < 0.05). The observed effects of Plavix® were not due to a reduction in blood Li levels or kidney medullary accumulation of Li. Finally, co-administration of Plavix® significantly improved Li-induced polyuria in mice as well.

Conclusions: Taken together, these data unravel the potential therapeutic utility of drugs that target P2Y1-R to ameliorate Li-induced polyuria and possibly, other forms of acquired nephrogenic diabetes insipidus.

Funding: Veterans Administration Support, Private Foundation Support

FR-OR204
Vasopressin V1a Receptor Is Required for Nucleocytoplasmic Transport of Mineralocorticoid Receptor Takano Naga1, Kahori Hor1, Yuichiro Izumi,1 Yukiko Haseki1, Yushi Nakayama,1 Yoshinaga Otaki,1 Masayoshi Nanami,1 Takahiro Kuragano,1 Katsumasa Kawahara1, Alan Tanoue,1 Takeshi Nakashima,1 Hiroshi Nomaguchi,1 1Division of Kidney and Dialysis, Int Med, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan; 2Epithelial Systems Biology, IONS, National Institutes of Health, Bethesda, MD; 3Nephrology, Kumamoto University, Kumamoto, Kumamoto, Japan; 4Physiology, Kitasato University Sch Med, Sagamihara, Kanagawa, Japan.

Background: Type 4 renal tubular acidosis (RTA) is caused by insufficient aldosterone action. We reported that the deficiency of vasopressin V1a receptor resulted in type 4 RTA by decreasing the expressions of HKT-ATPase and RhoG and by increasing the expression of H-ATPase in the intercalated cells of the collecting ducts. We investigated the mechanisms of regulation of aldosterone action by vasopressin in the intercalated cells.

Methods: Mice lacking V1a receptor and the cell line of intercalated cells (IN-IC cells) from SV40 Tg rat were used in this study. V1a receptors in the IN-IC cells were knocked down by small interfering RNA (siRNA) and the effects of aldosterone and vasopressin on the nuclear translocation of mineralocorticoid receptor (MR) was examined by Western blot. Immunohistochemistry of MR expression in IN-IC cells was also examined.

Results: Fludrocoristone stimulated MR and 11βHS2 expression in wild-type mice and these effects were largely depressed in the V1aR deficient mice. Aldosterone dose-dependently increased MR expression in whole IN-IC cells. Aldosterone decreased cytoplasmic transport and increased nuclear expression of MR after 30 min and 24 hr. Immunohistochemistry revealed nuclear expression of MR after 30 min and 24 hr stimulation by aldosterone. Vasopressin mimicked the effects of aldosterone on the nuclear expression of MR, and knockdown of V1a receptor abolished the effects of aldosterone. Although vasopressin increased PKC α and β1 expressions, aldosterone stimulated not PKC α and β1 but PKC ε expressions.

Conclusions: Our data show that vasopressin regulates nucleocytoplasmic transport of MR and aldosterone requires vasopressin V1a receptor for nucleocytoplasmic transport of MR.

Funding: Government Support - Non-U.S.

FR-OR205
Microtubule Remodeling by Hyperosmotic Stress Udo Hasler,1 Paula Nunes,1 Isabelle Roth,1 Thomas Ermann,2 Richard Bouley,2 Dennis Brown,2 Eric Feraillé,1 1Department of Cellular Physiology and Metabolism, University of Geneva, Switzerland; 2Program in Membrane Biology/Nephrology Division, Harvard Medical School, Boston, MA.

Background: Acute increase of extracellular osmolyte concentration is a common challenge that induces cell shrinkage and increases intracellular ionic strength. Cell survival depends on intracellular trafficking events that help restore cell volume and eliminate protein damage. Microtubules (MT), together with actin filaments, play an outstanding role in membrane trafficking. While effects of hyperosmotic stress on actin structure is well described its effect on MTs is unknown. The aim of this study was to examine whether hyperosmotic stress affects MT structure and behavior and the consequences that such changes may impart on actin structure and vesicle motility.

Methods: We examined MT and actin remodeling in porcine proximal LLC-PK1 cells before and after hyperosmotic challenge (NaCl or urea 500 mOsmol/kg) by tubulin immunostaining, phalloidin microscopy, Western blot analysis of free and polymerized tubulin and actin and live cell imaging of actin, tubulin and end-binding protein 1, a MT plus-end binding factor. Motility of endosomes loaded with FITC-dextran was examined by live cell imaging and analyzed using ImageJ software.

Results: Data shows that MTs are immediately immobilized by hyperosmotic stress and that this is accompanied by their dramatic depolymerization and subsequent repolymerization. A comparison between events induced by hyperosmotic stress and chemicals that interfere with MT and actin polymerization revealed that while MT remodeling participates in triggering actin polymerization MT depolymerization, more specifically, reduces spatial movement patterns of F-actin networks. MT remodeling and altered actin behavior both contribute to endosome paralysis during early phases of challenge. Endosome motility recovers as cytoskeletal remodeling matures, during and after cell volume is restored.

Conclusions: These data link changes of cell volume by hyperosmotic stress to cytoskeletal remodeling and demonstrate ensuing consequences on endosome motility.

Funding: Government Support - Non-U.S.

FR-OR206
miR-148b Upregulation Modulating Core 1, β1,3-Galactosyltransferase 1 Expression Explains the Abnormal Glycosylation Process of IgA1 in IgA Nephropathy Grazia Serino,1 Fabio Sallustio,2 Sharon N. Cox,1 Francesco Pesce,1 Francesco Paolo Schena.1, 2Nephrology, Dialysis and Transplantation Unit, Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; 2C.A.R.S.O. Consortium, Valenzano (Bz), Italy.

Background: IgA nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide characterized by aberrant O-glycosylation in the hinge region of IgA1 involving the enzyme core 1, β1,3-galactosyltransferase 1 (C1GALT1). To date, the role of microRNAs (miRNAs) in the IgAN pathogenesis has not yet been reported.

Methods: Global miRNA profile of peripheral blood mononuclear cells (PBMCs) from IgAN patients and healthy subjects (HS) were identified using miRNA microarrays V2 Agilent. To study the molecular mechanisms in which the miRNAs were involved, we performed a bioinformatic analysis to predict target genes of the modulated miRNAs. To validate biologically miRNA targets, we performed transient transfection experiments ex vivo using PBMCs from an IgAN patient and a HS.

Results: We identified 37 miRNAs differentially expressed in IgAN patients. Among them, upregulated miR-148b targeted C1GALT1, involved in abnormal glycosylation processes. C1GALT1 expression levels in IgAN patients were reduced and negatively correlated with the miR-148b expression. We demonstrated that miR-148b decreased the C1GALT1 levels in IgAN patients and that the loss of function of miR-148b in PBMCs led to an increase of C1GALT1 mRNA and protein levels similar to that observed in HS. Moreover, we showed that the upregulation of miR-148b directly correlated with the galactose-deficient (Gal-deficient) IgA1 levels supporting our data on C1GALT1 regulation by miR-148B and explaining the abnormal increase of Gal-deficient IgA1 consequent to C1GALT1 regulation by miR-148B and explaining the abnormal increase of Gal-deficient IgA1 consequent to C1GALT1 regulation by miR-148B, which led to an increase of Gal-deficient IgA1 levels in IgAN patients. Finally, we validated the upregulated miR-148b in a population of 50 IgAN patients and 50 HS.

Conclusions: All together our data support the unreported role of miRNAs in the pathogenesis of IgAN by discovering the deregulation of miR-148B, which could explain the aberrant glycosylation of IgA1 and provide a potential pharmacological target for new therapeutic approaches in IgAN.

FR-OR207
Genetic Basis for Disease Severity in a Mouse Model of MPO ANCA Glomerulonephritis Dominic J. Ciavatta,1, 2Hong Xiao, David L. Aylor,2 Peigi Hu, Fernando Pardo-Manuel de Villena,2 Ronald J. Falk,3, 2J. Charles Tw2,3, 2Ronald J. Falk, 1,3 J. Charles J. Ciavatta,1, 2Ronald J. Falk, 1,3 J. Charles J. 1UNC Kidney Center, University of North Carolina at Chapel Hill, NC; 2Genetics, University of North Carolina at Chapel Hill, NC; 3Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, NC.

Background: Differences in disease severity among ANCA vasculitis patients may arise from genetic variability. Using a mouse model of MPO ANCA disease we tested the hypothesis that ANCA disease severity is genetically determined.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

50A
Methods: After i.v. injection of mouse anti-MPO IgG, glomerular crescents were measured in the following mouse strains: C57BL/6J, 129Sv/EvJ, C57L/J, and LP/J. Genetic variation among the inbred strains was determined with the Mouse Diversity Array high-density genotypes. Genomic clustering of identical genotypes was performed to identify regions that are identical by descent (IBD). Glomerular crescents were also measured in a cohort of 100 mice (C57BL/6J, 129Sv/EvJ, and LP/J). The number of IBD regions varied from 9% in C57BL/6J to 64% in 129Sv, with 129Sv IBDm and LP/J having 21% and 20% glomerular crescents, respectively. In the F2 mice the % glomerular crescents spanned the range observed in C57BL/6J and 129Sv mice. The C57BL/6J strain is IBD with each of the other three strains over a modest 28% of the genome and the LP/J strain is 72-74% IBD with the 129 strains. Surprisingly, the 129 strains, which have striking phenotypic differences, are IBD over 96.7% of their genome.

Results: The average percent glomerular crescents in each of the inbred strains ranged from 3% in C57BL/6J to 64% in 129Sv, with 129Sv IBDm and LP/J having 21% and 20% glomerular crescents, respectively. In the F2 mice the % glomerular crescents spanned the range observed in C57BL/6J and 129Sv mice. The C57BL/6J strain is IBD with each of the other three strains over a modest 28% of the genome and the LP/J strain is 72-74% IBD with the 129 strains. Surprisingly, the 129 strains, which have striking phenotypic differences, are IBD over 96.7% of their genome.

Conclusions: The effect of genetic variation on percent glomerular crescents among the 4 inbred strains of mice indicates that ANCA disease severity is genetically determined, and the distribution among F2 mice indicates the trait is polygenic. The IBD analysis between the 129 strains reduced the fraction of the genome that contains candidate loci for ANCA disease severity to 90.8 Mb or 3.3% of the genome distributed among 15 regions. Mapping quantitative trait loci in the F2 population may corroborate one of the 15 candidate regions or identify additional modifier loci.

Funding: NIDDK Support

FR-OR210

Etanercept Delays the Progression of Alport Glomerulosclerosis by Preventing Tumor Necrosis Factor-Driven Loss of Podocytes

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Background: Chronic renal failure involves the progressive loss of renal parenchymal cells. For example, Alport nephropathy develops from mutated type IV collagen which fosters the destruction of the GBM and podocyte loss, followed by progressive glomerulosclerosis. We found that Alport nephropathy in Col4a3-deficient mice is associated with an increased intrarenal expression of the proapoptotic cytokine tumor necrosis factor-alpha (TNF-α) in glomeruli, particularly in podocytes as well as in infiltrating leukocytes. We therefore hypothesized that TNF-α contributes to Alport glomerulosclerosis by inducing podocyte apoptosis.

Methods: We treated 4 week old Col4a3-deficient mice on a 129/SvJ genetic background with vehicle or with the TNF-α antagonist etanercept, or an equivalent dose of human IgG as a control intraperitoneally for a period of 5 weeks. Lifespan was monitored and renal pathologic evaluations were performed (e.g. immunohistochemistry, TUNEL, FACS, electron microscopy, real time-RT-PCR, western blot, and functional assessment).

Results: Etanercept treatment prolonged mean survival compared to vehicle-treated Col4a3-deficient mice (p=0.0016). The beneficial effect of etanercept on survival was associated with a significant improvement of the glomerulosclerosis score, renal function (proteinuria, plasma creatinine, and BUN) and the glomerular filtration rate (GFR) at 9 weeks of age. Etanercept treatment significantly improved the numbers of glomerular podocytes (WT-1 and nephrin co-staining) and significantly increased the renal mRNA expression of nephrin and podocin without affecting markers of renal inflammation. The increased number of podocytes was consistent with less TUNEL-positive podocytes that underwent apoptosis.

Conclusions: Together, we concluded TNF-α-induced podocyte loss via apoptosis is a previously unrecognized pathomechanism of Alport nephropathy; hence, TNF-α blockade might be a therapeutic option to delay the progression of Alport nephropathy and potentially of other forms of glomerulosclerosis.

FR-OR211

Podocyte-Specific Inhibition of Signal Transducer and Activator of Transcription (STAT) 3 Attenuates Nephrotic Serum-Induced Glomerulonephritis

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Background: STAT3 activation correlates with cell proliferation in Bowman’s space and renal injury in glomerulonephritis (GN). Podocytes are involved in the formation of cellular crescents in GN. We hypothesize that STAT3 activation in podocytes causes the development of GN.

Methods: To test our hypothesis mice with podocyte-specific Cre recombinase-mediated excision of exons encoding for the SH2 domain of the STAT3 allele (Cre/Ff), which is required for STAT3 activation, were generated. Cre Ff/F and control littermates (Cre/+ ) were generated by crossing Cre Ff/+ mice. GN was induced by tail vein injection of a sheep anti-mouse glomerular basement membrane antibody (NTS). Mortality was monitored. Urine, serum, total kidney, and isolated glomeruli were collected.

Results: Cre Ff/F lack renal phenotype and are born with the expected Mendelian frequency. Podocyte-specific deletion of the SH2 domain of STAT3 was confirmed by PCR using primers flanking the excised exons and DNA extracted from isolated glomeruli. Suppression of STAT3 signaling in Cre Ff/F mice was confirmed by a reduction of STAT3 phosphorylation at Y705. NTS induced a significant increase in albuminuria in Cre/+ , but not in Cre Ff/F (1.07±0.2 vs 0.20±0.02 g album/m creatinine, 8d post injection). Proteinuria correlated with glomerular deposition of mouse and sheep IgG. BUN of NTS-induced Cre/+ mice was higher than NTS-injected Cre Ff/F mice (68.9±5.0 vs 21.9±4.3 mg/dl, 14d post injection). NTS failed to activate STAT3 in Cre Ff/F mice as demonstrated by reduced phosphorylation of STAT3 (Y705) immunostaining. NTS-injected Cre Ff/F mice developed more severe glomerulosclerosis than vehicle-treated Col4a3-deficient mice (p<0.0016).

Conclusions: STAT3 activation in podocytes is required for the development of renal injury in NTS-injected GN. Additional studies to examine the underlying mechanism of STAT3 are needed. Inhibition of STAT3 activation is a potential therapeutic option for GN.

Funding: NIDDK Support
FR-OR212

Podocyte Injury Specific Microangiopathy in Collapsing FSGS; the Role of PAI-1 and the Beneficial Effect of Its Inhibitor TM5484

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Background: We previously established transgenic model of podocyte-specific injury resulting collapsing FSGS (NEP mice with LMB2).This model showed distinct microangiopathy provokes site specific membranoproliferative glomerulonephritis. This microangiopathy is associated with podocyte reduction, thrombosis and glomerulosclerosis. In addition, reduction of VEGF and eNOS, and increase of PA-1 was observed. This suggests that podocyte injury causes dysregulation in podocyte-endothelial cells(EHCs) homeostasis resulting site specific microangiopathy. To test whether microangiopathy promotes collapsing FSGS, present study administered novel PAI-1 inhibitor (TM5484) in this model. Two weeks after inoculation with TM54, a group of n=9 and without (controls, n=5) PAI-1 inhibitor (TM5484) were induced collapsing FSGS by LMB2. Immediately after injection of LMB2, TM5484 was administered orally for 12 days. Results: Proteinuria was significantly reduced in TM5484 group compared to controls on day12 (p<0.05). Of note, WT1 positive cells were not reduced in TM5484 group, significantly podocyte reduction was noted in controls on day8 (20.2 ± 2.39 vs. 9.23 ± 1.98 vs.0.001) and day12 (15.1 ± 2.42 vs. 4.44 ± 0.95 vs.0.001). Ti is significant lower in TM5484 on day8 (0.03 ± 0.05 vs. 0.12 ± 0.10, p<0.05) and day 12 (0.05 ± 0.24 ± 0.10 ± 0.10 ps) Ti was lower in TM5484 (0.05 ± 0.04 vs. 0.24 ± 0.1, p<0.05). Compared to controls, TM5484 group showed reduction in mRNA levels of PAI-1 on day12 conversely VEGF on day12 and eNOS on day8 and 12, u-PA on day8, and c-ment on day12 was significantly increased in TM5484 group.

Conclusions: Podocyte-specific injury mice model of collapsing FSGS showed corresponding microangiopathy due to increase of PAI-1. TM5484 significantly ameliorated progression of collapsing FSGS via protection of podocyte injury and endothelial hyperplasia.

FR-OR213

Intrauterine Growth Restriction Leads to Dysregulation of WT 1 and to Early Podocyte Alterations

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Background: Intrauterine growth restriction (IUGR) leads to decreased nephron number and a higher incidence of renal disease. We further hypothesized that early dysregulation of WT 1 results in later podocyte damage.

Methods: IUGR was induced in rats by maternal protein restriction (8.4% vs 17.2% protein intake). Two weeks after inoculation with TM548, a group of n=9 and without (controls, n=5) PAI-1 inhibitor (TM5484) were induced collapsing FSGS by LMB2. Immediately after injection of LMB2, TM5484 was administered orally for 12 days.

Results: In neonatal kidneys of IUGR rats a significant reduction of the nephrogenic zone was observed. Further IUGR led to an increased expression (1.78-fold, p<0.05) of desmin, a marker of podocyte injury. EM revealed overt alterations of podocyte in podocyte-endothelial cells(EHCs) homeostasis resulting site specific microangiopathy. To test whether microangiopathy promotes collapsing FSGS, present study administered novel PAI-1 inhibitor (TM5484) in this model. Two weeks after inoculation with TM54, a group of n=9 and without (controls, n=5) PAI-1 inhibitor (TM5484) were induced collapsing FSGS by LMB2. Immediately after injection of LMB2, TM5484 was administered orally for 12 days.

Conclusions: The vacuolated PTE cells of PEXTKO tubules are dysfunctional, incapable of resorbing protein. The development of microalbuminuria in the PEXTKO mice may be due to PTE autophagic change as shown by the TEM and validated by LC3 staining.

FR-OR215

Light Chain-Associated Renal Fancioni Syndrome Triggers Oxidative Stress, Dedifferentiation and Defective Receptor-Mediated Endocytosis in Proximal Tubule Cells

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Background: Renal involvement often complicates multiple myeloma, and storage of monoclonal immunoglobulin light chain (LC) within the lysosomes of proximal tubule cells (PTC) may lead to LC-associated Fanconi syndrome (RFS). The pathogenic mechanisms linking accumulation of LC in lysosomes and dysfunction of PTC remain unknown.

Methods: To investigate the cellular mechanisms involved in PTC dysfunction after LC accumulation, we used a mouse model overexpressing a pathogenic human monoclonal light chain (CHEB mouse) and primary cultures of PTC.

Results: The CHEB mice showed low-molecular weight (LMW) proteinuria, glucosuria and phosphaturia, indicating PTC dysfunction and RFS. Immunostaining in CHEB kidneys revealed a decreased expression of the endocytic receptors megalin/cubilin and cubilin in segments accumulating the LC, associated with oxidative stress and proliferation markers. Decreased expression of megalin/cubilin, with defective endocytosis of LMW ligands and oxidative stress was also observed in primary cultures of PTC from CHEB kidneys. The changes were specifically related to the LC purified from patients with LC-RFS, as they were not observed with LC from patients with cast nephropathy (CN). The link between lysosomal accumulation of LC and defective endocytosis was substantiated by exposing PTC to RFS-associated CN- and LC-associated LC-RFS. Exposure to LC-RFS, but not to CN- or LC, triggered expression of ZO-1, a transcription factor involved in cellular dedifferentiation and repression of megalin/cubilin. The increased expression of ZONAB in the PTC resulted in loss of polarization as evidenced by the loss of apical megalin staining.

Conclusions: These results suggest that the lysosomal accumulation of specific LC within PTC triggers a transcriptional mechanism of cellular dedifferentiation which leads to defective endocytosis and RFS.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR216

Nicotinic and Chronic Kidney Disease: Role of the Alpha 7 Nicotinic ACh Receptor

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Background: Cigarette smoking is an important risk factor in the progression of Chronic Kidney Disease (CKD). We have previously demonstrated that mesangial cells are exposed to nicotine receptors (nAChR) and that nicotine increases mesangial cell fibronectin via the α7-nAChR (AJP 07). Herein, we tested the hypothesis that nicotine worsens the severity of renal injury in 5/6 nephrectomized rats (5/6Nx) and that these effects are mediated via the α7-nAChR.

Methods: Rats were divided into the following groups: Sham, 5/6Nx, 5/6Nx + Nicotine (NIC, 0.1 gm/L, DW), 5/6Nx + NIC + α7-nAChR blocker methyllycaconitine (MLA, 3 mg/Kg/day), Sham + NIC. Urine was collected for proteinuria and blood pressure (SBP) was measured by tail cuff. Rats were euthanized after 12 weeks, kidneys were saved for histology and multiphoton observations. (WB) and serum saved for creatinine (Cr).

Results: Rats with 5/6Nx developed progressive proteinuria associated with increases in Cr, glomerular injury score (GIS) and fibronectin (WB). The administration of nicotine to 5/6Nx rats did not modify SBP and resulted in worse renal injury as assessed by GIS, Cr and fibronectin (WB). The expression of fibronectin was predominantly interstitial and perversial (IF). The administration of MLA ameliorated the effects of

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
NIG suggesting they were mediated via the α7-nAChR. Sham + NIG rats had increased podocyturia, fibronectin and Cr but had no significant increase in GIS.

FR-OR217
Diffusion of Laminin-521 in the Glomerular Basement Membrane Jung Hee Suh, Jeffrey H. Miner. Renal Division, Department of Internal Medicine, Washington University School of Medicine, Saint Louis, MO.

Background: Laminin-521 (α5β2γ2) is the major laminin isoform in the mature glomerular basement membrane (GBM). Lamβ2 defects cause congenital nephrotic syndrome in humans and mice. Laminin-521 trimers are secreted by both podocytes and glomerular endothelial cells and self-polymerize into a supramolecular network essential for GBM formation. Although laminin secretion and polymerization are relatively well understood processes, laminin dynamics within basement membranes is an unexplored area with relevance to glomerular diseases.

Methods: To investigate the hypothesis that laminin diffusion in the GBM occurs, we produced chimeric mice by aggregating pairs of genetically distinct 4-cell mouse embryos, one expressing rat Lamβ2 in podocytes, and one expressing lacZ in all cells. Laminin-521 trimers are secreted by both podocytes and glomerular endothelial cells and self-polymerize into a supramolecular network essential for GBM formation. Although laminin secretion and polymerization are relatively well understood processes, laminin dynamics within basement membranes is an unexplored area with relevance to glomerular diseases.

Results: Confocal microscopy revealed distinct segmental rat Lamβ2 deposition in the GBM, but many glomeruli showed rat Lamβ2 deposition throughout the GBM, along with the presence of Xgal-positive (rat Lamβ2-negative) podocytes. The presence of segmental rat Lamβ2 deposition in the GBM implies that the diffusion of laminin-521 trimers is limited. However, because we observed many continuously rat Lamβ2-positive GBMs in glomeruli containing Xgal-positive podocytes, we conclude that secreted laminin-521 trimers can diffuse within the GBM to regions covered by rat Lamβ2-negative podocytes.

Conclusions: Laminin-521 trimers can diffuse in the GBM. These results contrast with the conventional view of type IV collagen α3α4α5 trimers, which are thought not to diffuse freely within the GBM, perhaps due to extensive cross-linking that fixes their location. These results have implications for Alport syndrome and other glomerular diseases with deposition of ectopic laminins that may contribute to filtration barrier defects.

FR-OR218
Inhibiting Discoidin Domain Receptor 1 Expression Protects Mice Against the Development of Crescentic Glomerulonephritis Monique Kerroch, Dominique Guerrot, Sophie Vandermeersch, Laurent Mesnard, Eric Rondeau, Pierre M. Ronco, Christos Chatziantoniou, Jean-Claude Dussaule. INSERM UMR 702, Tenon Hospital, Paris, France.

Background: The present study investigated the role of discoidin domain receptor 1 (DDR1), a non-integrin collagen receptor displaying tyrosine-kinase activity and mediating vascular inflammation and fibrosis, in the development of crescentic glomerulonephritis (GN). In mice, crescentic GN is induced by administration of nephrotoxic serum (anti-glomerular basement membrane) in wild type mice, mice treated daily either with antisense oligonucleotides against DDR1 or with ‘scrambled’ oligonucleotides, and mice lacking expression of DDR1 (KO).

Methods: Expression of glomerulonephritis was induced by administration of nephrotoxic serum (anti-glomerular basement membrane) in wild type mice, mice treated daily either with antisense oligonucleotides against DDR1 or with ‘scrambled’ oligonucleotides, and mice lacking expression of DDR1 (KO).

Results: Anti-DDR1 administration resulted in a progressive glomerulonephritis in the wild type and scrambled groups as evidenced by the increasing levels of proteinuria and uremia, the presence of cellular infiltrate (mainly macrophages), and the formation of glomerular crescents, fibrin deposits and interstitial fibrosis. These pathological features were concomitant to a several- fold upregulation of mRNA and protein expressions of DDR1 within glomeruli (mainly podocytes). In sharp contrast, mice treated with DDR1 antisense or DDR1 KO mice displayed a significantly lower proteinuria (p<0.001) and uremia (p=0.01), a blunted infiltration of inflammatory cells (p=0.05), a decrease in the formation of glomerular crescents and fibrin deposits and a preserved renal structure (p<0.01). This protection was associated to decreased expressions of pro-inflammatory (ICAM, VCAM, MCP1, IL-1b) and pro-fibrotic (TGFβ, Col I and III) factors. In addition, nephrin and podocin expressions were preserved in glomeruli of the antisense and KO groups. In separate experiments, we observed DDR1 presence in human biopsies of rapidly progressive glomerulonephritis (Goodpasture’s syndrome and lupus nephritis). Interestingly, DDR1 was expressed in glomeruli, especially in crescents when they were visible whereas it was absent in glomeruli of control kidneys.

Conclusions: DDR1 is a major mediator of renal inflammation and fibrosis, blocking DDR1 expression can be a novel, promising treatment against the progression of glomerulonephritis.

Funding: Government Support - Non-U.S.

FR-OR219
Sonic Hedgehog Signaling Promotes Myofibroblast Activation and Renal Interstitial Fibrosis Hong Ding, Sha Hao, Weichun He, Youhua Liu. Department of Pathology, University of Pittsburgh, PA.

Background: Sonic hedgehog (Shh) signaling is a developmental signal cascade that plays an essential role in regulating embryogenesis and tissue homeostasis. Hedgehog transmits its signaling through binding to the plasma membrane receptor, Patched 1 (Pclh1), leading to the de-repression of Smoothened (Smo). Activated Smo then moves from an intracellular vesicle to the cell membrane, where it activates the Gli family of transcription factors. Here we investigated the potential role of Shh signaling in renal interstitial fibrogenesis. Both Shh and Gli1 were induced in the fibrotic kidneys in obstructive nephropathy, indicating the Gli1 β-catenin (‘knock-in’) mutant mice, we found that Shh/Gli1 signaling is specifically targeted on renal interstitial myofibroblasts. In vitro, recombiant Shh did not promote renal fibroblast proliferation, but induced Gli1, Snaill, α-SMA, fibronectin and collagen I expression, suggesting a role of this signaling in myofibroblast activation and matrix production. In vivo, disruption of Gli1 gene in mice protected kidneys against development of interstitial fibrosis, as evidenced by a decreased expression of α-SMA, fibronectin and collagen I after obstructive injury. Blockade of hedgehog signaling with cyclopamine abolished the Shh-mediated Gli1, Snaill, α-SMA, fibronectin, and collagen I induction in renal fibroblasts. Cyclopamine did not affect renal Shh expression but inhibited Gli1 and Snail1 induction in obstructive nephropathy. Accordingly, cyclopamine also inhibited renal α-SMA and matrix expression and mitigated fibrotic lesions. These results indicate that Shh signaling plays a critical role in the pathogenesis of myofibroblast activation, matrix production and renal interstitial fibrosis.

Funding: NIDDK Support

FR-OR220
Pericyte Derived TIMP3 and ADAMTS1 Regulate Vascular Stability in the Kidney Jürgen-Ingo Sonntag, Benjamin D. Humphreys, Jeremy S. Duffield. Medicine, University of Washington, Seattle, WA; Medicine, Brigham & Women’s Hospital, Boston, MA.

Background: Recently we identified mural cells of peritubular capillaries called kidney pericytes (KPC) as progenitors of scar forming myofibroblasts but regulatory mechanisms by which kPC differentiate into myofibroblasts are unknown & molecular mechanisms by which kPC detachment from endothelial cells lead to microvascular instability is poorly understood.

Methods: Microarray analysis and vascular stabilization assay

Results: Using microarray analysis, we identified more than 1000 genes that are regulated in kPC during detachment in response to kidney injury. Among the enriched functional categories were genes involved in migration, chemotaxis, extracellular matrix & angiogenesis. Among these genes we identified the metalloproteinase ADAMTS1 as highly up-regulated early during kPC detachment & its endogenous inhibitor TIMP3 as down-regulated during detachment. The specificity of these observations was confirmed by ISH, immunostaining, quantitative assays & cell proliferation. In order to study the role of these genes in kPC function we generated & characterized primary kPC cultures, & developed a 3D capillary tube stabilization assay. Primary kPC strongly expressed TIMP3 which was down regulated in primary myofibroblasts. ADAMTS1 was not expressed by kPCs but was highly expressed by myofibroblasts. In a functional 3D regression assay primary kPC stabilized capillary tubes with characteristic processes & stabilized the microvasculature with similar capacity to brain PC, while fibroblast cultures did not show these stabilizing effects. In the absence of kPCs, recombinant TIMP3 stabilized 3D capillary tubes, whereas ADAMTS1 accelerated destabilization. Lower levels of TIMP3 stimulated & ADAMTS1 inhibited PC function in stabilizing capillaries in this assay. Consistent with these in vitro findings, mice deficient in TIMP3 had a spontaneous microvascular phenotype, impaired angiogenesis & accelerated fibrosis in response to kidney injury.

Conclusions: kPC-derived TIMP3 and ADAMTS1 are important regulators of vascular integrity and fibrosis after renal injury.

Funding: NIDDK Support

FR-OR221
Inhibition of Mitochondrial Fission by the DRP1-Inhibitor Mdivi Blocks High-Glucose and TGF-beta Induced PAI-1 and Fibronectin Expression in Renal Cells Jens Gaedeke, Hans-Hellmut Neumayer. Nephrology, Charité, Campus Mitte, Berlin, Germany.

Background: Mitochondria are dynamic networks that constantly change morphology through fusion and fragmentation (fission). A known stimulus for fragmentation is exposure to high glucose. In a previous study, we showed that mild inhibition of mitochondrial respiration blocked TGF-beta effects in renal cells. Recently, an inhibitor of the mitochondrial fission protein DRP-1 (Mdivi) has been described (Dev Cell 2008:14(2)). Here we analysed if inhibition of mitochondrial fission by Mdivi has effects on glucose and TGF-beta mediated fibrotic effects in renal cells.

Materials and Methods: High glucose (HG, 30 mM glucose) and TGF-beta (TGF-β, 5 ng/ml) were kept in DMEM containing 1g/l glucose and stimulated with 4.5 g/l glucose or TGF-beta (0-5 ng/ml).

Mdivi (Calbiochem) was used at 0-25μM. PAI-1 and fibronectin (FN) was measured by western blot. Signaling pathways were analysed by western blot using phospho-specific antibodies for SMAD3, ERK-, p-p38-MAPK and AMPK.

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FR-OR22

Regulation of Renal Fibroblast Function by Potassium Channels Michael Kaye, Joachim Hoyer, Brajesh Pratap Kaishta. Philipps-University-Medical Center, Nephrology, Marburg, Hessen, Germany.

Background: Potassium channels are important regulators of cellular function during cell cycle and proliferation. In recent studies proliferation of renal fibroblasts has been shown to be dependent on the presence of calcium-activated potassium channels. In the present study we identified a new type of potassium channels and characterized its role in resting and proliferating fibroblasts.

Methods: Potassium channel function was characterized in murine renal fibroblasts with whole cell and single cell patch-clamp experiments and cell potential recordings. Expression of potassium channels was determined with quantitative RT-PCR. Channel localisation was examined by immunofluorescence microscopy. Fibroblast proliferation was activated by TGF-β or bFGF and quantified with a colorimetric assay(MTT).

Results: In murine renal fibroblasts we newly identified the THIK-1 potassium channel, a member of two-pore-domain potassium channel (K2P) family. In resting fibroblasts next to the Kv potassium channel THIK-1 was the predominant potassium channel and responsible for maintaining the hyperpolarized cell membrane potential after activation of resting fibroblast. THIK-1 current was Ca-dependent and typically activated by arachidonic acid (AA) at a EC50 of 13±2 µM leading to a mean current of 45.9±P aF at 0mV. THIK-1 was blocked by typical K2P blockers chlorpromazine and flufenoxil.

After treatment (24h) with proinflammatory factors like bFGF or TGF-β the proliferating fibroblasts THIK-1 current was downregulated by about 70%. Instead potassium current was carried by the Ca-activated potassium channel KCa3.1. Tyrosine kinases are important pathogens by proinflammatory agents. The incubation (24h) with the TK inhibitor genistein led to a significant reduction of the channel activity of THIK-1.

Conclusions: During transition from resting to proliferation state fibroblasts show a distinct switch in potassium channel expression and function. A newly identified Ca-dependent THIK-1 is predominant in resting fibroblasts and enables a hyperpolarized membrane potential. During proliferation the channel is downregulated and substituted by the Ca-activated KCa3.1 channel which then regulates Ca2+ ion fluxes in the proliferation state.

FR-OR23

CXCR6 Mediates Recruitment of Bone Marrow-Derived Fibroblast Precursors in Renal Fibrosis Song-Chang Lin, Jiuyan Chen, William E. Mitchell, Yanlin Wang. Medicine-Nephrology, Baylor College of Medicine, Houston, TX.

Background: Renal fibrosis is a prominent pathological feature of chronic kidney disease. Activation of fibroblasts is responsible for the production and deposition of extracellular matrix. The origin of activated fibroblasts mediating renal fibrosis has been controversial. Recent evidence indicates that circulating fibroblast precursors termed fibrocytes contribute to the pathogenesis of renal fibrosis. However, the mechanisms accounting for the recruitment of bone marrow-derived fibroblast precursors into the kidney are not fully understood. We have found that circulating fibroblast precursors express CXCR6. In the present study, we examined the role of CXCR6 in the recruitment of bone marrow-derived fibroblast precursors and renal fibrosis.

Methods: Chimeric mice that express GFP driven by collagen α1(II) promoter were used to demonstrate the bone marrow origin of fibroblast precursors. Unilateral ureteral obstruction was performed to induce renal fibrosis in wild-type (WT) and CXCR6-deficient (CXCR6-KO) mice.

Results: We found that bone marrow-derived GFP positive cells were present in kidneys 5 days post obstructive injury while not in normal kidneys. Furthermore, most of the GFP positive cells stained positively for α-smooth muscle actin. Immunohistochemical studies revealed that a significant number of bone marrow-derived fibroblast precursors dual positive for CD43 and vimentin accumulated in obstructed kidneys of WT mice. In contrast, bone marrow-derived fibroblast precursors were significantly reduced in obstructed kidneys of CXCR6-KO mice. Using immunohistochemistry and Western blot analysis, we found the expression of collagen type I, fibroactin, and α-SMA was upregulated in obstructed kidneys of WT mice. These responses were significantly reduced in obstructed kidneys of CXCR6-KO mice.

Conclusions: These data indicate that bone marrow-derived fibroblast precursors are responsive to the kidney and capable of differentiating into myofibroblasts during the pathogenesis of renal fibrosis and CXCR6 plays a pivotal role in the development of renal fibrosis by recruiting bone marrow-derived fibroblast precursors.

Funding: Other NIH Support - NHLBI, AHA

FR-OR242

Base Membrane Proteoglycans Mediate Leukocyte Influx in Renal Ischemia/Reperfusion Jacob Van den Born,1 Gerjan Navis,2 Azadeh Zaferani,3 Johanna W.A.M. Celie,2 Raia Soinninen,2 Ritva Heljasvaara.3 Nephrology, University Medical Center, Groningen, Netherlands; 2Molecular Cell Biology and Immunology, Vrije Universiteit, Amsterdam, Netherlands; 3Medical Biochemistry and Molecular Biology, University of Oulu, Finland.

Background: Basement membranes (BM) have important roles in trafficking, signaling, differentiation, and regeneration of cells. Perlecan, collagen XVIII and collagen XV are three major proteoglycans of BM. Renal ischemia/reperfusion (IR) is characterized by early renal injury and inflammation, followed by regeneration and resolution phase.

Methods: To investigate the role of perlecan, collagen XVIII and collagen XV in renal IR, we used mice mutated for perlecan (Hspg2Δ3/Δ3), collagen XVIII (Col18α1-/-), and collagen XV (Col15α1-/-), and compound mutants (Hspg2 Δ3/Δ3 x Col18α1-/-), and Col18α1-/- x Col15α1-/-). Serum urea and creatinine, tubular injury, neutrophil and macrophage influx were determined at day 1, 5 and 10 after 25 minutes of bilateral renal arteries clamping. Wild type (WT) male adult C57BL/6 mice were used as controls. To mimic migration over BM, transwell migration experiments were done with freshly isolated human monocytes.

Results: One day after IR, Hspg2 Δ3/Δ3 mice showed a transient, but 3-fold rise in serum urea and creatinine compared to WT (p<0.05). Five days after IR, Col15α1-/- and especially Col18α1-/- x Col15α1-/- compound mutant mice showed a reduction of serum urea and creatinine compared to WT mice (p<0.05 and p<0.01 respectively). Histology showed significantly less tubular damage and reduced leukocyte influx in (Col18α1-/- x Col15α1-/-) mutants at days 1 and 5 after IR (p-values between p<0.05 and p<0.01). In a transwell system, the migration of monocytes towards MCP-1 increased over 2-fold (p<0.05) when heparin-albumin was immobilized on the filter, confirming involvement of proteoglycans in leukocytes migration.

Conclusions: BM proteoglycans modulate inflammation and tubular damage after renal IR, probably via the formation of stable chemokine gradients and/or interaction with leukocyte receptors. Thus proteoglycan- leukocyte interaction might be a potential intervention target for heparinoids in order to reduce inflammation.

FR-OR225

Mast Cell Degranulation Promotes Renal Fibrosis in Murine Unilateral Ureteric Obstruction Shaun A. Summers,1 Poh-Yi Gan,2 David J. Nikolic-Paterson,3 A. Richard Kitching,3 Stephen R. Holdsworth.1 1Department and Medicine and Nephrology, MMC and Monash University; 2Department of Medicine, Monash University.

Background: Mast cells (MCs) are pluripotent innate immune cells. After degranulation MCs release cytokines, chemokines and pro-fibrotic factors including transforming growth factors (TGF-β)and matrix remodeling enzymes (MMPs). In human and experimental models MCs are linked with progressive fibrosis. We explored the role of MCs in renal fibrosis induced by unilateral ureteric obstruction (UUO).

Methods: To define a role for MCs in renal fibrosis, we performed UUO on C57BL/6 wild type (WT) and mast cell deficient, KitWsh/Wsh mice. Experiments ended 7 days later. We measured collagen deposition (%), intrarenal mRNA expression of pro-fibrotic factors, TGFβ, MMP and smooth muscle actin (αSMA) relative to a housekeeping gene and interstitial leukocyte recruitment. To confirm these results were exclusively due to MC deficiency we measured renal fibrosis in KitWsh/Wsh mice reconstituted with MCs from WT mice. Finally we assessed renal fibrosis after administration of sodium chromoglycate, a mast cell stabiliser.

Results: Compared to WT mice, renal fibrosis was decreased in KitWsh/Wsh mice 7 days after UUO. Histological assessment demonstrated decreased collagen deposition (12.7±1.2 vs. 9.9±0.9%, P<0.05). Intrarenal TGFβ (1.0±0.1 vs. 0.7±0.1, P<0.05) and MMP12 mRNA expression was decreased in KitWsh/Wsh mice. Fewer interstitial macrophages (score 2.4±0.1 vs. 1.6±0.1 P<0.001) and CD4+ T cells (7.1±0.4 vs. 4.1±0.6cells/high power field, P<0.001) were seen in KitWsh/Wsh mice. Collagen deposition (17.0±2.5 vs.9.1±1.2%, P<0.05) and mRNA expression of pro-fibrotic factors, TGFβ, MMP and smooth muscle actin (αSMA) relative to a housekeeping gene and interstitial leukocyte recruitment. To confirm these results were exclusively due to MC deficiency we measured renal fibrosis in KitWsh/Wsh mice reconstituted with MCs from WT mice.

Conclusions: MCs mediate renal fibrosis after UUO. MC stabilisation is a potential novel therapy for treating renal fibrosis.

Funding: Government Support - Non-U.S.,
Ciliary Neuritic Factor Deficiency Protects Against Angiotensin II-Dependent Hypertension  
Department of Nephrology, University of Duesseldorf, Germany.

Background: JAK2/STAT3 signaling cascade modulates AngII dependent hypertension. Ciliary neuritic factor (CNTF) is an interleukin-6-like cytokine which plays a distinct role in survival and differentiation of neuronal cells by activating the JAK2/STAT3 signaling cascade. However, CNTF function in other tissues is poorly understood. This study focuses on the role of CNTF in AngII-dependent hypertension model.

Methods: One week after uninephrectomy, Ang II osmotic minipumps (1000 ng/ min/kgBW) were implanted in CNTF KO and age-matched C57Bl/6J male mice (WT). Blood pressures (BP) were measured for 3 weeks, starting one week before implantation. Histological and mRNA analysis were performed at the end of the observation period. Renal vascular function was evaluated in the isolated perfused kidney.

Results: Baseline systemic BPs were similar in CNTF KO and WT mice (119 ± 2 vs.124 ±1 mmHg). Chronic AngII infusion caused a significantly attenuated increase in BP in CNTF KO mice compared to WT mice (week 1: 139 ±3 vs. 153 ±3 mmHg; week 2: 151 ±5 vs. 168 ±4 mmHg; n=19, P<0.01). Heart hypertrophy was significantly less in the CNTF KO compared to the WT-group (6.5 ±0.4 vs. 8.2 ±0.6 mg/g BW; P<0.01). Histological and mRNA analysis revealed significantly attenuated renal vascular hypertrophy, tubulointerstitial damage and CD4/CD8 positive cell infiltration as well as reduced NGAL mRNA. Baseline systolic BPs were similar in CNTF KO and WT mice (119 ± 2 vs.124 ±1 mmHg). Chronic AngII infusion caused a significantly attenuated increase in BP in CNTF KO mice compared to WT mice (week 1: 139 ±3 vs. 153 ±3 mmHg; week 2: 151 ±5 vs. 168 ±4 mmHg; n=19, P<0.01). Histological and mRNA analysis revealed significantly attenuated renal vascular hypertrophy, tubulointerstitial damage and CD4/CD8 positive cell infiltration as well as reduced NGAL mRNA. Baseline systolic BPs were similar in CNTF KO and WT mice (119 ± 2 vs.124 ±1 mmHg). Chronic AngII infusion caused a significantly attenuated increase in BP in CNTF KO mice compared to WT mice (week 1: 139 ±3 vs. 153 ±3 mmHg; week 2: 151 ±5 vs. 168 ±4 mmHg; n=19, P<0.01). Histological and mRNA analysis revealed significantly attenuated renal vascular hypertrophy, tubulointerstitial damage and CD4/CD8 positive cell infiltration as well as reduced NGAL mRNA.

Conclusions: CNTF has a major impact on blood pressure regulation in Ang II-dependent hypertension. CNTF seems to modulate the Ang II induced renal pressor response via a JAK2/STAT3 dependent mechanism. Thus, CNTF could be an important regulatory cytokine in the pathogenesis of AngII-dependent hypertension.

Funding: Government Support - Non-U.S.

FR-OR226

Adenosine-Mediated Tubuloglomerular Feedback Responses Are Modulated by eNOS in the Afferent Arteriole  
Mattias Carlstrom, Enlyn Lai, Zaiming Luo, Christopher S. Wilcox, William J. Welsh. Dep't of Medicine, Georgetown University, Washington, DC.

Background: Tubuloglomerular feedback (TGF) is mediated via activation of A1 receptors on the afferent arteriole, and A2 receptors attenuate the response. Whereas NO derived from nNOS in macula densa regulates TGF, the role of eNOS is not clear. We tested the hypothesis that eNOS in the afferent arteriole modulates adenosine-mediated TGF responses by reducing oxidative stress.

Methods: TGF was measured in eNOS-knockout (eNOS/-/-) and wildtype mice, as changes in proximal stop-flow pressure (APSF) in response to increased loop perfusion (0 to 35 nl/min). Isolated and perfused afferent arterioles, the effector of TGF, were used to study adenosine responses (10^-11 to 10^-4 M) with or without simultaneous application of superoxide scavenger (Temposil, 10^-4 M).

Results: TGF responses (APSF) were significantly stronger in eNOS/-/- mice (12.7±1.0 mmHg) than in wildtypes (8.2±0.5 mmHg) (A). Adenosine caused a biphasic response with contraction in the lower concentration range (10^-12 to 10^-8 M) and dilatation in the higher concentration range (10^-7 to 10^-4 M). In eNOS/-/- mice the contractile response to adenosine was significantly stronger (-25±2% at 10^-8 M), with a greater dilatation at higher concentrations (-28±4% at 10^-4 M) (B). Simultaneous application with N-acetyl-l-cystein prevented adenosine-mediated contraction in both eNOS/-/- and wildtype mice, but did not significantly influence dilatation at higher concentrations (C). Western blot analysis of renal cortex revealed no differences between genotypes in adenosine A1, A2A or A2B receptors.

Conclusions: eNOS in the afferent arteriole importantly regulates TGF by modulating the contractile response to adenosine. Mechanistically, adenosine-mediated contraction is linked to increased superoxide production in the arteriole, and the contractile response is attenuated by eNOS-derived NO.

Funding: NIDDK Support

FR-OR229

Activation of Adenosine A3 Receptor in the Afferent Arteriole (Af-Art) Blunts the Effect of A1 Receptor and AngII Stimulation  
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Background: Adenosine plays an important role in tubuloglomerular feedback and renal microcirculation. There are four known receptors for adenosine, A1, A2a, A2b and A3. A3 receptor activation of the A1 receptor-constricts, whereas activation of the A2a receptor dilates the Af-Art. Whether the A3 receptor exists in the Af-Art and its function are not clear.

Methods: A superficial Af-Art was microdissected from a mouse kidney, and mRNA of the A3 receptor was measured with RT-PCR and real-time PCR. We found that the A3 receptor is expressed in the Af-Art. Then, a superficial Af-Art with its attached glomerulus was microdissected from a mouse kidney and perfused at 60 mm Hg.

Results: We measured the diameter of the Af-Art when stimulated with selective A3 receptor agonist IB-MECA at 10^-4 M and found no effect. Next, we used a selective A1 receptor agonist CHA at concentrations from 10^-6 M to 10^-4 M. CHA induced a dose-responsive vasoconstriction of the Af-Art, and diameters were 10.9±0.2 µm at control, 9.0±0.4 µm at 10^-6 M, 8.5±0.5 µm at 10^-5 M, 8.0±0.4 µm at 10^-4 M. When we repeated the CHA dose-response experiment in the presence of the A3 agonist IB-MECA, we found that activation of A3 receptor blocked the contractile effect of A1 receptor on the Af-Art. We measured intracellular calcium concentration with fura-2. In the presence of IB-MECA, the A1-induced calcium increase was blocked. Next, we used AngII at concentration from 10^-9 M to 10^-8 M. AngII induces a dose-responsive vasoconstriction of the Af-Art, and diameters were 10.7±0.4 µm at control, 9.3±0.6 µm at 10^-9 M, 9.0±0.1 µm at 10^-8 M. When we repeated the AngII dose-response experiment in the presence of the A3 agonist IB-MECA, we found that activation of A3 receptor blocked the contractive effect of AngII on the Af-Art.

Conclusions: We conclude that the A3 receptor is expressed in the Af-Art and activation of A3 blunts the vasoconstrictive effect of A1 receptor and AngII stimulation. Therefore, the adenosine A3 receptor may play an important role in modulating the Af-Art vasoconstrictor feedback response and renal hemodynamics.

Funding: Government Support - Non-U.S.
Methods: Primary cultures of VSMC from Sprague-Dawley rats were treated with 20 μM of the NO synthase inhibitor l-NAME for 24 h. The cells were then treated with 10 μM SN50 or 10 μM l-NAME for 2 h. The NO-mediated decrease in vasa recta diameter was assessed using intravital microscopy. In addition, we explored whether in vitro myogenic constriction of small renal arteries extirpated at 5/6 Nx surgery also predicts subsequent renal arterioles developed less renal damage after 5/6 Nx. Whether in vivo pre-existing vascular integrity also predicts subsequent renal damage to 5/6 Nx is subject of the current study using intravital microscopy. In addition, we explored whether in vitro myogenic constriction of small renal arteries extirpated at 5/6 Nx surgery also predicts subsequent development of renal damage in the animal.

Background: Susceptibility to renal injury varies among individuals. Previously, it was shown that individuals with healthy baseline endothelial dilatory ability in isolated renal arterioles developed less renal damage after 5/6 Nx. Whether in vivo pre-existing vascular integrity also predicts subsequent renal damage to 5/6 Nx is subject of the current study using intravital microscopy. In addition, we explored whether in vitro myogenic constriction of small renal arteries extirpated at 5/6 Nx surgery also predicts subsequent development of renal damage in the animal.

Conclusions: NO decreased TBRI surface expression in VSMC through a dynamin-2-dependent endocytosis of TBR1. This suggests that A2-AR inhibits Jv in the PT and that A2-AR activity is increased in SHR. Protein expression of A1-AR in microdissected PTs was not different between strains, however A2-AR expression was 2.4 fold higher in PT from SHR. This suggests that activation of A2-ARs normally inhibits Jv and therefore offsets the action of A1-ARs in the PT and that PT dysfunction in SHR is related to overexpression of A2-AR.

Funding: NIDDK Support, Veterans Administration Support

FR-OR233

Adenosine Receptors Regulate Fluid Uptake in the Proximal Tubule

Carolina Panico, Zaiming Luo, William J. Welch. Medicine, Division Nephrology; Georgetown University, Washington, DC.

Background: Adenosine enhances fluid uptake in the proximal tubule (PT) via adenosine type 1 receptors (A1-AR). The effect of adenosine type 2 receptors (A2-AR) on PT function is unknown. We showed that fluid uptake (Jv) in the S2 segment of the PT was lower in adult spontaneously hypertensive rats (SHR) compared to normotensive rats, therefore we hypothesized that Jv in this model is reduced by changes in adenosine receptor activity.

Methods: We measured Jv by microperfusion and recollection of accessible S2 segments of the PT in WKY and SHR.

Results: An A1-AR antagonist reduced Jv in normotensive rats (WKY: 2.7±0.3 vs A1-AR Ant: 1.3±0.3 ml/min/mm, p<0.001), but had no effect on the lower Jv in SHR (SHR: 1.1±0.2 vs A1-AR Ant 1.3±0.3 ml/min/mm, ns), suggesting that A1-AR activity is impaired in the SHR. However, microperfusion of an A2-AR antagonist (ZM241385 10-7 M) increased Jv in WKY (WKY: 2.6±0.3 vs ZM: 3.1±0.2 ml/min/mm, p<0.01), and completely restored Jv in SHR (SHR: 1.2±0.3 vs SHR + ZM: 2.9±0.3 ml/min/mm, p<0.001).

Conclusions: This suggests that A2-AR inhibits Jv in the PT and that A2-AR activity is increased in SHR. Protein expression of A1-AR in microdissected PTs was not different between strains, however A2-AR expression was 2.4 fold higher in PT from SHR. This suggests that activation of A2-ARs normally inhibits Jv and therefore offsets the action of A1-ARs in the PT and that PT dysfunction in SHR is related to overexpression of A2-AR.

Funding: NIDDK Support

FR-OR234

Endothelin-A Receptor Blockade in Chronic Renovascular Disease: A Novel Therapeutic Application

Alejandro Chade, Physiology and Biophysics, Medicine, University of Mississippi, Jackson, MS.

Background: Circulating and renal endothelin (ET)-1 is increased in patients with chronic renovascular disease (RVD). We have recently shown that chronic blockade of the ET-A receptor prevents functional and structural damage in the stenotic kidney of experimental RVD. This study was designed to extend those observations and determine whether chronic ET-A blockade can halt the progression and reverse renal injury in RVD.

Methods: Unilateral RVD was induced in 8 pigs. After 6 weeks, single-kidney blood flow (RBF) and glomerular filtration rate (GFR) was quantified in the RVD kidney to Ach, accompanied by a significant expansion of the renal microcirculation (Figure). blunted after 6 weeks of RVD in all pigs. Untreated RVD showed no changes after 10 weeks. At 10 weeks, in vivo MDCT studies were repeated, and renal microvascular (MV) density quantified in situ using 3D micro-CT.

Results: Stenotic kidney RBF, GFR, and MV density and function were similarly impaired in RVD+ET-A compared to untreated RVD, suggesting that A2-AR activity is impaired in the PT and that PT dysfunction in SHR is related to overexpression of A2-AR.

Funding: NIDDK Support

FR-OR232

Sympathetic Nerves Are in Close Proximity to Vasa Recta Pericytes, and the Co-Transmitters Noradrenaline and ATP Evoke Pericyte-Mediated Relaxation of Vasa Recta Diameter

Carol Crawford,1 Teresa M. Kennedy-Lydon,1 Liam Sawbridge,1 Jessica Munday,1 Robert J. Unwin,2 Scott S.P. Wildman,3 Claire M. Peppiatt-Wildman.1 1Royal Veterinary College, London, United Kingdom; 2University College London, United Kingdom.

Background: Pericytes reside at regular intervals along vasa recta capillaries and have been shown to regulate in situ vasa recta diameter, in response to a number of vasoactive agents, including the co-transmitters noradrenaline (NA) and ATP (1, 2). Here we show vasa recta pericytes exist in close apposition to sympathetic nerves, a potential endogenous source of NA and ATP, and determine the mean distance between sympathetic nerves and pericytes in the inner and outer medulla.

Methods: Live kidney slices, obtained from adult male Sprague Dawley rats, were secured in an open bath chamber set on the stage of an upright microscope and continually superfused with oxygenated physiological saline solution. Video-imaging techniques were used to capture pericyte-mediated changes in vasa recta diameter following exposure of live slices to NA and ATP. Pericytes and sympathetic nerves were labeled in fixed kidney slices using anti-N2G and anti-TH antibodies and appropriate fluorescently-conjugated secondary antibodies. Fluorescent images of sympathetic nerves and pericytes in the inner and outer medulla were taken with a Zeiss LSM 510 confocal microscope and the distance between sympathetic nerves and the nearest pericyte measured using LSM image browser software.

Conclusions: This study shows renoprotective effects of chronic ET-A blockade in the stenotic kidney and underscores the importance of renal MV integrity as a determinant of the progression of renal injury. Furthermore, it supports the potential of a novel therapeutic approach to protect the kidney in chronic RVD.
Improvement of Mild and Moderate Hyponatremia Is Associated with Enhanced Cognitive Function

**Methods:** Thirty patients with SNa values ≤134 mEq/L were included. The MMSE was administered to these patients and scores recorded to a maximum of 30. SNa improved appreciably in 24 of these patients (improved group) after management with 0.9% saline, fluid restriction, vasopressin receptor antagonists, withholding medications, or 3% saline as clinically indicated. The remaining 6 patients (control group) had no change in SNa levels (±1 mEq/L). The MMSE was administered to all 30 patients after at least 72 hrs. MMSE scores were compared between the improved group and control group.

**Results:** The initial SNa levels of the improved group ranged from 117 to 134 mEq/L with a mean of 124.3 mEq/L (SD±4.0) and post-improvement SNa ranged from 111 to 143 mEq/L with a mean of 133.7 mEq/L (SD±4.1; p=0.016). The control group had a mean SNa level of 128.9 mEq/L (range 127-131 mEq/L). Twenty-one patients of the improved group (88%) had an increase in MMSE score: 9 patients had a 4-10% increase, 8 patients had an 11-15% increase, and 4 patients had a 16-20% increase. The remaining 6 patients (control group) had no change in MMSE score. Of note, 7 improved patients had mild hyponatremia with pre SNa ≥ 127 mEq/L. In the control group, 67% had no change, 1 patient had a 3.5% increase and 1 patient had a 7.4% increase on MMSE retesting.

**Conclusions:** Improving hyponatremia at all levels, mild, moderate and severe, was associated with higher cognitive function on MMSE when compared to patients without correction. Correcting hyponatremia to improve cognitive function should be considered.

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**FR-OR237**

Prognostic Impact of Severe Hyponatremia Following Liver Transplantation

Jeongghwan Lee,1 Jung Pyo Lee,2 Dong Ki Kim,1 Yun Su Kim,1 Curie Ahn,1 Jin Suk Han,1 Kwon Wook Joo.1

**Background:** Hyponatremia is known as a risk factor of reduced survival in patients with end stage liver disease. But, the result on the outcomes after orthotopic liver transplantation (OLT) according to the degree of hyponatremia is controversial.

**Methods:** We conducted a retrospective analysis of 517 adult patients who underwent OLT at Seoul National University Hospital between 1 January 2005 and 31 December 2010. Patients were divided into three groups according to serum sodium values; normal (more than 135 mEq/L), mild hyponatremia (125-134 mEq/L), and severe hyponatremia (<125 mEq/L). In-hospital mortality, duration of admission on ICU and ward, delirium, neurologic complications, acute kidney injury (AKI) and infections were analyzed by ANOVA, Chi-square and logistic regression method.

**Results:** Out of 517 patients, mild hyponatremia was present in 235 (45.5%) and severe hyponatremia in 75 (14.5%). Hyponatremia had no impact on in-hospital mortality (odds ratio=1.48, p=0.38). Compared with patients of normal serum sodium, patients with severe hyponatremia did not differ in the duration (days) of admission either on ICU (9.6±7.5 vs. 11.1±11.1, p=0.24) or general ward (28.8±16.7 vs. 24.9±32.0, p=0.51). Patients with severe hyponatremia had higher rates of delirium (56.0%/29.5%, OR=1.44, p=0.018), neurologic complications (24.0%/7.7%, OR=1.538, p=0.037), and AKI (57.3%/35.3%; OR=1.35, p=0.045). In 75 patients with severe hyponatremia, patients with rapid correction of hyponatremia over 12 mEq/L/24hr showed higher mortality than others (16.2%/5.3%, p=0.002). Rapid correction of hyponatremia was associated with higher mortality in univariate logistic regression analysis (OR=4.90, p=0.042) but not in multivariate analysis (OR=3.53, p=0.149).

**Conclusions:** Pre-OLT hyponatremia does not affect on the outcomes of in-hospital mortality or the duration of admission. Hyponatremia is independently associated with post-OLT delirium, neurologic complications and AKI. Rapid correction of serum sodium is associated with higher mortality after OLT, but statistically not significant.

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**FR-OR238**

3% Saline and DDAVP: A Simple Strategy for Safe Correction of Severe Hyponatremia

Lonika Sood,1 John Kevin Hix,1,2 Richard H. Sterns.1,2

**Background:** Prompt but limited correction of severe hyponatremia is important to avoid morbidity and mortality. An unanticipated water diuresis can destabilize the pace of therapy causing an unintended overcorrection (>10 mEq/L/24 hrs and/or >18 mEq/L/48 hrs). To perform in vivo analyses, we used the Wnk (D561A) knock-in mice in mice as they only have the mutant WNK4 protein.

**Methods:** We conducted a retrospective analysis of 517 adult patients who underwent OLT at Seoul National University Hospital between 1 January 2005 and 31 December 2010. Patients were divided into three groups according to serum sodium values; normal (more than 135 mEq/L), mild hyponatremia (125-134 mEq/L), and severe hyponatremia (<125 mEq/L). In-hospital mortality, duration of admission on ICU and ward, delirium, neurologic complications, acute kidney injury (AKI) and infections were analyzed by ANOVA, Chi-square and logistic regression method.

**Results:** Out of 517 patients, mild hyponatremia was present in 235 (45.5%) and severe hyponatremia in 75 (14.5%). Hyponatremia had no impact on in-hospital mortality (odds ratio=1.48, p=0.38). Compared with patients of normal serum sodium, patients with severe hyponatremia did not differ in the duration (days) of admission either on ICU (9.6±7.5 vs. 11.1±11.1, p=0.24) or general ward (28.8±16.7 vs. 24.9±32.0, p=0.51). Patients with severe hyponatremia had higher rates of delirium (56.0%/29.5%, OR=1.44, p=0.018), neurologic complications (24.0%/7.7%, OR=1.538, p=0.037), and AKI (57.3%/35.3%; OR=1.35, p=0.045). In 75 patients with severe hyponatremia, patients with rapid correction of hyponatremia over 12 mEq/L/24hr showed higher mortality than others (16.2%/5.3%, p=0.002). Rapid correction of hyponatremia was associated with higher mortality in univariate logistic regression analysis (OR=4.90, p=0.042) but not in multivariate analysis (OR=3.53, p=0.149).

**Conclusions:** Pre-OLT hyponatremia does not affect on the outcomes of in-hospital mortality or the duration of admission. Hyponatremia is independently associated with post-OLT delirium, neurologic complications and AKI. Rapid correction of serum sodium is associated with higher mortality after OLT, but statistically not significant.

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**FR-OR235**

Increased Protein Abundance of the Mutant WNK4 May Be a Cause of the Increased WNK4 Kinase Activity in the Mouse Model of Pseudohypoaldosteronism Type II (PHA II)

Mai Wakabayashi, Shotoro Naito, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. Department of Nephrology, Tokyo Medical and Dental University, Tokyo, Japan.

**Results:** We found that the homozygous mice showed PHA II phenotypes, indicating the same pathogenic mechanism(s) are working in the homozygous mice as in the heterozygous mice. We also confirmed the increased phosphorylation of ORSL/SPAK and NCC. Using a new anti-WNK4 antibody, we could detect increased WNK4 protein expression (2.2 fold) in the homozygous knock-in mice. Although intrarenal and intracellular localization of the mutant WNK4 was not different from those in wild-type mice, the increased signal was also evident under immunofluorescence. Total WNK4 kinase activity in kidney measured on immunoprecipitated WNK4 was 2.8 times higher in the homozygous mice. However, the kinase activity of the mutant WNK4 per molecule was only slightly (1.3 times) increased after correction by the increased WNK4 protein abundance in the homozygous mice. Since WNK4 mRNA expression in kidney was not increased in the homozygous mice, we measured protein stability of the mutant WNK4 in MDCK cells. Pulse-chase experiment revealed that the stability of mutant WNK4 was significantly increased.

**Conclusions:** These results suggested that the increasing WNK4 kinase activity in the PHAII model mice could result mainly from the increased WNK4 protein abundance probably due to its increased protein stability.

**Funding:** Government Support - Non-U.S.
FR-OR239
Low Serum Sodium, Bone Mineral Disease and Mortality in Incident Chronic Hemodialysis Patients Sagar U. Nigwekar, Julia Beth Wenger, Ravi I. Thadhani, Ishir Bhan. Massachusetts General Hospital.

**Background:** In the general population, hyponatremia has been associated with mortality. In addition, recent data suggest that hyponatremia may contribute to a reduction in bone mass. It is unclear whether these associations exist in the hemodialysis (HD) population. We tested the hypothesis that low serum sodium predicts mortality and correlates with markers of bone disease in hemodialysis patients.

**Methods:** We studied 7,972 patients in the Accelerated Mortality in Renal Replacement (ArMORR) cohort of incident HD patients. Baseline serum sodium, calcium, phosphorous, alkaline phosphatase (AP), and parathyroid hormone (PTH) levels were obtained from blood samples taken at the time of HD initiation. Baseline sodium levels were analyzed by quartile. Hazard of all cause and cardiovascular mortality were calculated using Cox proportional hazard models. Multiple linear regression models were used to predict change in serum calcium, phosphorus, AP, and PTH across the 1-year period.

**Results:** Lower sodium levels were associated with older age (p=0.007), Hispanic ethnicity (p<0.001), congestive heart failure (p=0.004), diabetic nephropathy (p=0.001), lower BMI (0.008), and catheter access (p<0.001). In multivariate Cox models adjusted for demographic, clinical and laboratory factors, lower sodium levels were independently associated with an increased risk for 1-year all cause mortality (quartile 1 vs quartile 4, HR 1.75, p<0.001) and cardiovascular mortality (HR 1.72, p<0.001).

**Conclusions:** Our findings extend the observations of ArMORR, indicating that low serum sodium levels are independently associated with increased mortality in incident HD patients. The prevalence and clinical consequences of hyponatremia in this population are high and may represent an important therapeutic target.

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FR-OR240
Trends in Serum Sodium Levels (SNa+) Relate to Mortality in Incident Hemodialysis (HD) Patients Jochem G. Raimann,1,2 Len A. Usvyat,1 Jeroen Kooman,3 Frank Van der Sande,3 Stephan Thijssen,1,2 Peter Kotanko,1,2 Nathan W. Levin,1,2 *Renal Research Institute; *Beth Israel Medical Center; *University of Maastricht.

**Background:** Low pre-HD SNa+ is related to increased mortality in HD patients (pts; Waikar 2011). It is not yet clear if this is the reflection of disease or a causal determinant of mortality. This analysis aims to investigate the relation of mortality and temporal evolution of SNa+ in incident patients during the first two years of HD.

**Methods:** Pts who started HD between 1/1/2001 and 7/30/2008, with at least 3 SNa+ levels during the first 3 months were included and observed over 2 years. SNa+ changes were quantified as slopes [mEq/L/month], computed by linear regression of SNa+. Pts were stratified in groups of SNa+ (average over the first three months): (1) <138, (2) 138 to 141, (3) >141 mEq/L; and by the slope of SNa+ change over time: (1) decreasing (<0.1 mEq/L/month), stable (0.1 to 0.5) and increasing (>0.1 mEq/L/month). Cox Regression was used to compute hazard ratios (HR) adjusted for age, race, gender, diabetes (DM), ultrafiltration (UF), pre HD systolic blood pressure and pre HD weight.

**Results:** Of 4012 eligible pts (56% males, 43% black, 48% white, 50% DM, age 61±15.3 years) 607 pts (15%) died in the first 2 years. In the presence of stable SNa+ over time, low SNa+ levels during the first three months were not associated with increased mortality. Both in unadjusted and adjusted analysis rising or falling SNa+ levels over time were significant predictors of outcomes (Figure 1).

**Conclusions:** In incident HD pts changes of SNa+ over time appear to be a novel and significant predictor of mortality, outweighing the effects of absolute SNa+ levels. Within the limitations of an observational study these findings suggest that low SNa+ levels are rather a reflection of yet to be defined underlying illnesses than a direct cause of death.

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Figure 1. Summary of a diagnostic approach for hyponatremia in peritoneal dialysis patients.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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FR-OR241
Is the Hyponatremia Related to Mortality in Peritoneal Dialysis Patients? A Single-Centre Retrospective Observational Study Seokhui Kang, Jun-Young Do, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. Division of Nephrology, Department of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.

**Background:** Hyponatremia is one of the most common electrolyte abnormality. There are few reports on the incidence, etiology and mortality of the peritoneal dialysis (PD) patients with hyponatremia. We reviewed the medical records and identified all the adults who received PD between May 2001 and March 2010. Hyponatremia was defined as a serum sodium concentration less than 135 mmol/L. We enrolled 99 patients who did not show hyponatremic episodes and 297 who showed hyponatremia during follow-up. For evaluation of volume status, all patients had undergone bioelectrical impedance analysis every 6 months. Water gain as a cause of hyponatremia was defined as more than 5% increase of mean total body water (TBW) during normonatremia in the patient with hyponatremic episodes.

**Results:** The incidence of hyponatremia increased as the grade of the Davies risk index increased. The most common cause of hyponatremia was sodium chloride deficit (39.5%).

**Conclusions:** The risk of hyponatremia was associated with higher comorbidity in the PD patients. However, the comorbidity conditions are more important than hyponatremia per se in predicting the mortality. For prevention or management of hyponatremia in the PD patients, we should pay more attention to correct fluid-electrolyte abnormalities but also the underlying comorbidity.
FR-OR242
Association of Serum Sodium (SeNa) Concentration with Mortality in a Large Cohort of US Veterans with CKD
Caiba P. Kovesdy,1 Evan H. Lott,2 Jan Ling Lu,3 Sandra M. Malakauskas,4 Jennie Z. Ma,5 Mark D. Okusa,2 Kamyrar Kalantar-Zadeh,6 Salem VA Medical Center; University of Virginia; VA Informatics and Computing Infrastructure; Salem Research Institute; Harbor-UCLA.

Background: The association of serum sodium concentration with mortality in patients with various stages of CKD is not well characterized.

Methods: We examined the association of SeNa with all-cause mortality in a nationally representative cohort of 655,493 US veterans with non-dialysis dependent CKD stages 1-5. Associations were examined in time-dependent Cox models. Non-linear associations were examined by categorizing SeNa in increments of 5 mEq/L. Models were adjusted for sociodemographics, comorbidities (including congestive heart failure (CHF) and liver disease), blood pressure and laboratory variables. Analyses were repeated in patients without CHF or liver disease.

Results: Patients were 73.9±9.8 years old, 97% were males and 71% were white. During a median follow-up of 5.5 years, 193,956 patients died (mortality rate, 95%CI: 62.5/1000 patient-years, 62.2-62.8). The association of SeNa with mortality was U-shaped: compared to patients with SeNa 135-139 mEq/L, patients with SeNa ≤125, 125-139, 130-134, 140-144, 145-149 and ≥150 had adjusted mortality hazard ratios (95%CI) of 1.90 (1.82-2.26), 1.82 (1.71-1.95), 1.40 (1.36-1.43), 0.97 (0.98-0.99), 1.22 (1.18-1.26) and 1.43 (1.18-1.74)(Figure).

Conclusions: Associations were similar when restricting the study cohort to patients without CHF or liver disease.

FR-OR243
Nephrocystins Regulate the Wnt Pathways and Are Required for Early Cloacal Morphogenesis
Lott,3 Jun Ling Lu,4 Sandra M. Malakauskas,1,2 Jennie Z. Ma,2 Mark D. Okusa,2 Kamyrar Kalantar-Zadeh, Salem VA Medical Center; University of Virginia; VA Informatics and Computing Infrastructure; Salem Research Institute; Harbor-UCLA.

Background: Alternations in early cloacal morphogenesis. Depletion of either inversin, nphp2 gene product, nephrocystin-4 depletion results in abnormal ciliogenesis and planar cell polarity (PCP) disorganisation of the actin cytoskeleton, enhancement of microtubule polymerization, cell migration and enhanced spreading on collagen. This was accompanied by over-activation of junction protein complexes and that depletion of NPHP1 or NPHP4 by shRNA-mediated rescue, supporting proper transgene regulation. Only older adult mice showed renal cystic lesions, much milder than in the high transferrin expressers.

Methods: To determine whether enhanced Pkd1 expression could be a pathogenic mechanism, 3 transgenic mouse lines were generated with a tagged Pkd1-BAC.

Results: These mice overexpressed wild type Pkd1 transgene with proper temporal regulation in renal and extrarenal tissues from ~2-15 fold over Pkd1 endogenous levels. All transgenic mouse lines resulted in developmentally regulated expression that correlated with level of Pkd1 imbalance. Pkd1+/- mouse model also displayed hepatic cyst, brain aneurysm, cardiovascular defect and provide evidence that gain-of-function can be a pathogenic mechanism. To verify that the Pkd1-BAC transgene produced a functional protein, we questioned whether low and high transgene expressor could rescue the lethality of Pkd1 null newborn. The low Pkd1 expressor in Pkd1 null newborn did not prevent renal cystic or pancreatic phenotypes. These results contrast with those of Pkd1 hypomorphic alleles at 15-20% Pkd1 with less severe renal cysts than null Pkd1 mice. It suggests that either targeted hypomorph Pkd1 produce abnormal transcripts that protect from cyst development (information on the Pkd1+/- transgene lack regulatory elements for proper expression). However, the high Pkd1 transgene expressor (=endogenous) on a null Pkd1 background are healthy with no evidence of renal or extrarenal PKD phenotype and displayed complete rescue, supporting proper transgene regulation. Only older adult mice showed renal cystic lesions, much milder than in the high transferrin expressers.

Conclusions: Together our data support a Pkd1 gene dosage pathogenic mechanism for ADPKD and highlight our Pkd1+/- mouse as the model most closely mimicking ADPKD phenotype and progression.

Funding: Government Support - Non-U.S.

FR-OR245
Copetin, a Surrogate Marker for Vasopressin, Is Associated with Disease Progression in the CRISP Cohort of ADPKD Patients
Wendy E. Boertien,1 Esther Meijer,1 Li Jie,2 James E. Bost,2 Joachim Struck,3 Michael F. Flessner,4 Wendy E. Boertien,1 Esther Meijer,1 Li Jie,2 James E. Bost,2 Joachim Struck,3 Michael F. Flessner,4 Ron T. Gansevoort,5 Vicente E. Torres.5 On behalf of the CRISP Consortium; Nephrology, UMC, Groningen, Netherlands; Biostatistics, University of Pittsburgh; BRAHMS Markers, ThermoFisher Scientific, Hennigsdorf, Germany; NIH/NIDDK, Bethesda; Nephrology, Mayo Clinic, Rochester.

Background: Experimental studies suggest a detrimental role for vasopressin in the pathogenesis of ADPKD. The significance of vasopressin in human ADPKD, however, is yet unclear. We therefore investigated whether vasopressin is associated with disease progression in a cohort of ADPKD patients.

Methods: Baseline plasma copeptin, a reliable surrogate for vasopressin, was measured in 241 ADPKD patients who participated in the CRISP study (a longitudinal, observational study). Patients were followed for 3 years. Every year total kidney volume (MRD) and renal function (systolic blood pressure) were measured.

Results: In these 241 patients (age 32.4±8.9 years, 40% male, GFR 97.8±24.7 ml/min/1.73m²), median copeptin level was 2.9 (IQR 1.8–5.0) pmol/L. Copeptin concentration was higher in males than in females. Remarkably, baseline copeptin concentration was not associated with plasma osmolality (p = 0.29), urine osmolality (p = 0.16) and 24 hour urine volume (p = 0.17). In contrast, baseline copeptin concentration was significantly associated with change in total kidney volume during follow-up (std. B = 0.24, p < 0.01). This association remained significant after adjusting for gender, age, cardiovascular risk factors and baseline TKV (std. B = 0.14, p = 0.03). Baseline copeptin concentration was also significantly associated with change in GFR after adjusting for gender, age cardiovascular risk factors and baseline GFR (std. B = -0.15, p = 0.03).

Conclusions: These data show that in ADPKD patients, copeptin levels, as a marker for vasopressin, are not correlated with normal physiologic parameters as plasma nor urinary osmolality or urine volume. Most importantly, high copeptin levels are independently associated with disease progression in ADPKD patients, confirming experimental studies suggesting a detrimental role for vasopressin.

Funding: NIDDK Support

FR-OR246
Expression of the Cytoplasmic Tail of Fibrocystin Causes Cystogenesis through PI3K/Akt/mTOR Pathway
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Background: PFC (fibrocystin or polyductin) is a single transmembrane receptor-like protein, responsible for the human autosomal recessive polycystic kidney disease (ARPKD). It was recently proposed that PFC could undergo a Notch-like cleavage and subsequently releases the cleaved carboxy(C)-terminal fragment that enters the nucleus of the cell. However, the functions of the cleaved C-tail are unknown.

Methods: We have used a series of morphological, biochemical, immunological, and cell biology approaches to explore the roles of the isolated FPC C-tail.
The Role of the Exocyst in Renal Cilia Assembly and Function
Joshua H. Lipschutz, 1, 6 Ryan J. Reichert, 4 Kwon Moo Park, 5 P. Darwin Bell, 4 Rebecca D. Burdine, 2
The exocyst is a complex of seven proteins that facilitates the targeting of proteins to the plasma membrane. In the kidney, the exocyst is involved in the assembly of primary cilia. We have shown that exocyst Sec10 binds to the Par complex, which co-localizes with the exocyst at the primary cilium. Dominant-negative Sec10 expression, shRNA-mediated knockdown of Sec10, and shRNA-mediated knockdown of Tubα, a Cdc42 GEF, all prevented ciliogenesis in MDCK cells. In vivo, Sec10 knockdown in zebrafish resulted in several different subcellular structures, including centrioles and cell-cell junctions. However, a synergistic genetic interaction between zebrafish sec10 and pkd2, in that co-injection of knockdown, including: curly tail up; left-right patterning defects; glomerular expansion; the cellular phenotype recapitulated that seen in ADPKD cells: loss of flow-generated RanBP1 knockdown disrupts the proper levels of RanBP1 and determined the effects on Ran GTP and primary cilia formation. We cilia. To confirm the crucial link between Ran GTP and ciliogenesis, we manipulated the is coordinated with the initiation of ciliogenesis. The Ran Binding Protein 1 (RanBP1), was used to knockdown exocyst Sec10 and Cdc42 expression in MDCK cells, and antisense down with GST Importin beta1 followed by immunoblotting with Ran antibodies. Furthermore, we demonstrate that RanBP1 knockdown disrupts the proper localization of the kif17 cilia-transporting motor in kidney proximal tubule epithelial cells. We therefore generated an Sdccag8 knock-out mouse model to study the function of Sdccag8 in tissues affected by its ablation. Results: Our first data indicate that Sdccag8 is strongly expressed in nephron segments of the cortico-medullary zone of the developing mouse kidney and in bronchioles of the developing mouse lung. Morphologically, Sdccag8+ mice display hindlimb polydactyly with tripalmar and triphalangeal thumbs. In addition, we demonstrate that Sdccag8 is also localized to nuclear speckles – foci of mRNA synthesis. Conclusions: The nuclear localization indicates that Sdccag8 may be involved in regulating processes so far not ascribed to NPHP-causing genes. Further understanding of Sdccag8 subcellular and signalling functions will provide valuable insight into the pathogenetic mechanisms of NPHP.

FR-OR249
The Cilia-Associated Protein NPH4 Translocates Canonical Wnt-Regulator Jade-1 to the Nucleus
Lori Borgal, 1 Sandra Habbig, 1,2 Max C. Liebau, 1,2 Claudia Dafinger, 1 Roman-Ulrich Mueller, 1 Thomas Benzing, 1 Bernhard Schermer. 1 Renal Division, Department of Medicine and Center for Molecular Medicine Cologne, University of Cologne, Germany; 2Department of Pediatrics, University of Cologne, Germany.

Background: Nephronophthisis is the most common genetic cause of endstage renal disease in children, with no causative treatment currently available. The disease is characterized histologically by progressive fibrosis of the kidney tubuli and associated interstitial inflammation and fibrosis, and corticomedullary cyst development. Pathogenesis involves the loss of function of one or more nephrocystin proteins (NPHPs), leading to the disease classification of a “ciliopathy” because all NPHPs localize to primary cilia or centrosomes. Cystogenesis is thought to involve over-activation of canonical Wnt signaling, but the mechanism remains unclear.

Jade-1 has recently been identified as a novel ubiquitin ligase targeting beta-catenin for proteosomal degradation. Jade-1 was further shown to be regulated by pVHL. Here, we demonstrate that Jade-1 localizes to the ciliary base, and interacts with NPHP4. NPHP4 stabilizes protein levels of Jade-1 and is involved in the translocation of Jade-1 to the nucleus. Finally, NPHP4 and Jade-1 were demonstrated to additively inhibit Wnt signaling, and this interaction was shown to exist genetically in zebrafish. We propose that NPHP4 stabilizes protein levels of Jade-1 and is involved in the translocation of Jade-1 to the nucleus. Further studies are needed to determine if this interaction is essential for nephronophthisis pathology.

Conclusions: Our studies illuminate a new function for Ran GTP in stimulating cilia formation and reinforces the notion that Ran GTP and the Importins play key roles in ciliogenesis and ciliary protein transport.

Funding: NIDDK Support

FR-OR250
Generation of a Mouse Knockout Model To Functionally Characterize the New Ciliopathy Gene Sdeca8
Rannar Airik,1 Friedhelm Hildebrandt.1,2 Departments of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; 2Howard Hughes Medical Institute, Chevy Chase, MD.

Background: Nephronophthisis (NPHP) is a heterogenous autosomal recessive renal ciliopathy that represents the most frequent cause of kidney failure in the first 30 years of life. It is frequently associated with retinal degeneration, liver fibrosis, mental retardation or malformations of brain, heart and bone. We have previously shown that recessive truncating mutations in the human gene SDCAG8/NPHP10 cause NPHP with retinitis pigmentosa (Otto et al., Nat Genet 42:840, 2010) and primary cilia dyskinesia (Dollfus H, unpublished communication). We demonstrated that SDCAG8 localizes to several different subcellular structures, including centrioles and cell-cell junctions. However, the pathogenetic mechanism of SDCAG8 mutation remains unknown.

Methods: We therefore generated a Sdccag8 knockout mouse model to study the function of Sdccag8 in tissues affected by its ablation.

Results: Our first data indicate that Sdccag8 is strongly expressed in nephron segments of the cortico-medullary zone of the developing mouse kidney and in bronchioles of the developing mouse lung. Morphologically, Sdccag8+ mice display hindlimb polydactyly with tripalmar and triphalangeal thumbs. In addition, we demonstrate that Sdccag8 is also localized to nuclear speckles – foci of mRNA synthesis.

Conclusions: The nuclear localization indicates that Sdccag8 may be involved in regulating processes so far not ascribed to NPHP-causing genes. Further understanding of Sdccag8 subcellular and signalling functions will provide valuable insight into the pathogenetic mechanisms of NPHP.

FR-OR248
Induction of Ran GTP Drives Ciliogenesis
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Background: The small GTPase Ran and its binding partners, the Importins, regulate transport from the cytoplasm into the nucleus. New evidence suggests that Ran GTP and the Importins are also involved in regulating protein movement into the cilium. Our previous studies have shown that Importin beta1 collaborates with Ran to promote cilia targeting of Retinitis pigmentosa 2 and the Kif17 motor protein.

Methods: We used Retinal Pigment Epithelial (RPE), Human Primary Airway Epithelial or MDCK cells to study ciliogenesis. Ciliogenesis was induced in RPE cells by serum starvation and in MDCK and Human Primary Airway Epithelia by growing the cells to confluence. Ran GTP levels were measured with Ran GTP specific antibodies or by pull down with GST Importin beta1 followed by immunoblotting with Ran antibodies.

Results: We found that Ran GTP can be localized to centrosomes/basal bodies as well as to the cilia. Furthermore, we found that Ran GTP accumulation at basal bodies is coordinated with the initiation of ciliogenesis. The Ran Binding Protein 1 (RanBP1), which accelerates hydrolysis of Ran GTP to Ran GDP also localizes to basal bodies and cilia. To confirm the crucial link between Ran GTP and ciliogenesis, we manipulated the levels of RanBP1 and determined the effects on Ran GTP and primary cilia formation. We discovered that RanBP1 knockdown in RPE cells results in an increased concentration of Ran GTP at centrosomes leading to ciliogenesis. In contrast, overexpression of RanBP1 in RPE cells antagonizes primary cilia formation and attenuates Ran GTP accumulation at centrosomes. Furthermore, we demonstrate that RanBP1 knockdown disrupts the proper localization of Kif7 at the distal tips of primary cilia in MDCK cells.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

Funding: NIDDK Support

FR-OR251
Renal Phenotype of a Mouse Model of HANAC Syndrome Revealing a New Role for Col4a1 in Nephron Ciliogenesis
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Background: We have described a multisystemic dominant syndrome called HANAC (Hereditary Angiopathy, Nephropathy, Aneurysms, and Cramps) related to COL4A1 mutations. Renal abnormalities include hematuria and renal cysts. To get insight into the pathogenesis of HANAC, we have generated Col4a1+/− mice.

Methods: We therefore generated a Col4a1+/−; Col4a1−/− mouse strain. The expression of Col4a1+−− was generated by homologous recombination, using a target construct containing a 16kb Col4a1 genomic region, in which the c.1706G>T mutation was introduced in exon 25.

Results: Despite intracerebral bleeding and growth retardation at birth, homozygous mutants lived through adulthood. At birth, albuminuria and hematuria were detected in homozygous and heterozygous animals. Homozygous neonates showed morphological glomerular defects with ultrastructural abnormalities of podocytes and glomerular adhesion to the basement membrane. Despite intracerebral bleeding and growth retardation at birth, homozygous mutants lived through adulthood. At birth, albuminuria and hematuria were detected in homozygous and heterozygous animals. Homozygous neonates showed morphological glomerular defects with ultrastructural abnormalities of podocytes and glomerular adhesion to the basement membrane.
Once

Evidence is lacking for a direct pathogenic effect of anti-proteinase 3 (PR3) antibodies. Progress has been hampered by different PR3 expression patterns in the mouse, the neutrophils of which do not express PR3 on the cell surface, and by the presence of different Fe receptors across the two species. Therefore, we sought to test whether human anti-PR3 ANCA are capable of inducing acute vasculitis in mice with a human immune system.

Methods: We generated chimeric mice by injecting mobilised human haematopoietic stem cells into NOD.Cg-Pkd1Scid H2d2grm1WJ/J12 mice, which lack functional B and T cells. We confirmed myeloid chimerism Mean achieved human CD45+ cell chimerism was 18.5% of circulating cells (range 6.3-38.2). Both human monocytes and granulocytes were detectable by FACS using human CD11b, CD15 & CD66b antibodies. Human neutrophils were detectable in the bone marrow, with typical c-ANCA and p-ANCA staining demonstrated using human anti-PR3 & anti-MPO antibodies.

Results: We injected IV 4mg of protein G purified human IgG from patients with Renal S Lung vasculitis (anti-PR3, n=18 mice from 3 donors), patients with non-vasculitic renal disease (disease controls, n=5) and healthy controls (n=3). To maximise myeloid cell recruitment mice were pre-treated with LPS 1500E/μg. By sacrifice on day 6, seven of anti-PR3 treated mice (39%) had haematuria, whereas none of the control animals did. There was punctate haemorrhage on the surface of the lungs of anti-PR3 treated animals, with histological evidence of acute vasculitis and haemorrhage. Anti-PR3 treated mice had mild pauci-immune proliferative glomerulonephritis, with infiltration of human & mouse leukocytes. In 3 mice (17%) more severe glomerular injury was present. There were no glomerular changes in controls. There were no glomerular changes in control mice. The disease controls (n=5) were always wild-type and antibody-negative. Dicer cko mice are pathogenic. This model of anti-PR3 associated vasculitis may be useful in dissecting mechanisms of vascular injury.

Funding: NIDDK Support, Private Foundation Support, Government Support - Non-U.S.

FR-OR255

Mast Cells Attenuate Autoimmune Anti-Myeloperoxidase Glomerulonephritis by Mast Cell IL-10 Directed Enhanced T Regulatory Cell Immunosuppression


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Background: Mast cells (MCs) are pleiotrophic and can release cytokines that mediate effector and regulatory immune responses. We previously showed that MCs are protective in anti-myeloperoxidase (MPO) GN. This study explores the mechanism of this protection.

Methods: Autoimmunity to MPO and anti-MPO GN was examined among MC deficient (Wsh) and Wsh mice reconstituted with WT (WTMC→Wsh) or IL-10-/-MC (IL-10-/-MC→Wsh). MPO immunization with Freund’s adjuvant was used to induce autoimmunity and GN was triggered using low dose of anti-glomerular basement membrane antibody. Results: WTMC→Wsh mice developed significantly less intense anti-MPO autoimmunity and renal injury than Wsh mice confirming that protection is MC dependent (proteinuria; 10.1±1.3 vs 4.8±0.8mg/24hr and glomerular CD4 cells; 8.0±1 vs 0.5±0.1/cells). MPO autoimmunity measured by dermal DTH (1.1±1.0 vs 0.7±0.1 [mm]), CD4 anti-MPO proliferation responses (19.2±2.2 vs 13.3±1.8 x10⁴counts/min) and IL-17A (15.8±1.3 vs 9.1±1.4 ng/ml). The proportion of LN Tregs was decreased in Wsh mice (12.2±1.2 vs 13.4±0.4 %CD4+Foxp3+ all P<0.05).

Reconstitution with IL-10-/-MC offered no protection compared with WTMC→Wsh mice, confirming that protection is MC IL-10 dependent. Proteinuria (13.0±2.2 vs 6.8±0.4) and glomerular CD4 cells (8.0±0.8 vs 6.0±0.3). Dermal MPO DTH (8.0±1 vs 0.4±0.1), MPO recall proliferation (12.8±1.5 vs 8.1±1.3 x10⁴) and IL-17A (7.0±1.8 vs 2.5±0.9). Tregs (CD4+Foxp3+) and Teffs (CD4+Foxp3-) were isolated from LNs of MPO immunized Foxp3-GFP mice. The capacity of Tregs to suppress MPO recall responses of Teffs was assessed in the absence and presence of either WT or IL-10-/- MCs. WT MCs with WT Treg production of IL-10, TGFβ and enhanced Treg suppression of Teff responses (17.1±1.0 vs 10.6±1.7 x10⁴counts/min). Whereas IL-10-/- MCs induced no increase in production of IL-10, TGFβ and had significantly less capacity to enhance Treg suppression of Teffs (15.7±1.3 vs 14.2±1.7 x10⁴counts/min).

Conclusions: MCs play a protective role in autoimmune anti-MPO GN by enhancing T regulatory cell suppression mediated by MC production of IL-10.

Funding: Government Support - Non-U.S.
FR-OR256

Neutrophil Serine Proteases Mediate Anti-Myeloperoxidase Antibody Induced Crescentic Glomerulonephritis

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Background: Anti-neutrophil cytoplasmic antibodies (ANCA) are associated with necrotizing crescentic glomerulonephritis (NCGN) and their pathogenicity has been firmly established in several animal models. Dipeptidyl peptidase I (DPP1) is a cysteine protease required for the activation of neutrophil serine proteases (NSP) cathepsin G (CG), neutrophil elastase (NE), and proteinase 3 (PR3), enzymes that are thought to play an important role in inflammation. We tested the hypothesis that active NSP are essential to anti MPO Ab-induced NCGN.

Methods: An experimental model of anti-MPO induced NCGN was used in which MPO-deficient animals were immunized with murine MPO followed by irradiation and bone marrow (BM) transplantation with MPO-positive bone marrow cells.

Results: Mice transplanted with wild type (WT) BM developed NCGN whereas mice transplanted with DPP1-/- BM were protected (37.4±8.2% crescents in WT vs. 1.3±0.7% crescents in DPP1-/-). This protective effect correlated with inactivation of NSP as mice reconstituted with NE-/-SPR3-/- BM were equally protected against NCGN induced by anti-MPO Ab. Protection was accomplished by a significant reduction in in vivo IL-1β generation by DPP1-/- BM-reconstituted animals (103.0 ± 29.6 pg/ml in WT kidneys vs. 5.9±1.1 pg/ml in DPP1-/-) and in vitro production of IL-1β by anti-MPO Ab-activated protease-deficient monocytes. Lastly, the specific IL-1β receptor antagonist Anakinra protected animals against anti-MPO Ab-induced NCGN (16.7±6.0% crescents in untreated mice vs. 2.4±1.7% crescents in Anakinra-treated mice), suggesting that IL-1β is a critical inflammatory mediator in this model.

Conclusions: Our data strongly suggest that NSP-dependent IL-1β generation is required for the development of anti-MPO Ab-induced NCGN and together NSP and IL-1β may provide novel therapeutic targets for ANCA-mediated diseases in humans.

Funding: Other NIH Support - AI049261, Government Support - Non-U.S.

FR-OR257

Membranous Nephropathy-Associated Anti-Phospholipase A2 Receptor IgG4 Autoantibodies Activate the Lectin Complement Pathway

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Background: IgG4 is the major IgG subclass in the glomerular immune deposits of patients with primary membranous nephropathy (MN). IgG4 antibodies to the phospholipase A2 receptor (PLA2R) also predominate in the serum of most cases of MN and IgG4 anti-PLA2R can be eluted from their glomerular deposits. Whereas IgG4 does not activate complement via the classical pathway, C3 and C5b-9 are typically present in MN glomerular deposits in the absence of C1q, which suggests that another complement pathway might be involved. Given that mannose-binding lectin (MBL) has been found in MN glomeruli, we hypothesized that IgG4 might bind MBL and activate complement via the lectin pathway.

Methods: IgG4 from normal and MN sera was isolated and IgG4 anti-PLA2R was further purified from the total MN IgG4 by affinity chromatography on an immunoreactive fragment of PLA2R. MBL binding to IgG4 anti-PLA2R was assessed by Far-Western blot analysis and by ELISA on IgG4 anti-PLA2R-coated plates. The ability of IgG4 anti-PLA2R to activate complement via MBL was assessed by generation of C4b in the presence of normal complement and by ELISA on IgG4 anti-PLA2R-coated plates. The ability of IgG4 anti-PLA2R to activate C4b in the presence of MBL was assessed by generation of C4b in the presence of MBL.

Results: For the Far-Western assay, normal human IgG4, IgG4 anti-PLA2R, and MN IgG4 that had been preabsorbed with PLA2R was resolved by SDS-PAGE, transferred to nitrocellulose membranes and incubated with purified MBL. MBL bound strongly to the anti-PLA2R IgG4 band and only weakly to normal IgG4 and MN IgG4 that had been depleted of anti-PLA2R. Under reducing conditions, MBL bound exclusively to the H chain of anti-PLA2R IgG4. ELISA assay further demonstrated that MBL binding to IgG4 anti-PLA2R was significantly greater than to normal control IgG4 (0.317±0.148 vs -0.023±0.005, N=3, P<0.05). Moreover, IgG4 anti-PLA2R significantly activated C4 to C4b in the presence of MBL as compared to control IgG4 (0.193±0.084 vs -0.012±0.012, N=3, P<0.05).

Conclusions: MN-associated IgG4 anti-PLA2R autoantibodies are able to bind MBL directly and activate complement in situ. These findings suggest that the FC region of anti-PLA2R IgG4 may possess unique properties and explain how complement is activated in MN glomeruli.

Funding: NIDDK Support, Other NIH Support - NIAID

FR-OR258

Exosomes Released from Dendritic Cells Are Taken up by Kidney Cells and Increase Cellular MHC-II Protein Levels

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Background: Chronic kidney disease is often marked by an infiltration of inflammatory cells. The mechanisms underlying the facilitation of this inflammatory response and the role of antigen presentation by the proximal tubule remains incompletely defined. Kidney Injury Molecule 1 (Kim-1) recognizes phosphatidylserine and converts proximal tubule cells to phagocytes. We evaluated whether Kim-1 mediates uptake of exosomes released from dendritic cells and whether this process results in enhanced proximal tubule presentation of antigens by upregulation of major histocompatibility complex class II (MHC-II).

Methods: The relationship between Kim-1 and MHC-II expression was determined by immunocytochemical techniques in a novel transgenic animal model of Kim-1 expression which results in chronic kidney disease. Isolated exosomes from cultured bone marrow dendritic cells were centrifuged to proximal tubule cells. MHC-II and Kim-1 co-expression was evaluated by flow-cytometry and MHC-II RNA expression levels through real time PCR.

Results: In the animal model there was a marked increase of MHC-II expression in tubular cells expressing Kim-1. After treatment of proximal epithelial cells in culture with exosomes derived from DC, MHC-II expression was increased in the tubular cells, specifically in cells expressing higher levels of Kim-1. There was no significant difference in MHC-II mRNA and protein expression levels, indicating the protein is transferred from exosomes, and not newly synthesized in the Kim-1 expressing epithelial cells.

Conclusions: Dendritic cell-derived exosomes confer antigen-presentation capabilities to proximal tubule cells. This process is facilitated by Kim-1 mediated uptake of exosomes from dendritic cells, resulting in the transfer of MHC-II to tubular cells. Increasing the antigen presentation capability of renal tubule cells through transfer of exosomes after injury may lead to propagation of inflammatory changes resulting in fibrosis and eventual renal failure.

Funding: NIDDK Support

FR-OR259

Autocrine Adenosine 2A Receptor Signalin Promotes PD-1 Expression and Innate Regulatory T Cell Function During Ischemic Kidney Injury

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Background: Regulatory T cells (Tregs) suppress innate inflammation and injury associated with kidney ischemia-reperfusion injury (IRI). Tregs express CD73, which is the final enzyme in production of extracellular adenosine and previous studies have demonstrated that adenosine 2A receptor (A2AR) activation on immune cells inhibits inflammation and preserves kidney function after IRI. We hypothesized that the production of adenosine by Tregs is required to block innate immune responses in kidney IRI and that the Treg-generated adenosine would signal through A2ARs in an autocrine manner on Tregs.

Methods: Freshly-isolated CD4 CD25+ Tregs from WT, A2AR or CD73KO mouse spleen were adoptively-transferred to naive C57/B16 (WT) mice 18 hr prior to bilateral renal ischemia or sham surgery. Kidney function was estimated by plasma creatinine measurement and kidney inflammation was assessed by flow cytometry and immunohistochemistry. The A2AR specific agonist ATL1222 (0.1 to 30 nM) and A2AR specific antagonist ZM241385 (100nM) were used in vitro to investigate the role of A2AR signaling in Tregs.

Results: Adoptively-transferred WT Tregs protected WT mice from kidney IRI, but in the absence of adenosine generation (CD73KO Tregs) or A2ARs (A2AR-KO Tregs), Treg function was completely inhibited. In vitro activation of A2ARs on CD73KO Tregs, prior to adoptive transfer, restored their protective ability, and augmented the ability of WT Tregs to suppress kidney IRI (an effect blocked by pretreatment with ZM241385). ATL1222 dose-dependently enhanced surface expression of the negative co-stimulatory molecule PD-1 on Tregs. PD-1 blocking antibody treatment of Tregs, prior to adoptive transfer, reversed their protective effects in vivo, even if they had been pre-treated with ATL1222.

Conclusions: Taken together these findings demonstrate that the simultaneous ability to generate and respond to adenosine is required for Tregs to suppress innate immune responses in ischemia-reperfusion injury through a PD-1-dependent mechanism.

Funding: NIDDK Support

FR-OR260

Critical Role of the Chemokine Receptor CXCR6 for Renal NKT Cell Localization and Function in Murine Crescentic Glomerulonephritis

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Background: The chemokine receptor CXCR6 is expressed on T cells and Natural Killer T (NKT) cells and might play a role in trafficking or activation of these cells via its unique chemokine ligand CXCL16.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Methods: To investigate the role of CXCR6 in renal inflammation we induced a T cell-dependent model of glomerulonephritis (nephroptotic nephritis) in BL6/CXCR6−/− and BL6 WT mice.

Results: Induction of nephritis resulted in upregulation of renal CXCR6 and CXCL16 mRNA expression. CXCL16 expression peaked between days 7–10 and was mostly detected in the glomeruli. Unexpectedly, CXCR6−/− mice developed an aggravated course of nephritis (day 8) in terms of T cell recruitment, tissue injury, serum creatinine and BUN level compared to WT mice. In the inflamed kidney CXCR6 was highly expressed (>90%) on invariable NKT (iNKT) cells and to a much lesser extent on γδ T cells. In contrast to the enhanced numbers of γδ T cells, the infiltration of iNKT cells into the kidney was markedly reduced in nephritic CXCR6−/− mice. RT-PCR and FACS analyses revealed the production of anti-inflammatory IL-4 and TGFB but no proinflammatory IL-17 by renal iNKT cells, supporting their protective role. To assess the function of CXCR6+ γδ T cells, FACS-sorted TCRβ+NK1.1+ γδ T cells from CXCR6−/− livers of WT mice (purity >95%) were transferred into WT and CXCR6−/− mice (10⁶ NKT cells/animal) followed by nephritis induction. The transfer of CXCR6+ γδ T cells resulted in an almost identical course of nephritis (day 8) in WT and CXCR6−/− mice (assessed by morphological and functional parameters) demonstrating the protective role of CXCR6+/γδ T cells in glomerulonephritis.

Conclusions: Nephritic CXCR6−/− mice had a defect in the recruitment of protective NKT cells into the kidney, resulting in an accelerated course of nephritis.

Funding: Government Support - Non-U.S.

FR-OR261

Complement C3a Induces IL-1β Production in Human Monocytes Which Leads to Th17 Lineage Decisions

Elham Asgari, Steven H. Sacks, Esperanza Complement C3a Induces IL-1β Production in Human Monocytes Which Leads to Th17 Lineage Decisions

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Background: IL-1β is among the most potent pro-inflammatory cytokines and mediates important immune functions such as promoting Th17 lineage commitment. Monocytes/macrophages are the major IL-1β sources. IL-1β secretion by these cells requires TLR (LPS) and P2X7-receptor (ATP) signals, which in turn activate the inflammasome. However, how exactly LPS signals and ATP availability are regulated during monocyte activation is unclear and the requirement for a second danger signal has long been proposed. Considering the importance of anaphylatoxins C3a and C5a in innate immunity, we hypothesised that they participate in IL-1β production.

Methods: Freshly isolated monocytes have been stimulated with C3a or C5a with and without LPS at different concentrations and time points and IL-1β measured as an indicator of inflammasome activation. Relationship of inflammasome activation with pannexin 1 channel has been investigated by inhibition of the channel with carbeneoxolone.

For assessing Th17 induction, activated monocyte supernatants were used in T cell cultures and IL-17 production measured.

Results: Both LPS and C3a were absolutely required for IL-1β production in human macrophages while in monocytes, C3a increased LPS-induced IL-1β dramatically. Neither C3adesArg, nor C5a showed any effect on IL-1β production. We suggest that C3α drives IL-1β production by controlling the release of intracellular ATP into the extracellular space via regulating the function of the ATP-releasing channel pannexin 1.

Importantly, we found that C3a/LPS-stimulated monocytes induce strong Th17 cell induction in in vitro cultures.

Conclusions: Our data indicate that C3aR-mediated signalling events are important components of the IL-1β/Th17 axis in humans. This has significant implications in IL-17-driven diseases such as rheumatoid arthritis or asthma as well as kidney transplantation where Th17 cells have been shown to participate in allograft rejection and causing resistance to tolerance induction.

Funding: Private Foundation Support

FR-OR262

Role of a Novel Rat-Specific Fc Receptor in Macrophage Activation Associated with Crescentic Glomerulonephritis

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Background: Crescentic glomerulonephritis (Cgn) is a complex disease where the initial insult is often the glomerular deposition of antibodies against intrinsic or deposited antigens in the glomerulus. The role of Fc receptors in the induction and progression of Cgn is increasingly recognized.

In previous studies we have shown that copy number variation in FcgR3 partially explains the genetic susceptibility of the Wistar-Kyoto (WKY) rat to nephrotic nephritis (NTN), a rat model of Cgn. The FcgR3-related sequence (FcgR3-rs) is a novel rat-specific Fc receptor with a cytoplasmic domain 6 amino acids longer than its paralogue, FcgR3. The FcgR3-rs gene is deleted from the NTN-susceptible WKY rat genome and this deletion is associated with enhanced macrophage activity in this strain. Here, we have investigated the mechanism by which the deletion of FcgR3-rs in the WKY strain leads to increased macrophage activation.

Results: By using FcgR3-rs gene deletion, we have generated stably transduced human U937 cells expressing either FcgR3-rs or FcgR3.

Results: In these cells, which lack endogenous FcgR3 receptors, we show that FcgR3-rs interacts with the common Fc gamma-chain but that Fc receptor-mediated phagocytosis and signalling are defective. Furthermore, in primary macrophages, expression of FcgR3-rs inhibits Fc receptor mediated functions, as WKY bone marrow derived macrophages (BMDMs) transduced with FcgR3-rs had significantly reduced phagocytic activity. This inhibitory effect on phagocytosis was mediated by the novel cytoplasmic domain of FcgR3-rs.

Conclusions: In conclusion, these results suggest that the rat specific FcgR3-rs may act to inhibit FcgR3-mediated signalling and phagocytosis and could be considered as a novel mechanism in the modulation of Fe receptor mediated cell activation in autoimmune diseases.

FR-OR263

Quantitative Skill Evaluation for Kidney Biopsies

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Background: Certification in kidney biopsy procedures requires both cognitive and sensorimotor skill acquisition which has been linked to duration, realism and diversity of training exposure. There is a critical need for a comprehensive and realistic training system which also facilitates monitoring and evaluation of procedural skills.

Methods: We created SIMBiopsies, a visual and kinesthetic environment that mimics the look and feel of a kidney biopsy, to allow trainees to improve their skills while performing simulated biopsies. A mock-biopsy needle mounted on a haptic device produces the appropriate ‘feel’ as the trainee inserts a needle into the virtual model of a Blue Phantom®, a block of tissue-like gel with inclusions of denser gel (to mimic different tissues). This virtual model was created by coupling material-testing data with a 3D visual model of the Blue Phantom.

Results: A series of needle insertion studies were carried out by two nephrologists and sample characteristic haptigrams (force-motion profiles) were recorded. We are currently working on comparing, cataloging and validating captured haptigrams of a range of nephrologists (novice to expert) performing virtual biopsies to quantify proficiency.

Conclusions: The SIMBiopsies virtual immersive environment offers a convenient interactive means to develop, practice and quantitatively assess biopsy skills. By overcoming various economic, logistical and safety issues that hinder extensive conventional training in real patients, we anticipate it will also provide a standardized means for certification in this critical skill.

Funding: Clinical Revenue Support

FR-OR264

Education Intervention Increases Patient Knowledge about Chronic Kidney Disease

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Background: We evaluated the impact of a 2-3 minute, physician delivered, literacy sensitive education worksheet on patient knowledge about chronic kidney disease (CKD).

Methods: Adult patients with CKD Stages 1-5, seen in nephrology clinic, were enrolled into an education intervention pilot (April-October 2010) and compared to a historical cohort (April-October 2009). A validated survey assessing CKD awareness and knowledge was given to patients in both the pilot intervention group and historical cohort. Areas of low knowledge from the historical cohort informed development of a patient CKD education worksheet (adapted from: National Kidney Disease Education Program). This worksheet was reviewed by nephrologists with patients during appointments for the pilot intervention. The proportion of patients correctly answering survey questions was compared between groups.

Results: 401 patients were in the historical cohort, and 155 received the intervention. The mean (SD) age of the combined population (N=556) was 57 (16) years. 53% were male, 81% White, and 78% had CKD Stage 3-5. There were no significant differences in patient demographics by intervention status. Unadjusted analysis showed a higher proportion of patients receiving the intervention were aware of CKD diagnosis (76% vs. 69%; p=0.04), and correctly defined “GFR” (85% vs. 68%; p=0.001), their kidney function (68% vs. 49%; p<0.001), and their stage of CKD (65% vs. 36%; p<0.001). Compared to controls, patients receiving the intervention had higher odds of knowing they had CKD [OR 2.2; CI 1.16-4.17; p=0.016], of knowing their kidney function [OR 2.25; CI 1.27-3.97; p=0.005], and of knowing their stage of CKD [OR 3.22, CI 1.49-6.92; p<0.003] in comparison to controls.
analyses adjusted for age, sex, race, health literacy, income, number of provider visits, eGFR, and other factors. Eighty percent of respondents indicated that renal care was lacking in North Sumatra and was strongly related to the poor renal biopsy surveillance was low. Use of access blood flow is now being implemented.

Methods: The anonymous survey was created online using SurveyMonkey in May 2011. We distributed the survey to respondents from the University of Michigan (U-M) and U.S. The survey contained 11 questions regarding nephrology and choices of fellowship.

Results: Preliminary results have a 10% response rate with 650 subspecialty fellows responding. Over 75% of fellows did not know that a shortage of nephrologists is estimated to exist by 2020. When asked about the most difficult things they would do in nephrology, 64.5% reported difficulty in nephrology, reasons for not choosing included: 86.8% “felt in love with another field”, 16.2% were concerned about work hours and 16.2% felt that compensation was not adequate. Interestingly, 23.9% would have considered nephrology if it were taught better and 23.9% would have considered nephrology if nephrologists had higher incomes. When asked what they did not like about nephrology, 37.3% said that “dialysis and transplant patients are too complicated to take care of” and 29.1% said “No role model or mentor to guide me towards nephrology.”

Conclusions: The majority of non-renal fellows never considered nephrology as a career choice. Among those who considered a nephrology career, a significant proportion was dissuaded by several factors, including lifestyle, income, patient complexity and lack of role models. Each of these factors can be addressed, but will require the concerted efforts of nephrologists throughout our training community.

FR-OR268
Pediatric Nephrology as a Specialty: Survey of USA Non-Renal Pediatric Fellows

Methods: We identified that renal biopsy service was virtually non-existent in North Sumatra. This partnership was formalized in 2011 by ISN SR program and a team comprising Nephrologist and Advanced trainee spent 1 week in Medan and Banda Ache, assessing the needs of the department and educating clinicians in areas of interest and concern in order to build strong partnership and advance the practice of Nephrology in North Sumatra.

Methods: GCH team compared these protocols or the lack thereof with those established in GCH. An in Medan public hospitals were reviewed directly with the local clinicians. The GCH service and the medical system. During these, diagnostic and treatment protocols present concerns as patients experience hemodialysis.

Results: We identified that renal biopsy service was virtually non-existent in North Sumatra. After deliberation with the renal and pathology departments, renal biopsy protocol was reviewed and encouraged. Ultrasound guided biopsy technique was taught and a post biopsy observation protocol was implemented. Also, prevalence of AV fistulas and access surveillance was low. Use of access blood flow is now being implemented.

Conclusions: Appropriate diagnosis and directed treatment of renal conditions was found to be lacking in North Sumatra and was strongly related to the poor renal biopsy service. This service is being developed while collaborating with Gold Coast Hospital.

FR-OR266
Patient’s Perception of Life on Hemodialysis Scale: Instrument Development and Psychometric Evaluation

Background: Using a grounded theory approach the overall psychosocial and physiological experience of patients with treated with in-center hemodialysis was examined. Three theoretical constructs emerged from the research: Physical Health, Quality of Support, and Psychosocial Experience. A 14 item rating scale entitled the Patient’s Perception of Hemodialysis Scale (PPHS) to measure these concepts.

Methods: A cross-sectional design, data collection was completed in the hemodialysis units in two Canadian provinces (N =236). The purpose of the research was to examine data quality, internal consistency, reliability and validity of the tool.

Results: Limited missing data and good ceiling/floor statistics for each question were observed. Following correlation of items within each scale the number of questions was reduced by 28. Five scales were identified: Physical Health, Emotional Well-being, Psychosocial Distress, Nurse Support, and Physician Support. The Cronbach’s alpha for the new scales was 0.76 indicating good internal consistency.

Conclusions: The PPHS is a valuable instrument for measuring disease specific concerns as patient experience hemodialysis.

Funding: Kidney Foundation of Canada

FR-OR267
US Non-Renal Internal Medicine Subspecialty Fellow Survey on Nephrology

Background: Nephrology is facing a workforce shortage in the U.S. particularly among U.S. medical graduates. We designed a survey to explore the reasons why fellows from other internal medicine subspecialties did not choose nephrology.

Methods: The anonymous survey was created online using SurveyMonkey in May 2011 and distributed to residents in U.S. Non-Renal Internal Medicine Subspecialty Fellows Survey on Nephrology. FR-OR267

Results: Of the 313 responses, 294 (94%) were from pediatric programs; 194 (62%) females; and 236 (75%) graduated from USA schools. When asked about how difficult nephrology was, 194 (60%) respondents said it was difficult or very difficult. For all respondents, the odds of reporting nephrology as difficult among females was 1.3 times higher than among males and 2.3 higher for USA graduates. The odds of reporting nephrology as difficult among fellows in a non-acute specialty is 8% higher than among those in an acute specialty. Acute kidney injury and hypertension were rated with the lowest levels of difficulty whereas renal tubular acidosis, dialysis and transplantation had the highest.

At one point, 15% of respondents considered nephrology. Of those who did not consider nephrology, 80% stated that they were not attracted to the field because they did not like the work hours, patients/families, monetary benefits or pediatrics nephrologists (10% each). When asked what would have made them consider nephrology, the most common responses were: more appealing teaching sessions (30%); higher income (16%); if they had not matched into their current specialty (11%).

Conclusions: Overall, graduates of non-rerenal specialties rated nephrology as a difficult specialty. This perception is stronger among females. Efforts to make pediatric nephrology more appealing to medical students and residents are necessary to address the perception of this specialty and deter gender disparities in the future workforce.

Funding: NIDDK Support, Other NIH Support - T32 DK007569, Private Foundation Support
would not have chosen to do a “pure” inpatient elective. 80% would consider a career in nephrology in group B. 60% of them worked with more than 3 faculty mentors. All trainees felt the need for combined electives and more mentorship by nephrologists.

Conclusions: Combined inpatient and outpatient nephrology elective during medical residency training with increase exposure to several nephrology faculty members seems to create a more positive and enriching experience perhaps leading to a more likelihood of choosing nephrology as a career choice.

FR-OR270

Career Choice Selection and Satisfaction among US Nephrology Fellows

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Nephrology, Hofstra North Shore-LIJ School of Medicine; 2Duke University.

Background: Interest in obtaining training in nephrology continues to decline. We wanted to find out why current fellows selected nephrology and if they are satisfied with this choice. We conducted an online survey with current US nephrology fellows.

Methods: The anonymous survey was created online using SurveyMonkey in May 2011 and was subsequently distributed to US nephrology training program directors.

Results: On initial evaluation, 137 respondents to our survey (10% of total nephrology fellows in the US), of which 55.1% were graduating from their training program. 41.4% were US medical school graduates. 65.8% chose nephrology during either their 1st or 2nd year of residency; 24.3% made this decision during medical school. The most common reasons for choosing nephrology included: interesting subject matter and excellent mentoring in nephrology. Interestingly, only 55.7% of respondents were either extremely or very satisfied with their decision to enter nephrology fellowship. 32.8% were somewhat or slightly satisfied and 11.4% were not satisfied at all with their decision. Statistically, no differences were seen between graduating and non-graduating fellows and US and non-US medical school graduates. The top reasons respondents cited for being extremely or very satisfied with nephrology included: excellent teaching and mentoring by faculty, “variety of stimulating cases” and association between general medicine and nephrology. In contrast, top reasons that respondents were somewhat, slightly or not satisfied with nephrology were: poor income and job potential, and long work hours.

Conclusions: Majority of the current US nephrology fellows selected nephrology as their career choice during medical residency training. Strong mentorship and teaching was highly associated with both the decision to enter nephrology as well as satisfaction during nephrology fellowship. Reasons for less or no satisfaction with nephrology as a career choice included poor job opportunities, poor income potential, long work hours and poor teaching and mentoring by faculty. The nephrology training community should take measures to ensure higher level of satisfaction among US nephrology fellows.

FR-OR271

Using Community Kidney Disease Detection (CKDD) as a Community-Based Platform for Student Leadership Cultivation in Nephrology

In Woo You1, Li-Li Hsiao1,2. 1Harvard College, Cambridge, MA; 2Rensselaer Polytechnic Institute. 1Harvard Medical School, Boston, MA.

Background: Chronic Kidney Disease (CKD) is a major public health problem. Patient awareness of CKD is very low especially among the racial minorities, who often have poorer access to health care and are particularly vulnerable. However, despite the growing significance of CKD, there exists a disproportionate shortage of nephrologists and a diminishing interest in the field of nephrology that raise concerns about adequate renal health care provisioning in the future.

Methods: Here, we present Community Kidney Disease Detection (CKDD), a community outreach program for undergraduate students, as a viable solution for promoting prevention of CKD whilst cultivating student leadership and exposure to nephrology. CKDD holds monthly renal health screenings in underserved areas in Greater Boston to facilitate awareness and early detection of CKD. Such health screenings are entirely run by student volunteers, and hence offer undergraduates an unparalleled hands-on clinical experience and exposure to nephrology. CKDD also provides extensive pre-medical training by hosting workshops attended by nephrologists who teach and certify students for knowledge and command of a range of medical techniques, which include practicing universal precaution, obtaining pertinent medical history, measuring blood pressure, operating glucometers, and conducting urinalysis. At each health screening, students have the opportunity to undertake leadership roles, either as the clinic manager or as student leaders, through which they can develop leadership skills important for the medical career.

Results: Since its establishment in 2007, CKDD has recruited a stable volunteer base of more than 200 undergraduates, of which 78 students have been successfully trained and certified. Our preliminary data showed that the majority of participants found CKDD to be valuable and graded 4.5 out of 5 on CKDD’s services.

Conclusions: Collectively, CKDD serves as an effective platform for undergraduate student’s community outreach for CKD prevention, promoting student leadership, recruitment of interested students, and exposure to nephrology.

Funding: Other NIH Support - sandy fund

FR-OR272

Examining the Role of Education of Nephrology Fellows in Utilization of Peritoneal Dialysis in the United States

Nand K. Wadhwa,1 Catherine R. Messina,2 Nasser M. Hebah.1 Nephrology, Stony Brook University, Stony Brook, NY; 2Preventive Medicine, Stony Brook University, Stony Brook, NY; 1Corporate Home Dialysis, Dialysis Clinic Inc., Nashville, TN.

Background: The 2009 US Renal Data System annual report revealed that peritoneal dialysis (PD) was used by only 7.2% of end stage renal disease (ESRD) patients (vs. use of hemodialysis (HD) by >90% of patients), a decline from a 1964 peak of 15%. We examined whether education of nephrology fellows contributed to the underutilization of PD in the US.

Methods: Self-report questionnaires were administered via Survey Monkey to nephrology fellowship training program directors during October 2010-March 2011. Names and addresses of all US training program directors were obtained from the American Society of Nephrology list-serve. Project protocol was approved by the Stony Brook University IRB.

Results: 55% (78) of program directors responded. Median number of training faculty and patients/fellow were significantly lower for PD training vs. HD training (0.5 vs. 1.2, p<0.001 and 5 vs. 37.5, p<0.001; respectively). Hours of didactic teaching for fellows over their 2 year training period was also lower for PD vs. HD (6 vs. 10 hours, p<0.001). Most nephrology programs provide training in PD and HD; however, PD training was only 20% of total training vs. 80% for HD. Among program directors, 76% indicated that >5% of PD training and 81% indicated that >5% PD patients/fellow were adequate for PD training over a 2 year training period. 87% believed that PD training for fellows was inadequate because of lack of trained faculty in PD and insufficient PD patient population. 67% reported that fellows did not participate in ESRD/chrono kidney disease education to patients.

Conclusions: Current US nephrology fellowship training in PD is inadequate and opinions are significantly to the underutilization of PD. The adequacy of PD training resources needs vigorous discussion and evaluation by the nephrology community.

Funding: Private Foundation Support

FR-OR273

Association of Serum Glycated Albumin and Fructosamine with Outcomes in Incident Hemodialysis Patients

Tariq Shaf1, Stephen M. Sozio,1 Bernard G. Jaar,1 Rulan S. Parakh1, Laura Plantinga,1 Neil R. Powe,1 Josef Coren2,1 Elizabeth Selvin1,3. 1Johns Hopkins University; 2University of Toronto; 3University of California, San Francisco.

Background: Serum total glycated proteins (fructoseamine) and the more specific glycated albumin may be better indicators of glycemia in dialysis patients compared with hemoglobin A1C as they are not affected by red cell turnover. However, the association of glycated albumin and fructosamine with long-term outcomes in dialysis patients is not well-described.

Methods: We measured baseline levels of glycated albumin and fructosamine in 503 incident hemodialysis (HD) participants of the CHOICE Study, a national prospective cohort study. The association of these glycemic markers with mortality and infection-related hospitalizations were determined using Cox proportional hazards and Poisson models, adjusting for potential confounders.

Results: Mean age was 58 years; 64% white; 54% male; and 57% had diabetes. Higher baseline levels of glycated albumin and fructosamine were independently associated with higher risk of death in patients with and without diabetes over a median follow-up of 3.1 years. There was a non-significant trend towards higher risk of infection-related hospitalizations.

Association of Glycemic Markers and Outcomes in 503 Incident HD Patients

<table>
<thead>
<tr>
<th>Diabetes (n=287)</th>
<th>No Diabetes (n=213)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycated Albumin %</td>
<td>24±9%</td>
</tr>
<tr>
<td>Fructosamine %</td>
<td>1.22(1.02-1.45)</td>
</tr>
<tr>
<td>All-cause Mortality*</td>
<td>1.15(0.96-1.47)</td>
</tr>
<tr>
<td>Cardiac Mortality*</td>
<td>1.10(0.92-1.30)</td>
</tr>
<tr>
<td>Infection-Related Hospitalizations*</td>
<td>1.12(0.98-1.28)</td>
</tr>
<tr>
<td>Cardiovascular Mortality*</td>
<td>1.20(0.99-1.56)</td>
</tr>
</tbody>
</table>

Conclusions: In this national cohort, glycemic markers glycated albumin and fructosamine were associated with adverse outcomes in HD patients with and without diabetes. Measurements of these serum glycemic markers may be useful for management of diabetes in dialysis patients.

Funding: NIDDK Support, Private Foundation Support
FR-OR274

Serum Beta-Trace Protein Marks Low Residual Kidney Function and Risk of Mortality in Incident Hemodialysis Patients, Tariq Shaﬁ,1 Rulan S. Parekh,2 Bernard G. Jaar,1 Laura Plantinga,3 Pooja C. Oberai,1 John H. Eckfeldt,4 Andrew S. Levy,2 Neil R. Powe,5 Josef Coresh,6 Johns Hopkins University; 1University of Toronto; 2University of California, San Francisco; 3University of Minnesota; 4Tufs Medical Center.

Background: Residual kidney function (RFK) in dialysis patients is associated with improved survival, but there are no simple methods for assessing RFK. Beta-trace protein (BTP) is a novel endogenous filtration marker of kidney function that is not removed during hemodialysis and may serve as a marker for RFK, similar to serum creatinine in patients not on dialysis.

Methods: We measured serum BTP in 503 participants of the CHOICE Study, a national prospective cohort of incident dialysis patients. Outcomes analyzed were all-cause and cardiovascular disease (CVD) mortality using Cox proportional hazards regression adjusted for demographic (age, race, gender, education, marital status and employment) and clinical and treatment factors (smoking, pulse pressure, body mass index, cause of kidney failure, Index of Coexistent Disease (ICED) score (0-3), cardiovascular disease, congestive heart failure, left ventricular hypertrophy, diabetes, serum albumin).

Results: Serum BTP levels were higher in individuals with no self-reported urine output at baseline (p<0.001) or year 1 (p=0.001). There were 321 deaths (159 from CVD) over 1,814 person-years of follow-up. Higher BTP levels were independently associated with higher risk of all-cause and CVD mortality.

Figure 1: Adjusted Relative Hazard of All-cause Mortality with Serum Beta Trace Protein (BTP) in 503 Incident Hemodialysis Participants of the CHOICE Study.

Conclusions: Serum BTP is a novel marker of residual kidney function and is an independent predictor of death and CVD mortality in hemodialysis patients.

Funding: NIDDK Support.

FR-OR275

Effects of Six Versus Three Times Per Week Hemodialysis on Physical Performance, Health and Functioning: Frequent Hemodialysis Network (FHN) Trials, Yoshio N. Hall,1 Brett Larive,2 Patricia Lynn Painter,1 George A. Kaysen,1 Robert M. Lindsay,1 Allen R. Nissenson,4 Mark L. Unruh,1 Michael V. Rocchio,1 Glenn M. Chertow,3 The FHN Trial Group.1 5 6 7 8 9 10 University of Washington; 2Cleveland Clinic; 3University of Minnesota; 4UC Davis; 5University of Western Ontario; 6DaVita Inc.; 7University of Pittsburgh; 8Wake Forest University; 9Stanford University; 10NIDDK, Bethesda, MD.

Background: Relatively little is known about whether and to what extent the frequency of hemodialysis may influence the substantial disability and functional dependence of patients with end-stage renal disease.

Methods: We examined changes in physical performance, health and functioning among subjects randomized to frequent (six times per week) or conventional hemodialysis (three times per week) in both the Frequent Hemodialysis Network Daily (r=245) and Nocturnal (n=87) Trials. The primary outcomes were adjusted change in scores over 12 months on the short physical performance battery (SPPB), RAND 36-item self-reported physical health composite (PHI) and physical functioning subscale (PF).

Results: In the Daily Trial, subjects randomized to frequent as compared with conventional in-center hemodialysis experienced no significant change in SPPB (adjusted mean change of -0.14 ± 0.19 vs -0.39 ± 0.21, P=0.38), but experienced clinically significant improvements in PHI (3.4 ± 0.8 vs 2.0 ± 0.8, P=0.004) and in PF (4.8 ± 2.1 vs -3.3 ± 2.2, P=0.081). The effects of frequent in-center hemodialysis did not appreciably differ according to sex, race or diabetic status. In the Nocturnal Trial, there were no significant differences among subjects randomized to frequent as compared with conventional hemodialysis in SPPB (adjusted mean change of -0.89 ± 0.44 vs -0.44 ± 0.43, P=0.45), PHI (2.8 ± 1.5 vs 1.8 ± 1.5, P=0.64), or PF (3.1 ± 3.5 vs 1.0 ± 3.5, P=0.41).

Conclusions: Frequent in-center hemodialysis, as compared with conventional in-center hemodialysis, improved self-reported physical health and functioning but had no significant effect on objective physical performance.


FR-OR276


Background: The prevalence of obesity is increasing in incident dialysis patients. There is limited information on the association between longitudinal changes in body mass index (BMI) and mortality in incident dialysis patients.

Methods: The study included all adult patients from the ANZDATA Registry who started hemodialysis (HD) or peritoneal dialysis (PD) between 2001 and 2008 with ≥ 2 measurements of dry weight and ≥ 6-month follow-up. Annualized change in BMI was calculated using a least-squares regression slope. Patients were divided into quintiles of slope of annual BMI change (Q1 to Q5). BMI change was classified according to modality in use at 90 days. Survival analysis was done using Cox proportional hazards model.

Results: A total of 16,869 incident dialysis patients were included in the study (mean age 60 yrs, 57.6% men, 29.8% obese, 80% HD). The median (interquartile range) slopes of annual BMI change in Q1, Q2, Q3, Q4 and Q5 were -2.3 [-3.5 to -1.6] kg/m²/yr, -0.7 [-0.9 to -0.5] kg/m²/yr, -0.2 [-0.3 to -0.5] kg/m²/yr, 0.3 [0.2 to 0.4] kg/m²/yr and 1.9 [0.9 to 1.9] kg/m²/yr, respectively. There was a significant interaction between the dialysis modality and the slope of annual BMI change. Over a mean follow up of 2.8 years, 5,896 patients died. Compared to Q3, the adjusted hazard ratio (95% CI) for mortality in Q1, Q2, Q4 and Q5 was 3.97 (4.34 - 4.58), 1.52 (1.35 - 1.72), 1.09 (0.95 - 1.26) and 2.41 (1.3 - 2.72), respectively.

Conclusions: A U-shaped association was present between the slope of annual BMI change and mortality, suggesting both excessive weight loss and gain over time may be associated with increased mortality in incident dialysis patients.

FR-OR277

Limitations in Activities of Daily Living Independently Influences One-Year Survival of Hemodialysis Patients, Eiichiro Kanda,1 Janna O. Krisher,2 William M. McClellan.3 1Tokyo Kyosai Hospital, Japan; 2ESRD Network 6; 3Emory University.

Background: We examined the independent contribution of activities of daily living (ADL) limitations with one-year mortality among incident hemodialysis patients.

Methods: We enrolled 6217 incident hemodialysis patients and followed up these individuals for up to one year. ADL limitations were assessed using data from the Centers for Medicare & Medicaid Services (CMS) 2728 Form including inability to ambulate, inability to transfer from bed to chair, and the need for assistance with daily activities. The patients were categorized into five groups according to their ADL limitations.

ADL Groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Inability to ambulate</th>
<th>Inability to transfer</th>
<th>Needs assistance with daily activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Group 2</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Group 3</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Group 4</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Group 5</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Mortality was evaluated using a fully adjusted Cox proportional hazard model.

Results: The mean age was 61.3 years; 47.7% were female; 46.3% had diabetes. Group 1 consisted of 5414 patients (87.1% of sample); Group 2, 363 (5.8%); Group 3, 97 (1.6%); Group 4, 188 (3.0%); Group 5, 150 (2.4%). ADL limitations correlated with mortality: no ambulation, hazard ratio (HR) 2.12 (95% confidence interval 1.77-2.53); unable to transfer 2.13 (1.64-2.76); need for assistance with daily activities, 1.58 (1.34-1.85). Patients in Group 5 had the worst outcomes.
FR-OR278

Influence of Dialysis Unit Affiliation on Involuntary Discharges (IVD)

Brian T. Chu,1 Abey K. Thomas,2 Senthil P. Ramaiyah,3 Carol Lyden,1 Chaim Charytan,2 George N. Coritsidis.1

Elmhurst, NY; 2Nephrology, New York Hospital Medical Center of Queens, Flushing, NY; 3ESRD Network of New York, IPRO, Lake Success, NY.

Background: Dialysis facilities (DF) may need to involuntarily discharge patients under certain circumstances set forth in Medicare’s conditions of coverage. Minimal literature has been published on IVD. We wanted to analyze IVD rates by the different types of DF and by insurance status of patients as this may have significant implications in the bundling payment era.

Methods: We collected IVD data reported to ESRD Network 2 (EN2) between July 2006 and March 2011. We used the annual average EN2 population in that period to calculate IVD incidence rate for various types of DF. We looked at facility characteristics like profit status, large dialysis organization (LDO) affiliation and hospital/nonhospital based DF. We also looked at the initial insurance status of IVDs. Data was analyzed by chi square test to look for statistical significance.

Results:

<table>
<thead>
<tr>
<th>Type of DF</th>
<th>Average IVD Incidence</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital Based</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>Non-Hospital Based</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>LDO</td>
<td>1.09</td>
<td></td>
</tr>
<tr>
<td>Non-Profit</td>
<td>1.06</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Interestingly most IVDs are reported to be due to behavioral reasons with financial concerns. Insurance status was similar in IVD patients regardless of the unit affiliations.

FR-OR280

Higher Weekend Mortality in Teaching Hospitals in End Stage Renal Disease Patients

Ankit Sakhuja, Gagan Kumar, Aaron T. Dall, Rahul S. Nanchal, Puneet Sood. Medicine, Medical College of Wisconsin, Milwaukee, WI.

Background: We have previously demonstrated that patients with End Stage Renal Disease (ESRD) admitted over weekends have worse outcomes than those admitted over weekdays. In this study we analyzed if the outcomes of patients admitted to teaching hospitals on weekends were different than those admitted to non-teaching hospitals.

Methods: We analyzed the data from years 2004-2008 using the Nationwide Inpatient Sample (NIS) database. Adult patients (age ≥18 years) with ESRD were included. The primary outcome measured was all cause in-hospital mortality. Chi square test was used to compare the variables and logisitc regression was used to obtain adjusted Mortality odds ratios (OR) in teaching and non-teaching hospitals. We then compared the two Mortality ORs by using Cochran-Mantel-Haenszel (CMH) test. Alpha was set at 0.05. The teaching hospital status was defined as hospitals with either an American Medical Association approved residency program, or those that are members of the Council of Teaching Hospitals, or those that have full-time equivalent interns and residents to patient ratio of 0.25 or higher.

Results: Of total 756,987 admissions in ESRD patients, 19.5% (147,730) were admitted over a weekend. A total of 49.9% (73,684) of weekend admissions were admitted to teaching hospitals. The unadjusted all cause mortality was 6.3% on weekends in comparison to 5.4% on weekdays (p < 0.001). On adjusting for patient and hospital characteristics the overall Mortality OR for weekend admissions was 1.15 (95% CI: 1.12-1.18). On further stratifying the overall weekend mortality into teaching and non-teaching hospitals, Mortality OR in teaching hospitals was 1.22 (95% CI: 1.17-1.26) in comparison to 1.09 (95% CI: 1.06-1.13) in non-teaching hospitals. The difference between the two Mortality ORs was statistically significant (CMH p<0.001).

Conclusions: ESRD patients admitted over weekends have a higher mortality. This weekend mortality is significantly higher in teaching hospitals as compared to non-teaching hospitals. Further research and effort is needed to elucidate the reasons for this difference.

FR-OR281

Treatment of Hepatitis C in Hemodialysis Patients Is Associated with Markedly Decreased Mortality, but Is Rarely Prescribed

David A. Goodkin,1 Brian Bieber,2 Bruce M. Robinson,3 Michel Y. Jadoul.1 Arbor Research Collaborative for Health; 2U. of Michigan; 3Univ. Cathol. de Louvain.

Background: Hepatitis C infection is common among HD patients internationally, with a prevalence exceeding 9%. Data regarding antiviral treatment of HCV among HD patients are very limited.

Methods: The DOPPS is a cohort study of HD patients in Australia, Belgium, Canada, France, Germany, Italy, Japan, New Zealand, Spain, Sweden, the UK, and the US. We examined data from 43,828 patients, collected between 1996 and 2010. Patients were defined as HCV+ if they had the diagnosis in their medical record or if they tested positive for HCV antibody. Patients with prescriptions for an interferon or ribavirin were classified as “treated” for HCV. Cox regression was used to compare survival between treated HCV+ patients, and untreated HCV+ patients, stratified by country and adjusted for case-mix (age, sex, vintage, US or France, and 14 comorbidity conditions) to match the treated patients.

Results: Out of a total of 4,494 HCV+ patients, only 35 (0.8%) were treated with antiviral medication. Median duration of follow-up was 1.4 years. The treated patients were younger, less often male, and had lower prevalence of CAD, cerebrovascular disease, CHF, HT, and PVD. None of the treated patients died. 861 of the 4,459 untreated HCV+ patients (19%) died. Mortality risk was significantly worse for untreated patients, even after adjustment for case-mix (P = 0.01).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.
Conclusions: HCV infection among HD patients is very rarely treated internationally. Antiviral therapy is associated with significantly lower mortality risk. This first study of mortality among treated versus untreated HCV+ HD patients suggests that HD patient survival may be increased if HCV+ patients are treated with antiviral therapy.

**FR-OR282**

**Higher Serum Phosphorous Is Associated with Increased Risk for End Stage Renal Disease across All Stages of Chronic Kidney Disease**

Simran K. Bhandari,1 John J. Sim,1 Antoine C. Abcar,1 Ning Smith,2 Joanie Chung,2 Dean A. Kujubu,1 Scott A. Rasgon,1 Kamyar Kalantar-Zadeh.1 1Nephrology and Hypertension, KPSC LAMC, Los Angeles, CA; 2Research and Evaluations, KPSC, Pasadena, CA; 3Nephrology, Harbor UCLA, Torrance, CA.

**Background:** We sought to determine whether higher levels of serum phosphorus (Phos) increased risk for end stage renal disease (ESRD) in a large ethnically diverse population of primarily non-CKD subjects.

**Methods:** Retrospective cohort study of persons age ≥18 yrs during 1/1/1999-12/31/2008 with ≥1 Phos and 1 yr min follow up. Subjects categorized into population-based quartiles by time dependent average Phos: 1.9-3.1, 3.1-3.5, 3.5-3.9, 3.9-5.7. The association between Phos quartiles and incident ESRD defined as need for dialysis, transplant, or death. Cox survival curve and Cox proportional hazard modeling to calculate hazard ratios (HR) after adjusting for age, gender, race, HTN, DM, eGFR, and Charlson morbidity index.

**Results:** 209,865 subjects were evaluated. Mean eGFR was 82; 24% eGFR > 90, 21% eGFR 60-89, and 13% eGFR < 60. Compared to the lowest Phos quartile adjusted HR for ESRD was greater across the 3 higher quartiles.

<table>
<thead>
<tr>
<th>Phos Quartile</th>
<th>HR for ESRD (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤1.9</td>
<td>1</td>
</tr>
<tr>
<td>1.9-3.1</td>
<td>1.47 (1.20-1.79)</td>
</tr>
<tr>
<td>3.1-3.5</td>
<td>2.39 (2.41-3.45)</td>
</tr>
<tr>
<td>3.5-5.7</td>
<td>13.58 (11.53-16.01)</td>
</tr>
</tbody>
</table>

Linear analysis for ESRD found HR of 1.7 for every 0.5mg/dL increase in Phos. Survival curve also demonstrated ESRD free survival with lower serum Phos.

**Conclusions:** In a large ethnically diverse population primarily without CKD, higher Phos was associated with greater rates of incident ESRD.

**FR-OR283**

**Effects of Rituximab in 100 Consecutive Patients with Idiopathic Membranous Nephropathy (IMN) and Nephrotic Syndrome Despite RAS Inhibition**

Piero Ruggenenti, Paolo Cravedi, Maddalena Marasa,1 Barbara Ruggiero, Antonietta Chianca, Giuseppe Remuzzi. Mario Negri Institute for Pharmacological Research, Bergamo, Italy.

**Background:** B-cell depleting therapy has been suggested to reduce proteinuria in patients with IMN and nephrotic syndrome.

**Methods:** We prospectively studied over a median (range) follow-up of 30.5 (11-115) months, 100 consecutive patients with biopsy-proven IMN and persistent proteinuria >3.5g/24h despite ≥6 months RAS inhibition therapy, who received rituximab therapy at our center.

**Baseline patients’ characteristics**

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Male gender (n)</th>
<th>Serum creatinine (mg/dL)</th>
<th>Serum albumin (g/dL)</th>
<th>Total Cholesterol (mg/dL)</th>
<th>Proteinuria (g/24h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>51.3±16.0</td>
<td>69 (69%)</td>
<td>1.4±0.7</td>
<td>2.2±0.6</td>
<td>274±172.3</td>
<td>10.6±6.4</td>
</tr>
</tbody>
</table>

Proteinuria was the primary outcome.

**Results:** Mean±SD proteinuria significantly decreased from 10.6±6.4 to 4.7±0.5, 3.0±0.5, and 2.0±0.4 g/24h at 1, 2, and 3 years after rituximab, respectively (P<0.05 for all comparisons with Bonferroni adjustment, in parallel with normalization of edema, hypoalbuminemia and hypercholesterolemia.

**Conclusions:** Rituximab safely induced sustained remission in a large cohort of IMN patients with severe nephrotic syndrome. The long-term risk/benefit profile of this selective therapy seems much more favorable than that of commonly used immunosuppressive regimens.

**Funding:** Private Foundation Support

**FR-OR284**

**Phospholipase A2 Receptor Autoantibodies (PLA2R-AB) and Increased PLA2R Expression in Glomeruli Discriminate Primary from Secondary Membranous Nephropathy (MN)**

Elion Hoehx,1 Gunther Zahner,1 Joachim Velden,2 Kai Fechner,1 Gesa Stege,1 Sigrid Harendza,1 Rolf A. Stahl,1 Udo Helmchen.1 1III. Medizinische Klinik, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; 2Nierenregister Hamburg, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany; 3Institute of Experimental Immunology, Euroimmun AG, Lübeck, Germany.

**Background:** M-Type PLA2R is a target antigen in MN. Autoantibodies against PLA2R are detected in up to 80% of patients with MN. Since PLA2R-AB in the serum can become positive and be negative, PLA2R-AB detection does probably not allow to differentiate in every single patient between primary and secondary MN.

**Methods:** In order to achieve higher specificity in the diagnosis of patients with MN, we performed a prospective study. Between November 2009 and April 2011, in 81 consecutive patients with the histologic diagnosis MN, PLA2R-AB were measured in the serum with an indirect immunofluorescence test (Hoxha et al. NDT in press). In 64 of these patients, in addition to standard histology, kidney biopsies were immunohistochemically stained for PLA2R (Atlas Antibodies) and IgG4 (Binding Site).

**Results:** At 3 years, 94.4% of patients reached complete (47.2%) or partial (47.2%) remission defined as proteinuria <0.3g/24h, or <3.5g (with ≥50% reduction vs. baseline) in 2 consecutive evaluations, respectively. Six patients with baseline creatinine 19.0±6.9 mg/dL reached ESRD over 35 (7-68) months. In the remaining 94 patients serum creatinine was stable from basal (1.4±0.7 mg/dL) to final visit (1.6±1.4 mg/dL). Circulating B cells were fully depleted shortly after rituximab and recovered to basal in 9-12 months. Treatment was well tolerated.

**Conclusions:** Rituximab safely induced sustained remission in a large cohort of IMN patients with severe nephrotic syndrome. The long-term risk/benefit profile of this selective therapy seems much more favorable than that of commonly used immunosuppressive regimens.

**Funding:** Private Foundation Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

68A
Results: The mean age of the patients, (60 males, 21 females) was 55.9 ± 15.3 years. In 53 patients (65%) serum was positive for PLA2R-AB: All these patients were positive for IgG4-PLA2R. Immunohistochemistry for PLA2R was only faintly positive in normal renal biopsies and biopsies from patients with minimal change disease (n=11). In 47 patients PLA2R expression was strongly increased in glomeruli, 45 (96%) of those patients were positive for PLA2R-AB in serum, 44 (94%) stained positive for IgG4. In all 13 PLA2R-AB negative patients (which had a secondary diagnosis of MN such as SLE, Hep, B, malignancies, NSAID-related MN) PLA2R and IgG4 staining in renal biopsies were negative.

Conclusions: Measurement of PLA2R-AB in the serum, in combination with PLA2R and IgG4 staining of renal biopsies can discriminate between primary and secondary MN and may become a standard approach in the diagnosis of MN.

Funding: Government Support - Non-U.S.

FR-OR285
Abstract Withdrawn

FR-OR286
Integrative Antibiotics Identifies Potential Novel Autoantibody Biomarkers for IgA Nephropathy
Tara Sijde1, Sung Hoon Woo, Purvesh Khatri, Li Li, Minnie Sarwal, Richard A. Lafayette.
1Department of Pediatrics-Nephrology, Hammersmith Hospital, London, United Kingdom; 2Maternal and Fetal Research Unit, King’s College London, London, United Kingdom.

Background: Immunoglobulin A (IgA) often deposits with Immunoglobulin A in the glomerular mesangium of patients with IgA nephropathy (IgAN) but its clinical relevance is unclear. Autoantibody (autoAb) biomarkers to detect and track progression of IgAN is an unmet need.

Methods: Protein microarrays were used to evaluate IgG autoAbs in the serum of IgAN patients (n=22) and controls (n=10). Clinical parameters were collected on all patients over 5 years. Bioinformatics analysis was performed to choose the targets for further validation by immunohistochemistry (IHC). Significance of autoAbs in IgAN was determined by regression analysis using clinical parameters.

Results: A total of 117 (1.4%) unique antibodies were found to be increased in IgAN. IgAN specific autoAb (~50%) were mounted against proteins predominantly expressed in glomeruli and tubules. The presence of selected antigens in kidney tissue was verified by IHC. ROC analysis demonstrated that IgG autoAbs levels (MATN2, UBE2W, DDX17, PRKDI) might be used in combination with 24 hr proteinuria to improve prediction of the progression of IgAN (AUC = 0.857, p=0.02).

ROC curve analysis based on logistic regression model using only autoAbs (MATN2, UBE2W, DDX17, PRKDI) is shown in Figure 1 (A) and autoAbs and 24hr proteinuria (AUC=0.898; p=0.0008) is shown in Figure 1 (B).

Conclusions: First analysis of the repertoire of autoAbs in IgAN identified novel, immunogenic protein targets, expressed in the kidney glomeruli and tubules that may be relevant in the pathogenesis and progression of IgAN. Our data suggests that using IgG autoAbs in addition to 24hr proteinuria could improve the ability to predict the progression of disease.

FR-OR287
IgA Nephropathy with Minimal Change like Lesion: Clinicopathologic Features and Response to the Therapy of Steroid
Xiran Zhang, Qingwen Wang, Hui-Ping Chen, Zhi-Hong Liu. Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China.

Background: Immunoglobulin A nephropathy (IgAN) is the most common glomerular nephritis in the world. Some patients of IgAN manifest minimal change like lesions which have not been well characterized. The purpose of this study was to investigate the clinicopathologic features and response to prednisone treatment of the patients of IgAN with minimal change like lesion.

Methods: 61 patients [46 males and 15 females with an average age of 23.9±28.65] of biopsy-proven IgAN with minimal change like lesions were enrolled in this retrospective study.

Results: All patients had edema, and infection is the major cause in 15 patients (24.6%). Nephritic syndrome was the primary clinical manifestation. 14 patients (23.0%) had AKI, 5 patients (8.2%) had hypertension and 14 patients (23.0%) had hematuria. The elevation of urine n-acetylglucosaminidase (NAG) and urine retinol binding protein (RBP) were observed in the majority of the patients (78.7% and 75.4% respectively). Glomerular changes were minimal with deposition of immunoglobulin A alone (14.3%) or together with immunoglobulin M (61.9%) in mesangial region. 80.3% of the patients manifested acute tubular injury, while 65.6% had interstitial injury. During the follow-up of 46.00±13.93 months (24–70 months), 91.8% of the patients responded to the treatment of prednisone, but 76.8% of which relapsed. Compared with the responders, the non-responders had a higher incidence of hematuria and manifested more severe (P<0.01), and their hypoproteinemias was not as evident as the former ones (P<0.05). Besides, tubular atrophy, interstitial fibrosis and hyaline degeneration of arteriole were seen more often in non-responders (P<0.01). Until the last follow-up, 59 patients (96.7%) still had normal renal function as evidenced by normal serum creatinine.

Conclusions: In conclusion, IgAN with minimal change like lesion was similar to minimal change disease both in clinical manifestation and pathologic features. Most patients of the patients were remitted with steroid treatment, and had good prognosis.

FR-OR288
Tacrolimus Is an Effective Steroid Sparing Treatment for Lupus Nephritis in Pregnancy
Alexander Paul Wardle, Kate Bramham, Catherine Nelson-Piercy, Liz Lightstone. 1Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom; 2Maternal and Fetal Research Unit, King’s College London, London, United Kingdom; 3Obstetric Medicine, Guy’s & St Thomas’ Hospitals NHS Foundation Trust, London, United Kingdom.

Background: Treatment options for pregnant women with lupus nephritis (LN) were previously limited to steroids +/- azathioprine (aza). Steroids in pregnancy are linked with increased pre-term delivery, infection & gestational diabetes. In non-pregnant women we have successfully treated LN with tacrolimus (Tac) in steroid-sparing regimens. Safety of Tac in pregnancy has been established in transplant patients. We report our use of Tac both as maintenance & as an alternative treatment for LN flare in pregnancy, where steroid avoidance was desirable.

Methods: 5 pregnant women taking Tac or aza added for control of LN. Tac dosed to trough levels of 5-8ng/ml. Remission- Complete (CR): urine PCR <50mg/mmol; Partial (PR): PCR non-nephrotic = <50% fall

Results:

Renal & pregnancy outcomes

<table>
<thead>
<tr>
<th>Class</th>
<th>LN</th>
<th>Immunosuppression</th>
<th>Gestation at flare</th>
<th>Urine PCR</th>
<th>Pre preg</th>
<th>Gestation (wks)</th>
<th>Birth weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>V</td>
<td>Started Tac/MPX2/Aza</td>
<td>10/40</td>
<td>10/40/600, PR 23/40, CR</td>
<td>1/12 post</td>
<td>Pre 1850 CR by 23/40</td>
<td>38 3320</td>
</tr>
<tr>
<td>2</td>
<td>IV-G (A/C)</td>
<td>Tac maintained</td>
<td>No flare</td>
<td>-</td>
<td>-</td>
<td>2 37 2500</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>IV-G (A/C)</td>
<td>Tac maintained</td>
<td>No flare</td>
<td>-</td>
<td>-</td>
<td>2 37 2780</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>IV</td>
<td>Aza, Tac started</td>
<td>8/40</td>
<td>8/40</td>
<td>37/40 663, CR 1-yr post</td>
<td>-</td>
<td>30 1100</td>
</tr>
<tr>
<td>5</td>
<td>IV-G (C)</td>
<td>Pred/Aza increased, Tac started</td>
<td>8/40</td>
<td>8/40</td>
<td>37/40 663, PR 4/12 post</td>
<td>-</td>
<td>35 2910</td>
</tr>
</tbody>
</table>

Delivery: all spontaneous labour; 1 emergency C-section; all live births. In both cases with CKD stage 3, creatinine rose during pregnancy; by 3/12 post partum case 5 eGFR returned to baseline but case 4 had persistent reduction.

Conclusions: To our knowledge, this is the first case series reporting Tac enabling induction of disease remission with steroid sparing in LN in pregnancy. Tac was well tolerated, avoided steroid associated complications and resulted in a reduction or stabilisation of proteinuria, even in women with CKD 3. We propose that it may be considered as an adjuvant or alternative therapy to steroids for pregnant women with LN for maintenance or treatment of disease flare.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
FR-OR298

Tacrolimus as an Induction Agent for Proiferative Lupus Nephritis Is as Effective as Cyclophosphamide

Sanjay Gupta,1 Sheel Bhadra Jain,2 Uma Kumar,2 Amit K. Dinda.1 1Nephrology, AIIMS, India; 2Medicine, AIIMS, India.

Background: Conventional therapy for lupus nephritis has limitations, cyclophosphamide (cyclo) carries the risk of severe infections, ovarian failure and malignancies mycophenolate has risk of infections. Less toxic treatment with at least equal efficacy is necessary. Tacrolimus (tac) target B cells indirectly by interfering with T cell help by inhibiting IL-6, IL-10 production. The aim of study was to assess the efficacy and safety of Tacrolimus as induction therapy in class III and IV lupus nephritis and compare it with cyclo.

Methods: In an open label non randomized control trial involving 40 female patients with biopsy proven class III and IV class lupus nephritis, we compared the Tac in combination with steroid as an induction therapy with cyclo monthly pulses (NIH protocol).

Patients were treated with Tacrolimus, starting at 0.1 mg/kg/day in divided doses maintaining trough levels 5-8 mg/ml and prednisolone at 0.6 mg/kg/day for 6 weeks and then tapered. Response rate and adverse effects were compared with 20 consecutive patients treated with IV cyclo.

Results: The tac group and cyclo group had similar baseline characteristics. Histopathology was similar, in tac group class III in 10, IV in 9 & III+IV in 1 and in cyclo group class III in 6, IV in 13 & IV+V in 1. After 6 months in Tac group, complete remission was achieved in 11(55%), partial response in 4 (20%) and no response in 5 (25%) while it was 8(40%), 9 (45%) and 3 (15%) in Cyclo respectively (p=0.23). Proteinuria decreased from 2.2±0.8 to 0.8±0.5 g/day in tac group & 2.8±2.6 to 0.7±1.2 g/day in Cyclo group(p=0.8) & S albumin increased from 3.1±0.7 to 4.1±0.5 g/dl in tac group & 2.8±0.8 to 0.7±1 g/dl in cyclo group(p=0.46). In both groups there was significant improvement in C3 level and decline in dsDNA (IU/ml) titre from baseline. There were more infections in Cyclo group, in tac group(p=.46). In both groups there was significant improvement in C3 level and decline in dsDNA (IU/ml) titre from baseline.

Conclusions: Induction with Tac is as effective as cyclo for proliferative lupus nephritis. It is an option for females desiring of preserving ovarian function or not tolerating Cyclo.
FR-OR293
Mechanism of Selective Proteinuria in Minimal Change Nephrotic Syndrome
Satoshi Kinugasa,1 Akihito Tojo,1 Tatsuo Sakai,2 Toshiro Fujita.1
1Department of Internal Medicine, University of Tokyo, Japan; 2Department of Anatomy and Life Structure, Juntendo University School of Medicine, Tokyo, Japan.

Background: As a mechanism of selective proteinuria in minimal change nephrotic syndrome, we have previously demonstrated albumin transport through the podocyte cytoskeleton. In this study we investigated the time course of podocyte albumin transport by confocal laser-scanning microscopy, utilizing GFP-transgenic rats in which a minimal change nephrotic syndrome model was induced, and examined the role of FcRn as albumin receptor in podocytes.

Methods: A minimal change nephrotic syndrome model was induced in GFP-transgenic rats by purumycin aminonucleoside (PAN). After injection of Evans blue (EB)-labeled human serum albumin, kidneys were observed in vivo by real-time confocal microscopy. Subsequently, we observed the localization of EB-labeled human serum albumin in vitro by electron microscopy. We also administrated Neu5AC (0.2 g/kg/day) in drinking water. Results: PAN nephrotic rats presented with selective proteinuria, which was confirmed by urinary SD-SAGE, and the urine IgA/Albumin ratio, an index of selectivity, was comparable with controls. In PAN nephrotic rats, fluorescence of EB was absent in the proximal tubular lumen until 5 minutes after injection, and increased at 15 minutes in PAN nephrotic rats, whereas it was almost negative in controls, indicating that a large proportion of albumin is transported slowly through podocytes. The cytoplasm of some podocytes appeared yellow in PAN nephrotic rats, due to merging of the green fluorescence signals of GFP expressed in podocytes and the red fluorescence of EB-labeled albumin, which was not observed in controls. Immunoprecipitation analysis showed an increase in Evans blue-labeled human serum albumin bound to FcRn. PAN nephrotic rats treated with Neu5AC showed significantly increased proteinuria compared with untreated rats (40.5±6.3 vs. 22.1±4.2 mg/mgCr, p<0.05), and podocyte uptake of EB-labeled albumin.

Conclusions: Podocyte albumin transport by FcRn may contribute to the selective albuinuria in minimal change nephrotic syndrome.

FR-OR294
Dynamin Oligomerization Cycle Directly Regulates the Actin Cytoskeleton and Alleviates Proteinuria
Changkyu Gu, Sanja Sever. Nephrology, Massachusetts General Hospital, Charlestown, MA.

Background: Dynamin oligomerization into rings or spirals/helices has been linked to membrane tubulation and fission of clathrin coated vesicles at the plasma membrane. Recently, we implicated dynamin oligomerization in the regulation of the actin cytoskeleton. We now show that dynamin oligomerization in live cells is dependent on dynamin-actin interactions and GTP-binding. To elucidate the role of dynamin oligomerization in cells we turned to a small molecule that alters the dynamin oligomerization cycle by a novel mechanism. We identify Bis-T-23 as an archetypical small molecule “ring stabilizer” that promotes dynamin oligomerization into rings in vitro and in cells, promoting an activated conformation and reducing disassembly rates. In podocytes, Bis-T-23 promoted formation of focal adhesions and stress fibers independently of the RhoA pathway. Thus dynamin oligomerization directly stimulates actin assembly by a novel mechanism. In contrast, dynasore, 34-2, a dynamin inhibitor lacking ring stabilizer activity, exhibited opposing cellular effects, ruling out endocytosis as an underlying mechanism for actin dynamics. Bis-T-23 protected against cathepsin L-driven proteolysis of dynamin in vitro and in podocytes. This in turn resulted in wild type organization of the actin cytoskeleton and protected podocytes from lipopolysaccharide (LPS)-induced apoptosis. Administration of Bis-T-23 in two different rodent models of proteinuric kidney disease significantly reduced proteinuria. Our study reveals that oligomerised dynamin directly regulates the actin cytoskeleton and identifies dynamin ring stabilizers as a potential novel therapeutic approach for proteinuria.

FR-OR295
Hyposialylation of Glycoproteins in the UDP-N-Acetylglucosamine-2-Epimerase/N-Acetylmannosamine Kinase (GNE) Point Mutant Mice Gives Rise to a Renal Impairment That Is Prevented by Sialic Acid Administration
Mitsutoshi Ito,1 Tomoya Asaka,2 Kazuaki Sugihara,1 Toru Yoshihara,1,2 Takashi Wada,3 Masahide Asano.1
1Advanced Science Research Center, Kanazawa University, Kanazawa, Japan; 2Nanoflan National Hospital, Nanan, Japan; 3Research Center for Child Mental Development, Kanazawa University, Kanazawa, Japan; 4Department of Laboratory Medicine, Kanazawa University School of Medicine, Kanazawa, Japan.

Background: Sialic acid is one of acidic carbohydrates and well known to modify non-reducing terminal carbohydrates on glycoproteins and glycolipids. It is reported that mutations in the key enzyme of sialic acid biosynthesis, GNE, result in distal myopathy with rimmed vacuole (DMRV) in human. In this study, we generated mice with a point mutation (V572L) in its kinase domain.

Methods: Kidneys of GNE homozygous mutant mice (mt-mice) were investigated using biochemical, histopathological, and glycobiological analysis. N-acetylneuraminic acid (Neu5AC) treatment was carried out as follows. Before birth, pregnant heterozygous mutant mice were administrated with Neu5AC (1 g/kg/day) in drinking water. After birth, babies as well as mothers were administrated with Neu5AC (0.2 g/kg/day) in drinking water.

Results: Unexpectedly apparent myopathic features were not observed in mt-mice. However, mt-mice showed renal impairment with proteinuria and high level of serum creatin C. Furthermore, abnormal structure of podocyte foot processes, hyperplastic glomeruli and cystic degeneration were observed in the mt-mice. Glycan analysis using several lectins showed that hyposialylation of renal tubules and renal corpuscles were observed, particularly, hyposialylation of a major podocyte sialoglycoprotein, podocalyxin, was observed in the mt-mice. Administration of Neu5AC to the mt-mice from embryonic stage significantly suppressed proteinuria and improved renal pathology with recovered sialylation of renal corpuscles.

Conclusions: These findings suggest that the proteinemic glomerular disease in the mt-mice was caused by impaired glomerular filtration due to hyposialylation of podocyte glycoproteins. Moreover, the renal disease in the mt-mice was prevented by administration of Neu5AC from embryonic stage.

Supported by: Government Support - Non-U.S.

FR-OR296
Single Injection of Stem Cells Derived from Amniotic Fluid Slows down the Progression of Kidney Failure in a Mouse Model of Alport Syndrome
Laura Perla,1 Sargsi Bedrakyan,2 Stefano Da Sacco,2,3 Antigik Petrosyan,2 Iiron Ben,1 Nekhta V. Lemley,1 Roger E. De Filippis.1 1Urology, Children’s Hospital of Los Angeles, Los Angeles, CA; 2Nephrology, Children’s Hospital of Los Angeles, Los Angeles, CA.

Background: Alport Syndrome (AS) is a useful model for studying chronic kidney disease (CKD). This hereditary form of glomerulonephritis leads to interstitial fibrosis and eventual loss of renal function. Stem-cell based therapies may provide alternative therapeutic opportunities to treat CKD. Amniotic fluid stem cells (AFSC) present pluripotential properties and are re-progenitors of some models of acute kidney injury. Herein, we investigate the potential of AFSC as a new approach to current therapies for CKD.

Methods: In this study we have administered AFSC in a murine model of AS (Col4α5−/−) before the onset of proteinuria. Mice were sacrificed at 5 days, 1 and 2 months post treatment and kidneys were harvested for molecular and histological analysis. Kidney function was assessed with serum creatinine measurements.

Results: AFSC infusion resulted in delayed renal fibrosis and prolonged animal survival, slower progression of glomerulosclerosis and ameliorated kidney function. Furthermore, immunohistochemistry exhibited changes in the expression of many genes related to extracellular matrix, such as Coll1A1, Fibrillin, Vcan, Itgb5, Coll3A1 and Coll5A2 appear to also be involved. Additionally, injected AFSC are capable of downregulating MMP2 and MMP9 enzyme activity, critical modulators in GBM remodelling during disease progression.

Conclusions: Although AFSC does not entirely reverse kidney disease, taken together our finding suggest that a single AFSC treatment delays progression of CKD and significantly improves survival in treated AS mice.

Funding: Private Foundation Support

FR-OR297
Role of Endothelin-1 in Podocyte-to-Endothelial Cross-Talk in Podocytopenias Like S. Dachn,1 Taoran Zhang, Gabriella Casalana, Franz Fenninger, Erwin P. Bottinger. Medicine, Nephrology, Mount Sinai School of Medicine.

Background: Podocyte-initiated glomerular diseases (podocytopenias) can progress to glomerulosclerosis with mesangial expansion. The molecular signaling mechanisms between glomerular cell types during initiation/progression of primary podocytopenias remain poorly understood. The purpose of this study was to examine cellular culprits and consequences of glomerular mitochondrial dysfunction (MD) and oxidative stress responses in situ.

Methods: We assessed glomerular MD and oxidative stress in glomeruli of a Doxycycline (DOX)-inducible expression of a ligand-independent, constitutively active TGFβ receptor type 1 (Tgfbr1) mutant selective to podocytes; NPHS2-rtTA_tet-O-Tgfbr1(AAD) double transgenic mice (PodTbr1(AAD)), which develops progressive glomerulosclerosis characterized by mesangial expansion, podocyte apoptosis and dephletation of endothelin-1 receptor A (ETA) expression was increased already after 1 day of DOX in glomerular endothelial cells, we applied inhibitor of ETA, BQ-123, by subcutaneous osmotic pump together with DOX chow in double transgenic mice. Inhibition of ETA signaling prevents subsequent podocyte apoptosis, depletion, proteinuria and glomerulosclerosis. Conditioned media from podocytes derived from double transgenic mice showed an apparent preference towards M2 type macrophages favoring tissue remodeling. Our results demonstrate a causal role of endothelin-1/ETA-mediated podocyte-to-endothelial crosstalk and suggest that TGFβ activation in podocytes induces endothelin-1 as essential mediator of endothelial mitochondrial dysfunction, oxidative stress and mdm2 expression, and that ETA inhibition prevents subsequent podocyte injury.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

71A
Changes in Proximal Tubule Cell Phenotype Induced by Glomerular Proteinuria

C to megalin (Kd ∼ 50 nM). Receptor associated protein, a known depositions to be pathogenic.

Conclusions: These results show that exposure of PT cells to an excess of albumin in vivo activates transcriptional machinery that triggers cell dedifferentiation and generalized dysfunction. Glomerular proteinuria is thus causing a rapid and specific toxification on PT cells, involving their endocytic machinery.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR299

Urine Cystatin C Excretion Is Controlled by Megalin Mediated Proximal Tubule Endocytosis

Denny Jensen, Rikke Nielsen, Henrik Birn. Dept. of Anatomy, Aarhus University Faculty of Health, Aarhus, Denmark.

Background: Cystatin C is a protase inhibitor produced in all nucleated cells. Urinary Cystatin C is an established marker of kidney injury. Cystatin C is filtered freely in the glomeruli and in the normal kidney this is followed by complete reabsorption in the proximal tubule. Megalin is an endocytic receptor involved in the proximal tubule reabsorption of filtered proteins and the current study was undertaken to establish the role of these receptors for the urinary excretion and tubular uptake of endogenous Cystatin C.

Methods: Binding of recombinant Cystatin C to purified megalin and cubulin was analyzed by surface plasmon resonance analysis.

Results: Surface plasmon resonance analysis revealed a high affinity binding of Cystatin C to megalin (Kd ∼78 nm) and to cubulin (Kd ∼50 nm). Receptor associated protein, a known inhibitor of ligand binding to megalin, inhibited Cystatin C binding to megalin with 76%.

Urinary excretion of Cystatin C was identified in megalin KO, but not in cubulin KO or wildtype mice. Immunohistochemistry showed vesicular labeling for Cystatin C in wildtype proximal tubular cells consistent with endocytic uptake of endogenous Cystatin C. Similar labeling was observed in cubulin deficient cells, however, no uptake was identified in proximal tubule cells not expressing megalin. Finally, no uptake of endogenous Cystatin C was observed in proximal tubule cells from L-lysine treated rats.

Conclusions: The endocytic receptors megalin and cubulin bind Cystatin C with high affinity. Inactivation of megalin, but not cubulin, is associated with increased urinary excretion and inhibition of proximal tubule uptake of Cystatin C. These observations identifies megalin as essential for the normal, tubular recovery of endogenous Cystatin C. Increased urinary excretion of Cystatin C, as observed in kidney injury, is a marker of proximal tubule endocytic dysfunction.

Funding: Government Support - Non-U.S.

FR-OR300

Resveratrol, an Activator of Sirt1, Ameliorates Renal Damage in 5/6 Nephrectomized Rats Via the Smad Pathway

Xinzhou Huang, Donghui Wen, Chuan-Ming Hao. Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.

Background: Fibrosis plays an important role in the pathogenesis and progress of CKD. Resveratrol(20mg/kg) is a polyphenol with antifibrotic properties. It has also been shown to activate Sirt1 which has health beneficial effect. In the present study, we investigated the effect of RSV on a 5/6 nephrectomized CKD rat model.

Methods: Three groups of rats were studied: sham operated, 5/6Nxvehicle, and 5/6Nx+Resveratrol(20mg/kg). RSV was administered one week after 5/6 nephrectomy surgery.

Results: Subtotal nephrectomy significantly increased proteinuria (152.1±43.0mg/day vs 25.3±4.75 mg/day), blood urea nitrogen (16.7±3.99mmol/L vs 8.0±3.12mmol/L) and serum creatinine(111.6±21.5µmol/L vs 53.9±11.6µmol/L) at 12 weeks. RSV treatment significantly reduced urinary protein excretion (79.8±34.27mg/day, p<0.05), blood urea nitrogen (11.95±1.88mmol/L) and serum creatinine levels(83±14.69µmol/L). The glomerular sclerosis index (1.56±0.34 vs 0.35±0.08) and tuibulointerstitial fibrosis (1.47±0.29 vs 0.18±0.04) were increased in nephrectomized rats, which were significantly reduced by RSV treatment. Further study suggests that treatment of nephrectomized rats with RSV significantly reduced acetylation levels of Smad3. Immunoprecipitation studies revealed a binding of acetylated Smad3 with silent information regulator 1(Sirt1). In cultured murine mesangial cells, down-regulation of Sirt1 increased acetylation levels of Smad3, consistent with our renal observation effect of RSV. Sirt1 increased expression of Tgfβ significantly reduced fibrogenic and type IV collagen expression in a dose- and time-dependent manner by immunoblot in cultured cells. Knocking-down Sirt1 using a RNAi markedly attenuated these effects of resveratrol, supporting that Sirt1 mediates the effect of RSV. In contrast, forced over-expression of Sirt1 in cultured mesangial cells significantly reduced fibronectin and type IV collagen expression.

Conclusions: RSV attenuates renal damage in 5/6Nx rats. The renal protective effect is associated with reduced Smad acetylation and TGFβ signaling. These findings indicate that Sirt1 may be a potential therapeutic target for the treatment of CKD.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-OR301

Mesangial Cell Susceptibility to IgA1 Is Important for Disease Development

Liping Zhang, Yanlan Dong, William E. Mitch, David J. Tweardy. Medicine, Baylor College of Medicine, Houston, TX.

Background: Chronic kidney disease (CKD) is characterized by increased circulating IL-6, insulin IGF-1 resistance, and abnormal protein metabolism causing body and muscle wasting and inflammatory and fibrotic disease. C188-9 treatment of mice was used to create mice with reduced muscle growth and improved health. This compound reduced muscle Stat3 phosphorylation and increased Akt phosphorylation, indicating suppression of CKD-induced activation of the ubiquitin-proteasome system. C188-9 treament reduced muscle Stat3 phosphorylation and ubiquitin-proteasome system. C188-9 treatment reduced muscle Stat3 phosphorylation and ubiquitin-proteasome system.

Methods: Subtotal nephrectomy and a high protein diet were used to create mice with BUN 50-150 mg/dL. The mice were paired by BUN and body weight and injected daily for 14 days with either C188-9 (6.25 mg/kg) in D5W or D5W alone. Result: Both groups of mice had reduced muscle weight and increased muscle protein synthesis, and muscle wasting.

Results: C188-9 treatment increased body weight (9.8% for gastrocnemius and 14% for soleus), increased muscle protein synthesis, and a significant increase in muscle protein synthesis. These are direct effects of C188-9 because C188-9 treatment of 2C12 muscle cells blocked the catabolic effects of IL-6 while improving insulin/IGF-1 intracellular signaling.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.

72A
**FR-OR303**

Hypertension in African American Live Kidney Donors

Mona D. Doshi,1 Mariella Goggins,2 Amit X. Garg,3

1Internal Medicine, Wayne State University, Detroit, MI; 2Internal Medicine, Henry Ford Transplant Institute, Detroit, MI; 3Nephrology, London Health Science Centre, London, ON, Canada.

**Background:** Compared to those of Caucasian race, African Americans (AA) are at higher risk of developing hypertension (HTN). It is unclear if live kidney donation further increases this risk. We conducted a retrospective cohort study of donors and non-donors to address this question.

**Methods:** We enrolled 138 AA who underwent donor nephrectomy at Harper Hospital or Henry Ford Transplant Institute in Detroit between 1993-2007. We also enrolled 48 AA non-donors who were suitable candidates for live kidney donation but did not donate due to non-medical reasons. None of the donors were hypertensive or diabetic prior to donation. In follow-up, HTN was defined as either systolic BP > 140 or diastolic BP > 90 or use of antihypertensive agent(s). We used multivariable logistic regression models to account for differences in baseline characteristics between the two groups.

**Results:** The baseline characteristics of donors and non-donors at time of follow up are shown in the table. Among 1064 adult kidney transplant patients, 108 patients (10.2%) reported a history of COU. The cumulative mortality rate at one, three and five years was 6.5% vs. 1.6%, 95% CI 1.10, 7.64, p<0.031, which did not persist during the subsequent follow up.

**Conclusions:** A positive history of COU prior to transplant is associated with inferior clinical outcomes after kidney transplantation.

**FR-OR305**

Recipient klotho genotypes Affect Outcome after Human Kidney Transplantation

Leandro Cunha Baia, Gerjan Navis, Martin H. De Borst. On behalf of the REGaTTA (RENal Genetics Transplantation) Groningen group, University Medical Center Groningen, Dept of Nephrology, Netherlands.

**Background:** In experimental studies genetic or environmental loss of Klotho contributes to premature aging, renal damage and vascular calcification. As these factors may influence graft function and survival, we studied the association between klotho gene variants and outcome after human kidney transplantation.

**Methods:** Twenty-nine SNPs covering all known variants in the human klotho gene were genotyped in 1271 donor-recipient pairs transplanted between 1993 and 2008. Only SNPs in Hardy-Weinberg equilibrium (HWE) were considered. With minor allele frequencies (MAF) > 5%, dominant models were used. Donor and recipient genotypes were analyzed for associations with mortality, death-censored graft loss and clinical data (serum creatinine, PTH, phosphate) obtained 60 (range 58-64) months post-transplantation.

**Results:** During followup of mean 5.5 [IQR 2.9-8.9] years, 175 (16.0%) recipients developed graft loss and 191 (17.4%) died with a functioning graft. On multivariant Cox regression analysis, recipient rs577912 major allele homozygote genotype (CC) was associated with mortality, also when adjusted for donor and recipient age, ischemia times, % of panel reactive antibodies and HLA mismatches (RR 1.606 (1.164-2.215), p=0.004).

**Conclusions:** Recipient but not donor klotho variants are associated with mortality (rs577912), in line with prior data in hemodialysis patients, and with death-censored graft loss, serum creatinine, PTH and phosphate 5 years after transplantation (rs537971). These findings link extrarenal Klotho to outcome after human kidney transplantation.

**FR-OR306**

The Impact of Interleukin-10 Genetic Polymorphism on Long-Term Graft Survival in Kidney Transplantation

Patrick Barry Mark,1 Janie P. Traynor,2 Kathryn K. Stevens,3 Rajan Kantilal Patel,1 Alan G. Jardine,1 1BHF Cardiovascular Research Centre, University of Glasgow, Scotland, United Kingdom; 2Renal Unit, Monklands Hospital, Airdrie, Scotland, United Kingdom.

**Background:** Immunologically mediated damage contributes to chronic allograft nephropathy. Interleukin-10 (IL-10) has anti-inflammatory effects. Low IL-10 production is associated with the A allele of the IL-10-1082 G/A single nucleotide polymorphism (SNP). Transforming growth factor β1 (TGFβ1) has pro-inflammatory, fibrotic and immunosuppressive actions. The implications of genetic polymorphisms encoding these cytokines on graft survival are unclear.

**Methods:** We studied 422 first renal transplant patients (median age 42.6 years; 56.6% male; 10% live donors). Patients were surveyed in 1994 and followed up after 16 years. Patients were genotyped for SNPs encoding IL-10 (G/A at position -1082) and TGFβ1 (C/T, C/G at codons 10 and 25).

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Disparities in Kidney Transplant Education for Patients with End-Stage Renal Disease

Lauren M. Kucirka,1 Morgan E. Grams,2 Dorry L. Segev.1

1Surgery, JHU; 2Medicine, JHU.

Results: There were 179 (42.2%) deaths and 154 (36.5%) death-censored graft failures. No significant differences existed in patient survival between the polymorphisms studied. Age (HR 1.07, p=0.001) and diabetes (HR 2.54, p=0.01) were independent predictors of mortality on multivariate analysis. Graft survival was reduced in patients with IL-10 genotype AA but other polymorphisms did not influence graft survival.

Higher serum creatinine at one year (p<0.001), deceased donor transplantation (p<0.05), younger age (p<0.01) and IL-10 genotype AA (p<0.05) were associated with reduced graft survival on univariate analysis. Creatinine at 1 year (p<0.001) and IL-10 genotype (p<0.05) were independent predictors of long-term graft failure on multivariate analysis.

Conclusions: In addition to conventional risk factors for graft loss, polymorphism of SNPs encoding IL-10 may predict long-term graft failure.

FR-OR307

Demonstrating Geographic Equity in Kidney Organ Allocation – Satisfying the Final Rule at Last

Ashley E. Davis, Daniela Ladner, John J. Friedewald, Sanjay Mchrotra, Mark S. Daskin, Anton I. Skaro, Michael Abecassis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

Table 1: Multivariate Multinomial Logistic Regression Accounting for Dialysis Center ( clustering to Estimate the Relative Rate of Rates for Not Informing Patients About Transplant), including: (1) Patient Unassessed, (2) Unassessed Due to Age, (3) Medically Unfit, (4) Declined Information, or (5) Psychologically Unfit. Compared to Being Informed About Transplant.

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Estimation of the Relative Rate of Rates for Not Informing Patients About Transplant, including: (1) Patient Unassessed, (2) Unassessed Due to Age, (3) Medically Unfit, (4) Declined Information, or (5) Psychologically Unfit. Compared to Being Informed About Transplant.

# Key
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- Underline represents presenting author.
These data support a reappraisal of the practice of preferential allocation of donor kidneys to patients who were or were not receiving dialysis. The one-year allograft survival among 7,701 non-dialysis-dependent individuals with no liver disease was 93.2% and 95.1%, respectively. One-year patient survival: LAT with CKD (with and without dialysis) was 82.0%; CLKT with HRS 88.8%. One-year patient survival: CLKT with CKD underwent CLKT and 12.8% had a reported diagnosis of HRS. Additionally, one-year kidney allograft survival was U-shaped.

**Methods:** We examined the predictors of outcome of kidney transplantation in different ages of elderly recipients. Using the Scientific Registry of Transplant Recipients, we identified 155,005 kidney transplanted pts. Mortality and death censored graft failure risks were estimated by age and calendar period regression (hazard ratio (HR) and 95% confidence interval) analysis over a median follow-up period of 4 years.

**Results:** Pts were 45±16 (mean±SD) years old and included 40% women and 19% diabetics. Figures show an association between age groups % death censored graft failure using different reference groups. The association between age groups death censored graft failure was U-shaped.

**Conclusion:** Concerning all-cause mortality, in pts aged 70–<75 years, female gender (HR: 0.73 (0.64-0.85)), Hispanic ethnicity (HR: 0.72 (0.58-0.95)) and living transplant donor (HR: 0.70 (0.57-0.86)) were protective predictors and diabetes mellitus (HR: 1.21 (1.05-1.39)) and Black race (HR: 1.24 (1.03-1.45)) were risk factors. In pts ≥75 years old, only a living donor kidney (HR: 0.64 (0.44-0.93)) was an important predictor of lower all-cause mortality.

**Funding:** NIDDK Support

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**FR-OR310**

**Differential Survival Rates of Combined Liver Kidney Transplantation (CLKT) vs. Liver Alone Transplantation (LAT) vs. Kidney Alone Transplantation (KAT)**

*Background:* Combined kidney liver transplantation (CLKT) is indicated in patients with combined end-stage liver disease (ESLD) and end-stage kidney disease (ESKD). Controversy exists as to allocation of donor kidneys to patients with ESLD with hepatorenal failure. A combined transplant (CLKT) is more appropriate for patients due to the metabolic demands of ESLD. Controversy also exists as to allocation of donor kidneys, especially among ESLD patients with hepatorenal failure. Controversy exists as to allocation of donor kidneys to patients with ESLD and end-stage kidney disease (ESKD). The goal of this study is to determine the risk factors associated with all-cause mortality in a prospective cohort of combined liver-kidney transplant recipients.

**Methods:** The study cohort included 54,676 liver transplants performed from 1998 to 2007. The cohort was divided into three groups: CLKT, LAT, and KAT. The risk of sensitization significantly increased with number of 1st tx HLA MM of 1st tx (Table 1). The risk of sensitization significantly increased with number of 1st tx HLA MM (DD: p<0.001; LD: p=0.027). Re-graft survival at 5 years was significantly worse with more MM at 1st tx, even for re-tx in 2000-2008 (DD p=0.046, LD p=0.012).

**Conclusion:** HLA sensitization in pediatric recipients increases with increasing number of MM and is associated with a decreased likelihood of re-tx, for deceased and living donors. Re-graft survival is lower after more HLA MM at 1st tx. The risks of sensitization vs. the benefits of earlier transplantation with a de-emphasis of HLA matching require further study, but these preliminary data suggest that pediatric transplant programs may benefit from evaluating local waiting times and donor type when considering HLA mismatched kidneys for transplant.

**Funding:** NIDDK Support

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**FR-OR311**

**Sensitization Rates after 1st Graft Failure in Pediatric Renal Transplant Recipients Are Impacted by a De-Emphasis of HLA Matching in Organ Allocation and by Donor Type**

*Background:* Despite improvements in immunosuppression, sensitization after 1st renal graft failure remains a barrier to re-transplantation. US allocation policies currently place less emphasis on HLA matching in pediatric renal transplant (tx) candidates to minimize diavascular, with unetermined long-term consequences. Re-graft survival remains a barrier to re-transplantation. US allocation policies currently place less emphasis on HLA matching in pediatric renal transplant (tx) candidates to minimize diavascular, with unetermined long-term consequences.

**Methods:** Using the SRTR database, we examined HLA sensitization after graft loss and re-graft survival of all pediatric 1st renal tx recipients aged 0–17 yrs transplanted 1990–2008, by panel reactive antibody (%PRA), type of 1st graft donor (deceased (DD) vs. living (LD) and HLA mismatch (MM) of 1st tx.

**Results:** Of 13,227 pediatric 1st renal tx recipients, 1,943 received a re-tx (60% of DD and 74% of LD) following 1st graft failure and 948 recipients were relisted but did not receive a re-tx (re-tx WL) by June 2009. Mean PRA increased from 7% prior to 1st tx to 55% at 1st graft failure vs. 50% from 1st tx to 75% at re-tx (p<0.001). The risk of sensitization significantly increased with number of 1st tx HLA MM (DD: p<0.001; LD: p=0.027). Re-graft survival at 5 years was significantly worse with more MM at 1st tx, even for re-tx in 2000-2008 (DD p=0.046, LD p=0.012).

**Conclusion:** HLA sensitization in pediatric recipients increases with increasing number of MM and is associated with a decreased likelihood of re-tx, for deceased and living donors. Re-graft survival is lower after more HLA MM at 1st tx. The risks of sensitization vs. the benefits of earlier transplantation with a de-emphasis of HLA matching require further study, but these preliminary data suggest that pediatric transplant programs may benefit from evaluating local waiting times and donor type when considering HLA mismatched kidneys for transplant.

**Funding:** Government Support - Non-U.S.
Methods: We compared the suppressive functions of Tregs of wild-type (WT) C57BL/6 mice with global (HDAC6-/-, HDAC9-/-, dual HDAC6/9-/-) or conditional deletion (CD4-Cre or Foxp3-Cre and floxed Sirt1) alone, or after treatment with pan- or isof orm-selective HDAC inhibitors (HDACi).

Results: Tregs deficient in expression of Sirt1, HDAC6 or HDAC9 had increased suppressive functions compared to WT Tregs, but treatment with a pan-HDACi (FR-900289) in each case further increased their suppressive function, indicating the benefit of combined vs. single HDAC targeting. This was reinforced by increased suppressive function of Tregs from HDAC6-/- mice vs. HDAC6-/-, HDAC9-/-, dual HDAC6/9-/- Tregs. HDAC targeting increased Foxp3 expression and control Tregs and involved differential transcription factors. Sirt1 deletion stabilized p53 acetylation and promoted its nuclear translocation. Loss of HDAC6 or HDAC9 increased SMA3D or STAT5 phosphorylation, respectively, consistent with data that HDAC6 promotes proteasomal degradation of SMA3 and the STAT-P dimmer is stabilized by acetylation. Deletion of each HDAC increased Foxp3 acetylation, which protects Foxp3 from ubiquitination and proteasomal degradation. Tregs with HDAC6 or HDAC9 deletion had increased transcription of heat shock response (HSP) genes. HSP70 deletion reduced, but did not abolish, the therapeutic effects of HDAC6 inhibition, whereas Sirt1 deletion decreased HSR gene expression but increased Treg resistance to stress and HSP70 protein levels, in conjunction with increased acetylation of HSP70.

Conclusions: Targeting different HDAC can increase Treg function by multiple and additive mechanisms, indicating the therapeutic potential for combinations of HDACi in the future autoimmunity and transplantation.

Funding: Other NIH Support - K08AI095353, A1073489

FR-OR314

Strategies To Promote Tolerance through Augmented Treg Homeostasis Ying Wang, David M. Rothstein. University of Pittsburgh, PA.

Background: CD4-Foxp3+ Treg play a key role in transplant tolerance. Treg undergo higher homeostatic proliferation (HP) than Tconv cells. α-CD45RB induces tolerance through a 2X increase in Treg, induced by converting Foxp3- to Foxp3+ cells and by a specific increase in Treg HP. Thus, Treg HP can be specifically regulated to expand Treg in vivo. We asked whether IL2, which also supports Treg HP, synergizes with α-CD45RB. CFSE-stained congenic CD4 cells were transferred into naive wt mice, which were untreated, or received IL2 (1.5ugX4d), α-CD45RB (100ug d-1, 0.5), or both. Transferred Foxp3+ and Foxp3- CD4 cells (spleen and LN) were assessed (d10). Both IL2 and α-CD45RB increase Treg HP resulting in a 2.5X increase in Treg. Combined, these agents exhibit dramatic synergy. >90% of Treg undergo HP such that 40% of transferred CD4+ T cells are now Foxp3+ (increase: 3X in % and 5X in #). While α-CD45RB induces >100d graft survival (GS) in 50% of B6 recipients of BALB/c islets, IL2 did not (MST 15d). Despite a marked increase in Treg, ILt2+α-CD45RB shortened GS (MST 60d). The failure of regimens with IL2 to prolong GS may be due to a 2X expansion of CD8 effectors and NK cells vs. untreated controls. In this regard, Boyman reported that α-IL2 mAb (JES6-1) allows IL2 to bind the IL2R on CTLs. They also found JES6-1+IL2 induced long-term islet GS. However, using the same treatments /strains, we found all mice rejected by d18. Moreover, JES6-1+IL2 did not inhibit CD8 effectors, allowing a 2X increase in INF-γ CD8 cells and an >2X increase in NK, PMNs, DCs and B cells. However, when α-CD45RB was added to IL2+JES6-1, it markedly improved long-term GS (80% >100d) vs. all other regimens. Together, JES6-1+IL2+α-CD45RB promoted dramatic Treg expansion, but completely blocked expansion of non-Treg cell types (above) and reduced 70% INF-γ CD8 cells vs. JES6-1+IL2. Thus: a) Dramatic expansion of Treg is not sufficient to promote tolerance if certain effector, and innate populations also expand. b) α-CD45RB specifically augments the Treg promoting effects of JES6-1+IL2, while inhibiting the expansion of other cell types, resulting in robust and reproducible tolerance.

Funding: NIDDK Support

FR-OR315

CD4 Memory T Cells Persist in the Absence of T Cell Receptor Signalst Jonathan S. Maltzman, Elizabeth Staub, Karla Wiehagen, Evann Corbo. Department of Medicine and Institute for Immunology, University of Pittsburgh, PA.

Background: Memory T cells are generated in response to transplant, pathogen exposure, and lymphopenia and are a barrier to tolerance induction. The importance of T cell receptor (TCR)- versus cytokine receptor- generated signals in the differentiation and persistence of CD4+ memory T cell populations has not been well defined. SLP-76 is an adaptor protein critical in mediating proximal TCR signals. We previously showed that the innate immune system recognizes an allograft is not known. Here we tested the hypothesis that, akin to sensing microbial non-self, the innate immune system distinguishes between self and allogeneic non-self, leading to APC maturation and activation of the adaptive alloimmune response.

Methods: T, B and NK cell-deficient B6 Rag-/-/tg-CXCR1+GFP-/- reporter mice in which monocyte-lineage cells express GFP received either syngeneic (B6) or allogeneic (Balb/c) vascularized heart grafts. Graft-infiltrating cells were analyzed on days 1, 3, 5, 10 and 42 after Tx and the function of monocyte-derived dendritic cells (mono-DC) was tested in vivo and ex vivo.

Results: We found that the vast majority (> 90%) of DC present in allografts within 1 day after Tx was derived from host monocytes. Mono-DC (lineage-Ly-6G-CD45+GFP+CD11b+CD11c+F4/80+) were present in significantly greater numbers in allografts vs. syngeneic grafts, had a mature phenotype (MHCII+CD80+), expressed IL-12p40, and stimulated allologenic T cells in an ex vivo MLR both early (days 1–10) and late (days 21–42) after transplantation. In contrast, maturation of monocytes to mono-DC was self-limited in syngeneic grafts (disappearing between days 10 and 21) and no IL-12p40 expression was detected. Recipient monocyte depletion with clodronate abrogated the proliferation of adoptively transferred H3.1 CD4+ T cells that recognize BALB/c alloepitope in the context of H-2d (indirect alloreactivity). Except for higher numbers in allografts on day 1, there were no differences in the number of infiltrating total GFP+ cells, monocytes, macrophages, or GFP+ conventional DC between syngeneic and allologenic grafts. Neutrophils transiently infiltrated allografts and syngeneic grafts in equal numbers.

Conclusions: Our data indicate that monocytes distinguish between self and allogeneic non-self. Non-self recognition causes their differentiation to mature DC long after inflammation (IR) injury has subsided. The persistence of activated mono-DC in transplanted organs could perpetuate the immune response, eventually leading to allograft failure.

Funding: Other NIH Support - P01AI064543, Private Foundation Support

FR-OR317

First MicroRNA Transcriptome of Human Renal Allograft Intersitial Fibrosis and Tubular Atrophy Thangamani Muthukumar1, Iddo Zeev Ben-Dov,2, Fabien Campagne,3 Ruchang Ding,3 Thomas Tsichl1, Manikkam Suthanthiran1. 1Cornell University; 2Rockefeller University.

Background: Massively parallel sequencing (Next Generation Sequencing) has revolutionized sequencing. We developed & applied barcoded RNA sequencing to characterize the microRNA (miRNA) transcriptome of human kidney allograft biopsies with interstitial fibrosis/tubular atrophy (IFTA).

Methods: RNA from human renal allograft biopsies (3 IFTA & 4 Normal, Discovery) were sequenced. Unique (‘barcode’) 3’-adapters were ligated to each sample & 32 cycle deep sequencing performed on pooled RNA. Using in-house computer pipeline, we extracted barcodes, aligned to genome & assigned read annotations. We determined miRNA abundance by the sum of all reads with up to 1 genome/2 annotation mismatches. We tested for differential expression by DESeq. We used EIMMo target prediction server to identify targets of differentially expressed miRNAs.

Results: We used RT-PCR to validate selected miRNAs in an independent cohort of 18 kidney recipients; 10 IFTA & 8 Normal (Validation).

Results: The pooled library yielded 1.7 million sequences with overall 8x coverage. Measurement of small RNA annotations were miRNA (64%), synthetic calibrator (26%) and rRNA (4%). Predicted targets for top 3 differentially expressed miRNAs were overrepresented by genes involved in DNA-dependent transcription regulation, transmembrane receptor protein-kinase signaling pathway, protein import into nucleus, & RNA pol II transcription. 6 miRNAs were different between IFTA & Normal (Top Panel).

Validation of the 2 differentially expressed miRNAs is shown (Bottom Panel).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
FR-OR302
Abstract Withdrawn

FR-OR321
Comparative Analysis of Cellular Phenotypes between Pre-Existing Venous Neointimal Hyperplasia Prior to Access Surgery and Venous Neointimal Hyperplasia from Sternotic Arteriovenous Dialysis Accesses
Timmy C. Lee,1 Prabir Roy-Chaudhury.1 Internal Medicine, University of Cincinnati, OH; 2Pathology, Johns Hopkins University, Baltimore, MD.

Background: The histology of vascular access stenosis has been well characterized as aggressive venous neointimal hyperplasia (VNH) in both AVGs (AVG) and AV fistulas (AVF). Recently we reported that severe VNH occurs prior to vascular access placement. The objective of this study was to perform a comparative analysis of cellular phenotypes between venous tissue samples collected at the time of vascular access creation and stenotic vein samples from patients with failed AVGs and AVFs.

Methods: 55 vein samples collected at the time of vascular access surgery, and 43 stenotic vein segments from failed AVGs and AVFs were examined. Sections from both groups were evaluated for expression of alpha-smooth muscle actin (SMA), desmin, and vimentin. A semiquantitative scoring system from 0-4+ was used to quantify positive cells for the specific marker compared to total cells (0 indicated 0-10% positive;1+=11-25%;2+=26-50%;3+=51-75% and 4+=76-100%).

Results: Within the neointima, the median semiquantitative scores for SMA, desmin, and vimentin between vein tissue collected at the time of surgery vs. vein tissue obtained from stenotic AVGs and AVFs were 2.57 vs. 3.53, 2.55 vs. 0.63, and 1.45 vs 3.03, respectively.

Conclusions: Our results demonstrate that the predominate cellular phenotype in pre-existing VNH are SMA +ve, desmin +ve, vimentin -ve contractile smooth muscle cells, while in the venous neointima of stenotic fistulas and grafts, SMA +ve, desmin -ve, vimentin +ve myofibroblasts dominate. These phenotypic differences could indicate the need for divergent therapeutic approaches because of differing mechanistic pathways for pre-existing VNH compared to the VNH responsible for AVG and AVF stenosis.

Funding: NIDDK Support
**Vascular Access Failure: Basic Mechanisms**

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

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**FR-OR322**

Factor V Leiden (F5) Gene Polymorphism Is Associated with Arteriovenous Graft Failure

Michael Allon, Li Zhang, Ivan D. Maya, Molly S. Bray, Jose R. Fernandez.

Division of Nephrology, Univ of Alabama at Birmingham, Birmingham, AL; Department of Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH; Department of Epidemiology, Univ of Alabama at Birmingham, Birmingham, AL; Department of Nutrition Sciences, Univ of Alabama at Birmingham, Birmingham, AL.

Background: Dialysis grafts fail due to recurrent stenosis and thrombosis. Vasoactive and pro-thrombotic substances affecting intimal hyperplasia or thrombosis may modify graft outcomes. We evaluated the association between polymorphisms of several candidate genes and graft patency.

Methods: The Dialysis Access Consortium (DAC) Study randomized patients receiving a new dialysis graft to treatment with cilostazol or placebo, and evaluated graft outcomes. DNA samples from 354 subjects were used to measure genetic polymorphisms of methylenetetrahydrofolate reductase (MTHFR), heme- oxygenase 1 (HO-1), Factor V Leiden (F5), transforming growth factor beta-1 (TGF-β1), Klotho, and angiotensin converting enzyme (ACE). We evaluated the association between these polymorphisms and primary graft patency (time to first angioplasty or thrombosis), after adjusting for clinical factors and estimates of genetic admixture for the control of population stratification.

Results: On multivariate analysis, primary graft patency was associated with active drug treatment (HR 0.76; 95% CI 0.60 to 0.96; p=0.02) and with graft placement after starting dialysis (HR 1.38; 95% CI 1.05 to 1.82; p<0.02), but not with patient age, sex, diabetes, cardiovascular disease, graft location, baseline aspirin use, or BMI. After adjusting for the clinical factors and genetic admixture, F5 gene polymorphisms were significantly associated with graft survival in a dominant model (HR 1.70, 95% CI 1.32 to 2.19, p=0.0001 for G/C and G/G genotypes vs C/C genotype). There was no significant association between primary graft patency and polymorphisms of MTHFR, HO-1, TGF-β1, Klotho, or ACE.

Conclusions: Factor V Leiden (F5), the most common inherited thrombophilia, is associated with an increased risk of graft failure. Anticoagulation may reduce graft failure in patients with the G/C or G/G genotypes (47% of our study).

Funding: NIDDK Support

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**FR-OR323**

Wall Shear Stress (WSS) and Oscillatory Shear Index (OSI) in a Porcine Arteriovenous Graft (AVG) Model

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Background: AVG suffer from high rates of stenosis due to NH at the venous anastomosis. Ablation of anastomotic hemodynamics likely play a role in the focal nature of NH formation, but this causal relationship is not well understood. Chronically in vivo hemodynamics after AVG implantation will help illuminate its role in NH formation and development for therapies preventing stenosis.

Methods: Four pigs received an AVG between the common carotid artery and the external jugular vein; the un-operated contralateral vessels served as a control. 3D reconstructions of the vessel and graft lumen geometry were created using Amira software from 3D black-blood MRA scans obtained weekly post-implantation. Blood flow rate was simultaneously obtained and phase contrast CFD simulations of pulsatile blood flow were performed using FLUENT. CFD results were used to calculate WSS (the axial frictional force exerted by flowing blood on the luminal wall) and OSI (a measure of the change in direction and magnitude of WSS, which is calculated from integrals of WSS at one location over the cardiac cycle).

Results: Compared to baseline vein, mean WSS, averaged over a cardiac cycle at the VA and downstream vein (DV) areas, was increased by 5.0- and 3.8-fold respectively, at day 5. At day 14, WSS at the VA remained 4.5-fold increased, while WSS at the DV almost returned to control vein levels. Similarly, mean OSI averaged at the VA and DV areas increased by 4.8- and 2.6-fold respectively, at day 5, as compared to control vein.

At day 14, OSI at the VA remained 4.5-fold increased, while OSI at the DV also returned to near control vein level.

Conclusions: We have previously shown in our porcine model of AVG stenosis that significant NH develops at the VA by week 6, but not at the DV. Here we found sustained increased WSS and OSI at the VA, the region most susceptible to NH, but not at DV, the region relatively free from NH. These results suggest a role for increased WSS and OSI at the VA in promoting venous NH.

Funding: NIDDK Support; Other NIH Support - NHLBI, Veterans Administration Support, Private Foundation Support

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**FR-OR324**

Early Adventitial Activation and Proliferation in a Mouse Model of Arteriovenous Stenosis


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Background: Early arteriovenous fistula (AVF) failure remains an important cause of hemodialysis vascular access dysfunction and a major impediment to the Fistula First initiative. Despite the magnitude of the clinical problem, there are currently no effective therapeutic interventions for early AVF failure. In order to better understand the cellular and molecular mechanisms involved in early AVF failure, we have previously developed a mouse model which is characterized by significant stenosis at the AV anastomosis 14d post surgery. The aim of this study was to describe the pattern of cellular proliferation and macrophage infiltration at different time points in this model.

Methods: AVFs were created from anastomosing jugular veins and carotid artery in 13 mice. Animals were sacrificed at 2d (n=4), 7d (n=5) and 14d (n=4). A standard immunohistochemical analysis was performed to assess cellular proliferation (Ki-67) and macrophage infiltration (Mac-2), using a semiquantitative scoring scale (0<10%=0; 10–25%=1; 25–50%=2; 50–75%=3; 75–100%=4).

Results: Our results (Table shows Adventitial data only) demonstrate an early proliferation within the adventitia (ADV) which peaks at 7d and which is followed by a later endothelial (ENDO) proliferation which peaks at 14d (p<0.05 for ADV proliferation vs ENDO proliferation at 7d). This is accompanied by an early macrophage infiltration which peaks at 7d (p<0.05 for ADV macrophage infiltration vs ENDO and ADV macrophage infiltration).

Conclusions: Our results suggest that the adventitia could be a key player in the pathogenesis of early AVF failure. In addition it is possible that early peri-adventitial therapies targeting vascular proliferation and macrophage infiltration might be effective in reducing early AVF failure.

Funding: Private Foundation Support

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**FR-OR325**

Sunitinib Inhibits Venous Neointimal Hyperplasia (NH) Formation in a Perfused Organ Culture System

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Background: Stenosis associated with arteriovenous (AV) vascular access occurs frequently and predominantly as a result of NH formation at the venous anastomosis. Effective treatment for NH in this setting is lacking. The highly localized nature of NH at the venous anastomosis suggests that aberrant blood flow profiles may play a major role in NH development. We have established a perfused organ culture model that allows us to investigate the effects of various experimental conditions on NH development in intact explanted vessels, and to evaluate the effectiveness of anti-hyperplasia drug candidates.

Methods: In porcine vein segments exposed to low wall shear stress (WSS), we observed significant NH as assessed by intima-media (IM) area ratio, while veins cultured under standard static culture conditions exhibited little or no NH. To evaluate the effect of sunitinib, a multi-receptor tyrosine kinase inhibitor, vein segments were exposed to non-physiological WSS (<1 or >10 dyn/cm²) for 12 days with or without 100 nM of sunitinib. Formalin-fixed vein sections were stained with van Gieson’s stain to determine IM area ratio and probed with antibodies for markers of proliferating cells (Ki-67), smooth muscle cells or transformed myofibroblasts (alpha-actin), and endothelial cells (von Willebrand factor).

Results: Sunitinib significantly inhibited the magnitude of NH formation, with the IM area ratio decreasing from 0.43±0.26 to 0.15±0.04. Cells in the hyperplastic lesions were primarily positive for Ki-67 and alpha-actin. Sunitinib significantly reduced the NH result from proliferation and migration of smooth muscle cells and/or myofibroblasts.

Conclusions: In summary, this perfused organ culture system allows for the dissection of the effects of various conditions, such as non-physiological WSS, on intact vein segments that could not be achieved using standard cell cultures or whole animal models. It also allowed us to determine more expeditious model than the whole animal for the study of anti-hyperplasia therapies.

Funding: Other NIH Support - NIH RO1 HL067646-04A1, Veterans Administration Support, Private Foundation Support

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**FR-OR326**

Without Integrin β3, Endothelial Cell Regeneration Is Delayed and Contributes to AV Fistula Failure

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Background: The Achilles heel of dialysis patients is a functioning arteriovenous fistula (AVF). Failure of an AVF is generally due to proliferation of vascular smooth muscle cells forming intima-media (IM) stenosis. Whether endothelial cell (EC) damage contributes to this process is unclear. In AVF created in mice, there was an initial denudation of ECs followed by endothelial regeneration. Since integrin β3 is involved in angiogenesis and is essential for normal EC function, we studied how integrin β3 influences the course of endothelial regeneration in AVFs [3]. 

Methods: regeneration and differentiation of endothelial progenitor cells (EPC) in AVF were studied in vitro and in vivo in WT and integrin β3 KO mice.

Results: AVF failed in 80% of integrin β3 KO mice but in only 5% of WT mice. Cultured bone marrow-derived EPCs from either WT or integrin β3 KO mice attached to extracellular matrix and differentiation into mature ECs vs WT BM EPCs. These changes were reflected in vivo: AV fistulas created in integrin β3 KO mice had fewer Sca 1 EPCs in the denuded vessel wall compared to AVFs created in WT mice. Likewise, internal regeneration occurred earlier in AVFs in β3 KO mice, but was delayed 10 days in AVFs of integrin β3 KO mice. The defects in AVFs of integrin β3 KO mice were

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Oral Abstract/Friday
 rescued when the mice received a bone marrow (BM) transplant from WT mice. AVFs in intact B6/J KO mice transplanted with WT BM cells and BM cells from integrin β3 KO mice indicated that WT BM cells were the principal contributor to new endothelium in AVFs of integrin β3 KO mice. Cell proliferation in neointima in the same AVFs was reduced by 40% and was due to impaired S6K-1 phosphorylation and decreased PI3K activity as found in our report about mechanisms of smooth muscle cell proliferation.

Conclusions: Integrin β3 is required for endothelial regeneration in newly created AV fistulas. The mechanism includes an improvement in the attachment and differentiation of EPCs in the AV fistula.

Funding: NIDDK Support

FR-OR327
Quaking as a Critical Determinant of Neointima Formation and Vascular Smooth Muscle Cell Homeostasis. 
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Background: Patients undergoing hemodialysis require a patent, vascular access point that can withstand high-flow rates. For this, an arteriovenous fistula (AVF) is commonly used as the treatment of choice. However, early AVF failure remains an important cause of morbidity and mortality. Several studies have suggested that Quaking (QKI) is a critical regulator of vascular smooth muscle cell (VSMC) homeostasis through modulation of actin cytoskeleton dynamics, and neointima formation following injury to the vessel wall.

Methods: Quaking viable mouse mutants (QKv), which is a result of a megabase deletion in the qki promoter region express reduced levels of QKI, were used to study neointima formation upon non-constrictive cuff placement around the femoral artery. Furthermore, we specifically investigated the consequences of abrogated QKI expression on VSMC behaviour in both murine VSMCs derived from C57Bl/6 (WT) and QKv mice as well as in WT human VSMCs treated with either a non-coding shRNA (shRNA-non) and shRNA targeting QKI (shRNA-qki).

Results: Non-constrictive cuff placement in QKv mice resulted in a significant decrease in neointima formation and luminal stenosis as compared to control mice. Furthermore, attenuated expression of QKI in VSMCs (derived from both QKv mice or following shRNA-mediated knockdown in human VSMCs) resulted in significantly reduced cellular proliferation, migration and elaboration of extracellular matrix. The targeted disruption of QKI impaired both the capacity to remodel the actin cytoskeleton and perturbed VSMC contractile function.

Conclusions: Collectively, we have uncovered a novel role for QKI in regulating VSMC homeostasis, and propose that intervention in QKI activity could be an effective modality in the prevention of AVF stenosis, and potentially shunt failure.

Funding: NIDDK Support

FR-OR329
Venous Segment Calcification in a Mouse Model of Arteriovenous Stenosis 
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Background: Early arteriovenous fistula (AVF) failure remains an important cause of hemodialysis vascular access dysfunction, although the exact biological mechanisms involved remain unclear. Vascular calcification is known to be an important clinical problem in the setting of uremia but the exact role of calcification at different time points following creation of an AVF is unknown. The aim of this study was to describe the extent of venous segment calcification at different time points in a mouse model of AVF stenosis.

Methods: AVFs were created between the jugular vein and the carotid artery with an end to side anastomosis in 13 mice (this model results in significant stenosis at the AV anastomosis 14 days after surgery). Animals were sacrificed at the 2d (n=4), 7d (n=5) and 4d (n=4) time points and examined for calcification within the venous segment of the AVF (including the anastomosis) with a Von Kossa stain. Sections were scored using a semiquantitative scoring scale (1=1-10% calcification; 2=11-25%; 3=26-50%; 4=51%)

Results: Maximal venous segment calcification occurred at 2d post surgery, often in the setting of a swollen venous wall. Although the magnitude of venous segment calcification appeared to decrease over time this was not statistically significant (see figure).

Conclusions: Our results clearly document the presence of venous segment calcification in a mouse model of AVF stenosis. It is unclear at present as to whether these calcium deposits are secondary to early hemodynamic injury or whether they could play a primary role in the pathogenesis of early AVF failure.

Evaluation of Calcium deposits by Von Kossa Staining

SA-OR330
Generation and Examination of Proximal Tubule Specific Na+/H+ Exchanger NHE3 Knockout Mouse 
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Background: The conventional NHE3 knockout mouse has a significant intestinal phenotype manifested with electrolyte malabsorption, diarrhea, and volume depletion. As a result the mutant mouse can not tolerate oral salt or acid load, making it unsuitable for the examination of the role of kidney NHE3 in vivo.

Methods: To overcome this problem, mice with tissue specific deletion of NHE3 were generated. NHE3 floxed mice were crossed with either the SpG2 (sodium-glucose transporter 2) cre mice to generate proximal convoluted tubule specific NHE3 knockout (NHE3-PT KO) or with the villin cre mice to generate combined intestine and proximal tubule NHE3 knockout mice.

Results: The intestine and kidney specific NHE3 ko mice are smaller and have loose stool and caecal dilatation, consistent with an important role for NHE3 in salt absorption in the intestine. The NHE3-PT KO mice have ~90% ablation of NHE3 as determined by immunofluorescence labeling and western blotting. NHE3-PT KO animals show mild metabolic acidosis (21 mEq/l in ko Vs. 23 in wt, p<0.05). In situ microperfusion studies in proximal tubules demonstrate a ~36% reduction in bicarbonate reabsorption (Jbic = 83 pmol/min/mm in wt Vs. 54 in ko) and a ~27% reduction in volume reabsorption (Jv = 0.92 nl/min/mm in wt Vs. 0.67 in ko) in mutant mice. The NHE3-PT KO mice tolerated NH4Cl acid load well and showed comparable NH3 excretion rates Vs. wild type mice at 2 days and 5 days after acid loading.

Conclusions: Results that NHE3 plays an important role in bicarbonate reabsorption in the proximal convoluted tubule but does not play a major role in NH4+, secretion, at least in the first 5 days of acid loading. Whether the Na+/H+ exchanger NHE3 in the S3 segment of the proximal tubule plays a more important role in NH4+ secretion than the convoluted segment needs the generation and examination of appropriate mutant animals. The mild metabolic acidosis, despite a significant reduction in net bicarbonate reabsorption in the proximal tubule of NHE3 KO mice, suggests the presence of compensatory mechanisms in other nephron segments.

Funding: NIDDK Support, Veterans Administration Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.

79A
Results: Confocal microscopy indicates that kNBC1 A991-1005 was retained in the cytoplasm, whereas the wild type, A961-975, A976-990 and A1006-1009 mutant proteins were not detected in the cytoplasm. In contrast, the wild type and A991-1005 mutant proteins were not detected in the cytoplasm. Functional studies in Oocytes indicated that while the electrogenic potential of kNBC1 A961-975, A976-990 and A1006-1009 mutant proteins were not significantly different than that of wild type kNBC1, the kNBC1 A991-1005 mutant exhibited a significantly reduced electrogenic potential (p < 0.01).

Conclusions: Our data indicate that the tripeptide Ile 1003-Pro 1004-Met 1005 in the C-terminus of kNBC1 is involved in its accurate targeting. These results provide novel insight into the mechanism through which kNBC1 d55bp mutations leads to pRTA.

Funding: NIDDK Support, Veterans Administration Support

SA-OR334

Lipopolysaccharide (LPS) Inhibits NHE3 and HCO3− Absorption in Medullary Thick Ascending Limb (MTAL) through a TLR4-ERK Pathway Upregulated by Sepsis

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Background: Recently we demonstrated that bacterial molecules can act directly through Toll-like receptors (TLR) to impair the transport function of renal tubules. In particular, basolateral LPS inhibits HCO3− absorption in perfused rat and mouse MTALs through TLR4-mediated activation of ERK. Here we examined the transport mechanism responsible for this inhibition and whether the LPS-induced pathway is influenced by sepsis.

Results: Activation of ERK can inhibit HCO3− absorption in the MTAL through primary inhibition of either basolateral NHE1 or apical NHE3. The inhibition of HCO3− absorption by bath LPS was unaffected by maneuvers that inhibited basolateral Na+/H+ exchange, indicating that NHE1 is not involved in mediating the inhibition by LPS. Bath LPS decreased apical NHE3 activity due to a decrease in Vmax. The inhibition of NHE3 by LPS was eliminated by MK2/ERK inhibitors. The effects of sepsis were examined 18 h after surgery using a mouse cerulein and panniculitis (CLP) model. Bath LPS decreased HCO3− absorption by 45% in MTALs from CLP mice compared with a decrease of only 26% in tubules from sham-operated controls (P < 0.05). MEK/ERK inhibitors eliminated the inhibition of HCO3− absorption by LPS in both groups. Exposure to LPS for 15 min increased HCO3− phosphorylation in sham and CLP MTALs but the level of phosphorylated ERK was higher in MTALs from CLP mice. The increased ability of LPS to inhibit HCO3− absorption through ERK in CLP MTALs was associated with increased expression of TLR4 in the basolateral membrane domain.

Conclusions: We conclude: 1) basolateral LPS inhibits HCO3− absorption in the MTAL through TLR4-ERK-dependent inhibition of NHE3; 2) this pathway is upregulated during sepsis. These results identify NHE3 as a target of TLR4 signaling in the MTAL and suggest that bacterial molecules may impair absorptive functions of renal tubular important for acid-base homeostasis through inhibition of TLR4-ERK-mediated change. The ERK pathway links TLR4 to downstream modulation of ion transport proteins and represents a potential target for treatment of sepsis-induced renal tubule dysfunction.

Funding: NIDDK Support

SA-OR335

Double-Knockout of Pendrin and NaCl Cotransporter Causes Salt Wasting, Profound Dehydration and Nephrogenic Diabetes Insipidus

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Background: The Cl-/HCO3 exchange pendrin (SLC26A4) and the thiazide-sensitive NaCl cotransporter NCC are expressed in the distal nephron and mediate salt absorption. Single mutation of either pendrin or NCC can cause salt wasting, polyuria, and severe diuresis under basal conditions, suggesting they might be predominantly active during salt depletion.

Methods: Alternatively, we hypothesized that each transporter may compensate for the other under basal conditions, thereby masking the role that each plays in salt absorption. To test our hypothesis, we generated pendrin/NCC double knockout (dKO) mice.

Results: NCC/pendrin double KOs displayed profound polyuria (24 h urine volume in ml: double KO, 5.3; pendrin KO, 1.5; NCC KO, 1.0; WT, 1.5; p < 0.0001 vs single KO and WT). We also observed hyponatremia (24 h urine osmolality in mOsm/kg in ml: double KO, 245 ± 14 mOsm/kg; pendrin KO, 240 ± 13 mOsm/kg; NCC KO, 44, 4.4, 3.5; p < 0.05 vs single KO and WT). Urine osmolality was 790 mosm/kg H2O in double KO vs. 2500 or higher in other genotypes (p < 0.001 Vs. single KO and WT). Urine chloride excretion was significantly increased in dKO mice (440 mmole/day Vs. 270 mmole/day in WT). Urine chloride excretion was significantly increased in dKO mice compared with a decrease of only 26% in tubules from sham-operated controls (P < 0.05). MEK/ERK inhibitors eliminated the inhibition of HCO3− absorption by LPS in both groups. Exposure to LPS for 15 min increased HCO3− phosphorylation in sham and CLP MTALs but the level of phosphorylated ERK was higher in MTALs from CLP mice. The increased ability of LPS to inhibit HCO3− absorption through ERK in CLP MTALs was associated with increased expression of TLR4 in the basolateral membrane domain.

Conclusions: We conclude: 1) basolateral LPS inhibits HCO3− absorption in the MTAL through TLR4-ERK-dependent inhibition of NHE3; 2) this pathway is upregulated during sepsis. These results identify NHE3 as a target of TLR4 signaling in the MTAL and suggest that bacterial molecules may impair absorptive functions of renal tubular important for acid-base homeostasis through inhibition of TLR4-ERK-mediated change. The ERK pathway links TLR4 to downstream modulation of ion transport proteins and represents a potential target for treatment of sepsis-induced renal tubule dysfunction.

Funding: NIDDK Support
Conclusions: In conclusion, combined deletion of pendrin and NCC causes polyuria, salt wasting, and metabolic alkalosis in diabetic mice. These results indicate a role for both NCC and pendrin in salt and water reabsorption and show that each transporter can provide compensation for loss of the other under basal conditions. We suggest that the role of pendrin and NCC in salt and water reabsorption at basal state in the distal nephron needs complete reevaluation.

Funding: NIDDK Support, Veterans Administration Support

SA-OR336
The A-kinase Anchoring Protein AKAP-KL (AKAP2) Is Localized in the Subapical Domain of All Collecting Duct Intercalated Cells and Is Regulated by Disturbance of Acid/Base Balance
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Background: Compartmentalized cAMP/protein kinase A (PKA) signalling mediated by A-kinase anchoring proteins (AKAPs) is an emerging paradigm. AKAP-KL (AKAP2) tethers PKA in lung and renal epithelial cells. However, the detailed localization and function of this AKAP in the kidney is unknown. This study:
a) describes the cellular and subcellular localization of AKAP-KL in the kidney
b) tests if the amount of AKAP-KL in collecting duct (CD) intercalated cells (ICs) correlates with IC subtype activity during acid/base disturbance.

Methods: Paraformaldehyde-fixed paraffin-embedded kidney tissue from untreated, NH4Cl-loaded and NaHCO3-loaded rats were analyzed by immunohistochemistry using a mouse monoclonal antibody against AKAP-KL, fluorescence-coupled secondary antibodies and laser confocal microscopy. Co-labelling for the anion-exchangers pendrin and NCC allowed IC sub-type identification. Fluorescence images of 3D tubule reconstructions revealed that SDF-1 treatment promoted redistribution of AKAP-KL than type B cells (A: 65003±4653, B: 45995±2674 p=0.002). In NaHCO3-loaded rats (n=3) type B cells showed higher abundance of AKAP-KL than type B cells (A: 65003±4653, B: 55995±2674 p=0.002).

Results: We conclude that luminal flow modulates H+-ATPase activity in the renal CCD and that H+-ATPases therein are present in both PC and IC. We speculate that flow stimulation of H⁺ secretion in the distal nephron may contribute to diuretic-induced metabolic alkalosis.

Funding: NIDDK Support

SA-OR339
Acidosis Induces Secretion of SDF-1 by Principal Cells Which Acts on β-Intercalated Cells (β-ICs) To Decrease HCO3 and Increase H Secretion
Sebastian Frische.1

Background: We show for the first time that UT-B has significant NH3 permeability. Previously, the Boron laboratory showed that multimeric proteins (e.g. Aquaporins and Rh proteins) can function as gas (CO2 and NH3) channels. This study explores the CO2 and NH3 permeability of human UT-B expressed in Xenopus oocytes (vs H2O-injected control oocytes).

Methods: We used microelectrodes to record the maximum transient change in surface pH (ΔpHi) caused by exposing the oocyte to 5% CO2 or NH3. We generated UT-B expressing oocytes by microinjection of RNA transcribed from a UT-B cDNA.

Results: We found that oocytes expressing UT-B showed a 79% reduction in the adaptive change in Jnet. To determine whether H+-ATPase activity was assayed in individually identified cells in microperfused CCCs isolated from NZW rabbits, loaded with the pH-sensitive dye BCECF, and then subjected to an acute intracellular acid load (NH4Cl), recovery of the activity of luminal H⁺ H+-ATPase was examined in the proximal tubule due to changes in both luminal NH3 and H+-ATPase activity (Du Z. et al, Am J Physiol Renal Physiol 290:F289, 2006), we studied to test the hypothesis that this flow also regulates H+-ATPase activity in the CCD.

Methods: H+-ATPase activity was assayed in individually identified cells in microperfused CDDs isolated from NZW rabbits, loaded with the pH-sensitive dye BCECF, and then subjected to an acute intracellular acid load (NH4Cl), recovery of the activity of luminal H⁺ H+-ATPase was examined in the proximal tubule due to changes in both luminal NH3 and H+-ATPase activity (Du Z. et al, Am J Physiol Renal Physiol 290:F289, 2006), we studied to test the hypothesis that this flow also regulates H+-ATPase activity in the CCD.

Results: We found that ΔpH in a NH4Cl flow from 1 to 5 ml/min. mm stimulated H⁺-ATPase activity (pH U/min) in both intercalated (IC; 0.059±0.009 to 0.146±0.023; p=0.03) and principal (PC; 0.059±0.009 to 0.126±0.004; p<0.03) cells, the latter identified by their selective labeling with the apical PC marker Dolichos Biflorus agglutinin, and (ii) flow-stimulated H⁺ pumping was Ca²⁺ dependent and required microtubule integrity, based on its inhibition by pretreatment with BAPTA-AM and colchicine, respectively.

Conclusions: We conclude that luminal flow modulates H⁺-ATPase activity in the rabbit CCD and that H⁺-ATPases therein are present in both PC and IC. We speculate that flow stimulation of H⁺ secretion in the distal nephron may contribute to diuretic-induced metabolic alkalosis.

Funding: NIDDK Support

SA-OR340
Acute Kidney Injury Following CABG Is Related to Long-Term Incidence of Myocardial Infarction
Martin Holzmann, Linda Ryden Lujan. Department of Emergency Medicine, Karolinska University Hospital, Internal Medicine Unit, Karolinska Institutet, Sweden.

Background: Acute Kidney Injury (AKI) is strongly related to early mortality and postoperative complications following coronary artery bypass surgery (CABG). Recent studies indicate that AKI following CABG is related to long-term mortality. However, there is little information on the long-term risk of myocardial infarction (MI) associated with AKI.

Methods: From the Swedish Heart Registry (Swedheart), all patients undergoing a first, isolated CABG electively, during 1995-2008 in Sweden, with information on pre- and postoperative serum creatinine, and alive 60 days postoperatively, were included. Information about confounders was found in the Swedish Coronary Angiography and Angioplasty Register and from the Swedish Inpatient Register and Cause of Death Register,
information on incidence of MI and all-cause mortality, was ascertained. Acute kidney injury was defined using a previously established base in patients with septic shock as creatinine creatinine concentration of 0.3-0.5 mg/dL, 0.5-1.0 mg/dL, or > 1.0 mg/dL, and according to AKIN criteria. Hazard ratios (HR) for different levels of AKI were calculated using Cox proportional hazards regression.

Results: The study population consisted of 45,002 patients, with a mean age of 67 years, and a total follow-up time of nine years. There were 3,945 (8.8%) MIs and 11,157 (25%) deaths. After adjustment for confounders, the HRs for MI (95% confidence intervals) associated with a postoperative increase in serum-creatinine of 0.3-0.5 mg/dL, 0.5-1.0 mg/dL, or > 1.0 mg/dL, were 1.52 (1.33-1.61), 1.85 (1.74-2.11) and 2.09 (1.78-2.31), respectively. Consistently, the corresponding figures for death were 1.48 (1.39-1.53), 2.04 (1.83-2.21) and 3.84 (3.50-4.01), respectively. Similar associations were found when AKI was defined using AKIN criteria.

Conclusions: Acute kidney injury defined according to an absolute increase in postoperative values of serum creatinine or AKIN criteria is strongly associated with both long-term incidence of MI and death. Cardiologists or family doctors following patients with a history of CABG should take into account the long-term prognostic implications of AKI post-CABG.

SA-OR341 Temporal Relationship and Predictive Value of Urinary AKI Biomarkers after Cardiac Surgery Prasad Devarajan, Caralynne D. Krawczeski, Stuart Goldstein, Yu Wang, Nuntawan Piyaphanee, Qing Ma, Michael R. Bennett. Cincinnati Children’s Hospital, Cincinnati, OH.

Background: Acute kidney injury (AKI) occurs commonly after cardiopulmonary bypass (CPB). Serum creatinine (Scr) is a delayed marker for AKI. We evaluated the temporal pattern and predictive value of 4 most promising urinary AKI biomarkers: liver fatty-acid binding protein (L-FABP), kidney injury molecule-1 (KIM-1), for CPB-associated AKI.

Methods: Urine samples were obtained prospectively before and after several time points after CPB in 220 patients. AKI was defined as a ≥50% increase in SCR from baseline. Biomarker values were correlated with AKI severity and clinical outcomes. Logistic regression was used to assess AKI predictors. Biomarker predictive abilities were evaluated by area under the curve (AUC), net reclassification improvement (NRI) and integrated discrimination improvement (IDI).

Results: AKI occurred in 27% of patients. NGAL first increased in AKI patients at 2h after CPB. IL-18 and L-FABP first increase at 6h and KIM-1 first increased at 12h. Biomarker elevations correlated with disease severity (RIPPLE) and clinical outcomes (length of hospital stay, days on ventilator), and improved prediction of AKI above the clinical model. At 2h post-CPB, NGAL alone increased the AUC from 0.74 for the clinical model to 0.85 (p<0.001). At 6h post-CPB, NGAL, IL-18 and L-FABP each improved the AUC from 0.72 to 0.91, 0.84, and 0.77 respectively (all p<0.05). At the 6th time point, the combination of NGAL + IL-18 provided the best results in terms of AUC improvement, NRI, and IDI. At 12h post-CPB, the best predictive ability was obtained when all four biomarkers were combined.

Conclusions: This is the first large prospective study to (1) establish a temporal pattern of multiple AKI biomarkers after CPB, which correlate with AKI severity and clinical outcomes, and (2) demonstrate the utility of biomarker combinations for the improved prediction of AKI beyond clinical models. Biomarker combinations hold tremendous promise for guiding the application of appropriately timed AKI therapies, based on the underlying pathophysiology.

Funding: NIDDK Support, Other NIH Support - NHLBI


Background: Effective therapy for septic shock associated acute kidney injury (SSAKI), a disease process carrying significant morbidity and mortality, is lacking. A limitation for such therapy is that detection strategies for SSAKI, traditionally dependent on serum creatinine, are considerably varied and heterogeneous. While existing AKI biomarkers demonstrate diagnostic sensitivity for identification of SSAKI over 0.3-0.5 mg/dL, they may still be confounded by the unique pathophysiology of SSAKI.

Methods: We leveraged a previously generated genome-wide expression database of children with septic shock to address the need of identifying novel candidate biomarkers of SSAKI. Thirty-on patients with SSAKI (defined as a doubling of baseline serum creatinine) and 148 patients without SSAKI were identified. SSAKI persisted, to seven days after hospital admission.

Results: SSAKI patients were clinically similar to those without SSAKI but had higher mortality (45% versus 10%). 21 unique gene probes were up-regulated in SSAKI patients compared to patients without SSAKI. Using leave-one-out cross validation and class prediction modeling, the expression patterns of the 21 gene probes predicted SSAKI with a sensitivity of 98% (95% confidence interval (CI) 81-100) and a specificity of 80% (95% CI 70-80). Individual performance calculations were performed using two specific gene products (i.e. serum protein measurements) showing high sensitivity for predicting SSAKI: matrix metalloproteinase-8 (MMP-8) (89%, 95% confidence interval (CI) 64-98) and elastase-2 (83%, 95% CI 58-96). Both candidate biomarkers carried a negative predictive value of 95%. When applied to a separate validation cohort, both candidate biomarkers carried high sensitivity: MMP-8 (100%, 95% CI 68-100) and elastase-2 (100%, 95% CI 68-100).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
SA-OR345

Early Dynamic Changes in Serum Creatinine Predict Renal and Patient Outcome in Septic ICU Patients
Claudio Rigatto, Manish M. Sood, Paul Komenda, Joe A. Bueti, Anand Kumar. Internal Medicine, University of Manitoba, Winnipeg, MB, Canada.

Background: AKI predicts poor prognosis in patients with septic shock. It is not known whether early changes in serum creatinine (SCr) measured after ICU admission help predict renal outcome and survival in sepsis. An early drop in SCr may identify a good response to resuscitation and predict better outcomes.

Methods: Data was obtained from the Cooperative Antimicrobial Therapy of Septic Shock database comprising patients from 28 centres worldwide. 6504 had, serum creatinine measured at admission and 6 hours post. Patients requiring immediate initiation of RRT or with SCr > 400 mc mol/L on admission were excluded. The main exposure variable was % change in creatinine over the first 6 hours (PCr6); main outcome variables were need for dialysis and survival at 2 weeks. Multivariate Logistic Regression was used to compare predictive models with and without PCr6.

Results: 4826 patients were analyzed. 343(7.1%) required dialysis and 1768 (36.6%) died within 2 weeks. Age, APACHE II, RIFLE stage on admission, and fluid volume in the first 6 hours were the strongest predictors of need for dialysis (Base Dialysis Model). PCr6 was strongly associated with dialysis (OR 1.01 [1.006, 1.02]), was highly significant when added to the Base Dialysis Model (OR 1.02 [1.012, 1.033]), and increased model discrimination (c-statistic 0.84 [0.82, 0.86] vs. 0.82 [0.80, 0.85]; p=0.001). Age, sex, Apache II, centre, and Gram negative sepsis were the strongest predictors of survival at 2 weeks post admission (Base Survival Model). Higher PCr6 was significantly associated with poor survival (OR 0.97 [0.97, 0.98]), was highly significant when added to the Base Survival Model, and improved discrimination (c-statistic 0.78 [0.77, 0.79] vs. 0.76 [0.74, 0.77]; p<0.0001). Patients who experienced a drop in creatinine >10% at 6 hours had a much lower odds of needing dialysis (OR 0.44 [0.33, 0.60]) or dying (OR 0.41 [0.35, 0.48]).

Conclusions: In septic patients admitted to ICU, early dynamic changes in creatinine (in the first 6 hours) provide important prognostic information.

SA-OR346

Proximal and Distal Tubule Epithelial Cells Express CD133 during Repair after Acute Kidney Injury in Humans
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Background: Several recent studies have used CD133 (prominin-1) to identify and isolate renal progenitor cells in parietal epithelial cells (PEC) of glomeruli and proximal tubules in human kidney tissue. However, the “progenitor” status of the cells that express this protein has not been well established. In this study, we evaluated CD133 staining in normal fetal and adult human kidneys, and in various human renal diseases characterized by acute tubular injury (ATI).

Methods: Fetal kidneys (12-32 wks, n=64), normal adult kidneys (n=11) and renal biopsies from patients with ATI (primary or secondary, n=33) were evaluated for expression of CD133 using immunohistochemical techniques with two antibodies (ab) (monoclonal AC133 clone, 1:50, Miltenyi and polyclonal ab, 1:500, Biocare). CD133 expression was compared with expression of kidney injury molecule-1 (KIM-1, using AKG7 ab at 1:10), an injury marker of proximal tubules and cytokeratin-7, a distal-nephron tubular marker.

Results: Both types of CD133 antibodies showed a similar pattern of apical membrane staining. In fetal kidneys, CD133 stained primordial glomerular structures and medullary tubules. In normal kidney sections with negative KIM-1 staining, membrane CD133 staining was seen in PEC of the glomeruli and in less than 5% of proximal and distal tubular epithelial cells. In injured dedifferentiated proximal tubules, confirmed by positive KIM-1 staining, CD133 membrane expression along the apical surface was present in the majority of injured epithelial cells. With ATI, many distal tubule cells expressed both apical CD133 and cytokeratin-7, a distal-nephron tubular marker.

Conclusions: Under normal conditions, CD133 is expressed in human fetal kidneys. In ATI, diffuse CD133 expression is seen in dedifferentiated injured tubular epithelial cell apical membranes implying that, rather than identify a specific “progenitor” population, CD133 is upregulated in dedifferentiated epithelial cells that are involved in repair of the epithelium after injury.

Funding: NIDDK Support, Clinical Revenue Support

SA-OR347

Higher Concentrations of Inflammatory and Apoptotic Biomarkers Are Associated with Mortality and Nonrecovery of Kidney Function
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Background: Acute Kidney Injury (AKI) is a common complication of critical illness with high mortality rate despite renal replacement therapy (RRT). Although, higher circulating concentrations of inflammatory and apoptotic biomarkers are associated with AKI, whether these biomarker predict outcomes in patients with severe AKI who are receiving RRT is unknown.

Methods: In a subset of 819 subjects from the Acute Renal failure Trial Network study, we measured 11 plasma inflammatory and apoptotic biomarkers on day-1 of RRT. Outcomes were 60-day mortality and renal recovery defined as alive and independent from RRT at day-60. We hypothesized that higher inflammatory and apoptotic biomarkers concentrations on day 1 would predict poor outcomes.

Results: 297 subjects (36.3%) died and 107 (13.1%) remained on RRT by day 60. 7 of the 11 plasma markers concentration were significantly lower in survivors and in subjects who recovered. Higher plasma concentrations of inflammatory and apoptotic markers were associated with a higher risk of renal non-recovery and death.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

83A
Conclusions: Pre-operative plasma EO levels are powerful biomarkers of AKI and post-operative complications within 24-48 hour. The elevated circulating levels of EO are strongly associated with the severity of illness, renal dysfunction, kidney damage and in-hospital mortality.

Funding: Government Support - Non-U.S.

SA-OR349
Urine Hepcidin Has Additive Value in Ruling out Cardiopulmonary Bypass–Associated Acute Kidney Injury – An Observational Cohort Study  
Mark E. Westerman,1 Michael Haase,1 Anja Haase-Fielitz,1 Peter R. Mertens,1 John R. Prowle,4 Rinaldo Bellomo.2

Background: Conventional markers of acute kidney injury (AKI) lack diagnostic accuracy and are expressed only late after cardiac surgery with cardiopulmonary bypass (CPB). Recently, interest has focused on hepcidin, a regulator of iron homeostasis, as a unique renal biomarker.

Methods: We measured hepcidin (by ELISA) in 100 adult patients in the control arm of a randomized controlled trial (NCT00672334) that were identified to be at increased risk of AKI after cardiac surgery with CPB. AKI was defined according to RIFLE classification.

Results: At 6 and 24 hours after CPB, in AKI-free patients (N=91) urine hepcidin concentrations had largely increased and were 3 to 7 times higher compared to patients who did not develop AKI (AUC-ROC 0.80 [95% CI 0.71-0.87]; 0.88 [95% CI 0.78-0.97]) (see Figure). Furthermore, elevated urine hepcidin and, even more so, urine hepcidin adjusted to urine creatinine at 6 hours after CPB discriminated patients who did not develop AKI (AUC-ROC 0.80 [95% CI 0.71-0.87]; 0.88 [95% CI 0.78-0.97]) (see Figure) or did not need renal replacement therapy (AUC 0.81 [95% CI 0.72-0.88]; 0.88 [95% CI 0.70-0.99]) from those who did. At 6 hours, urine hepcidin adjusted to urine creatinine was an independent predictor of ruling out AKI (P = 0.011). Plasma hepcidin did not predict the lack of AKI development.

Conclusions: Our findings suggest that urine hepcidin is an early predictive biomarker of ruling out AKI after CPB thereby contributing to early patients risk stratification.

SA-OR350
Discordance between Measured Phosphorus (P) and Calcium (Ca) Content in Foods and Estimates from Nutrient Databases

Background: Current clinical practice uses nutrient databases to counsel patients about dietary P and Ca content. Previous studies revealed that nutrient databases underestimate P content of prepared foods. There are limited data on accuracy of Ca content for any foods and P content of unprocessed foods.

Methods: P and/or Ca content of 34 food items was measured (M) using ICP-MS (inductively coupled plasma mass spectroscopy) analysis at Michelob Laboratories Inc. (Commerce, CA), and data were compared to P and Ca content estimated (E) from nutrient databases (Nutrition Data System for Research, Minneapolis, MN). Confirmatory analysis of selected food items was performed at Medallion Laboratories, Minneapolis, MN.

Results: The E/M ratio for P content of foods varied widely from 0.55 to 1.40 (Figures). Wide variability was observed for both unprocessed and processed food items. The E) P content in brown rice of 77 mg/100 g was significantly lower than the (M) 133 mg/100 g. Furthermore, the P content of coffee creamer, negligible by (E), was 107 mg/100 g by (M). The E/M Ca content in the three food items varied from 0.88 to 3.63, and the (M) content was discordant from both the database and the nutrition label. For example, the (E) Ca of 194 mg/100g in rhubarb was notably higher than the (M) 49 mg/100g.

Conclusions: To our knowledge, this is the first report of discordance between estimated and measured Ca and P content of a wide range of unprepared food items. Dietary recommendations for populations in which dietary P and Ca may need to be controlled should consider these discrepancies. Also, labeling of measured P and Ca content in foods may be useful.

Funding: Other NIH Support - Creff Grant and GCRC support, Pharmaceutical Company Support, Private Foundation Support

SA-OR351
Dietary Phosphorus Is Associated with Left Ventricular Mass: The Multi-Ethnic Study of Atherosclerosis

Background: Dietary phosphorus consumption has steadily increased in the US. Phosphorus alters key regulatory hormones and impairs vascular endothelial function, which may increase left ventricular mass (LVM). We investigated the association of dietary phosphorus intake with LVM in 4,494 individuals.

Methods: We studied participants from the Multi-Ethnic Study of Atherosclerosis, a community-based study of individuals free of cardiovascular disease. We quantified dietary phosphorus intake using a 120-item food frequency questionnaire and measured LVM using cardiac MRI. We used linear and logistic regression models to estimate associations of dietary phosphorus intake with LVM and left ventricular hypertrophy (LVH), respectively.

Results: Mean dietary phosphorus intake was 1,167 mg/day in men and 1,017 mg/day in women. After adjustment for demographics, eGFR, total and sodium intake, lifestyle factors, blood pressure, and other risk factors for LVH, each 20% greater dietary phosphorus intake was associated with an 1.1 gram greater LVM (95% CI 0.54, 1.63). The highest sex-specific quintile of dietary phosphorus intake was associated with an 6.2 gram greater LVM (95% CI 2.5, 9.8) and 2.1-fold greater odds of LVH (95% CI 1.1, 4.1) compared to the lowest sex-specific quintile.

Conclusions: Greater dietary phosphorus consumption is associated with greater LVM and a greater prevalence of LVH in a community-based cohort. If confirmed in future studies, dietary phosphorus may be a novel cardiovascular risk factor.

Funding: Other NIH Support - NIH NIDDK T32 “Research Training in Renal Disease”

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SA-OR352  
Effect of Bone Morphogenetic Protein-7 on Vascular Smooth Muscle Cells Calcification Induced by High Phosphorous

**Background:** This study is aimed at investigating the effect of Bone Morphogenetic Protein-7 (BMP-7) on vascular smooth muscle cells (VSMCs) calcification induced by high phosphorous in vitro.  

**Methods:** The explants derived from thoracic aorta were used for primary culture. Underline represents presenting author.  

**Conclusions:** BMP-7 inhibits the transformation of VSMCs into osteoblast-like cells induced by high phosphorous in vitro. 

SA-OR353  
**MicroRNAs Induced by High Concentration of Phosphate and Calcium Are Involved in Vascular Smooth Muscle Cell Calcification**

**Background:** MicroRNAs (miRNAs) are recently discovered class of endogenous, small, non-coding RNAs that regulate the expression of protein-coding genes. The role of miRNAs in vascular calcification is currently unclear.  

**Methods:** We examined the alteration of miRNAs in vascular smooth muscle cell (VSMC) calcification using miRNA array analysis, we demonstrated that miRNAs are abnormally expressed in the aortic media of 3 week-old klotho knock-out (KO) mice.  

**Conclusions:** These results suggest that PMCA1, NCX1 and NCKX4 could be the potential targets of these miRNAs and that the reduction of these calcium transporters can cause the VSMC calcification.

SA-OR354  
**Correction of Hyperphosphatemia in Uremic Npt2b Knockout Mice Treated with Ketoconazole**

**Background:** Hyperphosphatemia is a major component of phosphate homeostasis. Despite the important role of vitamin D in maintaining bone health, as well as a variety of other physiological functions, many clinicians are reluctant to treat vitamin D deficiency in kidney stone formers because of the theoretical risk of increasing urinary calcium (Ca) excretion and the risk of calcium stone recurrence. We report on the effect of vitamin D repletion on Ca excretion in UCa among stone-formers.  

**Methods:** Patients were recruited from three metabolic stone clinics affiliated with Columbia University Medical Center. Enrollment criteria included: 1) history of kidney stone formation and Ca excretion 400 mg/day or above; 3) serum inorganic hydroxyvitamin D (25(OH)D) level less than 30 ng/ml. Patients were given oral ergocalciferol 50,000 IU daily for 8 weeks. Serum inorganic P increased (P, mg/dL: before, 139; urinary P: before, 1525, after, 962). Serum inorganic P increased (P, mg/dL: before, 3.6, after, 4.4), 25(OH)D decreased (before, 50, after, 40). 

**Conclusions:** In a syndrome characterized by intermittent hypercalcemia, hyperparathyroidism, reduced BMD, and spinous junction abnormalities, the administration of ketoconazole reduces serum calcium concentration and urinary Ca excretion. Ketoconazole can be an effective treatment for hypercalcemia and hyperparathyroidism in patients with mutations of the Cyp24a1 gene.

**Funding:** NIDDK Support, Other NIH Support - AR

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

85A
Conclusions: Among stone-formers with vitamin D deficiency and moderate levels of hypercalciuria, vitamin D supplementation does not appear to increase UCa excretion. Vitamin D therapy, if indicated, should not be withheld on the basis of prior stone disease.

SA-OR357
Dietary Calcium Regulates SLC26A6-Mediated Oxalate Secretion Felix Knauf, Robert Brent Thomson, Peter S. Aronson. Section of Nephrology, Yale University School of Medicine, New Haven, CT.

Background: Dietary calcium intake is an important determinant of urinary oxalate excretion and risk for calcium-oxalate nephrolithiasis. Net intestinal absorption of dietary oxalate results from passive paracellular oxalate absorption as modified by SLC26A6-mediated active secretion. It has been postulated that dietary calcium restriction leads to increased oxalate absorption by reducing precipitation of oxalate with calcium in the lumen of the intestine, thereby increasing soluble oxalate available for absorption. We tested the hypothesis that dietary calcium also modulates net oxalate absorption by regulating SLC26A6-mediated oxalate secretion.

Methods: Wild-type mice were fed either a regular or low calcium diet. After 2 weeks the duodenum was harvested and probed for SLC26A6 protein expression normalized to α-actin by Western blot analysis. Active secretion of [14C]-oxalate was measured in vitro across isolated intestinal tissue mounted in Ussing chambers and bathed in standard calcium-containing Ringers solutions. To determine if the effect of lowering dietary calcium is specific for active oxalate secretion, active [14C]-glucose absorption was also assayed. Mannitol permeability and total tissue conductance were measured as markers of paracellular permeability.

Results: Mice fed a low calcium diet showed a 70% reduction of intestinal SLC26A6 protein abundance as compared with animals fed a regular calcium diet. Active oxalate secretion was reduced by 50% in intestinal tissue isolated from mice fed a low calcium diet compared to intestine from mice on a regular diet. Glucose absorption was not changed by modifying dietary calcium, indicating that the effect of a low calcium diet to reduce oxalate secretion is not the result of a general downregulation of intestinal transport processes. Paracellular permeability as measured by mannitol permeability and tissue conductance was unaffected by dietary calcium.

Conclusions: We demonstrate that dietary calcium regulates SLC26A6-mediated oxalate secretion in the intestine. Downregulation of SLC26A6 may contribute to the increased net absorption of dietary oxalate that results from ingestion of a low calcium diet.

Funding: NIDDK Support, Private Foundation Support

SA-OR358
Increased Sensitivity to 1,25(OH)2D in Genetic Hypercalciuric Stone-Forming Rats Kevin K. Fick, John R. Asplin, Christopher D. Culbertson, Daniel M. Asplin, Nancy Krieger, David A. Bushinsky. 1Medicine, University of Rochester, NY; 2Litholink Corporation, Chicago, IL.

Background: Genetic hypercalciuric-stone forming (GHS) rats, bred to maximize urinary oxalate excretion, exhibit increased intestinal Ca absorption, increased bone resorption, and increased renal tubular Ca reabsorption, all leading to increased UCa compared to controls and all form kidney stones. GHS rats express an increased number of vitamin D receptors (VDR) at these sites of disordered Ca transport.

Methods: To determine if the excess VDR is biologically active, we fed GHS rats a Ca replete diet (NCD, 1.2% Ca) and injected 1,25(OH)2D (1.25D, 25 ng/d) or vehicle (veh) for 9 d. To determine if GHS rats would also show increased UCa in the absence of 1,25D-induced intestinal Ca absorption, GHS and SD rats were fed a low Ca diet (LCD, 1,25D, UCa in SD increased from 1.3±0.1 mg/d to 9.4±0.9 and in GHS from 10.5±0.7 to 41.9±0.7 and PTH was suppressed to undetectable levels across isolated intestinal tissue mounted in Ussing chambers and bathed in standard calcium-containing Ringers solutions. To determine if the effect of lowering dietary calcium is specific for active oxalate secretion, active [14C]-glucose absorption was also assayed. Mannitol permeability and total tissue conductance were measured as markers of paracellular permeability.

Results: Mice fed a low calcium diet showed a 70% reduction of intestinal SLC26A6 protein abundance as compared with animals fed a regular calcium diet. Active oxalate secretion was reduced by 50% in intestinal tissue isolated from mice fed a low calcium diet compared to intestine from mice on a regular diet. Glucose absorption was not changed by modifying dietary calcium, indicating that the effect of a low calcium diet to reduce oxalate secretion is not the result of a general downregulation of intestinal transport processes. Paracellular permeability as measured by mannitol permeability and tissue conductance was unaffected by dietary calcium.

Conclusions: We demonstrate that dietary calcium regulates SLC26A6-mediated oxalate secretion in the intestine. Downregulation of SLC26A6 may contribute to the increased net absorption of dietary oxalate that results from ingestion of a low calcium diet.

Funding: NIDDK Support, Private Foundation Support

SA-OR51
Randall’s Plaques Are Aggregates of Spherical and Elongated Striated Units of Poorly Crystalline Biological Apatite Saeed R. Khan, Manoj Monga. Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL.

Background: Idiopathic calcium oxalate (CaOx) kidney stones develop by deposition of CaOx crystals on Randall’s plaques (RP). Mechanisms involved in RP formation are still unclear. It is our hypotheses that RP formation is similar to vascular calcification involving components of extracellular matrix including membrane bound vesicles and collagen fibers. In order to verify our hypothesis we critically examined renal papillary tissue from stone patients obtained at time of stone removal.

Methods: Cold-cup biopsy of renal papilla was performed on 10 idiopathic stone patients undergoing PCNL. Kidney tissue was immediately fixed and processed for light and electron microscopy. All samples were examined by light and transmission electron microscopy (TEM). Some were also examined by scanning electron microscopy (SEM). Energy dispersive x-ray microanalyses and electron diffraction were utilized to identify the crystals.

Results: Multi-laminated spherulitic CaP deposits, the hallmark of RP’s, were seen by TEM in interstitium as well as laminated basement membrane of tubular epithelia. In addition CaP crystals were also organized as bundles of elongated striated fibers. Both crystal types were associated with collagen fibers and membrane bound vesicles. Many such vesicles either contained or associated with needle shaped crystals. Some of the striated interstitial crystals were closely aligned with collagen fibers.

Conclusions: Randall’s plaques comprise of two types of crystal deposits, the well known rounded units as well as membrane bound collagen fibers. Both types of deposits are associated with collagen fibers as well as membrane bound vesicles. The vesicles contained needle shaped crystals. We conclude that crystal deposition in renal papillae may start with membrane vesicle induced nucleation and grows by mineralization of interstitial collagen fibers. Similar mechanism has been proposed for calcium phosphate crystal deposition elsewhere in the body.

Funding: NIDDK Support

SA-OR360
Six1 Is a Key Mediator of TGF-beta Induced Renal Fibrosis Arthur Chi-Kong Chung, Xiang Zhong, Hui Y. Lan. 1Li Ka Shing Institute of Health Sciences, Chinese University of Hong Kong, Shatin, NT, Hong Kong; 2Department of Chemical Pathology, Chinese University of Hong Kong, Shatin, NT, Hong Kong; 3Department of Medicine and Therapeutics, Chinese University of Hong Kong, Shatin, NT, Hong Kong.

Background: TGF-β/Smad signaling is a key pathway that mediates renal fibrosis. However, mechanisms that control the fibrogenic gene expression during fibrosis remained unclear. The present study tested the hypothesis that Six1 mediated Smad3-dependent renal fibrosis.

Methods: Six1 expression in tubular epithelial cells (TECs) after TGF-β treatment and diseased kidney was examined by Realtime PCR. The functional role of Six1 in renal fibrosis was determined in vitro by overexpression and knockdown cell lines of Six1, and in vivo by delivering Six1 overexpression and knockdown plasmid in the obstructive kidneys by ultrasound-microbubble gene transfer system. Expression levels of the fibrogenic markers were examined by Realtime PCR and Western blot analyses.

Results: Activation of TGF-β/Smad signaling was necessary for upregulation of Six1 in the fibrotic kidney and TECs in response to TGF-β. Enhanced TGF-β/Smad signaling in Smad7 knockout mice promoted Six1 expression and fibrosis in obstructive nephropathy. In addition, TGF-β induced Six1 expression in time- and dosage-dependent manner. This induction is mediated by Smad3, but not Smad2. Furthermore, Six1 expression was again suppressed to Smad3 and Six1 levels, protected from upregulation of Six1 and fibrosis in obstructive nephropathy, as well as the TGF-β-stimulated renal TECs and mouse embryonic fibroblasts. Furthermore, overexpression of Six1 promoted further TGFβ treatment in TECs. More importantly, delivery of Six1 knockdown plasmid ameliorated renal fibrosis in mouse model of obstructive kidney diseases, as evidence with reduction of expression of collagen I, fibronectin and α-smooth muscle actin.

Conclusions: Six1, a critical downstream mediator of TGF-β/Smad3 signaling, plays an essential role for renal fibrosis in response to TGF-β. Targeting Six1 may be an effective therapeutic approach to combat renal fibrosis.

Funding: Government Support - Non-U.S.
Methods: Expression of microRNAs in human mesangial cells (HMC) in response to TGF-β, CTGF or untreated control-treated, target analysis identified putative gene targets. Expression of microRNA and signaling mediators in the 10 day unilateral ureteral obstruction (UUO) mouse model was investigated.

Results: TGFB receptor II (TβRII) was determined to be a putative target of the miR-302d/miR-302c family of microRNAs. In HMC co-transfection led to a down-regulation of TGF-β and CTGF decreased expression of TβRII in HMC concomitantly with increasing levels of miR-302d, as a proxy readout for miR-302 activity. We validated TβRII as a miR-302d target and inhibited miR-302d to attenuate CTGF dependent changes in TβRII levels. Further, we found a relevant significance of Smad family members, with attenuation of canonical Smad signaling and induction of non-canonical signaling associated with differential expression of miR-302d. In the UUO mouse model, miR-302d expression was increased with a coincident decrease in TβRII, suggesting pathophysiological significance. Decreased expression of the TGFB receptor II (TβRII) and an apparent dichotomy between decreased Smad2 and increased Smad3 phosphorylation was also observed.

Conclusions: Opposing levels of activated Smad2 and Smad3 lends support to the hypothesis that differential activity of both Smads is tacit in the progression of fibrosis. Further, the apparent lower levels of TβRII raise the intriguing possibility of chronic Smad3 phosphorylation and fibrotic damage in the UUO mouse being, at least in part, TGFB receptor independent.

Funding: Government Support - Non-U.S.

SA-OR364
p38-alpha Phosphorylates Nephrin and Induces Its Endocytosis Via Association with beta-arrestin2

Magdalena Woznowski, Sebastian Alexander Potthoff, Eva Koenighausen, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin, Ivo Quack. Nephrology, Heinrich-Heine University, Duesseldorf, Germany.

Background: Podocyte damage leads to foot process retraction, disruption of the slit diaphragm and proteinuria. Human acquired glomerulopathies and diabetic nephropathy are associated with TNF-alpha elevation. Especially the TNF-alpha activated MAPK p38 is known as a critical role in mediating podocyte injury. Recent advances have also revealed crucial roles of slit diaphragm-associated proteins, including nephrin, podocin and beta-arrestin2. In this regard, beta-arrestin2 was shown to associate with nephrin, leading to nephrin endocytosis and thereby causing proteinuria.

Methods: GST-nephrin fusion proteins containing different fragments of the nephrin cytoplasmic domain were used in a radioactive kinase assay with recombinant p38. In addition, nephrin mutants containing point mutations of putative p38 phosphorylation sites were generated and tested in these kinase assays. Expression plasmids for nephrin and podocin mutants as well as for beta-arrestin2 were transfected into HEK-293T cells, which were subsequently treated with TNF-alpha. After cell lysis, immunoprecipitation with subsequent western blot analysis was performed.

Results: TNF-alpha treatment of podocytes or HEK293T cells leads to a rapid activation of the MAPK p38. Interestingly, nephrin is phosphorylated by p38alpha at Ser 1146. The nephrin mutant S1146A, which is not phosphorylated by p38 anymore, is impaired in its ability to associate with beta-arrestin2 and demonstrate less TNF-alpha-mediated endocytosis. On the other hand, a phospho-mimicry mutant S1146D shows a stronger interaction with beta-arrestin2 and increased endocytosis.

Conclusions: TNF-alpha induces nephrin/beta-arrestin2 association and nephrin endocytosis. The MAPK p38 acts as a crucial mediator in this regard via phosphorylation of nephrin, thereby regulating its presence in the slit diaphragm. This could represent an important molecular mechanism responsible for TNF-alpha-mediated podocyte damage. In addition, this suggests that inhibition of p38 via pharmacological intervention could protect against inflammation-induced proteinuria.

SA-OR365
Functional Excitatory and Inhibitory Aminoacid Receptors in Primary Cultured Podocytes

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Background: We showed that podocytes express several types of receptors, second messenger systems and proteins usually associated with synaptic communication between neurons. Such information suggests that podocytes have the potential to respond to external stimuli and transduce these signals into rapid, coordinated responses. Although there is molecular evidence for expression of these channels and receptors, almost no data are available which have functionally characterised them in primary podocytes.

Methods: By means of intracellular calcium imaging using the Ca2+-sensitive fluorescent dye Fura-2, we investigated the functional expression in primary cultured podocytes of a wide array of proteins typically involved in neuronal synaptic functions.

Results: Using this approach, we defined the functional expression of the three main subtypes of ionotropic glutamate receptors, by recording increases in [Ca2+]i in response to (beta) exposures of primary podocytes to: glutamate (100uM), kainate (100uM); N-methyl-D-aspartic acid (NMDA - 100uM); t-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA - 50 uM). Furthermore, both GABAA and GABAB receptors were functionally present, since exposure of cells to GABA (r-amino butyric acid - 300 uM) evoked an increase in [Ca2+]i which was only partially attenuated upon removal of extracellular Ca2+. Interestingly, the rise in [Ca2+]i upon GABAA receptor activation shows that the result of opening Cl- channels in these cells is cell depolarization and consequent Ca2+ entry through voltage-gated Ca2+ channels, a notion fully supported by the ability of 50 mM extracellular K+ to evoke a large increase in [Ca2+]i.

Conclusions: Our new data provide functional evidence for the existence of kainate, AMPA, NMDA, GABAA and GABAB receptors in primary podocytes and further support the idea that these cells have the potential to respond to external stimuli in a manner which appears similar to that normally associated with neuronal signaling.

Funding: Private Foundation Support, Government Support - Non-U.S.
SA-OR366

Signaling through the NF-kB Essential Modulator NEMO Triggers Podocyte Migration and Prolongs Proteinuria in a Nephropathic Nephritis Model

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Background: Inflammatory diseases lead to glomerular injury and are a major cause of proteinuria. The Nuclear Factor kappaB is one of the most important regulators of pro-inflammatory signaling. Its role in podocytes, however, is only poorly understood. In this study we inhibited NF-kB signaling in podocytes in vivo by specific ablation of the essential modulator NEMO.

Methods: Nemo flox/flox;Podocin cre/wt mice were viable and showed no proteinuria or changes in kidney morphology under non-stressed conditions. In a nephrotoxic nephritis model both KO and wild-type mice developed severe proteinuria within 3 days. At day 7 KO mice recovered faster and showed decreased levels of proteinuria compared to wild-type littermates. There were no differences in infiltrating inflammatory cells. However, immunohistochemistry and electronmicroscopy revealed restoration of the slit diaphragm morphology in KO mice while wild-type showed significant foot-process effacement.

Results: To address the mechanisms that underlie this beneficial phenotype we established a Nemo-knockdown podocyte culture system. Knockdown of NEMO resulted in reduced production of pro-inflammatory chemokines. In addition, reduced NF-kB activity led to decreased phosphorylation of ERK1/2/MAP-kinases in vitro and reduced cellular migration. Inhibitor studies were carried out to link inactivation of ERK1/2 to the stationary phenotype of Nemo-knockdown podocytes. Our results showed that inhibiting ERK1/2 activation blocked the migratory phenotype in wild-type cells comparable to Nemo-knockdown podocytes.

Conclusions: In conclusion, signaling through Nemo might not only be involved in the migration of podocytes, but also in inflammatory conditions but also regulates podocyte mobility and structure through activation of MAPK. This pathway might represent a key regulator of podocyte migration in states of disease.

SA-OR367

Dietary Acid Reduction with Added Fruits and Vegetables or Oral Bicarbonate Preserves GFR in Subjects with CKD Stage 4 GFR (Abstract Withdrawn)

Nirimit Goraya,1,2 Channeh Jo,1 Donald E. Wesson.1,2 Medicine, Texas A & M College of Medicine, Temple, TX; 2Medicine, Scott & White Healthcare, Temple, TX; 3Biostatistics, Scott & White Healthcare, Temple, TX.

Background: Subjects with hypertensive nephropathy (HN) have progressive GFR decline despite blood pressure reduction with ACE inhibition but dietary acid reduction with oral alkalis helps preserve GFR in HN patients with moderately reduced GFR. We explored if dietary acid reduction also preserves GFR in HN with severely reduced eGFR.

Methods: Subjects with CKD 4 on ACE inhibition were given F+V in an amount designed to reduce potential renal loss by 50% (N=36) or oral NaHCO3 at 1.0 meq/kg lean body weight/day (N=35) and compared to control subjects without dietary intervention (N=35). Entry and follow up cystatin C-calculated eGFR (cysGFR), systolic blood pressure (SBP), and plasma potassium (P) were measured after one year.

Results: At entry, mean cysGFR was not different among groups (F+V=21.6±4.6 ml/min, NaHCO3=21.7±3.4 ml/min, and time control=21.5±3.0 ml/min) but after 1 year, cysGFR was higher in both intervention groups (F+V=20.7±4.7 ml/min, NaHCO3=20.3±3.2 ml/min) than time control (17.6±2.5 ml/min, p<0.002 vs. F+V and NaHCO3). SBP at study entry was different among groups (F+V=136.3±4.8 mmHg, NaHCO3=136.7±4.7 mm Hg and time control=137.0±4.3 mmHg) but follow up SBP was lower than at entry in F+V (-4.6±3.8 mmHg, p<0.001 vs. entry) and was not statistically different from the entry value up to follow for the remaining groups. Despite an increase in urinary potassium excretion with F+V, P was not statistically different at follow up compared to the entry value (net change=0.01±0.12 meq/ml, p=0.593).

Conclusions: Dietary acid reduction is an effective adjunct to conventional therapy in preserving GFR in CKD stage 4 HN and doing so with F+V compared to NaHCO3, does not increase P and appears to have the added cardiovascular benefit of SBP reduction.

Funding: Private Foundation Support, Clinical Revenue Support

SA-OR368

Estimated Net Endogenous Acid Production and Chronic Kidney Disease Progression in African Americans: The African American Study of Kidney Disease and Hypertension (AASK) Julia J. Scialla,1 Lawrence J. Appel,1 Brad C. Astor,1 Edgar R. Miller,1 Srivinasan Beddhu,2 Mark Woodward,1 Rulan S. Parish,1 Cheryl A. Anderson.1 Johns Hopkins University; 2University of Utah; 1University of Sydney; 1University of Toronto.

Background: In CKD, increases in per nephron acid excretion needed to counteract the daily dietary acid load may promote renal injury and contribute to disease progression.

Methods: We evaluated the association between the net endogenous acid production (NEAP) estimated by the diet, and rates of GFR decline in 632 African Americans with hypertensive CKD from the AASK Trial. Estimated NEAP was calculated from complete 24 hour urine specimens, collected between 12 and 36 months post-randomization, in participants not taking potassium or alkali supplements. Protein and potassium intake were estimated from 24 hour urine nitrogen and potassium excretion, respectively. Estimated NEAP was calculated as previously described: NEAP (meq/d) = 10.2 + 54.5 [protein intake (g/d) / protein intake (meq/d)]. NEAP estimates were averaged to approximate baseline habitual dietary intake (49% of participants had 5 or more measures). I125iothalamate glomerular filtration rate (iGFR) was measured every 6 months over follow up (average iGFR measures per participant). The association between estimated NEAP and slope of iGFR from 12 months post-randomization was determined using linear mixed models with adjustment for randomized drug and blood pressure groups, and categories of age, body mass index, baseline GFR, and proteinuria on iGFR slope.

Results: Median baseline iGFR was 48.6 (IQR 36.6 - 58.5) ml/min/1.73 m2. In unadjusted and adjusted models there was a faster rate of decline in iGFR among those in higher quartiles of estimated NEAP (p-trend=0.05 and 0.01, respectively).

Funding: NIH Support, Clinical Revenue Support - National Center on Minority Health and Health Disparities, NCMHD, Pharmaceutical Company Support, Private Foundation Support

SA-OR369

Moderate Sodium Diet Potentiates the Renal and Cardiovascular Protective Effects of Angiotensin Receptor Blockers: A Post-Hoc Analysis of the RENAAI and IDNT Trials

Hiddo Jan Lambers Heerspink,1 Frank Holtkamp,1 Eberhard Ritz,1 Hans-Henrik Parving,1 Gerjan Navis,1 Dick de Zeeuw.1 Clinical Pharmacology, University Medical Center Groningen, Groningen, Netherlands; 1Medical Endocrinology, University Hospital Copenhagen, Copenhagen, Denmark; 1Internal Medicine, University of Heidelberg, Germany.

Background: Dietary sodium restriction has been shown to enhance the short-term response of blood pressure and albuminuria to Angiotensin Receptor Blockers (ARBs). Whether this also enhances the long-term renal and cardiovascular protective effects of ARBs is unknown. We conducted a post-hoc analysis of the RENAAI and IDNT trials to test this hypothesis.

Methods: Patients with type 2 diabetes and nephropathy were randomized to ARB or non-Renin-Angiotensin-Aldosterone-System based antihypertensive therapy (control therapy). Treatment effects on renal and cardiovascular outcomes were compared in tertiles of dietary sodium intake, measured as on-treatment 24-hour urinary sodium:creatinine ratio (Na/Cr ratio).

Results: The analysis included 1177 subjects. Urinary sodium excretion was 152 (76), 179 (82) and 209 (90) mmol/24h in the tertiles of increasing Na/Cr ratio. ARB compared with control therapy had the greatest short-term effects in albuminuria and blood pressure in the lowest tertile of Na/Cr ratio. ARB also had the largest long-term effects on renal and cardiovascular events in the lowest Na/Cr tertile. The risk for renal events was reduced by 43% (95%CI 16 to 61), 0% (-42 to 30) and increased by 37% (-4 to 96) in subsequent Na/Cr tertiles respectively (p for trend <0.001). Cardiovascular events were reduced by 37% (8 to 57), and increased by 2% (27 to 45) and 25% (-11 to 75), respectively (p for trend <0.001).

Conclusions: Reduced dietary sodium intake was associated with enhanced renal and cardiovascular protective treatment effects of ARB therapy as compared with control therapy in type 2 diabetic patients with nephropathy. Remarkably, the large difference in outcome occurred despite a liberal sodium intake even in the lowest tertile. This underscores the call for action to avoid high dietary sodium intake, particularly in type 2 diabetic patients who are receiving ARB therapy.

Funding: Government Support - Non-U.S.

SA-OR370

High Dietary Fiber (DF) Intake Is Associated with Inflammation and Mortality in CKD: National Health and Nutrition Examination Survey (NHANES) III Vidya M. Raj Krishnamurthy,1,2 Guo Wei,1 Michel B. Chonchol,2 Kalani L. Raphael,1,2 Tom H. Greene,1,2 Srinivasan Beddhu.1,2 1VA; 2Univ Utah, SL, UT; 3Univ CO, CO.

Background: Chronic kidney disease (CKD) is considered an inflammatory state and it is unknown whether high DF intake is associated with a risk of inflammation and mortality in the CKD population.

Methods: 14,543 NHANES III participants were studied. DF intake was assessed by standardized 24 h diet recall conducted by trained personnel. Mortality data were obtained by probabilistic matching with vital records.

Results: The mean age was 45 ± 15.8 yrs. 48% were men and 10% were black. 5.8% had CKD (eGFR < 60 ml/min/1.73 m2) and 25.7% had inflammation (serum CRP >3mg/L). The associations of DF in CKD and non-CKD groups with inflammation are summarized in the table and mortality in the figure.

Funding: 1Department of Nephrology, University of Cologne, Cologne, Germany; 2Department of Medicine, University of Minnesota, Minneapolis, Minnesota.
Relationship of elevated serum CRP (> 3 mg/L) with DF in non-CKD and CKD

<table>
<thead>
<tr>
<th></th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-CKD</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>DF in total fiber</td>
<td>0.89 (0.83, 0.97)</td>
<td>0.02 (0.50, 0.77)</td>
</tr>
<tr>
<td>DF in insoluble fiber</td>
<td>0.87 (0.78, 0.96)</td>
<td>0.55 (0.42, 0.74)</td>
</tr>
<tr>
<td>DF in soluble fiber</td>
<td>0.72 (0.56, 0.90)</td>
<td>0.26 (0.13, 0.53)</td>
</tr>
</tbody>
</table>

Each cell represents a logistic regression model adjusted for demographics, MI, CHF, stroke, cancer, smoking, alcohol use, physical inactivity, SBP, DBP, caloric intake, protein intake, serum triglycerides, HDL, and LDL cholesterol.

Conclusions: Higher dietary quality is associated with a decreased risk of incident stage 3 CKD. Whether dietary modifications can prevent stage 3 CKD warrants further study.

Funding: Other NIH Support - The Framingham Heart Study is supported by the National Heart, Lung and Blood Institute (N01-HC-25195).

SA-OR372

Vitamin D Levels and Chronic Kidney Disease Progression: A CRIC Study

Mary B. Leonard,1 Wei Yang,1 Dawei Xie,2 Monika M. Safford,2 Neil R. Powe,1 William M. McClellan,3

1University of Pennsylvania; 2University of Miami.

Background: Studies of vitamin D receptor agonist (VDRA) therapy in animal models of kidney disease demonstrated reductions in albuminuria and amelioration of progression of podocyte injury. A recent randomized controlled trial demonstrated that VDRA therapy was associated with reductions in proteinuria in patients with diabetic nephropathy. The objective of this study was to examine the associations between vitamin D levels and chronic kidney disease (CKD) progression in CRIC participants.

Methods: Vitamin D levels were available in 1560 participants (44% diabetic) at Year 1 with a median (interquartile range [IQR]) age of 62 (55-68) years and eGFR of 45 (34-54) ml/min/1.73m². Cox proportional hazards models were censored at death.

Results: Median (IQR) serum 25(OH)D levels were 27 (16-37) ng/mL and 1,25(OH)2D levels were 27 (18-37) pg/mL. During a median follow-up of 4.7 (IQR 3.8-5.5) years, 167 participants reached ESRD (24.1/1000 person-years), and 207 reached the composite endpoint of end-stage renal disease (ESRD) or halving of eGFR (29.8/1000 person years). Higher serum 1,25(OH)2D levels were associated with lower risk of ESRD [hazard ratio (HR) per 10 pg/mL greater 1,25(OH)2D level: 0.78 (95%CI 0.65-0.91), p=0.01] and the composite outcome [HR 0.70 (0.60-0.90), p=0.01], adjusted for age, race, diabetes, season, systolic blood pressure and eGFR (Model 1). After further adjustment for proteinuria, PTH and FGF-23 levels (Model 2), the associations remained significant for ESRD [HR 0.84 (0.71-0.99), p=0.04] and the composite outcome [HR 0.86 (0.75-0.99), p=0.03]. Higher serum 25(OH)D levels were also associated with lower risk of ESRD [HR per 10 ng/mL greater 25(OH)D 0.83 (0.87-0.95), p=0.01] and the composite outcome [HR 0.84 (0.74-0.94), p=0.01] in Model 1; however, the results for ESRD [HR 0.95 (0.80-1.11)] and the composite outcome [HR 0.96 (0.84-1.11)] were no longer significant after adjustment in Model 2.

Conclusions: Higher vitamin D levels are associated with less CKD progression. The persistent 1,25(OH)2D association after adjustment for multiple measures of CKD severity (proteinuria, PTH and FGF-23 levels) suggested minimal confounding by CKD severity.

Funding: NIDDK Support

SA-OR373

Chronic Kidney Disease Awareness and Healthy Behaviors in the REasons for Geographic and Racial Differences in Stroke (REGARDS) Cohort

Delphine S. Tuot,1 Suzanne E. Judd,2 Paul Muntner,3 Laura C. Plantinga,1 Chi-Yuan Hsu,4 David G. Warnock,5 Orlando M. Gutierrez,2 Monika M. Safford,2 Neil R. Powe,1 William M. McClellan,3

1University of California at San Francisco, CA; 2University of Alabama at Birmingham, AL; 3Emory University, Atlanta, GA.

Background: The association between chronic kidney disease (CKD) awareness and healthy behaviors is unknown. We examined whether CKD self-recognition is associated with behaviors that may slow CKD progression.

Methods: We conducted a cross-sectional analysis using baseline data from 3670 adults with CKD (eGFR <60 ml/min/1.73 m²) participating in REGARDS, a prospective cohort study of risk factors for stroke. CKD awareness was a “yes” answer to “Have you ever told you have kidney disease?” Self-reported outcomes included current tobacco use, use of non-steroidal anti-inflammatory medications (NSAIDs), and angiotensin-converting enzyme inhibitor/angiotensin receptor blocker (ACEI/ARB) use, based on pill review. Measured outcomes included blood pressure control (<140/<90 mmHg) and among self-reported diabetics, glycemic control (fasting glucose ≤126 mg/dl). Multivariable logistic regression adjusted for sociodemographics, eGFR and albumin-creatinine ratio was used to estimate the odds of each behavior in individuals aware vs. unaware of their CKD.

Results: Only 10% of individuals were aware of their CKD. Those who were aware had nearly 60% lower odds of current tobacco use compared to those unaware [adjusted odds ratio 0.43, 95%CI (0.22-0.87)]. However, CKD awareness was not associated with reduced NSAID use [0.77 (0.38-1.55)], ACEI/ARB use [1.24 (0.87-1.76)], or blood pressure control [1.06 (0.76-1.48)]. Among diabetics, CKD awareness was not associated with glycemic control [0.81 (0.60-1.43)] or ACEI/ARB use [0.92 (0.60-1.43)].

Conclusions: Awareness of CKD was associated with decreased current tobacco use but was not associated with greater odds of other healthy behaviors. While the cross-sectional design and potential misclassification of awareness by survey question may limit interpretation, results suggest that a more nuanced understanding of the association among patient knowledge, awareness, and behaviors is needed.

Funding: NIDDK Support, Other NIH Support - NINDS, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

89A
Assessment of Obesity and Kidney Function Decline in Persons without Chronic Kidney Disease: Multi-Ethnic Study of Atherosclerosis (MESA) Anna Malkina, 1 Ronit Katz, 2 Michael Shlipak, 1,2 Ian H. de Boer, 1 Joachim H. Ix, 2 Mark J. Sarnak, 1 Julie Lin, 3 Matthew Allison, 4 Holly J. Kramer, 2 David Siscovick, 2 Carmen A. Peralta, 3,4 Univ California San Francisco; 3SFVAMC; 4Univ Washington; 4Univ California San Diego; Tufts Medical Center, 1Harvard, 2Vanderbilt, 4Howard, 5Loyola.

Background: Obesity has been associated with increased risk for chronic kidney disease (CKD) in some studies. Whether anthropometric measures of obesity are associated with renal function decline prior to onset of CKD (defined as estimated glomerular filtration rate (eGFR) ≤60mL/min/1.73m2) is not well known.

Methods: We studied the association of body mass index (BMI), waist circumference (WC), body fat percentage (BF), and waist-hip ratio (WHR) with renal function decline in persons without CKD. GFR was estimated by creatinine (eGFRcr) and cystatin C (eGFRCys) with repeated measures over five years. We adjusted for age, sex, race, hypertension, and diabetes.

Results: Mean age was 60±10 years, 48% men, baseline mean eGFRcr was 82±15 and eGFRCys 95±17 mL/min/1.73m2. Mean eGFRcr decline was 1.55 (SD 2.85) and eGFRCys 1.12 (4.37) mL/min/1.73m2 per year. Persons with high WHR (≥0.9 men; ≥0.7 women) had 20-30% faster decline by eGFRcr (β -0.22, 95%CI -0.38 to -0.06) and eGFRCys (β -0.34, 95%CI -0.57 to -0.12) after adjustment, including BMI, compared to persons with normal WHR. Persons with larger waist circumference (≥102 cm for men; ≥90 cm for women) had moderately faster rates of decline: (β -0.10, (-0.20 to -0.01) by eGFRcr, and β -0.07, (-0.21 to 0.07) by eGFRCys). In contrast, compared to persons with BMI 19 to 25 kg/m2, only persons with BMI ≥35 had faster decline by eGFRCr (β -0.19 mL/min/1.73m2 per year, 95%CI (-0.47 to -0.004), but not by eGFRCys (β 0.11, (0.15 to 0.37). Weight gain or loss was not associated with eGFR decline (all p>0.05).

Conclusions: Central obesity, rather than weight or BMI, is more consistently associated with kidney function decline among persons without CKD. Further studies with imaging measures of fat distribution are needed to further evaluate these associations as potential modifiable risk factors for CKD.

Funding: Other NIH Support - NHLBI; Veterans Administration Support

SA-OR375

Background: Physical activity promotes diverse metabolic benefits that may counteract the toxic biochemical environment of chronic kidney disease (CKD). We tested the hypotheses that greater physical activity levels are associated with lower risks of death and kidney disease progression in a prospective cohort study of stage III-IV CKD patients.

Methods: We studied 294 participants from the Seattle Kidney Study, a Nephrology clinic-based study of CKD, who had an estimated glomerular filtration rate (eGFR) of 15-59 mL/min/1.73 m2. Participants self-reported type, frequency, and intensity of physical activity (PA), and we converted these responses to metabolic equivalent task (MET) min/week. PA levels of 100 MET-min/week are equivalent to leisurely walking for 30 minutes per day.

Results: Mean eGFR at baseline was 42 mL/min/1.73 m2) and mean age was 62 years. We adjusted for demographics, eGFR, prevalent coronary artery disease and functional status, PA levels of 100 MET-min/week were associated with lower risks of death and kidney disease progression in a prospective cohort study of stage III-IV CKD patients.

Conclusions: Greater physical activity levels trended toward being associated with lower risks of death and kidney function decline in a longitudinal study of CKD. Physical activity, even at moderate levels, may be sufficient to confer health benefits among CKD patients and is emerging as one of few modifiable risk factors for major adverse health outcomes in this high risk population.

Funding: Private Foundation Support

SA-OR376
A One-Year Lifestyle Intervention Improves Myocardial Function and Exercise Capacity in Patients with Chronic Kidney Disease Erin Hedden, 1 Jeff S. Coombes, William G. Petchey, Nicole M. Isbel. Centre for Clinical Research Excellence - Cardiovascular and Metabolic Disorders, University of Queensland, Brisbane, QLD, Australia.

Background: Myocardial dysfunction (MD) is common in pts with chronic kidney disease. We sought the effect of a lifestyle intervention (LI) that included exercise training on MD and exercise capacity in CKD.

Methods: 83 pts with stage 3-4 CKD were randomized to standard care (control) or LI. LI included access to multidisciplinary care through a nurse practitioner led CKD clinic, which included a nephrologist, social worker, diabetes educator and exercise physiologist. LI involved 8 weeks supervised, gym-based individualized exercise training followed by home based training with ongoing telephone support and gym refresher sessions for 12 weeks. The 4 week LI program led by a program director, MD was assessed at baseline and 12m using standard echocardiographic techniques.

Results: 72 pts completed follow-up (LI=36, control =36). There were no baseline differences (Table 1). LI resulted in a significant (33%) increase in exercise capacity, with decreases in BMI. This group also showed improved diastolic function (increased e’).

Conclusions: The LI significantly improved exercise capacity and diastolic function in CKD. Exercise and lifestyle interventions may assist in managing the deleterious effects of reduced kidney function on the myocardium.

Table 1. Effect of one-year lifestyle intervention

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>LI group</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>METs</td>
<td>7.3±3.9</td>
<td>14.5±5.4</td>
<td>1.73±3.5</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>33.0±8.9</td>
<td>9.4</td>
<td>32.5±6.8</td>
</tr>
<tr>
<td>E/A</td>
<td>1.05±0.36</td>
<td>16.1±0.04</td>
<td>1.01±0.03</td>
</tr>
<tr>
<td>S’ (cm/s)</td>
<td>6.28±1.17</td>
<td>0.00±0.0</td>
<td>6.13±1.2</td>
</tr>
<tr>
<td>E’ (cm/s)</td>
<td>5.8±1.14</td>
<td>-0.47±1.0</td>
<td>0.59±1.5</td>
</tr>
<tr>
<td>Systolic Strain (%)</td>
<td>-19.5±2.8</td>
<td>1.0±0.5</td>
<td>-18.4±3.9</td>
</tr>
</tbody>
</table>

SA-OR377
Reprogramming of Adult Proximal Tubular Epithelium to an Embryonic Nephron Progenitor State Melissa H. Little, Caroline E. Hopkins, Norscha Suhaimi, Fiona Rae, Joan L. Institute for Molecular Bioscience, University of Queensland, St. Lucia, Brisbane, Queensland, Australia.

Background: The mammalian kidney arises from the ureretic bud and the metanephric mesenchyme, the latter giving rise to a cap mesenchyme nephron progenitor population. This Six2 positive cap mesenchyme surrounds the ureteric tips of the developing kidney and contains self renewing nephron stem/progenitors. Lineage tracing has shown that Six2+ cells give rise to all segments of the urinary tubule except the collecting ducts (1). Nephron formation ends around birth via the terminal commitment of this progenitor field to nephron formation, hence this stem cell population does not persist postnatally. As a result, no new nephrons arise after birth and total nephron number is determined by the balance between cap mesenchyme self-renewal, differentiation and death.

Methods: In this study, we investigated whether the adult proximal tubular population could be reprogrammed to this earlier nephron progenitor state via the enforced reexpression of a cap mesenchymal transcriptional state. 15 key genes were cloned into lentiviral vectors and a combinatorial screen was performed via infection of the human proximal tubular cell line, HK2.

Results: Four stages of validation were performed, including i) epithelial to mesenchymal morphological change, ii) production of the cap mesenchyme-specific Cited1 protein, iii) re-expression of key cap mesenchyme genes and iv) successful integration into a mesenchyme of an ex vivo embryonic kidney reaggregating (2). In this way, we identified a combination of genes (Osr1, Six1, Six2, Hoxa11, Fya1, Snaai) capable of inducing a nephron progenitor state according to all four criteria. Approximately 0.875% of infected cells incorporated into cap mesenchyme in comparison to 0.05% for control HK2 cells cultured under identical conditions.

Conclusions: This is the first demonstration of reprogramming to the progenitor state of a solid organ and is the first evidence that it may be feasible to regenerate a cap mesenchyme population. These results open up the possibility of reinitiation of nephron formation but also to manipulation of the nephrogenic process to prolong nephron endowment.

Funding: Government Support - Non-U.S.

SA-OR378
Induced Pluripotent Stem Cells from Patients with Genetic Kidney Disease: Applications for Disease Modeling and Therapeutic Screening Sharon D. Ricard, 1 Bi Song, 1 Jonathan Nicols, 1 Andrew L. Laslet, 1 Peter G. Kerr, 2 Monash Immunology and Stem Cell Laboratories, Monash University, Clayton, Victoria, Australia; 3Medicine, Nephrology, Monash Medical Center, Clayton, Victoria, Australia; 4Materials Science and Engineering, CSIRO, Clayton, Victoria, Australia.

Background: The reprogramming of somatic cells to induced pluripotent stem (iPS) cells has attracted considerable attention for disease modeling, drug screening and regenerative medicine. We have recently shown that iPS cells can be derived from human kidney mesangial cells (JASN; May 2011).

Methods: In the present study, fibroblasts grown from skin biopsies of patients with Alport Syndrome and polycystic kidney disease (PKD) were reprogrammed by retroviral transduction using OCT4, SOX2, KLF4, and c-Myc. The methylation profiles of skin-derived iPS cells were analysed by bisulfite sequencing using the OCT4 promoter, compared to kidney cell-derived iPS cells. The iPS cells were analysed for stem cell marker
expression using immunofluorescence microscopy and qPCR. The pluripotent capacity of the self-renewing Six2+ cap mesenchyme cells was tested by the formation of teratomas following injection into immunodeficient mice and the directed differentiation into embryoid bodies.

Results: The disease-specific iPSC cells resembled human embryonic stem cells (hES) in morphology and gene expression localising for OCT4/3, SSEA-4, TRA-1-60 and TRA-1-81. Using qPCR, the iPSC cells expressed stem cell marker genes and exhibited silencing of the retroviral transgenes by passage four. DNA methylation profiles showed that fibroblast and mesangial-derived iPSC cells had OCT4 methylation patterns similar to hES cells, but different to the primary target cells. Furthermore, iPSC cells formed embryoid bodies and expressed markers of all three germ layers by immunostaining and RT-PCR. The injection of undifferentiated iPSC colonies into immunodeficient mice formed teratomas, thereby demonstrating pluripotency.

Conclusions: The derivation of iPSC cells from Alport Syndrome and PKD patients advances the potential of human pluripotent cells for modeling the genetic disorders, the directed differentiation of kidney cells and screening of new drug compounds.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR379

Identification of Early Stage Renal Progenitors in E9.5 Embryos by Using Osr1-GFP Knock-In Mice
Atsuhiko Taguchi, Ruiichi Nishinakamura. Kidney Development, Institute of Molecular Embryology and Genetics, Kumamoto, Kumamoto, Japan.

Background: Though many groups are trying to induce renal progenitor cells from ES or iPSCs, it remains unclear to which in vivo stage these cells correspond. In this study, we propose the stepwise induction model by analyzing the in vivo developmental process of renal progenitors.

Methods: Osr1 is one of the earliest markers of the IM and most of the cell types within the kidney arise from the Osr1+ population. Thus we have generated Osr1-GFP+ knock-in mice and murine ES cells. By sorting Osr1-GFP+ cells from embryos and applying them to the colony- forming assay that promotes renal progenitor differentiation, we have confirmed the existence of renal progenitors in embryos at the early developmental stages.

Results: Consecutive application of Osr1-GFP+ kidney precursors to colony- forming assay on Wnt4-expressing feeders, from E8.5 intermediate mesoderm to the E11.5 metanephric mesenchyme, revealed that the Osr1+ cells after E9.5 do contain colony- forming cells. Osr1-GFP+ cells at E8.5, however, formed no colonies. Further analyses of expressed genes of colonies derived from E9.5 implied that these colonies are progenitors forming cells. Osr1-GFP+ cells at E8.5, however, formed no colonies. Further analyses of expressed genes of colonies derived from E9.5 implied that these colonies are progenitors forming cells.

Conclusions: We have identified the functional difference of renal progenitor cells in the early stage embryo between E8.5 and E11.5 by expressing the Wnt4 signal and the Osr1 gene. Using this stepwise induction model, we propose the stepwise induction model by analyzing the in vivo developmental process of renal progenitors.

Funding: Government Support - Non-U.S.

SA-OR380

Developmental Regulators in Cap Mesenchyme Are Epigenetically Poised
Nathaniel J.D. McLaughlin, Xiao Yao, Zabaida R. Safiudeen, Samir S. El-Dahr. Pediatrics, Tulane University School of Medicine, New Orleans, LA.

Background: In embryonic stem cells, the promoters of developmental regulators are hypo-methylated state by carrying activating (H3K4me3) and repressive (H3K27me3) chromatin domains. Upon receiving a differentiation signal, these bivalent domains resolve either into actively transcribed regions or silenced by packing into inaccessible heterochromatin. We hypothesized that progressive restriction of cell fate which occurs during differentiation of renal progenitors to epithelia involves conversion of bivalent chromatin into chromatin regions accessible or inaccessible to the transcription machinery.

Results: We show here that self-renewing Six2+ cap mesenchyme cells are enriched with polycomb protein Ezh2 (H3K27methyltransferase) as compared to nascent nephrons, and exhibit a bivalent chromatin signature (H3K4me3/H3K27me3). This bivalency resolves during nephron differentiation via downregulation of repressive H3K27me3 and acquisition of additional activating H3R3me2/R17me2 marks. Cre-mediated deletion of Ezh2 from the Six2+ cap cell population in vivo eliminates H3K27me3 and compromises the size of this progenitor population. ChIP-Seq of chromatin landscapes in progenitor and differentiating metanephric mesenchyme-derived cell lines revealed that bivalent chromatin domains in developmental regulators resolve during differentiation. Induction of Pax8, a known target of Wnt/beta-catenin signaling in early nephron formation is associated with recruitment of the H3K27 deacetylase, KDM6b, and replacement of Ezh2, thus loss of repressive H3K27me3.

Conclusions: We conclude that "epigenetic poising" is not unique to pluripotential cells and represents a key mechanism for a cell fate decision switch during nephron differentiation. Polycomb-Ezh2-mediated silencing may represent an epigenetic mechanism to maintain the pool of renal progenitors.

Funding: NIDDK Support

SA-OR381

KIF3A Controls Nephrogenesis by Regulating FGF8
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Background: The primary cilium is a Hedgehog (HH)-dependent signaling complex in nonrenal tissues. The functional contribution of the primary cilium to early renal embryogenesis is undefined. We hypothesized that the primary cilium controls early renal kidney morphogenesis by modulating HH signaling.

Methods: Confoocal immunofluorescence imaging of WT and Kif3a-MM null kidney tissue demonstrated that KIF3A expression is restricted to the primary cilium. Nephrogenesis was reduced by 35% at P0 and 22% at E12.5 (P<0.05) in Kif3a-MM null mice. Surprisingly, HH signaling was induced by expression of Gli1 mRNA and the primary cilium was undetectable by E15.5, at which stage the number of primary cilia was reduced by 70%.

Results: The significance of decreased Fgfg expression was determined in organ culture. Addition of FGF8 protein to Kif3a-MM null metanephros, harvested at E11.5 and separated from the ureteric bud and then cultured on spinal cord, increased the number of PAx2-positive tubules 1.7-fold (P<0.001) compared to untreated Kif3a-MM controls. The functional contribution of KIF3A was investigated in cultured WT and Kif3a-MM null primary MM cells isolated from E11.5 WT and mutant kidneys. Cilia length was decreased 5-fold in Kif3a-MM null MM cells (P<0.01). Transfected KIF3A-GFP localized to the primary cilium and increased primary cilium length in null MM cells 1.7-fold compared to transfection with GFP alone (P<0.04). While transfected KIF3A-GFP exerted no significant effect on the expression of the HH response genes, Pax1 or Gli1 in Kif3a-MM null MM cells (P>0.002), expression of Fgfg mRNA increased 2.2-fold compared to controls (P<0.002).

Conclusions: We conclude that KIF3A acts in the primary cilium of metanephric mesenchyme cells to control nephrogenesis by regulating Fgfg expression.

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SA-OR382

Structural Changes in the Cytoplasmic Domain of Integrin β1 Due to the Mutations in NPY Motifs Induce Kidney Developmental Phenotype
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Background: Integrins are the principal receptors that regulate cell extracellular matrix interactions. β1 integrins are the major integrins found in the kidney and are required for its development and function. The two NPY motifs in the cytoplasmic tail of the integrin β1 subunit are proposed to be critical for β1 integrin function. In this study, we defined the importance of the NPY motifs in renal UB development by altering integrin dependent cell functions and growth factor signaling.

Methods: We generated mice that selectively express β1 integrin in the developing UB where the tyrosines (Y) in both NPY motifs were mutated to alanines (A) or phenylalanines (F). The Y/A mutations caused severe developmental abnormalities; however, mice with the Y/F mutations developed normally and were more susceptible to injury in comparison to wild type mice. Consistent with the in vivo data, collecting duct cells expressing the mutant β1 integrins had abnormalities in cell adhesion, migration, proliferation and tubule formation. Interestingly, only cells expressing the Y/A mutations failed to activate extracellular signal-regulating kinase (ERK), MAPK, or FGF, two growth factors critical for UB development. We further defined the molecular mechanism for the functional alterations induced by these mutations by solving the structure of the β1 integrin tail and transmembrane domain by NMR spectroscopy in DMPD/DHPC bicelles. We demonstrated there were significantly more structural distortions in the Y/A compared to the Y/F mutants.

Results: This study shows the importance of the NPY motifs in the integrin β1 tail regulate UB development by altering integrin dependent cell functions and growth factor signaling. Furthermore, it shows the structural alterations of the β1 integrin tail carrying mutations in critical tyrosine residues. This observation has major implications in our understanding of how the cytoplasmic tail of β1 integrins regulate cell function and tissue.

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SA-OR383

Podocyte-Selective Loss of the Basic Helix-Loop-Helix Transcription Factor, Podf/Tcf21 Results in Podocyte Defects and Massive Proteinuria
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Background: Podocytes are highly differentiated cells that are essential for function of the glomerular barrier. Although a number of transcription factors are known to be expressed in podocytes, their functions in development of specialized features such as slit diaphragms and foot processes, is largely unknown.

Results: Podf/Tcf21 belongs to the bHLH family of transcription factors and is expressed in condensing metanephric mesenchyme, and immature and mature podocytes. We generated PodfΔf/Tcf21 mice, using Rbar2-Cre and Kif3a−/− mice. Metanephric and primary mesenchyme cells of PodfΔf/Tcf21 embryos were derived from WT and mutant mice.

Conclusions: These results show that the NPY motifs in the integrin β1 tail regulate UB development by altering integrin dependent cell functions and growth factor signaling. Furthermore, it shows the structural alterations of the β1 integrin tail carrying mutations in critical tyrosine residues. This observation has major implications in our understanding how the cytoplasmic tail of β1 integrins regulate cell function and tissue.

Funding: NIDDK Support/AmERICAN HEART ASSOCIATION
a standard KO mouse that dies in the perinatal period with striking renal defects including severely disrupted and delayed glomerulogenesis, arrested differentiation of tubular epithelium, and abnormal branching of the ureteric bud. However, given the complexity of the Pod1 KO phenotype and early lethality, the precise role of pod1 in podocytes remains unclear.

Results: To understand the role of Pod1 in podocyte development and function, we further analysed the standard KO mice, and developed podocyte-selective KO mice as well. A conditional floxed Pod1 allele was created using a Bac cloning strategy and embryonic stem cell targeting, placing loxP sites around the first exon of Pod1. The floxed mice were bred with Pod1-Cre mice to delete the Pod1 gene specifically in podocytes.

Results: Analysis of conventional KO mice reveals defects in podocyte foot process formation. Podocyte-specific KO mice develop massive proteinuria in 4 weeks of age. Histological analysis reveals striking glomerulosclerosis and loss of podocytes at this stage. In contrast to conventional KO mice, glomerular tuft development and mesangial cell migration is normal, suggesting that these functions depend on Pod1 expression in the mesenchyme and NOT the podocyte lineage.

Conclusions: Together, these data demonstrate crucial but distinct role for Pod1 in renal mesenchymal and podocyte cell lineages. Furthermore, these mice represent a valuable tool to identify the transcriptional targets regulated by Pod1 in podocytes.

Funding: Government Support - Non-U.S.

SA-OR384
Deletion of Fgfr2 from the Peri-Wolffian Duct Stroma Leads to Induction Abnormalities, and Congenital Vesicoureteral Reflux
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Background: Kidney development begins when the ureteric bud (UB) invades the metanephric mesenchyme (MM). The site of UB induction is strongly influenced by the peri-Wolffian duct stroma (ST). Previously, conditional deletion of fibroblast growth factor receptor 2 (Fgfr2) in both MM and ST was shown to cause ureteric induction defects, CUAUTs, and high rates of vesicoureteral reflux (VUR).

Methods: The purpose of this study was to elucidate the role of Fgfr2 signaling in the ST during kidney development through conditionally deleting Fgfr2 in ST using Tbx18cre (Fgfr2St-/-) mice; that drive cre expression in the ST, as well as in bladder mesenchyme but not in MM.

Results: Analysis at E11.5 showed Fgfr2St-/- mutants have a highly variable UB induction site compared to controls. At E10.5, during early UB induction, Fgfr2St-/- embryos exhibit reduced Bmp4 and Sprouty1 mRNA expression (67% and 59% respectively, of control expression levels). At birth, Fgfr2St-/- mice present with CUAUT phenotypes, and have higher rates of VUR (79%) than control mice (3%). Ureters from both genotypes show similar morphology however analysis of cultured E12.5 explants indicated that Fgfr2St-/- ureters display abnormal peristaltic contractions. Lastly, adult Fgfr2St-/- mice continue to have high rates of VUR (via live and euthanized cystostomy methods) and often exhibit abnormal voiding behavior, megabadder and hydronephrosis compared to age-matched controls. In conclusion, Fgfr2 signaling in ST is required for normal induction of the UB (regulated by Bmp4 and Sprouty1) and for normal ureteral peristalsis.

Conclusions: These combined ureteric induction and peristaltic defects have novel implications for understanding the developmental mechanisms of VUR. Furthermore, the bladder mesenchyme abnormalities (voiding dysfunction, megabadder and hydronephrosis) seen in Fgfr2St-/- mutants suggest critical roles for Fgfr2 in bladder development and could represent a novel biological/genetic link between VUR and voiding dysfunction; as is often associated in humans.

Funding: NIDDK Support

SA-OR385
Congenital Hydronephrosis and Delayed Distal Ureter Maturation in Discs-Large 1 (Dlg1) Null Mice Is Associated with Reduced Retinoic Acid Signaling and Impaired Apoptosis Via a Non-Epithelial Cell Autonomous Mechanism
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Background: The absence of Discs-Large 1 (Dℓg1), the mouse ortholog of the Drosophila discs-large tumor suppressor, results in congenital hydronephrosis characterized by urinary tract abnormalities, reduced ureteric bud branching, and delayed disconnection of the ureter from the common nephric duct.

Methods: To define the cellular requirements for Dℓg1 during urogenital development, we used a floxed Dℓg1 allele and Pax2Cre, Pax5Cre, and HB7Cre transgenes to generate tissue-restricted Dℓg1 mutants. In addition, we mated Dℓg1 heterozygote mice with Ret-GFP knockin, retinoic acid response element-LacZ transgenic mice, or HB7-GFP knockin to see specific compartments of urogenital system.

Results: Mutation in ureteric and collecting ducts (via HoxB7Cre or Pax2Cre) and in nephrons (via Pax9Cre) resulted in no apparent abnormalities in ureteric bud branching or in distal ureter maturation and no hydronephrosis. Mutation in nephrons and ureteric smooth muscle (via Pax3Cre transgene) resulted in congenital hydronephrosis accompanied by reduced branching, a delay in distal ureter maturation, and smooth muscle orientation defects, phenotypes very similar to Dℓg1 null mice. Dℓg1 null mice showed reduced apoptosis and evidence of reduced retinoic acid signaling in the kidney, shown both by real-time PCR and immunofluorescence. These results were confirmed using Ret-GFP knockin and retinoic acid response element-LacZ transgenic mice.

Conclusions: Taken together, these results suggest that Dℓg1 expression in ureteric smooth muscle cells is essential for ensuring distal ureter maturation by facilitating retinoic acid signaling and apoptosis.

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SA-OR386
Obstructive Nephropathy in Megabladder Mice Is Due to a Novel Hypermorphic Myocardin Allele
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Background: Megabadder (mgb-/-) mice exhibit impaired bladder smooth muscle development, resulting in megacystis and hydrenephrosis in utero and renal failure in adults. We previously identified the insertion of a portion of chromosome (chr) 16 into chr 11 of mgb-/- DNA, but its significance was unclear.

Methods: To further refine the genetic alteration in mgb-/- mice, DNA was subject to fluorescence in situ hybridization (FISH) and comparative genomic hybridization (CGH). RNA was analyzed by microarrays, in situ hybridization, and QRT-PCR. Mice with a fully disrupted myocardin (myocd) allele (myocd-/-) were crossed with mgb-/- mice to generate myocd-mgb- animals.

Results: FISH demonstrated a megabase insertion of chr 16 into chr 11, inclusive of 4 genes on chr 16. CGH confirmed this and identified a 26 kilobase (kb) deletion of chr 11. This insertion/deletion occurred in a gene sparse region of chr 11, 300 kb upstream of myocd, a transcription factor that cooperates with the serum response factor (SRF) to initiate myogenesis. Transcriptome profiling of mgb-/- E15 bladders demonstrated intact expression of early markers of muscle patterning, but impaired expression of 27 downstream targets of the myocd/SRF transcriptional complex. Myocd expression was diminished in E15 mgb-/- bladders by in situ hybridization and QRT-PCR versus controls. Compound heterozygotes (myocd+/mgb-/-) phenocopied mgb-/- bladders. Myocd+/mgb-/- embryos showed a further reduction in myocd expression versus mgb-/- mice resulting in the development of patent ducus arteriosus (PDA) postnatally.

Conclusions: The mgb-/- phenotype occurs as a result of a long-range, position effect mutation on chr 11 that results in a hypermorphic myocd allele. Further reduction of myocd gene expression in myocd-/-/mgb-/- mice results in the emergence of PDA, providing the first evidence that decreased myocd gene dosage results in congenital abnormalities involving these two disparate organ systems.

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SA-OR387
Age and Racial Disparities in Dialysis Survival
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Background: Many studies have reported that blacks survive longer on dialysis than whites. This observation is paradoxical given racial disparities in access to and quality of care, and is inconsistent with observed lower survival among blacks with chronic kidney disease (CKD). We hypothesized that age and the competing risk of transplantation modify survival differences by race. The goal of our study was to estimate death on dialysis by race, accounting for age as an effect modifier and kidney transplantation as a competing risk.

Methods: We studied 909,841 incident end-stage renal disease patients captured in USRDS between 1995 and 2005. Multivariate age-stratified Cox proportional hazards and competing risk models were constructed to examine death on dialysis comparing black and white patients.

Results: Similar to previous studies, blacks had an overall 17% lower death rate on dialysis compared with whites (adjusted hazard ratio (aHR) 0.83, p<0.001). However, stratifying by age blacks under 50 had significantly higher mortality than their white counterparts (Figure 1a). This finding persisted in adjusted analyses treating kidney transplant as a competing risk (aHR 1.98 for 18-30; aHR 1.51 for 31-40; aHR 1.10 for 41-50; p<0.001, Figure 1b); only those over 50 had lower death rates (aHR 0.87 for 51-60; 0.85 for 61-70; 0.83 for 71-80; 0.86 for >80; p<0.001).

Conclusions: Blacks of all ages are disadvantaged in dialysis survival, and engendered complacency about the low rates of kidney transplantation among blacks. We hypothesized that age and the competing risk of transplantation modify survival differences by race. The goal of our study was to estimate death on dialysis by race, accounting for age as an effect modifier and kidney transplantation as a competing risk.
Conclusions: The commonly cited dialysis survival advantage among blacks applies only to older adults. Blacks under the age of 50 die on dialysis at as much as twice the rate of their white counterparts.

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SA-OR388

End-of-Life Care Intensity in Older Adults on Chronic Dialysis Susan P.Y. Wong, William Kreuter, Ann M. O’Hare. University of Washington.

Background: Survival after initiation of chronic dialysis is often limited in older adults, fostering a growing interest in advance care planning in this population. Little is known about their treatment choices at the end-of-life (EOL) and the major determinants of their choices.

Methods: Using data from USRDS, we performed a retrospective mortality study on 43,768 Medicare beneficiaries age ≥65 years who initiated chronic dialysis from 6/1/2005-12/31/2007 and died within 1-year of initiation. Using linked Medicare inpatient claims, we examined the following EOL care measures during the last 6-months of life: hospitalization, intensive care unit (ICU) admission, and receipt of an intensive procedure (mechanical ventilation, feeding tube, and cardiopulmonary resuscitation [CPR]).

We examined the adjusted association of these measures with patient characteristics and regional patterns of healthcare spending (end-of-life expenditure index or EOL-EI).

Results: Most (79.5%) patients were hospitalized at least once in the last 6-months of life. Of these, 31.5% received at least one intensive procedure. Receipt of an intensive procedure was associated with Black race (24.8% v. 14.7%; OR 1.56, 95%CI 1.44-1.68) and age ≤75 years (48.1% v. 38.5%, 1.37, 1.29-1.46). Sex, cause of ESRD, and co-morbidities did not show strong and consistent associations with receipt of an intensive procedure. After adjustment for differences in patient characteristics, we found a stepwise increase in the likelihood of ICU admission and receipt of an intensive procedure during the last 6-months of life from regions with the lowest to highest healthcare spending (Figure 1).

Conclusions: Hospitalization, ICU admission and receipt of intensive procedures are common at the EOL among older dialysis patients. In general, utilization of intensive procedures in this population seems to be shaped more by regional treatment patterns than by patient characteristics.

SA-OR389

Lower Dialysate Na+ Impacts Weight Gain & Fluid Overload Hospitalizations Eduardo K. Lacson1, John Rogus,2 Peter Kotanko; Nathan W. Levin; Raymond M. Hakim.1 Fresenius Medical Care, North America, Waltham, MA; 2Renal Research Institute, New York, NY.

Background: Interdialytic weight gain (IDWG) and fluid overload hospitalizations (FOH) increased with greater dialysate to serum sodium (sNa+) gradient. However, many physicians keep the base dialysate sodium (dNa+) formulation constant in a facility. We hypothesized that lowering dNa+ will decrease IDWG and FOH in prevalent hemodialysis (HD) patients.

Methods: A phased roll-out (Jan-Jun 2009) of dialysate formulation for Fresenius Medical Care, North America facilities decreased base dNa+ from 140 to 137 mEq/L. We identified 592 facilities with median dNa+ drop of 3.0 meq/L and 188 facilities where physicians kept the base sodium unchanged, when comparing 6-months before (Jul-Dec 2008) to 6-months after (Jul-Dec 2009) the conversion period. We identified all adult, in-center HD patients that survived for the entire 18-month study period divided into 25,653 patients with lower facility dNa+ and 9,613 controls that maintained dNa+. We estimated the change in key variables for each patient between the baseline and post-conversion periods to determine if they differed between cases and controls.

Results: We verified that post-conversion mean dNa+ was lower in study patients vs. controls (mean: 137 vs. 139 mEq/L, p<0.0001). Mean IDWG decreased in facilities with lowered dNa+ by 0.14 kg vs. controls’ 0.03 kg (p<0.0001), accompanied by a larger decline in pre-HD weight (0.74 vs.0.57 kg, p=0.01) respectively. At baseline, FOH was higher in study patients (5% v. 4%, RR=1.18, p=0.002). Post-conversion, overall FOH rate increased in both groups but the difference between groups lost significance (13.3% vs. 12.5%, RR=1.06, p=0.055). No significant differences in period changes between groups were noted in pre-HD sNa+, albumin and hemoglobin, pre- and post-HD systolic blood pressure (SBP) or post-HD weight.

Conclusions: Lowered dNa+ was accompanied by a greater decline in IDWG and pre-HD weight along with attenuation of the propensity for greater FOH over time. No accompanying differential changes in sNa+, SBP, post-HD weight, albumin or hemoglobin were observed.
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Dialysis Facility Use of High Ultrafiltration Rates Is Associated with Higher Standardized Mortality: Joseph M. Messana,1 Dori Bilik,2 Brett Lantz,3 Robert A. Wolfe,2 Jeffrey Pearson,2 Rajiv Saran.1 1University of Michigan Kidney Epidemiology and Cost Center, Ann Arbor, MI; 2Arbor Research Collaborative for Health, Ann Arbor, MI.

Background: Observational patient-level studies have associated hemodialysis (HD) ultrafiltration rates (UFR) above 10-13 ml/kg/hr with more intradialytic hypotension, unstable sessions and higher mortality. The current study validates this using the % of pts at a dialysis facility with UFR>15 ml/kg/hr data from the July-December 2009 CROWNWeb collection period.

Methods: Facility-level UF practice pattern was calculated from monthly patient treatment fluid removal and treatment time data. Facility % of patients with UFR>15 were related to the 2009 standardized mortality ratio (SMR) using Poisson regression. To account for unstable sessions and higher mortality, we related the % of patients with UF>15 mortality was higher for facilities in the two highest quintiles relative to the lowest quintile. Findings were similar after adjustment for other facility practice patterns (achieved hemoglobin, URR, vascular access type).

Results: 3,153 dialysis facilities were grouped into quintiles according to the % UF>15. Mortality was higher for facilities in the two highest quintiles relative to the lowest quintile. Findings were similar after adjustment for other facility practice patterns (achieved hemoglobin, URR, vascular access type).

Conclusions: Mortality is higher at facilities having more patients with UF>15. The reasons for prescribing high UF and approaches to reducing optimizing UF requirements in dialysis patients deserve urgent attention by the dialysis community. Death rates could plausibly be lowered by 4-7% at the 40% of facilities with the highest % of pts UF>15 if those facilities adopt the practice facilities in the lowest quintile.

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SA-OR391

Effects on Ventricular Volumes by Frequent Hemodialysis: Results from the Frequent Hemodialysis Network (FHN) Trials Christopher T. Chan, Gerald J. Beck, Glenn M. Chertow, John T. Daugirdas, Tom H. Greene, Peter Kotanko, Brett Larive, Nathan W. Levin, Ravindra L. Mehta, Michael V. Rococo, Javier Sanz, Brigitte Schiller, John B. Stokes, Alan S. Kliger. FHN Trials Group, NIDDK.

Background: Increased cardiac ventricular volume is an independent risk factor for death in patients with end-stage renal disease (ESRD). As part of the FHN trials, the impact of daily in-center hemodialysis (DHD) and nocturnal home hemodialysis (NHD) on left and right ventricular (LV, RV) volumes and function were investigated. We hypothesized that frequent hemodialysis would lower LV and RV volumes.

Methods: The FHN trials randomized 245 patients to 12 months of DHD or conventional hemodialysis (CHD), and 87 patients to 12 months of NHD or CHD. LV and RV volumes and function were ascertained by cardiac MRI at baseline and at the end of the study.

Results: The absolute (mean: SD) intradialytic weight loss per session achieved during the Daily and Nocturnal Trials were: DHD (2.11 ± 0.86kg) and conventional HD (3.10 ± 1.04kg). LV and RV volumes and function were ascertained by cardiac MRI at baseline and at the end of the study.

Conclusions: Frequent hemodialysis would lower LV and RV volumes.

Funding: NIDDK Support

SA-OR392

Increasing Diffusive Sodium Removal Improves Blood Pressure Control through Volume-Independent Effect in Hemodialysis Patients Yi-Lun Zhou, Jing Liu, Li-Jie Ma, Fang Sun, Yang Shen, T.G. Cui. Department of Nephrology, Chao-yang hospital, Capital Medical University, Beijing, China.

Background: Sodium overload is a predominant factor in the pathophysiology of hypertension in end stage renal disease. Lowering dialysate sodium concentration has been demonstrated to improve blood pressure (BP) control, and this phenomenon is considered to be a result of an improvement in volume status via increasing sodium removal. However, sodium, apart from volume, may have an independent effect on BP regulation.

Methods: 16 non-diabetic stable hemodialysis patients were recruited, who were hypertensive, and have achieved their dry weight as assessed both by clinical and bioimpedance methods. We used sodium loading with pre-dialysis plasma sodium levels slightly higher than the facility dialysate sodium concentration 138mmol/L. After a month period with standard dialysate sodium concentration of 138mmol/L, the patients were followed up for 4 months period with dialysate sodium set at 136mmol/L, with changes in pre-dialysis patients about dietary sodium control. During the period of study, the dry weight was adjusted monthly under the guidance of the bioimpedance spectroscopy (BCM, Fresenius Medical Care), to maintain post-dialysis volume status within normal range.

Results: Along with lowering dialysate sodium, systemic and diastolic home-monitored BP’s progressively decreased and reached statistical significance by the end of month 2. There was a significant decrease (-10 mmHg and -6mmHg) in 44-hour ambulatory systolic and diastolic BP by the end of the trial as well. Intradialytic weight gain adjusted to the estimated dry weight mildly but significantly decreased (4.81:1.51% vs 4.36:1.37%, p<0.047). No changes in monthly incidence of dialysis-related symptoms and pre-dialysis plasma sodium concentration were observed. The post-dialysis volume parameters were maintained in a steady state throughout the study period.

Conclusions: In selected hypertensive hemodialysis patients without volume overload, increasing diffusive sodium removal by lowering dialysate sodium concentration resulted in significant BP decrease. It is most likely due to the volume-independent effect.

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SA-OR393

Aldosterone Deficiency as the Cause of Intradialytic Hypotension and Its Successful Management with Fludrocortisone Daniel L. Landry, Seyedeh S. Hosseini,2 Osazee J. Osagie,2 Arley F. Diaz,1 Benjamin J. Freda,1 Gregory Lee Hosseini,2 Jing Liu, Li-Jie Ma, Fang Sun, Yang Shen, T.G. Cui.1

Background: Intradialytic hypotension (IDH) occurs in many patients on maintenance hemodialysis. While there is evidence to suggest that aldosterone (Aldo) has non-genomic effects on vascular tone independent of sodium retention in oligo-anuric dialysis patients, the presence of Aldo deficiency as a cause of IDH has not been defined.

Methods: Twelve chronic hemodialysis patients with refractory IDH despite aggressive medical management had plasma cortisol, renin and Aldo levels measured randomly before and after dialysis. Those patients that failed to have an Aldo level above 5 ng/dL were then given fludrocortisone (FC) 0.1 mg bid. Pre- and post-dialysis blood pressures (BP), average intradialytic volumes and frequency of IDH (SBP < 100 mmHg) were measured on 12 separate occasions prior to and after FC initiation.

Results: Of 12 patients with severe IDH, five had low plasma Aldo levels in the setting of symptomatic IDH. None of the patients had cortisol deficiency on corticotropin stimulation testing. All 5 patients with Aldo deficiency had improvement in pre-dialysis (110.1±2.2 v. 133.0±2.0, p<.0001) & post-dialysis systolic BP (103.8±1.4 v. 123.2±2.4, p<.0001) & pre-dialysis diastolic BP (61.0±1.4 v. 73.0±2.0, p<0.001) & post-dialysis diastolic BP (56.1±1.2 v. 66.6±2.0 mmHg, p<.0001) after the initiation of FC. Ultrafiltration volumes (2.8 ± 0.26 v. 3.6 ± 0.40 liters, P=0.0012) and the frequency of IDH events (2.9 ± 0.27 v. 0.13 events per treatment, p<0.0001) improved as well. Three of the 7 patients with normal adrenal hormones were also empirically tried on FC with no improvement in any of the above-mentioned parameters.

Funding: Other U.S. Government Support
Conclusions: Aldo deficiency is a clinical entity present in some chronic hemodialysis patients. It is a readily treatable condition, hereby, this study supports the premise that Aldo – in the absence of changes in sodium balance in oligo-anuric dialysis patients - has direct effects on vascular resistance and deserves further investigation in the chronic dialysis population.

SA-OR394

Acetate-Free Biofiltration Reduces Intracellular Hypertension in the Long-Term: A Randomized Controlled European Multicenter Trial

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Background: Intracellular hypertension (IH) is a common complication of conventional bicarbonate hemodialysis (BD) and it may contribute to the high cardiovascular morbidity and mortality among dialysis patients, a risk that can be contained by the use of convective therapies.

Methods: We have analysed data from a controlled randomised trial to evaluate whether acetate-free biofiltration (AFB), a hemodiafiltration technique with buffer-free dialysate, a high-flux membrane and sterile bicarbonate infusion in post-dilution mode which improved dialytic cardiovascular stability in short-term nonrandomized studies, may influence long-term IH rate, predialysis Sistolic Blood Pressure(SBP), Left Ventricular Mass Index(LVMI), cardiac ejection fraction by cardio computed tomography (CCT).

Results: The study enrolled 371 patients, 194 on BD and 177 on AFB. During a 3-year follow-up, the IH rate did not change for BD (3.2 to 3.1 session/month, p=0.0001), equal to a significant IH risk reduction for AFB (incidence rate ratio 0.81 [95% CI 0.76-0.86], p=0.0001). Median predialysis SBP did not change for BD, and decreased by 7 mmHg for AFB(p=0.01). Median LVMI increased for BD (4.2 g/m²), and decreased for AFB (2.2 g/m²), with no significant differences within or between each group. There was no difference in annual cardiovascular mortality and mortality between BD and AFB (8.8% and 6.9% vs 9.0% and 5.9%, p=0.001). AFB, however, reduced long-term case fatality (cardiovascular death among patients who had a nonfatal cardiovascular event) by comparison with BD (27% vs 59%, p=0.019).

Conclusions: In conclusion, compared to BD, AFB reduced both the IH rate and predialysis SBP in the long-term. It does not affect, however, LVMI, cardiovascular morbidity and mortality, but it contains the risk of cardiovascular death after a nonfatal cardiovascular event, possibly thanks to the better intradialytic cardiovascular stability.

SA-OR395

Short-Term Versus Long-Term Effects of Depressive Symptoms on Cardiovascular and Non-Cardiovascular Mortality in Incident End-Stage Renal Disease Patients

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Background: The present study assessed the impact of depressive symptoms on mortality among incident hemodialysis (HD) patients. It is unknown whether short-term mortality differs from long-term mortality and whether this depends on the cause of mortality.

Methods: We included 398 HD patients (175 males, mean age 67±16 years) who started HD for an average of 6 months. Depressive symptoms were assessed with the Mood Disorder Questionnaire (MDQ). Cardiovascular and non-cardiovascular deaths were identified using national death registers. Cox regression analyses were used to examine survival time. Results: The adjusted hazard ratio for mortality (HR) for patients with DS compared to patients without DS was 1.43 (95% CI, 1.08-1.88) for cardiovascular mortality and 2.07 (95% CI, 1.26-3.46) for non-cardiovascular mortality. DS posed a strong risk factor for non-cardiovascular mortality at the short-term (HR=2.82, 95% CI, 1.58-5.05), medium-term (HR=2.02, 95% CI, 1.36-3.01) and long-term (HR=1.74, 95% CI, 1.18-2.58), whereas the association between DS and cardiovascular mortality was not observed during the first 6 months of follow-up (HR=1.03, 95% CI, 0.49-2.15).

Conclusions: DS at the start of dialysis therapy pose a risk factor for cardiovascular and/or non-cardiovascular mortality, depending on the length of follow-up. The cause-specific mortality rates over time may help clinicians to understand the association between DS and survival in dialysis patients.

Funding: Other NIH Support - This work was supported by grants from Baxter Healthcare, the Dutch Kidney Foundation and the Dutch National Health Insurance Board. The funding sources were involved in neither the collection, interpretation, and analysis of the data, nor the decision for the writing and submission of this report for publication, Pharmaceutical Company Support

SA-OR396

An Important Novel Association between Circulating Endothelial Progenitor Cells and Residual Kidney Function in Peritoneal Dialysis Patients

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Background: Bone marrow-derived circulating endothelial progenitor cells (EPCs) plays an important role in vascular repair & maintaining vascular homeostasis. Given the increasing recognition of the significance of residual kidney function (RFK) in end-stage renal disease (ESRD) patients receiving long-term peritoneal dialysis (PD), this study aimed to determine the association, if any, between circulating EPCs (defined as cells that are both positive for CD34 and kinase insert domain receptor IDO) and RFK in PD patients.

Methods: Forty-one ESRD patients (mean age: 58 ± 8 years) receiving long-term PD treatment (mean ± SD duration on PD: 22 ± 27 months) were recruited with flow cytometric analysis of circulating CD34+KDR+CD45− cells & assessment of RFK, urea (Ku/V) and creatinine clearance (CrCl), clinical and biochemical data.

Results: The median (interquartile range) residual glomerular filtration rate (GFR) was 2.5 (0.7, 4.9) ml/min per 1.73 m². The total weekly Kt/V and CrCl were 2.17 ± 0.51 and 57 ± 41 L/wk per 1.73 m², respectively. On univariate analysis, circulating CD34+KDR+CD45− cells showed significant association with total CrCl (<0.40; p=0.009) and was contributed by its association with RFK (β=0.41; p=0.008) but not with PD CCI (β= −0.19; p=0.23). Using multiple linear regression analysis and adjusting for confounding covariates, a significant and independent association remained between circulating CD34+KDR+CD45− cells and RFK (β= 0.60; p<0.005).

Conclusions: RFK but not PD clearance showed an independent, positive association with circulating CD34+KDR+CD45− cells in PD patients. These data provide important novel evidence that reduced level of circulating EPCs may be the missing link between low RFK and increased cardiovascular events and mortality. Further investigation is needed to confirm these data and to determine the exact cause and effect relationship between circulating EPCs and RFK in PD patients warrant further investigation.

Funding: Government Support - Non-U.S.

SA-OR397

Low Vitamin E Levels, Cerebrovascular Events and Mortality in Hemodialysis Patients

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Background: Hemodialysis patients suffer from increased oxidative stress and experience an exceedingly high mortality as compared to the general population. Trials with the antioxidant vitamin E have shown controversial results. Considering the different causes of death in the dialysis population, this study investigated the effect of vitamin E on specific clinical outcomes in hemodialysis patients.

Methods: In 1046 diabetic hemodialysis patients (participants of the 4th study) alpha-tocopherol was measured by RP-HPLC. By regression analyses, hazard ratios (HR) were determined for pre-specified endpoints according to baseline alpha-tocopherol levels: sudden death (n=134), myocardial infarction (n=172), stroke (n=89), combined cardiovascular death (n=193), all-cause mortality (n=1038), and cause specific mortality in the cerebrovascular events (n=193). Low alpha-tocopherol levels particularly increased the risk of ischemic stroke by 3fold (HR 2.56 [1.43-4.31]), while they did not associate with hemorrhagic stroke [HR 1.10 (0.17-7.32)]. Furthermore, all-cause mortality was significantly increased [HR 1.31 (1.01-6.91)].

Conclusions: Low alpha-tocopherol was strongly associated with stroke, particularly ischemic stroke, in diabetic hemodialysis patients, and contributed to an increased mortality. Whether vitamin E supplementation decreases the occurrence of ischemic stroke, requires further evaluation.

SA-OR398

Oxidized High-Density Lipoprotein as a Risk Factor for Cardiovascular Events in Prevalemt Hemodialysis Patients

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Background: The present study assessed the impact of oxidized high-density lipoprotein (oxHDL), dysfunctional HDL, on mortality and cardiovascular disease (CVD) events in prevalent HD patients and compared oxHDL to interleukin-6 (IL-6), a strong predictor for CVD events in HD patients.

Methods: This prospective study examined a cohort of prevalent HD patients (n=412). Blood samples were obtained from predialysis uremic lipids, high sensitive C-reactive protein (hsCRP), IL-6, oxidized low-density lipoprotein, N-terminal pro B-type natriuretic peptide, inter-cellular adhesion molecule 1 (ICAM-1), myeloperoxidase, adiponectin, and oxHDL.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

95A
Carotid intima-media thickness (CIMT) was assessed at baseline and the 3-year follow-up. Nutritional status was assessed by subjective global assessment (SGA), body mass index, and geriatric nutritional risk index (GNRI). After the baseline assessment, study patients were prospectively followed-up (mean observational period, 40 months).

Results: At baseline, patients with high oxLDL had a worse nutritional state and higher C-reactive protein (CRP), low lipoprotein receptor (LDLr) pathway, which was inhibited by Rapamycin, an inhibitor of mammalian target of Rapamycin (mTOR). This study was to investigate whether inflammation accelerates lipid accumulation in the tissues of radial arteries in hemodialysis patients and its underlying mechanisms.

Methods: Thirty-one hemodialysis patients receiving arteriovenous fistula were divided into two groups by the plasma level of C-reactive protein: Control (n=16), inflamed group (n=15). Hematoxylin-eosin staining and Oil Red O staining were used to check foam cell formation and lipid droplets accumulation using the tissues surgically removed from radial artery. Immunohistochemistry and immunofluorescent staining were used to check protein expressions related with intracellular cholesterol trafficking.

Results: There was a significant increase in lipid accumulation in the radial artery in inflamed group compared to control, which was correlated with the increased protein expressions of LDLr, sterol regulatory element binding protein-2 (SREBP-2), and SREBP in inflamed group compared to baseline and a larger increase in CIMT at the 3-year follow-up. High oxLDL did not predict all-cause mortality; however, it was significantly associated with CVD-related mortality and composite CVD events, particularly with concomitant high IL-6. Those associations were confirmed in multivariate Cox hazard models adjusted with confounding variables.

Conclusions: A high oxLDL level may be associated with CVD events and CVD-related mortality, particularly with concomitant high IL-6 in prevalent HD patients.

SA-OR399

The Activation of mTOR Pathway Induced by Inflammation Accelerates the Progression of Atherosclerosis in Hemodialysis Patients Kun Ling Ma, Jing Liu, Min Gao, Xiaoliang Zhang, Bi-Cheng Liu. Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.

Background: Chronic inflammation is an independent risk factor in the progression of atherosclerosis (AS) in hemodialysis patients. Our previous studies in vivo and in vitro demonstrated that inflammation accelerated the progression of AS via the dysregulation of low lipoprotein receptor (LDLr) pathway, which was inhibited by Rapamycin, an inhibitor of mammalian target of Rapamycin (mTOR). This study was to investigate whether inflammation exacerbates lipid accumulation in the tissues of radial arteries in hemodialysis patients and its underlying mechanisms.

Methods: Thirty-one hemodialysis patients receiving arteriovenous fistula were divided into two groups by the plasma level of C-reactive protein: Control (n=16), inflamed group (n=15). Hematoxylin-eosin staining and Oil Red O staining were used to check foam cell formation and lipid droplets accumulation using the tissues surgically removed from radial artery. Immunohistochemistry and immunofluorescent staining were used to check protein expressions related with intracellular cholesterol trafficking.

Results: There was a significant increase in lipid accumulation in the radial artery in inflamed group compared to control, which was correlated with the increased protein expressions of LDLr, sterol regulatory element binding protein-2 (SREBP-2), and SREBP in inflamed group compared to baseline and a larger increase in CIMT at the 3-year follow-up. High oxLDL did not predict all-cause mortality; however, it was significantly associated with CVD-related mortality and composite CVD events, particularly with concomitant high IL-6. Those associations were confirmed in multivariate Cox hazard models adjusted with confounding variables.

Conclusions: A high oxLDL level may be associated with CVD events and CVD-related mortality, particularly with concomitant high IL-6 in prevalent HD patients.

SA-OR400

Pharmacologically-Relevant Concentrations of Intravenous Iron Preparations Promote Endothelial Injury and Monocyte Adhesion/Infiltration Vajiniath (Vijay) S. Kamanna, 1 Shobha H. Ganji, 1 Nosratola D. Vaidyan, 2 Krankins, University of California, Irvine, CA; 1Medical Research Service, VA Health Care System, Long Beach, CA.

Background: Intravenous iron (IV Fe) preparations are widely used in the management of anemia in ESRD populations. With recent implementation of bundling reimbursement policy, the use of these agents in ESRD patients has dramatically increased to lower the cost of anemia treatment by limiting the use of expensive ESA products. In many instances iron preparations are administered on a routine basis with insufficient attention to the status of total body iron stores or the underlying inflammation which can be aggravated by iron. Endothelial injury and dysfunction are critical steps in the pathogenesis of atherosclerosis, thrombosis and cardiovascular disease. Non-transferrin-bound iron avidly promotes oxidative stress which is a major mediator of endothelial damage and dysfunction. In fact, administration of these agents has been shown to raise markers of oxidative stress in ESRD patients. This study was undertaken to assess the effect of pharmacologically-relevant concentrations of IV iron preparations on endothelial cells and monocytes and interaction thereof in vitro.

Methods: Morphological changes in human aortic endothelial cells (HAEC) were examined by phase contrast microscopy. Cell viability was tested by a cell growth kit. Monocyte adhesion to HAEC was done using fluorescently labeled monocytes. The study documents the potential adverse effects of IV iron preparations on endothelial cells and their interactions with monocytes. These monocytes can contribute to endothelial dysfunction, atherosclerosis, thrombosis and cardiovascular disease which are the major cause of premature death in ESRD population.

Conclusions: Our results indicate that iron use and iron levels in dialysis patients influence endothelial biology and should be carefully monitored.

Funding: Private Foundation Support

SA-OR401

Intradialytic Protein Supplementation Attenuates Dialysis-Associated Inflammation and Reduces Co-Morbid Disease Risk Emily Tomakoy,1 Pei-Tzu Wu,1 Rae Yong Chung,1 Peter Fitschen,1 Brandon Kistler,1 Barbara Yudell,1 Elizabeth Jeanes,2 Shane Phillips,2 Ken Wilund,2 1Kinesiology and Community Health, University of Illinois, Urbana, IL; 2Physical Therapy, University of Illinois, Chicago, IL.

Background: Inflammation may be both cause and consequence of protein malnutrition, cardiovascular disease, and bone disease in dialysis patients; improving nutritional status may reduce inflammation and the risk for these conditions. This study examines the effects of intradialytic protein intake on circulating inflammatory markers and disease risk factors during a single dialysis session and also over a 6-month period.

Methods: Hemodialysis patients were randomly assigned to the following groups: whey protein (n=20), soy protein (n=15) or placebo (n=10). Blood was drawn immediately after start of dialysis treatment and three hours later on two days one week apart and analyzed for plasma interleukin-6 (IL-6) levels. On day 1, patients did not receive a study beverage but on day 2 consumed their assigned beverage. A subset of 19 patients continued with the beverage at every dialysis session for 6 months; blood was collected monthly and analyzed for standard clinical chemistries.

Results: All groups had a day 1 increase in IL-6 levels during dialysis indicating an acute inflammatory effect of a single dialysis session; this increase was attenuated in the groups consuming protein on day 2 compared to the placebo group (p<0.05). For the 6 month intervention, regression modeling showed a significant increase in albumin levels over time in the whey protein group (p<0.05). Alkaline phosphate levels declined in the protein groups compared to placebo (p<0.05) suggesting improvement in bone and vascular calcification disease risk. Furthermore, there were no significant group differences for plasma phosphorus, calcium, or potassium, suggesting neither soy nor whey protein intake during dialysis treatment adversely affects these clinically-relevant minerals.

Conclusions: These data support the safety and efficacy of intradialytic protein supplementation for attenuating increases in dialysis-associated inflammation and reducing co-morbid disease risk over a six-month period.

Funding: NIDDK Support

SA-OR402

Effect of Brazilian Nut Supplementation on Antioxidant and Inflammatory Status in Hemodialysis Patients Denise Mafra,1 Milena Barcelo Stockler-Pinto,2 Julie Lobo,2 Cristina Moraes,1 Najla Elias Farage,1 Gilson Teles Boaventura,1 Denis Fouque,2 Maria Therza Batista Wady,1 Wellington Seguins da Silva,2 Olaf Malm.1 1Clinical Nutrition, Federal University Fluminense, Niteroi, Rio de Janeiro, Brazil; 2Instituto de Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 1Nutrition, RenalCor Clinic, Rio de Janeiro, Brazil, Brazil; 2Nephrology, Hospital Edouard Herriot- Université Claude Bernard, Lyon, Rhone, France; 3Osvaldo Cruz Foundation (FIOCRUZ), Rio de Janeiro, Brazil.

Background: Cumulative evidence indicates that oxidative stress and inflammation frequently occurs in hemodialysis (HD) as a result of a decrease of antioxidant defenses and an overproduction of reactive oxygen species (ROS). Dietary intake of selenium (Se) has been associated with increased activity of glutathione peroxidase (GSH-Px) and, Se also plays an important role as an anti-inflammatory agent. The richest known food source of Se is the brazilian nut (Bertholletia excelsa, family Leichyiaceae), found in the Amazon region. The aim of this study was to evaluate the effect of Brazil nut supplementation on GSH-Px, TNF-α and IL-6 levels in HD patients.

Methods: Forty HD patients (57.5% men, 53.3 ± 16.1 yr) from RenalCor Clinic at Rio de Janeiro, Brazil were studied. All patients received 1 nut (around 5g) a day for three months. The GSH-Px, TNF-α and IL-6 levels were determined by ELISA.

Results: The cytokines levels were above the normal values and the activity of GSH-Px was below the normal values before nut supplementation. After 3 months supplementation, cytokines levels decreased and the activity of GSH-Px increased significantly (p< 0.001).

Parameters before and after nut supplementation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before supplementation</th>
<th>After supplementation</th>
</tr>
</thead>
<tbody>
<tr>
<td>GSH-Px (mmol/mL)</td>
<td>3.46 ± 5.2a</td>
<td>403 ± 8.4</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>871.1 (21.8-20.2)b</td>
<td>13.5 (12.8-22.2)</td>
</tr>
<tr>
<td>TNF-α (ng/mL)</td>
<td>21.0 ± 6.3b</td>
<td>13.9 ± 8.7</td>
</tr>
<tr>
<td>p&lt; 0.001</td>
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Conclusions: Our results indicate that brazilian nut increases the antioxidant status and plays an important role as an anti-inflammatory agent in and HD patients.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Effect of Phosphate Binders on Serum Inflammatory Profile, Soluble CD14 and Endotoxin Levels in Hemodialysis Patients: A Prospective, Randomized, Controlled Trial
Juan F. Navarro Gonzalez,1 Mercedes Muñoz,2 Carmen Mora,2 Patricia García-García,2 Nieves Del Castillo Rodríguez,1 Javier Donate,1 Violeta Cazaña,1 Javier García-Pérez.1,2 Nephrology, Hospital University Nuestra Señora de Candelaria; Research Unit, University Hospital Nuestra Señora de Candelaria; Clinical Biochemistry, University Hospital Nuestra Señora de Candelaria.

Background: Hyperphosphatemia and subclinical endotoxemia are important sources of inflammation in hemodialysis (HD). Pro-inflammatory cytokines are strong correlates of soluble CD14 (sCD14) concentrations, an independent predictor of mortality in this population. We evaluated the effects of calcium acetate and Sevelamer hydrochloride on serum inflammatory profile, endotoxin concentrations and sCD14 levels in HD patients.

Methods: This prospective, randomized, open-label, parallel design trial included 59 stable HD patients, 30 receiving Sevelamer and 29 calcium acetate. Serum levels of inflammatory parameters (high-sensitive C-reactive protein (hs-CRP), tumor necrosis factor-α (TNF-α), interleukin (IL)-1, 6, 10, and 18), as well as serum endotoxin and sCD14 concentrations were measured at baseline and after 3 months of therapy.

Results: Serum IL-6 increased in patients receiving calcium acetate, whereas hs-CRP and IL-6 significantly decreased in subjects treated with Sevelamer, with IL-10 experienced a trend to increase (p=0.05). Serum endotoxin and sCD14 levels did not change after treatment with calcium acetate. However, these parameters decreased by 22.6% and 15.2%, respectively (p<0.01), in patients receiving Sevelamer. Multiple regression analysis showed that variation in serum endotoxin concentrations was the strongest factor associated with IL-6 change, whereas the only variables independently associated with changes in sCD14 levels were the variations in serum IL-6 and endotoxin concentrations.

Conclusions: Administration of the non-calcium phosphate binder Sevelamer to maintenance HD patients is associated with a significant decrease in hs-CRP and IL-6.

Mechanism of Increased Renal Angiotensin II in Glomerular Disease
Taiji Matsusaka,1 Fumio Niimura,1 Akihiro Shimizu,1 Akihiko Saito,2 Akira Nishiyama,3 Tekuni Ichikawa,1,4 (Tokai University, Japan; 3Keio University, Japan; 4Kagawa University, Japan; 5Vanderbilt University.

Background: Earlier studies by others demonstrated that intrarenal angiotensin II (II) is increased in various kidney diseases independently of plasma II.

Methods: We explored the mechanism of this using 2 types of tissue-specific angiotensinogen (Agt) knockout (KO) mice.

Results: Kidney Agt KO showed 85.6% decrease in renal Agt mRNA; however, renal Agt protein was unaffected. Nevertheless, urinary Agt/creatinine was significantly decreased by 47%, indicating that Agt protein synthesized in the kidney is immediately secreted into the urine.

Liver Agt KO mice were near-completely deficient in Agt mRNA and protein in the liver, and in plasma Agt, as well. Remarkably, in the kidney of liver Agt KO, Agt protein was undetectable. Immunostaining of Agt in mosaic proximal tubule-specific megalin KO mice revealed that Agt uptake is completely dependent on megalin. Prior to assessing Agt content next, we verified the RIA method by observing undetectable Agt in whole body liver Agt KO. With this method, we found that renal Agt content is similar in kidney KO (n=9; 286±38 fmol/g) and control mice (n=8, 315±15), whereas both liver KO (n=8) and kidney liver dual KO (n=10) have lower Agt (110±16, 74±4; both p<0.05).

We, then, assessed the impact of glomerular sieving dysfunction on renal Agt and Agt mRNA expression using the immunotoxin-inducible podocyte injury model. After selective podocyte injury, renal Agt protein was dramatically increased. Renal Agt content was also increased upon podocyte injury (364±33 fmol/g, n=10), compared with mice without injury (155±27, n=10).

Conclusions: These results indicate that 1) liver Agt is the major source of renal Agt, 2) a fraction of circulating Agt, which is originated in the liver, is filtered through the glomerulus, delivered into the urinary space and reabsorbed by PTCs via megalin, and 3) glomerular damage increases the leakage of Agt into the urinary space, which leads to increase in both renal Agt and II. The data also offer one mechanistic explanation for the notion that proteinuria is a significant risk factor for the progression of glomerular diseases.

Funding: Government Support - Non-U.S.

Mesenchymal Stem Cells Infusion After Revascularization of Atherosclerotic Renal Artery Stenosis Improves Renal Tubular Transport Function
Van Solingen,1,2 Hetty C. de Boer, 1,2 Roel Bijkerk, 1,2 Eric P. Van der Veer, 1,2 van Solingen,1,2 Hetty C. de Boer, 1,2 Roel Bijkerk, 1,2 Eric P. Van der Veer,1,2 Alexander F. Schaapheider,2 Paul Quax,1,2 Ton J. Rabelink.1,2, Nephrology, LUMC, Leiden, Netherlands; 2Einhoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, Netherlands; 3Surgery, LUMC, Leiden, Netherlands.

Background: Stromal cell-derived factor-1 (SDF-1) expression has been shown to be upregulated in the kidney following ischemia reperfusion injury and may serve a reparative response by recruiting CXCR4-positive cells to the kidney. As SDF-1 mRNA contains a potential seed sequence for microRNA-126 (miR-126), we investigated the regulatory role of this endothelial cell (EC) enriched miR in SDF-1 expression and peripheral progenitor mobilization in mouse models of ischemia-reperfusion.

Methods: Using miR-reporter constructs and Western blots, we demonstrated that SDF-1 mRNA is a direct target of miR-126 in HUVEC. Using a transwell system and conditioned medium harvested from HUVEC incubated with antagonim-126 or scramblmir we assessed the effect of miR-126 on chemotaxis of CD34+ cells. Finally, we injected mice with antagonim-126 and performed either hind limb ischemia (HLI) or ischemia-reperfusion injury of both kidneys (IRI) to assess the effect of miR-126 silencing on progenitor cell mobilization.

Results: In vitro experiments showed that silencing of miR-126 in HUVEC enhanced SDF-1 expression and stimulated migration of CD34+ cell. After IRI or HL, qRT-PCR on whole kidney lysates and immunohistochemistry in the ischemic hind limb displayed elevated levels of SDF-1 in the animals injected with antagonim-126 or scramblmir. We assessed the effect of miR-126 on chemotaxis of CD34+ cells. Finally, we injected mice with antagonim-126 and performed either hind limb ischemia (HLI) or ischemia-reperfusion injury of both kidneys (IRI) to assess the effect of miR-126 silencing on progenitor cell mobilization.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: We demonstrate that silencing of endothelial miR-126 can enhance the expression of SDF-1 in an ischemia-dependent way, leading to the mobilization of SCA-1 Lin progenitor cells into the circulation.

Funding: Other NIH Support - Netherlands Heart Foundation: NHS2006B145

SA-OR407
Chronic Perturbation of the Endothelial Surface Glycocalyx Results in Proteinuria without Morphological Changes of the Glomerulus
Martijn Dane,1,2 Daniel R. Potter,1 Ton J. Rabelink,1 Hans Vink,1 Bernard Van Den Berg,3 Nephrology, LUMC, Leiden, Zuid-Holland, Netherlands; 2Fysiology, MUMC, Maastricht, Limburg, Netherlands.

Background: The glycocalyx is a layer of proteins and glycans that covers the luminal surface of the endothelium and plays a critical role in regulating fluid and solute transport across the vessel wall. In this study, we investigated whether chronic perturbation of the glycocalyx affects proteinuria in the absence of morphological changes of the glomerulus.

Methods: Male rats were randomized to either control (n=10) or glycocalyx-depleted (n=10) groups. Rats in the glycocalyx-depleted group were injected with hyaluronidase (hyal) for 2 weeks to damage the glycocalyx. The glycocalyx function was assessed using confocal microscopy. Kidney function was measured as urine output, proteinuria, and blood pressure.

Results: Rats in the glycocalyx-depleted group showed a significant increase in proteinuria (0.27 ml ± 0.04 SD vs. hyal-inact 0.51 ml ± 0.11). Pooled urine samples after hyaluronidase showed a 5-fold increase in proteinuria. The glycocalyx function was assessed using confocal microscopy, and the results showed a significant decrease in glycocalyx function in the glycocalyx-depleted group.

Conclusions: Chronic glycocalyx damage can lead to increased proteinuria without morphological changes of the glomerulus. These findings suggest that the glycocalyx plays a critical role in regulating proteinuria.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR408
In End-Stage Renal Disease Serum Amyloid A Accumulates in Plasma and HDL of Patients Leading to Diminished Anti-Inflammatory Capacity of HDL
Tao Huang, Markus Tolle, Mirjam Schuchardt, Markus van der Giet. Med. Klinik mit SP Nephrologie, Charite - Campus Benjamin Franklin, Berlin, Germany.

Background: Serum amyloid A (SAA) is an acute-phase protein that accumulates in plasma and HDL in patients with chronic kidney disease. SAA has been shown to contribute to the inflammatory state in these patients. However, the role of SAA in the anti-inflammatory capacity of HDL is not well understood.

Methods: Serum and HDL were isolated from patients with end-stage renal disease (n=10) and healthy donors (n=10). SAA content in plasma and HDL was measured using ELISA. The anti-inflammatory capacity of HDL was assessed using a human monocyte cell line (THP-1).

Results: SAA content in plasma and HDL of patients with end-stage renal disease was significantly higher than in healthy donors. The anti-inflammatory capacity of HDL from patients was significantly lower than in healthy donors. SAA was found to inhibit the induction of monocyte chemoattractant protein-1 (MCP-1) by HDL.

Conclusions: SAA accumulation in plasma and HDL in patients with end-stage renal disease leads to a diminished anti-inflammatory capacity of HDL. This finding has important implications for the management of chronic kidney disease.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR409
Knockout of the Vascular Endothelial Glucocorticoid Receptor Accelerates Atherosclerosis in a Mouse Model
Julie Goodwin,1 Xinbo Zhang,2 Jun Yu.2 Pediatrics, Yale University School of Medicine, New Haven, CT; 2Cardiology, Yale University School of Medicine, New Haven, CT.

Background: Atherosclerosis is a major risk factor for cardiovascular disease, and the therapeutic potential of alterations in glucocorticoid metabolism in cardiovascular disease has recently become a focus of investigation. The glucocorticoid receptor (GR) is a key regulator of the inflammatory response and is known to play a role in the development of atherosclerosis.

Methods: Mice with knockout of the endothelial glucocorticoid receptor were bred onto a high-fat diet (HFD) to induce atherosclerosis. Control and knockout animals were fed an atherogenic diet for 14 weeks. The effect of the knockout on atherosclerosis was assessed by measuring aortic area and cholesterol content.

Results: Mice with knockout of the endothelial glucocorticoid receptor had significantly worse atherosclerosis compared to control animals. The knockout mice had a 50% increase in aortic area and a 2-fold increase in cholesterol content.

Conclusions: Knockout of the endothelial glucocorticoid receptor Accelerates Atherosclerosis in a Mouse Model.

Funding: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Private Foundation Support

SA-OR410
Type-I Interferon Enhances Arterial Intimal Hyperplasia
Mark S. Segal, Pui Lee, Laura Sautina, Yanpeng Diao. Medicine, University of Florida, Gainesville, FL.

Background: Type-I interferon (IFN-I) is known to enhance intimal hyperplasia in various models of arterial injury. However, the mechanisms by which IFN-I enhances intimal hyperplasia are not well understood.

Methods: Mice with type-I interferon knockout (Ifn-1−/−) were bred onto a high-fat diet to induce arterial injury. Control and knockout animals were perfused after 4 weeks of injury. The extent of intimal hyperplasia was assessed by histological staining.

Results: The extent of intimal hyperplasia was significantly lower in knockout animals compared to control animals. The knockout animals had a 50% reduction in intimal hyperplasia.

Conclusions: Knockout of the type-I interferon receptor accelerates arterial intimal hyperplasia.

Funding: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Private Foundation Support

SA-OR411
Knockout of the Vascular Endothelial Glucocorticoid Receptor Accelerates Atherosclerosis in a Mouse Model
Julie Goodwin,1 Xinbo Zhang,2 Jun Yu.2 Pediatrics, Yale University School of Medicine, New Haven, CT; 2Cardiology, Yale University School of Medicine, New Haven, CT.

Background: Cardiovascular complications, and in particular atherosclerosis, are a major source of morbidity and mortality in patients with chronic kidney disease. The therapeutic potential of alterations in glucocorticoid metabolism in cardiovascular disease has recently become a focus of investigation. The glucocorticoid receptor (GR) is a key regulator of the inflammatory response and is known to play a role in the development of atherosclerosis.

Methods: Mice with knockout of the endothelial glucocorticoid receptor were bred onto a high-fat diet (HFD) to induce atherosclerosis. Control and knockout animals were fed an atherogenic diet for 14 weeks. The effect of the knockout on atherosclerosis was assessed by measuring aortic area and cholesterol content.

Results: Mice with knockout of the endothelial glucocorticoid receptor had significantly worse atherosclerosis compared to control animals. The knockout mice had a 50% increase in aortic area and a 2-fold increase in cholesterol content.

Conclusions: Knockout of the endothelial glucocorticoid receptor accelerates atherosclerosis.

Funding: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Private Foundation Support

SA-OR412
Type-I Interferon Enhances Arterial Intimal Hyperplasia
Mark S. Segal, Pui Lee, Laura Sautina, Yanpeng Diao. Medicine, University of Florida, Gainesville, FL.

Background: Type-I interferon (IFN-I) is known to enhance intimal hyperplasia in various models of arterial injury. However, the mechanisms by which IFN-I enhances intimal hyperplasia are not well understood.

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Results: The extent of intimal hyperplasia was significantly lower in knockout animals compared to control animals. The knockout animals had a 50% reduction in intimal hyperplasia.

Conclusions: Knockout of the type-I interferon receptor accelerates arterial intimal hyperplasia.

Funding: National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Private Foundation Support

Figure 1: Confocal analysis of the effect of IFN-1 on neointima lesion after 4 weeks injury. Dual immunohistochemical stain of K-37 (marker of proliferation, red nuclei) and SM-MG (marker of SMC, green cytoplasm) indicates that an increased number of proliferating yellow orange SMC within neointima in IFN-1 treated animals (lower panel) than control (upper panel). Note: Only the cells with red nuclei and green cytoplasm were identified as proliferating SMC. Most of the red nuclei without green cytoplasm identified as leukocytes (CD45 staining cells, orange area), were not shown. All sections were counterstained with DAPI (blue). Object: 4x.
MicroRNA-21 and TGF-β in Diabetic Glomerulopathy

Jennifer Yi-Chun Hasegawa, Shu Wakino, Koichi Hayashi.

MicroRNA-21 and TGF-β signaling and podocyte apoptosis. Pima Indians exhibit high rates of type 2 diabetes mellitus and DN. MicroRNAs (miRs) are implicated in mediating development of DN. We previously showed that miR-21 inhibits renal cell apoptosis in cultured renal cells and in vivo. We examined regulation of miR-21 expression in human and murine models of DN and further explored miR-21 functions in miR-21 null mice.

Methods: Total RNA was extracted from micro-dissected glomeruli of kidney biopsy tissues from 26 diabetic Pima Indians. Small RNA fraction was obtained from the flow-through of silica-membrane RNA columns. MiR-21 expression was quantified using Taqman qRT-PCR (Applied Biosystems) and correlated with urinary albumin-creatinine ratio (ACR) using Pearson correlation. Mice ubiquitously deficient for miR-21 were generated by cre-mediated deletion of the floxed pre-miR-21 coding sequence were crossed with Albumin-TGFβ1 transgenic mice. PAS-staining was used for histologic analysis. Albuminuria in mice was determined semi-quantitatively.

Results: MiR-21 exhibited high relative expression in glomeruli and excellent correlation with ACR (Correlation coefficient = 0.8, FDR = 0, p < 0.001). TGFβ1/miR-21 null mice exhibited strongly increased deposition of PAS-positive material, and loss of glomerular capillary loops. In TGFβ1/miR-21 null mice albuminuria was already increased at 2 weeks of age and exceeded 5mg albumin/1g creatinine at 4 weeks of age in over 50% of mice but was less than 0.5g/g in all wildtype littermates.

Conclusions: MiR-21 expression is abundant in glomeruli and associated with ACR in DN. MiR-21 null mice exhibited progressive glomerular injury induced by TGFβ1. We propose that miR-21 expression increases with injury as an attempt to protect against acute kidney injury (JBC 2010) and diabetic nephropathy (DN) (oral presentation at ASN 2010). In this study, we newly developed PT-specific conditional Sirt1-deficient mice to investigate if its role in the initiation and progression of DN. Methods: We generated PT-specific Sirt1 deficient mice (conditional knockout mice, CKO) by crossing Sirt1floxflox mice with KAP-Cre mice. Wild-type (WT) or CKO mice were injected with saline (control) or streptozotocin to be rendered DN. The phenotypes of four groups of mice, WT+Sal, CKO+Sal, WT+STZ, CKO+STZ were analyzed 24 weeks after the treatment.

Results: Sirt1 expression was specifically reduced in PTs in CKO. Immnostaining showed that Sirt1 expression in PTs and podocytes was reduced in WT+STZ compared with that in WT+Sal. This reduction was further prominent in CKO+STZ. Urinary analysis demonstrated that the increase in urinary albumin excretion (AUE) in WT+STZ was further augmented in CKO+STZ. PAS staining and electron microscopy showed the increase in mesangial matrix accumulations or GBM thickness in WT+STZ mice were not altered in CKO+STZ. However, the increase in podocyte foot process effacement in WT+STZ as compared with WT+Sal was further enhanced in CKO+STZ. Real time PCR assay after laser microdissection of glomeruli demonstrated that a tight junction protein, Claudin-1 was elevated in WT+STZ as compared with WT+Sal and that this increase was further enhanced in CKO+STZ. Correspondingly, slit diaphragm proteins, nephrin, podocin, synaptopodin were further enhanced in CKO+STZ. Furthermore, CKO+STZ had no effect on blood glucose levels and pancreatic islet morphology.

Conclusions: The present study revealed that deficiency of Sirt1 in PT had unfavorable effects on the phenotype of podocytes including the foot process effacement, the downregulation of slit diaphragm proteins and the upregulation of tight junction protein, Claudin-1. Sirt1 in PT is the safeguard against the initiation and progression of DN-induced podocytes de-differentiation through the novel mechanism of PT-podocyte communication.
In vitro assay, high glucose medium significantly induced mRNA expression of FGFRs in mouse podocyte cell line (138%). Microalbuminuria in AKITA+FGF2 was significantly increased on day 10-15 (231.7 ± 64.8 / 88.6 ± 11.7 mg/gCr). In contrast, microalbuminuria was not induced in control mice. Next, the FGF antagonist was evaluated as a therapeutic agent to the accelerated diabetic nephropathy using puromycin aminonucleoside and AKITA. When PAN (10 mg/animal/day i.v., day 0-10) was administrated to AKITA, microalbuminuria in AKITA+PAN was significantly increased on day 5-10 (AKITA+FGF2 52.7 ± 64.8 mg/gCr). In contrast, microalbuminuria was not increased in those treated by FGF antagonist.

**Conclusions:** Both FGF2 and FGF receptors were induced under hyperglycemia and play a crucial role of podocyte injury in DMN.

**Funding:** Government Support - Non-U.S.

**SA-OR418**

**Translationally Controlled Tumor Protein Is Associated with Podocyte Hypertrophy in a Mouse Model of Type 1 Diabetics**

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**Background:** Translationally controlled tumor protein (TCTP) is thought to be involved in various intracellular processes by regulating mammalian target of rapamycin complex 1 (mTORC1) signaling. Because diabetes characteristically induces hypertrophy in podocytes in the early stage of the disease, mTORC1 signaling has been implicated in this process. TCTP may have a role in the pathogenesis of podocyte hypertrophy in diabetic nephropathy.

**Methods:** We examined the changes in TCTP expression in experimental diabetic glomeruli. To characterize the role of TCTP in podocyte hypertrophy, we conducted in vivo and in vitro assay of silencing of TCTP through the hydrodynamic injection of TCTP shRNA expressing lentivirus (LV-shTCTP).

**Results:** Glomerular expression of TCTP were higher in DM mice compared with C mice. Double immunostaining for TCTP and synaptopodin revealed that podocytes were the main cells responsible for this increase. TCTP knockdown by RNA interference ameliorated the activation of mTORC1 downstream effectors and the overexpression of cyclin dependent kinase inhibitors (CKIs) in diabetic glomeruli. Light microscopic examination revealed a marked glomerular hypertrophy in DM mice compared with C mice. In contrast, transfection of LV-shTCTP in DM mice attenuated the glomerular hypertrophy. The mean volume of podocyte measured by an unbiased stereological technique using optical dissector were significantly higher in DM mice compared with control, whereas numerical density of podocytes were lower in diabetic mice. These morphologic changes were significantly ameliorated by the LV-shTCTP treatment. Electron microscopy of glomeruli revealed that podocytes of DM mice were enlarged with mild thickening of GBM, whereas the foot process was mostly intact. However, the diabetes-induced hypertrophy of podocytes, but not the GBM thickening, was substantially reduced in DM+LV-shTCTP mice.

**Conclusions:** These findings suggest that TCTP may play an important role in the process of podocyte hypertrophy under diabetic conditions via the regulation of mRNA translation and the induction of cell cycle arrest.

**Funding:** Private Foundation Support

**SA-OR419**

**Transcriptional Analysis of Human Diabetic Kidney Disease**

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**Background:** Diabetic kidney disease (DKD) is the single leading cause of kidney failure in the United States. The aim of this study was to provide an unbiased catalogue of gene expression changes in human diabetic kidney samples. We used a microarray platform for transcriptional profiling of human nephrons.

**Methods:** Affymetrix expression array was used to identify differentially regulated transcripts in 22 microdissected human renal glomerular and 22 tubular samples. Stringent statistical analysis was used to identify differentially expressed transcripts in control and diseased glomeruli and tubuli. Two different web-based algorithms were used to define differentially regulated pathways.

**Results:** The DKD samples were significant for racial diversity, decreased eGFR (DKD stage IV < 10 ml/min/1.73 m²), and was associated with increased glomerulosclerosis.

**Conclusions:** Our studies identified multiple novel genes and pathways that may play a role in the pathogenesis of DKD or could serve as biomarkers.

**Funding:** Other NIH Support - NIH (U.S. Government), Private Foundation Support
SA-OR420
Mir-21 Is a Key Mediator in the Development of Diabetic Kidney Injury in db/db Mice and In Vivo Under Diabetic Conditions
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Background: Our recent studies found that mir-21 plays a pathological role in TGf↓β-induced renal fibrosis. In the present study, we hypothesized that mir-21 may also act as a key mediator and therapeutic target for diabetic nephropathy.

Methods: mir-21 expression was examined in diabetic kidney of db/db mice. The functional role and therapeutic potential of mir-21 in diabetic kidney injury was examined in db/db mice treated by overexpressing or down-regulating of mir-21. In addition, role and mechanisms of mir-21 in diabetic renal injury was examined in vitro under diabetic conditions in a rat mesangial cell (MC) line / NRK52E tubular epithelial (TEC) line by overexpressing or down-regulating of mir-21.

Results: Renal mir-21 was markedly upregulated (two folds increased) in db/db mice, which was associated with the development of renal fibrosis including collagen I and IV (a 1.5-fold increased), and the severity of microalbuminuria (p<0.01). Similar results were also evidenced in high glucose-stimulated MC and TEC. The functional role of mir-21 was further examined in vitro that knockdown of mir-21 suppressed, but overexpression of mir-21 enhanced, high glucose-induced Col1, Col IV, and fibronectin expression (p<0.05). More importantly, ultrasound-microbubble-mediated gene transfer of mir-21 knockdown plasmid into the diabetic kidney of db/db mice at week 10 resulted in a significant improvement of microalbuminuria (p<0.01) and renal fibrosis (p<0.05) after 20 weeks, revealing a therapeutic potential for diabetic nephropathy by targeting mir-21.

Conclusions: In conclusion, mir-21 is a key mediator for the development of diabetic kidney disease. Targeting mir-21 may represent a novel and effective therapy to combat diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-OR421
Identification of the Site of Endothelin A Receptor Antagonist-Induced Fluid Retention
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Background: Endothelin (ET) receptor antagonist-induced fluid retention has been responsible for discontinuation and/or failure of several clinical trials. ETB receptor inhibition causes fluid retention though reduction of the known natriuretic effects of renal tubules. The protein kinase mammalian target of rapamycin, mTOR, has been shown in the native collecting duct (Oxidative Stress Responsive 1) kinase phosphorylate several renal ion cotransporters, including NCC, NKCC2 and NKCC1, increasing cotransporter activity. Knock-in of a majority of SPAK expressed in the thick ascending limb is truncated at the N-terminus, by performing RT-PCR on a broad panel of tissues, and named it KS-SPAK. Next, we examined the effect of mTOR on current in the native collecting duct (NIH DK085101 NIDDK Support, Other NIH Support - NHLBI, Pharmaceutical Company Support)

Methods: To better understand the effect of mTOR on current in the native collecting duct, we examined the effect of PP242 using in vitro microperfusion of collecting ducts. Cortical collecting ducts were harvested and subjected to microperfusion according to standard techniques. Potential difference (PD) measurements were made following treatment with PP242 (1 µM, N = 7) or rapamycin (0.1 µM, N = 5).

Results: We found that urine volume (ul) was increased by 56% (609 +/- 55 vs 953 +/- 101, p<0.048), sodium excretion increased by 67% (13.3 +/- 2.4 vs 22.2 +/- 3.0, p<0.034), total sodium excreted (mmol) was increased by 167% (117.1 +/- 24.6 vs 42.9 +/-11.1 p = 0.014). The Urine sodium to potassium ratio increased by 62% (0.20 +/-0.03 vs 0.33 +/- 0.13 p = 0.026), suggesting ENaC inhibition, possibly due to decreased SGK1 phosphorylation. In the microperfusion data, PD (mV) decreased from 1.9 +/- 2.76 to 4.92 +/- 1.29 with 242 treatment (p = 0.03 paired t-test vs control), and recovered to 6.98 +/- 1.71 (p = 0.11). Effects were amiloride sensitive, indicating ENaC dependence. Rapamycin had no effect on PD.

Conclusions: Together, with cell culture and in vitro data, these data strongly suggest that mTOR — in particular mTOR complex 2— regulation of SGK1 plays a physiologically important role in ENaC regulation in the kidney tubules. (NIH DK056695)

Funding: NIDDK Support

SA-OR424
Impaired Phosphorylation of Na-K-2Cl Cotransporter by OSR1 Deficiency Manifests Hypotension and Bartter-Like Syndrome
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Background: Na+-K+2Cl- cotransporters (NKCC1/KCC2), including NKCC1 and renal-specific NKCC2 and CCl, play pivotal roles in the regulation of blood pressure and renal NaCl reabsorption. Oxidative stress-responsive kinase-j (OSR1) is a known upstream regulator of NKCC1/KCC2.

Methods: We generated a novel global and kidney tubule-specific (KSP) OSR1 knockout mice to elucidate the physiologic role of OSR1 in vivo, particularly on blood pressure and kidney function.

Results: Although global OSR1- mice were embryonically lethal, OSR1+ mice had lower blood pressure associated with reduced phosphorylated (p-)NKCC1 abundance in aortic tissue but intact renal Na+ reabsorption. KSP-OSR1 mice were normotensive but primarily exhibited impaired Na+ reabsorption in the thick ascending loop (TAL) on a low Na+ diet accompanied by remarkably decreased expression of p-NKCC2 and a blunted response to furosemide, a NKCC2 inhibitor. The expression of total SPAP and p-SPAP was decreased, and NaCl intake increased parallel to that of total NCC and p-NCC despite unchanged total NKCC2 expression. KSP-OSR1 mice also manifest hypokalemia with renal K+ wasting and hypercalciuria, associated with reduced ROMK1, enhanced ENaC (β), and epithelial Ca2+ channels (TRPV5 and 6).

Conclusions: These results suggest that globally, OSR1 is involved in the regulation of blood pressure and renal tubular Na+ reabsorption mainly via the activation of NKCC1 and NKCC2. In the kidneys, NKCC2 but not NCC is the main target of OSR1 and the reduced p-NKCC2 in KSP-OSR1 mice may lead to a Bartter-like syndrome.

Funding: Government Support - Non-U.S.

SA-OR442
The Role of mTOR and SGK1 in Mediating Aldosterone Regulation of ENaC In Vivo
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Background: SGK1 is a key mediator of aldosterone activation of ENaC in the kidney tubules. The protein kinase mammalian target of rapamycin, mTOR, has been shown in tubular cells and in vitro to phosphorylate SGK1, and stimulate ENaC.

Methods: To determine the importance of this effect in vivo, we monitored Na+ balance in mice following intraperitoneal injection of the mTOR inhibitor PP242 (30mg/kg; n = 10). To better understand the effect of mTOR on current in the native collecting duct, we examined the effect of PP242 using in vitro microperfusion of collecting ducts. Cortical collecting ducts were harvested and subjected to microperfusion according to standard techniques. Potential difference (PD) measurements were made following treatment with PP242 (1 µM, N = 7) or rapamycin (0.1 µM, N = 5).

Results: We found that urine volume (ul) was increased by 56% (609 +/- 55 vs 953 +/- 101, p<0.048), sodium excretion increased by 67% (13.3 +/- 2.4 vs 22.2 +/- 3.0, p<0.034), total sodium excreted (mmol) was increased by 167% (117.1 +/- 24.6 vs 42.9 +/-11.1 p = 0.014). The Urine sodium to potassium ratio increased by 62% (0.20 +/-0.03 vs 0.33 +/- 0.13 p = 0.026), suggesting ENaC inhibition, possibly due to decreased SGK1 phosphorylation. In the microperfusion data, PD (mV) decreased from 1.9 +/- 2.76 to 4.92 +/- 1.29 with 242 treatment (p = 0.03 paired t-test vs control), and recovered to 6.98 +/- 1.71 (p = 0.11). Effects were amiloride sensitive, indicating ENaC dependence. Rapamycin had no effect on PD.

Conclusions: Together, with cell culture and in vitro data, these data strongly suggest that mTOR — in particular mTOR complex 2— regulation of SGK1 plays a physiologically important role in ENaC regulation in the kidney tubules. (NIH DK056695)

Funding: NIDDK Support

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Underline represents presenting author.

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SA-OR425

T60M Mutation in Thiazide-Sensitive Na+/Cl- Cotransporter (NCC) Causes Defective NCC Expression and Reverses Gordon Syndrome
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Background: Defective phosphorylation of the thiazide-sensitive sodium-chloride cotransporter (NCC) on T60 residue, the most important phosphate acceptor site of SPAK/OSR1 kinases, abolished its functional activity without affecting surface expression in Oocytes. T60M-NCC mutation is common in Asian patients withGitelman’s syndrome (GS).

Methods: To explore the physiopathological importance T60 residue of NCC in vivo, we created T58M-Ncc knock-in mice to model human GS with T60M-NCC.

Results: NccT58M mice exhibited typical features of GS with a blunted response to thiazide, confirming that Ncc function was markedly diminished. In the kidneys, total and p-Ncc (T55, T58, and S71) of NccT58M mice were remarkably reduced despite normal abundance of Ncc mRNAs, and immuno-gold staining also showed that T58M-Ncc almost located in the sub-apical region of the DCT cells, suggesting that phosphorylation of T60 may play a pivotal role in the NCC trafficking/sorting. In MDCK cells, NCC interacts with adaptor protein (AP)-3, a lysosomal sorting-related protein. An increased T60M-NCC-AP3 interaction with an attenuated T60M-NCC expression which could be reversed by AP3 knockdown was observed. Furthermore, phenotype of Wnk4G46A/Nck knock-in, a Gordon syndrome animal model with an activated Spak/Osr1-Ncc phosphorylation signifying and increased p-Spak/Osk1-Ncc expression featuring the mirror image of GS, was reversed by C57B6/6 NccG46A knock-in mice with a reduced total and p-Ncc expression.

Conclusions: Our results indicated that phosphorylation of NCC T60 is involved in its trafficking/sorting and thus activity in vivo. Defective NCC phosphorylation by T60M could reduced its expression by an enhanced AP3-associated lysosomal degradation, and reverses the phenotype of Gordon syndrome.

Funding: Government Support - Non-U.S.

SA-OR426

TheCalcineurinInhibitorTaclorolimusActivatestheRenalSodiumChlorideCotransporterToCauseHypertension
Ewout J. Hoorn,1 Stephen B. Walsh,2 James A. McCormick,3 Antje Furstenberg,2 Chao-Ling Yang,2 Tom Roeschel,4 Alexander Paliege,3 James P. Conley,1 Sebastian C. Bachmann,4 Robert J. Unwin,1 David H. Ellison.1 Erasmus Medical Center, Rotterdam, Netherlands; 2UCL, London, United Kingdom; 3OHU, Portland; 4Charité University, Berlin, Germany.

Background: Calcineurin inhibitors (CNIs) are immunosuppressive drugs, which are used widely to prevent rejection of transplanted organs and treat autoimmune disease. Hypertension, hyperkalemia and acidosis often complicate their use. These side effects resemble familial hyperkalemic hypertension, a genetic disease characterized by overactivity of the renal sodium chloride co-transporter (NCC) and caused by mutations in WNK kinases. We hypothesized that CNIs induce hypertension by stimulating NCC.

Methods: The effects of the CNI tacrolimus on NCC were studied in mice and kidney transplant patients by characterizing renal tubular function, and by using immunoblotting and immunofluorescence.

Results: In wild-type mice, tacrolimus caused salt-sensitive hypertension, hyperkalemia, and acidosis. In kidney homogenates, tacrolimus increased phosphorylated NCC and the NCC regulatory kinases WNK3, WNK4, and SPAK. Immunofluorescence confirmed that NCC co-localized with calcineurin. The functional importance of NCC was confirmed by showing that tacrolimus had no effect on blood pressure in NCC knockout mice, while SPAK expression was similar to control. Immunohistochemistry revealed normal morphology of distal convoluted tubule, with lower NCC expression. Under low salt diet or AngII infusion, no increased expression or phosphorylation of pNCC-T58S or pSPANK-S373 was observed.

Conclusions: Our observations reveal that absence of NCC in vivo precludes the NCC and SPAK phosphorylation promoted by low salt diet or AngII infusion, suggesting that this peptide hormone action on NCC occurs by signaling through a WNK4 dependent mechanism.

Funding: Private Foundation Support

SA-OR428

Role and Mechanism of WNK1-OSR1/SPAK Cascades in the Regulation of Sodium Homeostasis in Mice
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Background: WNK1 is a ubiquitous protein kinase of which increased expression causes hypertension at least partly by increasing renal Na reabsorption. In vitro, WNK1 phosphorolyses and activates Ste20-related protein kinases OSR1 and SPAK, which in turn activate NCC and NKCC2. Alternatively, others reported that WNK1 activates these transporters independently of OSR1/SPAK. We examine the in vivo role and mechanism of WNK1 regulation of Na transport.

Methods: Mice with global inactivation of Wnk1 are embryonic lethal with cardiovascular (CV) developmental defects. We generated mice with conditional knockout of Wnk1 in the kidney by crossing homozygous exon2-flox mice with transgenic mice carrying a kidney-specific Cre. Mouse lines that allow conditional tissue-specific expression of constitutive-active (CA) OSR1 or SPAK were generated by targeting respective cDNA preceded by floxed transcriptional terminator to the ROSA26 locus.

Results: Mice with conditional KO of WNK1 in the kidney exhibited increased 24-h urinary Na excretion (168 ± 26 vs 131 ± 12 µmol, p < 0.01) and lower blood pressure (SBP 114 ± 1 vs 123 ± 3 mmHg, p < 0.05) versus wild type when fed a normal Na diet ad lib. Conditional KO mice had defect in conserving Na during transition from pair-fed normal Na diet (0.4%) to Na-deficient diet (0.01%) (24-h Na excretion 1 and 2 days after Na-deficient diet: 29 ± 4 and 3 ± 0.11 in KO vs 13 ± 0.8 and 1.3 ± 0.3 µmol in WT, p < 0.01). Mice with global deletion of Osrl exhibited CV developmental defects similar to mice with global Wnk1 deletion, but with ~0.5 day delay in onset (begins at embryonic day E10.5 vs E11). Endothelial-specific expression of CA-OSR1 rescued lethality from global deletion.

Conclusions: These results indicate that WNK1 plays an important role in regulation of renal Na reabsorption. Similar timing and developmental defects between Wnk1 and Osrl deletion indicate potential interactions of WNK1 and OSR1 signaling cascades. These studies will investigate which ion transporters are regulated by WNK1 and OSR1, and whether CA-OSR1/SPAK can complement developmental and renal defects of WNK1 deficiency.

Funding: NIDDK Support

SA-OR429

Pathophysiology of Familial Hyperkalemic Hypertension Related to Deletions of the First Intron of WNK1
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Background: Mutations in the serine-threonine kinase WNK1 are responsible for Familial Hyperkalemic Hypertension (FHHt), an autosomal dominant form of hypertension associated with hyperkalaemia and hyperchloremic metabolic acidosis. WNK1 gives rise to a long ubiquitinous isoform, L-WNK1, and a shorter isoform lacking a functional kinase domain and expressed exclusively in the distal nephron, KS-WNK1. FHHt mutations are large deletions of the first intron of the gene, but the consequences of these deletions on the expression of L-and KS-WNK1 and on renal ion transport are unclear.

Methods: In order to elucidate the mechanisms underlying the deregulation of renal ion handling in WNK1-related FHHt patients, and thereby the role of WNK1 on blood pressure control and ion homeostasis, we generated WNK1+-/ΔΔ knock-in mice harboring a deletion of the first intron of WNK1.

Results: WNK1+/ΔΔ mice display high blood pressure, hyperkalaemia and hyperchloremic metabolic acidosis. We show that L-WNK1 expression is increased in the kidneys and convoluted Tubule (CDCT), and, to a lesser extent, in the connecting tubule, whereas KS-WNK1 expression is not modified upon deletion of WNK1 first intron. As

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Underline represents presenting author.

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expected from in vitro studies, the modification of L-WNK1 expression leads to increased excretion of potassium and cholride in the DCT. In addition, blood pressure and metabolic disorders are reversed by administration of hydrochlorothiazide. Finally, the increased sodium transport in the DCT is associated with a decreased activity of the epithelial sodium channel (ENaC), as illustrated by a blunted diuretic response to amiloride and hyperkalemia and acidosis.

Conclusions: Our study demonstrates that, by increasing L-WNK1 expression in the DCT, the deletion of WNK1 first intron leads to the stimulation of NCC activity and the development of fibrosis. The contribution of L-WNK1 activation in the CNT remains to be defined.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-OR430
RDX5791, a Non-Systemic NHE3 Inhibitor, Normalizes Blood Pressure and Renal Function in Uremic Rats
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Background: Excess dietary Na+ exacerbates hypertension and accelerates cardiac and renal dysfunction in CKD patients. RDX5791 is a potent, non-systemic inhibitor of the intestinal Na+ transporter NHE3 and shifts Na+ excretion from urine to feces. The current study evaluated the effects of RDX5791 on systemic hemodynamics and organ damage in uremic rats fed a 4% NaCl diet for four weeks.

Methods: At study inception, 5/6th nephrectomized rats were functionally matched to a vehicle group (n=24) or one of three groups of prophylactically dosed RDX5791 (0.3, 1, or 3 mg/kg/d; n=12/group). At mid-study, the half the vehicle group was functionally matched to one of three new vehicle treatment groups or begin 3 mg/kg/d RDX5791 treatment (therapeutic arm). Diastolic and systolic blood pressure (DBP, SBP) and serum and urine chemistry were measured weekly or biweekly. Extracellular fluid volume (ECFV) and total body water (TBW) were monitored via bioimpedance spectroscopy. At endpoint (day 28), rats were sacrificed and morphological analyses of heart and kidney were performed.

Results: Compared to vehicle rats, prophylactic RDX5791 (1 mg/kg/d) reduced DBP by 26% (144 to 107 mmHg), SBP by 21% (204 to 161 mmHg), albuminuria by 78% (97.1 to 21.4 mg/dl), left ventricle/ibia length ratio by 20% (27.7 to 22.1 mm/g), ECFV/TBW (43.5% to 41.5%), and weight of remnant kidneys, (1.88 to 1.43 g) which were analyzed to compare the effects of fibrotic injury. Importantly, the cardio-renal effects of RDX5791 were dose-dependent. In the therapeutic arm, RDX5791 reduced established albuminuria, hypertension and the expanded ECFV to values similar to those in prophylactically-treated animals.

Conclusions: RDX5791 reduces Na+ transport in the DCT, associated with reduced activity in the hyperkalemia and acidosis.

Funding: Government Support - Non-U.S.

SA-OR431
Renal Allograft Rejection and Delayed Graft Function Regulated by miRNAs
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Background: Aim of this study was the identification of deregulated miRNAs in renal biopsies from patients who developed acute vascular rejection, acute interstitial rejection, antibody mediated rejection (ABMR) and delayed graft function (DGF). Methods: Sixty-five selected post-transplant FFPE biopsy samples (30 acute cellular rejection (15 Banff-I, 15 Banff-II), 11 ABMR, 14 DGF, 10 protocol biopsies) were analyzed. Affymetrix GeneChip® miRNA Arrays holding 904 unique small RNA sequences based on the Sanger miRBASE version 11 were used for miRNA profiling. Differentially regulated miRNAs were identified by Student’s t-test and Bonferroni correction. First experimentally validated targets according to miRTarBase were used as basis for functional interpretation. Second predicted target genes were identified using in-silico approaches (miRror – combination of databases like TargetScan and miRand, miTALOS) and also interpreted on the functional level.

Results: Patients with acute rejection (ARE), ABMR and DGF discriminate from the control group (protocol biopsies) in the unsupervised cluster analysis and also in the principal component analysis suggesting miRNAs play an important role in rejection and DGF. Seven miRNAs are up-regulated in DGF. Four up-regulated and 17 down-regulated miRNAs are identified in acute rejection episodes compared to control group and six miRNAs are up-regulated in DGF and ABMR and miR-663 is up-regulated in both rejection groups.

Conclusions: These preliminary data suggest that a distinct miRNA signature is associated with rejection and delayed graft function. A detailed analysis of the miRNAs and target genes will be performed at the meeting. The identified miRNAs and target genes may provide novel insights in the molecular regulation of acute rejection and delayed graft function and the identified molecules may serve as novel diagnostic markers and therapeutic targets.

Funding: Government Support - Non-U.S.

SA-OR432
Urinary miR-210 as a Mediator of Acute T-Cell Mediated Rejection in Renal Allograft Recipients

Background: microRNAs (miRNAs) are small ribonucleotides regulating gene expression. microRNAs are present in the blood in a remarkably stable form. We tested whether miRNAs are also detectable in urine and may serve as new predictors of outcome in renal transplant patients with acute rejection.

Methods: We profiled urinary miRNAs of stable transplant patients and transplant patients with acute rejection. The results were validated in a validation cohort of 62 patients with acute rejection, 19 control transplant patients without rejection and 13 stable transplant patients with urinary tract infection by quantitative RT-PCR.

Results: miR-10a, miR-10b and miR-210 were strongly deregulated in urine of patients with acute rejection. We confirmed these data in urine of a validation cohort of 62 patients with acute rejection, 19 control transplant patients without rejection and 13 stable transplant patients with urinary tract infection by quantitative RT-PCR. miR-10b and miR-210 were down-regulated and miR-10a up-regulated in patients with acute rejection compared to controls. Only miR-210 differed between patients with acute rejection when compared to stable transplant patients with urinary tract infection or transplant patients before/after rejection. Low miR-210 levels were associated with higher decline in GFR one year after transplantation.

Conclusions: Selected miRNAs are strongly altered in urine of patients with acute renal allograft rejection. miR-210 levels identify patients with acute rejection and predict long-term kidney function. Urinary miR-210 may thus serve as a novel biomarker of acute kidney rejection.

Funding: Government Support - Non-U.S.

SA-OR433
Impact of Inflammation in Protocol Biopsies Taken at Week 6 after Transplantation on Fibrosis in Biopsies Taken at One Year
Willy Aasebo,1 Hallvard Holdaas.2 1Akershus University Hospital; 2Oslo University Hospital - Rikshospitalet.

Background: To assess if inflammation in protocol biopsies taken 6 weeks after kidney transplantation predict inflammation and fibrosis one year after transplantation.

Methods: All single kidney transplant recipients that had a protocol biopsy taken at week 6 and week 52 (n = 157) were included. Recipients with donor specific antibodies at transplantation, AB0 incompatibility transplantations and recipients with indication biopsies taken between week 4 and 8 were excluded. The immunosuppressive protocol consisted of induction therapy with two doses of basiliximab, thereafter calcineurine inhibitors + mycophenolate + steroids. All biopsies were classified according to Banff criteria. Recipients with rejection in protocol biopsies at week 6 received treatment with Solu-medrol and temporarily increased oral steroids dosages.

Results: Of the 100 biopsies that were negative at week 6, 69 were also negative at one year (69%), 26 had borderline and 5 had rejection. In the Borderline-group at week 6 (n = 43), 20 were negative at one year (47%), 19 had borderline and 4 had rejection. Of the 14 recipients with subclinical rejection at week 6 (9%), 10 had negative biopsies at one year (71%), 3 had borderline and 1 had rejection. Fibrosis/atrophy in biopsies at one year according to inflammation in protocol biopsies taken at week 6 after transplantation. N = 157.

Conclusions: Inflammation in protocol biopsies at week 6 does not predict interstitial fibrosis or tubular atrophy at one year in low-risk single kidney transplant recipients. Recipients with subclinical rejections at week 6 had less tubular atrophy than the other groups and low degree of inflammation at one year. This finding indicates that identifying and treating these 9% of the recipient may be important.

Funding: Non-U.S.

SA-OR434
Microcirculation Endothelial Cell Cycling Is Selectively Increased in Antibody-Mediated Rejection of Kidney Transplants, but Not in Other Inflammatory Disorders
Stephen Adedayo Osaain, Declan G. de Freitas, Jessica Chang, Michael Mengel, Philip F. Halloran, Banu Sis. ATAGC, University of Alberta, Edmonton, AB, Canada.

Background: Antibody-mediated rejection (ABMR) is dominated by microcirculation inflammation and endothelial injury, caused by donor specific antibodies. We hypothesized that ABMR is associated with a greater endothelial repair response than other diseases in kidney transplants.

Methods: We related microcirculation endothelial cell cycling (MECC) to histopathology lesions, diagnoses, and whole-genome microarrays in 100 kidney transplant and 40 normal implantation biopsies. We performed double immunostaining for Ki-67 and CD31 to identify capillaries with cycling endothelial cells. We quantified MECC by counting number of Ki-67/CD31+ glomerular and peritubular capillaries in the entire cortical area of the biopsies.

Funding: Non-U.S.
Results: Transplant biopsies showed higher numbers of capillaries with cycling endothelial cells than controls (p<0.003). MECC was higher in ABMR than other diseases.

Increased MECC correlated with donor specific HLA antibody (p=0.003), microcirculation lesions (glomerulitis, capillaritis, transplant glomerulopathy), and C4d staining (p<0.05). Furthermore, transcript sets representing the molecular burden of active ABMR (endothelial cell, NK-cell, and macrophage-associated transcripts as well as interferon gamma regulated transcripts) correlated with increased MECC (p<0.05). Within the ABMR biopsies, increased MECC correlated with higher grades of glomerulitis (p=0.01) and peritubular capillaritis (p=0.08). Interestingly, endothelial cycling was not increased in TCGR, suggesting that T cells cross the microcirculation in TCGR without damaging the capillaries.

Conclusions: The endothelial repair response is selectively increased in kidneys with ABMR, reflecting the burden of active microcirculation injury.

SA-OR435
Ischaemic Glomeruli Are Increased in Protocol Biopsies after Transplantation, in Association with Peritubular Capillary (PTC) Loss, Inflammation and IF/TA
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Background: Chronic transplant dysfunction is a major cause of renal allograft loss, preceded by inflammation and IF/TA in the renal biopsy. Recently we showed that PTC loss occurs within the three months after transplantation, and predicts lower renal function at 1 year. We hypothesized that capillary loss also occurs in the glomerular capillary bed, resulting in more ischemic glomeruli.

Methods: Protocol biopsies of 89 patients taken 0, 3, and 12 months (M0, M3, M12) after renal transplantation were studied. Recipients received kidneys from living (n=23), deceased after brain death (n=26) or deceased after cardiac death (n=40) donors. Biopsies were scored according to Banff09 with a total inflammation (t) score. Ischemic glomeruli were scored as percentage of the total glomerular number. In a subgroup of 48 patients PTC number was determined as described (Steegh et al. JASN 2011). Statistical analysis was performed by T-test, Pearson correlations and multivariate analysis.

Results: Compared to M0, the mean number of ischemic glomeruli at M3 and M12 increased significantly from 7.86% to 20.64% and 23.79% (0-3 months p<0.001, 0-12 months p<0.001). The progression of ischemic glomeruli correlates with PTC loss at M3 (r=0.397, p=0.030). Increase in ischemic glomeruli correlates with t and IF/TA scores at M3 and M12. Significant predictors for increased ischemic glomeruli at M12 are donor type (c2=0.165, p=0.005).

Conclusions: This study shows for the first time that the loss of PTCs is associated with an increase of ischemic glomeruli at M3 after transplantation. We hypothesize that interstitial and glomerular microvasculature loss is interrelated and may be initiated by similar mechanisms, leading to decreased renal function and worse prognosis for allograft survival.

SA-OR436
Kidney Transplant Fibrosis Is Not Dependent on Peritubular Capillary Loss
Stephen Adebayo Osasan, Jessica Chang, Declan G. de Freitas, Michael Mengel, Philip F. Halloran, Banu Sis. ATAGC, University of Alberta, Edmonton, AB, Canada.

Background: It has been suggested that progressive loss of peritubular capillaries (PTC) contributes to kidney fibrosis. We hypothesized that kidney transplant fibrosis is associated with and dependent on microcirculation loss.

Methods: We related PTC density to histopathology lesions, diagnoses, and post-transplant time in 100 kidney transplant biopsies for cause with 40 normal implantation biopsies as control. We performed CD31 immunostaining to identify the PTCs and counted total number of CD31+ PTC’s in the entire cortical area of the biopsies. The PTC density was calculated by dividing the total number of PTC’s by the number of ocular grid areas (0.2mm2).

Results: The PTC density was lower in transplant biopsies compared to controls, but did not differ among diagnoses in the transplant cases.

SA-OR437
Systematic Review & Meta-Analysis of Graft Outcomes Associated with Donor Specific Antibodies (DSA) & Negative Crossmatch

Background: The introduction of solid phase methods provides increased sensitivity and specificity for the detection of DSA. However, the clinical relevance of DSA in the presence of negative CDC and flow crossmatches is unclear.

Methods: We identified 415 studies for review using the search term “antibodies OR solid-phase assay OR luminox OR antibody mediated rejection” AND kidney AND crossmatch” which investigated allograft outcomes in patients with DSA detected by Luminex but not by cytotoxic or flow crossmatches. In addition, abstracts presented at the ASN & NKF between 2005 – 2010 were hand-searched for inclusion. 54 studies were identified for further review and 17 studies were extracted for review of full text. Ultimately, 5 retrospective studies with data comparing the incidence of AMR and graft survival in DSA positive and DSA negative groups detected by Luminex alone were pooled for analyses.

Results: All 5 studies reported the incidence of AMR, but only 4 studies reported the occurrence of graft failure. The time to follow-up was variable (range 9 months-4 years). Our pooled analysis included 104 DSA positive patients and 676 patients without DSA. There was an overall increased risk of AMR associated with the DSA positive group compared to patients without DSA (RR=1.149, 95% CI 1.017–1.299, p=0.026). The heterogeneity between the studies was not significant (I2=63.6%, p= 0.06). However, no increased risk of graft failure was detectable (RR=1.06, 95% CI 0.944–1.91, p=0.325) and there was no heterogeneity between studies (I2=0%, p=0.69).
Conclusions: Our systematic review and pooled analysis underlines the paucity of outcomes data associated with preformed DSA detected by luminex but suggests an increased risk of AMR and no significant impact on overall graft survival in the short term.

SA-OR438

The Serum p-Cresol and Indoxyl Sulfate Correlated with Cardiovascular Disease in Mild-to-Moderate Chronic Kidney Disease of Renal Transplant Recipients Cheng-Hau Chen,1,2,3 Chi-Hung Cheng,1,4 Ming-Ju Wu,1,4 Tung-Min Yu,1,2 Ya-Wen Chuang,1 Kuo-Hsiung Shu.1,4

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Background: Cardiovascular disease (CVD) is the major cause of death after renal transplantation (RTx). The p-cresol (pCS) and indoxyl sulfate (IS), anionic uremic toxins, markedly accumulated in serum and deteriorated of chronic kidney disease (CKD), are associated with CVD.

Methods: We recruited 95 RTx recipients with mean follow-up duration 6.3 ± 4.9 years. Their mean age was 48.7 ±14.1 years; and 56.8% (54/95) male patients. According to the eGFR staging, we divided RTx recipients into stage 1-2 group and stage 3-5 group, then, examined pCS and IS and CVD events in these two group RTx recipients.

Results: There was no significant difference in CKD staging is significantly different in the follow-up duration (5.0 ± 3.2 vs. 7.1 ± 5.6; P = 0.037). The serum pCS (2.4 ± 3.1 vs. 5.2 ± 6.8 mg/dL, P = 0.020) and IS (0.7 ± 1.0 vs. 2.7 ± 3.4 mg/dL, P = 0.001) was also significantly different between groups.

<table>
<thead>
<tr>
<th>Stage 1-2</th>
<th>Stage 3-5</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age of RTx (yrs)</td>
<td>31.3 ± 15.2</td>
<td>30.7 ± 13.8</td>
</tr>
<tr>
<td>Follow-up duration (yrs)</td>
<td>5.0 ± 3.2</td>
<td>7.1 ± 5.6</td>
</tr>
<tr>
<td>HS (mg/dL)</td>
<td>13.4 ± 1.6</td>
<td>14.2 ± 2.2</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>2.4 ± 0.3</td>
<td>2.1 ± 0.6</td>
</tr>
<tr>
<td>Serum p-cresol</td>
<td>0.7 ± 1.0</td>
<td>2.7 ± 3.4</td>
</tr>
</tbody>
</table>

Interestingly, the CV event was correlated with the serum levels of pCS and IS between the CKD groups.

Conclusions: These findings suggest that serum pCS and IS may help to predict CVD risk in different CKD stage recipients. Whether pCS and IS is a modifiable cardiovascular risk factor in RTx recipients remains to be proven.

SA-OR439

Longer CMV Prophylaxis May Prevent Low Level CMV Reactivation but May Not Prevent Primary CMV Infection Christine M. Ribic,1 Sundus A. Lodhi,2 Jon A. Gregg,3 Kenneth E. Lamb,1 Herwig-Ulf Meier-Kriesche.1

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Background: Whether longer valganciclovir (VG) prophylaxis prevents primary CMV disease after kidney transplantation (tx) is still debatable even after the published IMPACT trial and especially considering the subsequent editorials. For this reason, we analyzed data from our own center where we have been using extended VG prophylaxis since 2005.

Methods: This single centre retrospective study evaluated 690 tx recipients who received VG prophylaxis with a mean follow-up of 2.6 (SD+/−2.35) years. CMV event-free survival was calculated from the time of completion of prophylaxis and the primary endpoint was defined as positive CMV viremia identified by quantitative PCR (≥200 copies/ml) or positive pp65 antigenemia.

Results: There was no significant difference in CMV event-free survival (p=0.68) in high-risk (D+/R-) tx recipients who received prophylaxis for a median duration of 227 (n=55) versus 125 days (n=63) (Fig 1A). Likewise, when changing the endpoint to different CMV PCR thresholds (100, 200, 250, 500, 1000, 5000 or 10,000 copies/ml of plasma), there was also no significant difference between long and shorter prophylaxis.

Conversely in intermediate-risk patients, there were significantly (p= 0.004) higher rates of CMV viremia in those patients who received shorter prophylaxis for a median duration of 186 (n=263) versus 116 (n=292) days (Fig 1B). However this effect was driven by low level viremia results as the reduction in events in the longer prophylaxis group did not persist for the endpoint of CMV PCR ≥500 copies/ml.

Conclusions: In summary, our data suggests that longer prophylaxis may not impact the cumulative incidence of primary CMV infection but may prevent low level CMV reactivation.
SA-OR40

Significance of Persistent Asymptomatic EBV Viral Load in Pediatric Renal Transplant (TX) Recipients
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Background: Children, in particular EBV naïve at TX, are at risk of developing post-transplant lymphoproliferative disorder (PTLD). Currently, many centers are prospectively monitoring EBV viral load (VL) by real time quantitative PCR (qPCR) and intervening (reducing immunosuppression [IS], therapy with antiviral drugs in response to + VL to prevent PTLD. However, acute rejection (AR) may occur due to reduction in IS causing graft failure [GF]. Outcome of these strategies is not clear.

Methods: EBV asymptomatic VL subregistry was created within NAPRTCS to study prevalence of PTLD on AR and GF in pts with persistent + VL for 6 months and in those with high VL (>10^6 copies/ml) over the time determined by negative qPCR to compare to EBV -VL pts from the same centers.

Results: 14 centers enrolled 425 children (63% [18.4] with +VL and 362 [85.2%] with -VL) from 2005 onwards. Of 60 with +VL and complete data, 40% were <5 yrs age at TX, 31: M:29, 68.3 Caucasian and 8.3% Blacks, 79.3% and 75% were EBV and CMV naïve at TX. Of donors, 3.3% and 45% were EBV and CMV naïve. Thymoglobulin induction was used in 43.3% and 35% in EBV +VL and -VL pts (p<0.05). Asymptomatic +VL developed 6-9 months after TX, 50% with +VL had reduction in IS and 10% treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 and 5.9-8.8 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/39 (7.7%) treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/60 (5%) with +VL had reduction in IS and 1.3% with +VL. PTLD developed 6.4±6.5 months after TX, 50% with +VL had reduction in IS and 10% treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/60 (5%) with +VL had reduction in IS and 1.3% with +VL. PTLD developed 6.4±6.5 months after TX, 50% with +VL had reduction in IS and 10% treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/60 (5%) with +VL had reduction in IS and 1.3% with +VL. PTLD developed 6.4±6.5 months after TX, 50% with +VL had reduction in IS and 10% treated with antivirals. PTLD developed in 3/60 pts with +VL and 4/362 pts with -VL, 6.7-8.1 months after TX (5% vs 1.1% P=0.06). Of pts with high VL 3/60 (5%) with +VL had reduction in IS and 1.3% with +VL.

Conclusions: These results show the first to document strong activation of HB viral signaling during renal fibrosis, and they suggest pancreatic signaling of epithelial Hh ligand to stromal pericytes and myofibroblasts. The ability of Hh to trigger pericyte proliferation in culture suggests a possible role for HB signaling in pericyte and myofibroblast proliferation in the development of renal fibrosis. These studies introduce the HB signaling pathway as a novel therapeutic target in renal fibrosis.

Funding: NIDDK Support

SA-OR41

Endothelial HIF Signaling Modulates Renal Fibrosis
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Background: Hypoxia and increased activity of hypoxia-inducible factor (HIF) is commonly found in chronic kidney disease. We previously identified tubular epithelial HIF-1α as a promoter of renal fibrosis, however the role of HIFs in other renal cell types remains unclear. Here we used a genetic approach to investigate the role of endothelial HIF-1α and HIF-2α in tubulointerstitial chronic renal fibrosis.

Methods: To delete HIF-1α and -2α in endothelial cells Vascular endothelial growth factor (VEGF) is a major mediator of angiogenesis in renal disease. To determine the role of VEGF in endothelial HIF signaling, we generated a conditional VEGF knockout mouse line (VEGFfl/fl mice). We then crossed this line with a creERT2, iCre mouse line to delete VEGF specifically in endothelial cells. We then assessed renal fibrosis in these mice using established renal fibrosis models, including unilateral ureteral obstruction (UUO) and streptozotocin-induced diabetic nephropathy (STZ-DN).

Results: Endothelial HIF-1α/-2α double mutants developed and aged normally, had no defects in renal morphology and function and possessed intact renal vasculature. When we exposed double mutants to 12 days of UUO, the group compared to controls (n=4-6, P<0.03). Capillary density was reduced by 32% in collagen deposition compared to controls based on Sirius Red staining (n=10-11, P<0.03). Capillary density observed in the control group was similar to that observed in the double mutant group. In addition, mutant UUO kidneys showed a 2.4-fold increase in F4/80+ve cell infiltration. No difference in collagen accumulation was found when UUO experiments were performed with either endothelial HIF-1α or HIF-2α single mutants. However, capillary density by CD31 and cabinet staining was decreased by 32% in endothelial HIF-2 single mutants compared to controls (n=5-6, P<0.03).

Conclusion: Our data show that endothelial HIF-1α and HIF-2 are dispensable under physiologic conditions, whereas both endothelial HIF-1α and HIF-2 in concert suppress the fibrotic response to chronic kidney injury. Furthermore, we identified HIF-2 as a critical regulator of the capillary maintenance during fibrosis. Our data provide new insights into the cell type-specific role of individual HIF transcription factors in renal disease.

Funding: NIDDK Support

SA-OR42

Macrophage HIF Suppresses Renal Inflammation in Chronic Kidney Disease
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1Medicine, Vanderbilt University, Nashville, TN; 2Medicine, Mount Sinai Medical Center, New York, NY; 3Medicine, Vanderbilt University, Nashville, TN.

Background: Hypoxia has been implicated as an important microenvironmental factor involved in the development of fibrosis in chronic kidney disease (CKD), and hypoxia-inducible factor (HIF) has been shown to be a key regulator in this process. Our previous studies showed that deletion of HIF-1α in proximal tubule epithelial cells decreases renal fibrosis, indicating that HIF-1 activation in epithelial cells is fibrogenic. However, systemic pharmacologic HIF activation in certain animal models can slow CKD progression indicating cell-type specific dependence of HIF with regard to renal fibrogenesis.

Methods: We used a genetic approach and studied the effect of HIF activation (HIF-1 and HIF-2) in the unilateral ureteral obstruction (UUO) mouse model. HIF was activated by global deletion of von Hippel-Lindau tumor suppressor gene (Vhl) using inducible Cre-loxP-mediated gene targeting system (Ucc-Cre). We measured HIF expression (HIF-1α), accumulation of HIF-mediated genes (TIE2, VEGF), and renal fibrosis scores in UUO kidneys in Vhl+/+ and Vhl−/− mice using immunohistochemistry, qPCR, and Masson’s trichrome staining. HIF-1α, TIE2, and VEGF expression were increased in UUO kidneys of Vhl−/− mice compared to Vhl+/+ mice. These results are the first to document strong activation of Hh signaling in renal fibrosis, and they suggest a possible role for Hh signaling in pericyte and myofibroblast proliferation in the development of renal fibrosis. These studies introduce the Hh signaling pathway as a novel therapeutic target in renal fibrosis.

Funding: NIDDK Support

Key: TX - Tuesday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

106A
Iron Irritates Kidney Inflammation in a Model of Chronic Renal Failure Induced by Persistent Expression of Kidney Injury Molecule-1
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Background: Chronic renal failure is characterized by tubular degeneration, fibrosis and often tissue iron deposition. In humans and rodent models of progressive kidney disease, Kidney Injury Molecule-1 (Kim-1) is persistently expressed in the proximal tubular (PT) and released into the urine.

Methods: A transgenic mouse was created, using Cre-Lox technology, which overexpresses Kim-1 in the PT. Transgene was confirmed for iron staining. Tubular Kim-1 expression and surrounding inflammatory cells were characterized by immunofluorescence. Primary PT cells were treated with media containing bovine Holo-Transferrin and 100 µM ferric ammonium citrate to model tissue iron overload. MHC-II expression was evaluated by flow cytometry. RAW macrophage activation was evaluated by TNFα production after exposure to conditioned media from Kim-1 expressing cells.

Results: The Kim-1 transgenic mouse develops progressive kidney disease with fibrosis, anemia, cardiac hypertrophy and suffers a uremic death. Urinary transferrin levels, tubule-interstitial inflammation and heme oxygenase-1 are increased. There are large amounts of iron deposition in PT cells and surrounding macrophages. We studied the role of this iron maldistribution in the modulation of inflammatory changes leading to renal failure. In primary cultures of mouse PT cells, addition of transferrin upregulated MHC-II levels. Iron loading of cells also eventually resulted in cell death of PT cells. Conditioned media from transferrin-treated tubular cells produced a marked induction of TNFα production by macrophages.

Conclusions: Increased PT cell iron is associated with upregulation of MHC-II in vivo. In vitro, increased cellular iron uptake results in increased cellular MHC-II expression, and eventual iron-loaded cell death. Iron-enriched PT cells release pro-inflammatory agents which increase activation of macrophages and may contribute in an important way to progression of chronic kidney disease.

Funding: NIDDK Support, Private Foundation Support

Platelet-Derived Growth Factor Receptor Signaling Activates Pericyte-Myoﬁbroblast Transition in Progressive Kidney Fibrosis
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Background: Pericytes are the major source of scar producing myofibroblasts following kidney injury; however, the mechanisms of this transition are unclear.

Methods: We examined Collagen 1 (α1)-GFP reporter mice (pericytes and myofibroblasts express GFP) following ureteral obstruction or ischemia-reperfusion injury and focused on the role of platelet-derived growth factor (PDGF)-receptor (PDGFR) signaling in these two different injury models.

Results: Pericyte proliferation was noted after injury with reactivation of α-smooth muscle actin expression, a marker of the myofibroblast phenotype. PDGF expression increased in injured tubules, endothelium, and macrophages after injury, whereas PDGFRα subunits α and β were expressed exclusively in interstitial GFP-labeled pericytes and myofibroblasts. When PDGFRα or β activation was inhibited by receptor-specific antibody following injury, proliferation and differentiation of pericytes decreased. The antibodies also blocked the de novo transcription of PDGFR, transforming growth factor-β1, and chemokine CCL2. They also reduced macrophage infiltration and fibrosis. Imatinib, a PDGFR tyrosine kinase inhibitor, attenuated pericyte proliferation and kidney fibrosis in both fibrogenic models.

Conclusions: PDGFR signaling is involved in pericyte activation, proliferation and differentiation into myofibroblasts during progressive kidney injury. Hence, pericytes may be a novel target to prevent kidney fibrosis by means of PDGFR signaling blockade.

Funding: Government Support - Non-U.S.

Angiotensin II Induces Progressive Renal Injury by Activating the TGF-beta/Smad Pathway through Activation of Sustained EGRF-ERK Signaling
Jun-Chen Chen,1,2 Jia-Kang Chen, Raymond C. Harris.1 Medicine, Vanderbilt University, Nashville, TN.

Background: Although it is well recognized that angiotensin II can mediate progressive renal fibrosis, the underlying mechanisms are not completely understood.

Methods: We generated renal proximal tubule specific EGRF receptor knockout mice (EGRF^-/-) by crossing EGRF^-/- mice with GtCre mice and chromically isolated angiotensin II (Ang II) into these mice and their wild type littermates (WT). Blood pressures were similar in the two groups.

Results: In WT, Ang II infusion increased TGF-beta expression and Smad3 phosphorylation in isolated renal proximal tubule epithelial cells, decreased E-cadherin expression, up-regulated vimentin, N-cadherin and snail expression and increased tubulointerstitial fibrosis. Ang II also markedly induced EGRF phosphorylation at Tyr485, a Src-dependent phosphorylation site. In Ang II-infused WT, Tyr485-phosphorylated EGRF associates with the adaptor proteins Shc and Grb2 in vivo. In LLC-PK1 cells stably transfected with AT1R (AT/1/C4), Ang II stimulated ROS production, TGF-beta de novo synthesis and Smad3 phosphorylation. Antioxidants or siRNA knockdown of Src kinase or EGRF blocked Ang II-activated ERK activation, inhibited TGF-beta A and B signaling. In vivo, EGRF^-/- mice had decreased ERK signaling, TGF-beta expression and Smad3 phosphorylation, and significantly less tubulointerstitial fibrosis in response to Ang II perfusion. Administration of a specific EGF tyrosine kinase inhibitor, erlotinib, to the wild type FVB/NJ mice, had a similar inhibitory effect on Ang II-induced renal cortical fibrosis in vivo.

Conclusions: This study demonstrates that ROS-dependent Src activation-mediated prolonged-EGRF-ERK signaling, inducing TGF-beta de novo synthesis and Smad3 signaling activation, is a novel molecular mechanism underlying Ang II-induced progressive renal injury.

Funding: NIDDK Support, Veterans Administration Support

NADPH-Oxidase 4 Knock-Out Mice Display Increased Tubular Apoptosis and Interstitial Fibrosis in the Unilateral Obstructed Model
Stellier Khodu

Background: Kidney interstitial fibrosis is correlated with chronic kidney disease (CKD) progression. NOX4 is the major kidney NADPH-oxidase expressed mostly in the tubular compartment. In the kidney NOX4 is expressed at lower levels. NOX isoforms are involved in apoptosis and prosurvival pathways as well as in hypoxia signaling and may therefore play a role in mediating apoptosis.

Methods: We studied unilateral ureteral obstruction (UO) in wild type and NOX4 knock-out (KO) mice as well as in NOX4/2 double-KO mice to decipher the role of these enzymes in kidney fibrosis progression. mCdc21 cells were specifically used to examine mediated apoptosis.

Results: NOX4 was expressed in the proximal tubule and collecting duct whereas NOX2 was expressed at low levels along the nephron. Kidney development was not altered by the absence of NOX2 and NOX4. Interstitial fibrosis assessed by Sirius red staining and collagen Western blot after 7 and 14 days UUO was lower in NOX4 and NOX2/NOX4 mice compared to wild type. Tubular apoptosis was significantly enhanced in NOX4 and NOX2/NOX4 KO mice compared to wild type. Peritubular capillary density and VEGF expression assessed by Western blot were significantly lower in UO kidneys of NOX4 and NOX2/NOX4 KO mice compared to wild type. Oxidative stress was increased in the interstitium of obstructed kidneys of NOX4 KO mice whereas it was attenuated in NOX2/NOX4 KO animals. In mCdc21 cells NOX4 siRNA silencing led to apoptosis in the presence of TGF-B.

Conclusions: We demonstrate that NOX4 deletion is deleterious in the UUO model and increases tubular cell apoptosis under conditions of tubular cell stress. NOX4 deletion decreases kidney peritubular vascularisation via decreased tubular VEGF production in UUO. NOX2 has a different role from NOX4 and does not compensate for the absence of NOX4. The role of NOX4 in tubular cell might be different from other cell types. These effects of NOX4 may explain enhanced kidney fibrosis in UUO NOX4 KO mice.

Funding: Private Foundation Support, Government Support - Non-U.S.

Inhibition of microRNA-21 as a Therapeutic Strategy for Kidney Fibrosis
Jeremy S. Duffield,1 Deidre Mackenna,2 Phong T. Tran,3 Kara Kersjes,2 Aaron Chang,2 B. Nelson Chau,2 Medicine, University of Washington, Seattle, WA; 2Regulus Therapeutics, Lo Jolla, CA; 3Medicine, Harvard Medical School, Boston, MA.

Background: Fibrosis is an underlying pathology involved in disease progression and is associated with poor prognosis in a variety of chronic kidney diseases (CKD). microRNAs are ~22 bp evolutionarily conserved non-coding RNAs that regulate multiple mRNA targets and their dysregulation has been implicated in multiple disease processes including fibrosis. In particular, miR-21 is up-regulated in cardiac, lung & liver fibrosis & oligonucleotide-mediated inhibition of miR-21 is effective in mouse models of heart & lung fibrosis. We investigated if antagonizing miR-21 could represent a novel therapeutic approach for CKD.

Methods: We used mouse models of kidney injury with fibrosis, genetic and oligonucleotide silencing of miR-21 in vivo, and bioinformatics to study miR-21 in kidney disease.

Results: miR-21 was upregulated in two different models of kidney fibrosis: unilateral ureteral obstruction (UUO) and unilateral ischemia-reperfusion injury (IRI). Genetic deletion of miR-21 prevented kidney fibrosis in both models as measured by fibrotic gene expression, quantitative histology and biochemical analyses. To assess the therapeutic potential of inhibiting miR-21, high affinity oligonucleotide-based anti-miRs were administered to mice prophylactically or therapeutically in UUO or IRI models. These compounds delivered effectively to renal tubule epithelium and were detected in various interstitial cell types, including pericytes and interstitial myofibroblasts. Anti-miR-21 prevented fibrosis in both models that largely phenocopied miR-21 deletion. To investigate the role of miR-21 in fibrosis, we evaluated mRNA expression profiles of miR-21 deficient kidneys. Surprisingly, the expected global de-repression of potential miR-21 target mRNAs Key: TH - Thursday; FR - Friday; SA - Saturday; OR- Oral Abstract; PO - Poster; PUB - Publication Only.
T Lymphocytes Lacking Sphingosine Kinase 2 Attenuate Folic Acid Induced Kidney Fibrosis in Mice

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Background: Epidemiological studies indicate some survivors of AKI who regain renal function have a progressive decline in kidney function leading to end stage renal disease (ESRD). Sphingosine-1-phosphate (SIP) is generated by phosphorylation of sphingosine by sphingosine kinases (SphK1 and SphK2).

Methods: WT, SphK1-/- and SphK2-/- mice were administered Folic Acid-FA (250 mg/ kg) and followed for 14 days. mRNA changes were measured with RT-PCR and protein changes with IHC and western blots.

Results: SphK2-/- mice exhibited lower fibrosis (trichrome staining) compared to WT or SphK1-/- mice at day 14 post FA. Compared to kidneys of FA treated SphK1-/- mice, SphK2-/- mice expressed lower levels of mRNA encoding TGF-β-SMA (30.8%, p<0.01) and fibronectin (36.9%, p<0.01). Likewise, kidney sections of FA-treated SphK2-/- mice displayed lower levels of collagen and fibronectin immunoreactivity compared to SphK1-/- mice. FA-treated SphK2-/- mouse kidneys had reduced infiltration of macrophages (45.1%, p=0.05) and neutrophils (27.9%, p=0.01) compared to SphK1-/- mice. SphK2-/- CD4+ T cells have a hyper-proliferative response with significantly higher amounts of IFN-γ compared to WT or SphK1-/- cells. In fibrosis, IFN-γ is anti-fibrotic; therefore, we tested the role of CD4+ T cells from SphK2-/- mice transferred into WT and SphK1-/- mice. Transfer of SphK2-/- CD4+ T cells to either WT or SphK1-/- mice attenuated the progression of fibrosis at day 14 in the recipients.

Conclusions: We conclude that: 1) FA induces fibrosis to a similar degree in WT and SphK1-/- mice, 2) the lack of SphK2 attenuates FA-induced fibrosis possibly due to hyper-proliferative T cells. Understanding the function of SphK2 may contribute further to our understanding of the pathogenesis of fibrosis and development of a SphK2 selective inhibitor may lead to a new therapeutic agent that impede the progression of kidney disease to ESRD.

Funding: NIDDK Support

Vascular Access Failure: Clinical Studies

SA-OR452
Progress of the Hemodialysis Fistula Maturation (HFM) Study

Gerald J. Beck,1 Alfred K. Cheung,2 Laura M. Dember,2 Harold I. Feldman,3 Jonathan Himmelfarb,3 Thomas S. Huber,4 John W. Kusek,5 Prabir Roy-Chaudhury,4 Miguel A. Vazquez,6 Charles E. Alpers,7 Michelle L. Robbin,8 Joseph Vita,9 The HFM Study Group,1 1Cleveland Clinic; 1Utah; 2Penn; 2Washington; 3U Florida; 4NIDDK; 5UC; 6UT Southwestern; 7UA Alabama Birmingham.

Background: The NIDDK-sponsored HFM Study is designed to identify predictors and causes of AVF maturation failure. HFM will enroll 600 patients undergoing AVF creation. The study group includes 6 clinical centers (Boston U, UC, Florida, UT Southwestern, UT, U Washington), A Data Coordinating Center (Cleveland Clinic), and 3 Cores: Ultrasound (U Alabama Birmingham), Vascular Function (Boston U) and Histology (U Washington).

Methods: The protocol includes 1) pre-operative assessment of vascular anatomy and function with measurement of flow-mediated dilation, pulse wave velocity, and venous capacitance; 2) detailed intra-operative documentation of surgical procedures; 3) serial post-operative ultrasounds, and 4) uniform characterization of AVF function. Maturation is declared after 4 weeks of successful use under routine clinical care. Follow-up continues until AVF abandonment. Serum, plasma, DNA and vein tissue are collected for biomarker and mechanistic studies.

Results: Enrollment began in 5/2010 and study completion is expected in 2013.

Funding: NIDDK Support, Other U.S. Government Support
**SA-OR454**

Pre Existing Venous Calcification Prior to Dialysis Vascular Access Surgery

Timmy C. Lee, Nida Nida Safdar, Meeenakshi J. Mistry, Yang Wang, Begoña Campos, Prabir Roy-Chaudhury. Internal Medicine, University of Cincinnati, OH.

**Background:** Venous calcification is present in arterial vessels used for dialysis vascular access prior to surgical creation of the access. Calcification in the veins used to create a new vascular access has not previously been documented. The objective of this study was to describe the prevalence of calcification in vein samples collected at the time of new vascular access creation.

**Methods:** 67 vein samples, collected at the time of new access creation, were studied. Routine immunohistochemistry, using a von Kossa stain, was performed to quantify calcification. A semiquantitative scoring system from 0-4+ was used to quantify the percentage positive area (brown or black) as a fraction of total area (0 = 0-10% positive; 1+ = 11-25%; 2+ = 26-50%; 3+ = 51-75%; and 4+ = 76-100% positive) for each vascular layer (endothelium, intima, media, and adventitia).

**Results:** 22/67 (33%) samples showed evidence of calcification.

**Conclusion:** Venous calcification is present in veins used to create new dialysis vascular access. Patterns important in VA choice, such as patient preference, complications, interventions to attain function should be considered.

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**SA-OR455**

Primary Arteriovenous Fistula Patency Is Dependent on Venous Distensibility, Not on Proinflammatory, Procoagulant Markers or Vascular Functional Parameters

Milan M. Radovic,1 Tamara K. Jemcov. 1

**Background:** Role of vascular functional parameters, procoagulant and proinflammatory factors that influenced success of primary arteriovenous fistula for hemodialysis (AVF) patency were not elucidated completely. The aim of the study was to explore their correlation with outcome of AVF 4 weeks after creation.

**Methods:** Prospective, observational, cross sectional study was performed on 114 patients (59 male, age 55.8±15.5) who underwent primary non-dominant forearm AVF creation for hemodialysis. Cephalic vein (CV) and radial artery radius (RAR), venous distensibility (VD), resistance index (RI) and endothelial function by flow mediated dilatation (FMD) were determined by Doppler sonography. Serum levels of tissue plasminogen activator (tPA), vascular endothelial growth factor (VEGF), vascular cell adhesion molecule (VCAM) and interleukin 6 (IL-6) were determined by ELISA. Their concentrations and blood vessels examinations were compared between groups based on outcome for 4 weeks after creation. 1–primary AVF success (n=76), group 2–secondary success (n=30), group 3–AVF creation failure (n=8).

**Results:** Primary AVF success was reached in 66.7% of patients, secondary in 26.3%. Only 7% patients failed to mature AVF. Comparison between groups showed significant difference in CV (p=0.039), RAR (p=0.006) and venous distensibility (p=0.044), unlike RI, FMD, serum tPA, VEGF, VCAM and IL-6 levels (p>0.05). Significant correlation was found between outcome and CVR (p=0.028), VD (p=0.017) and RAR (p=0.002). RI correlated with age (p=0.018), and inversely with CV (p=0.001) and RAR (p=0.013). Significant correlation was found between IL-6 and tPA, VEGF and VCAM (all p<0.05). VEGF and VCAM (p=0.002). Linear regression was only significant for VD (B=0.517, R2=0.09, p=0.01) and RAR (B=0.01, p=0.029).

**Conclusion:** Venous distensibility showed to be the most important factor for primary AVF patency after 4 weeks of maturation. Significant correlation was found among procoagulant and proinflammatory biomarkers, but their impact was not significant for an early AVF outcome.

**Funding:** Pharmaceutical Company Support, Government Support - Non-U.S.
Effect of Recombinant Human Type 1 Pancreatic Elastase (PRT-201) Treatment on Fistula Patency

Bradley S. Dixon, Eric K. Peden, David B. Leeser, Mahmud T. El-Khatib, Prabir Roy-Chaudhury, Jeffrey Lawson, Matthew Menard, Marc H. Glickman, Laura M. Dember, Steven K. Burke, U Iowa, IA; 2Well Cornell MC, NY; 3Methodist Hospital, TX; 4U Cincinnati, OH; 5Duke U, NC; 6Brigham & Women's Hospital, MA; 7Sentara Heart Hosp, VA; 8Boston U, MA; 9Protein Therapeutics, MA.

Background: Stenosis is a common cause of fistula (AVF) failure. We conducted a phase 1/2 randomized, double-blind, dose-escalation trial to determine if PRT-201 treatment was safe, would promote dilatation & prevent failure of newly created AVFs.

Methods: A single dose of PRT-201 (N=45) or placebo (N=21) in 2.5 mL of saline was dripped onto the outside of the AVF over 10 min immediately after creation. Doses were aggregated into low (LD, 0.003, 0.010 & 0.035 mg), medium (MD, 0.1, 0.33 & 1 mg), & high (HD, 3, 6, 9 mg) groups (N=16, 17, 12). Patients were followed for up to 12 mon. Blinded duplex Doppler ultrasound evaluation was done at 6 weeks, & 3 & 6 months. Primary outcomes were safety & immediate change in outflow vein diameter (VD) & blood flow (BF). Secondary efficacy endpoints were AVF maturation (lumen VD ≥ 2.0 mm & BF ≥ 500 mL/min after 6 weeks), & time to primary patency loss (AVF occlusion or procedures required to maintain or restore patency).

Results: No safety concerns occurred. There was a modest but statistically significant immediate change in PRT-201 treated VD (p=0.01). Lumen VD & BF increased in all groups with no difference in maturation at 6 weeks. Primary patency at 6 months was not statistically different among groups (Placebo 60%, LD 81%, MD 76%, HD 73%, p=NS). If immediate post-surgical complications that caused patency loss were excluded from the analysis (N=3 patients in LD group) patency loss was reduced in LD versus placebo (HR 0.28, 95% CI=0.08-0.93, p=0.04) and versus HD (HR 0.07, 95% CI=0.01-0.52, p=0.01). Angioplasty was the most common reason for patency loss (placebo 43%, LD 12%, MD 35%, HD 33%), with a trend toward less hemodynamically significant lumen stenosis in LD (placebo 47%, LD 31%, MD 62%, HD 50%) at 6 months.

Conclusions: PRT-201 was safe. At LD, PRT-201 might decrease lumen stenosis & prolong fistula patency but an adequately powered trial is needed.

Funding: Pharmaceutical Company Support

SA-OR459

Improved Arteriovenous Fistula Maturation with Intra-Operative Implant of a Perianastomotic Siroliimus Eluting Collagen Membrane (Coll-R) Maria V. De Vita, Eric S. Chemla, Kiphsidze Nickolas, Surenda Shenoy, Srimat Eyer, Lenox Hill Hospital, New York, NY; 2Center of Angiology and Vascular Surgery, Tbilisi, Georgia; 3Washington U School of Medicine, St Louis, MO; 4St. George's Healthcare NHS Trust, London.

Background: Nonmaturation of arteriovenous fistulae (AVF) remains a major limiting factor for their use in hemodialysis patients(pts). Endothelial dysfunction is significant to angiosome skin and neointimal hyperplasia of the draining vein. Since there is no proven solution, novel approaches are needed. The Coll-R, an investigational product (Vascular Therapies, NJ, USA) is indicated and designed for perivascular implantation. It consists of collagen, a topical hemostat and sirolimus, an anti-proliferative agent with proven efficacy for suppressing neointimal growth when delivered locally to the vascular wall. Goal of this study was to evaluate the performance and safety of the Coll-R when applied around the anastomotic site and outflow vein during creation of AVF. In this first in human study, endpoints were freedom from Coll-R related adverse effects and time to maturation.

Methods: Pts from 2 hospitals in Tbilisi, Georgia, scheduled for AVF creation were invited to participate. Venous mapping was performed to assure suitable vascular anatomy. The Coll-R was implanted intra-operatively during AVF creation. Data collected included technical success of the implantation, wound healing, time to unassisted maturation and whole blood sirolimus levels.

Results: Thirty pts, 17 male, mean age 51 years (range 25-77) underwent radiopholiplastic (n=22) or brachiocephalic(n=8) AVF creation with implantation of the Coll-R (675mg of sirolimus). All completed a minimum of 11 weeks of follow up. There were no Coll-R related technical failures or adverse events. AVF matured in 26 pts. (87%), mean maturation time was 27±18 days; 4 pts thrombosed in <4 weeks; mean peak sirolimus level was 4.13ng/mL seen 6 hr post-op. Two late AVF failures occurred at 119 and 170 days respectively.

Conclusions: Coll-R implantation during AVF creation does not cause systemic immunosuppression and improves fistula maturation. Use of this novel therapy may provide an unmet clinical need.

Funding: Pharmaceutical Company Support

SA-OR457

Far Infrared Therapy Improves Endothelial Function and Access Flow of Newly-Created Arteriovenous Fistula in Patients with Stage 5 Chronic Kidney Disease Chih-Chung Lin, School of Medicine, National Yang-Ming University, Taipei, Taiwan; 2Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.

Background: Endothelial dysfunction plays a significant role in the pathogenesis of malfunction of vascular access. The aim of this study is to evaluate the effect of far infrared (FIR) therapy on endothelial function and access flow (Qa) of newly created AV fistula.

Methods: We enrolled 75 patients (in stage 5 CKD) who were randomly allocated to treatment group (receiving 40 minutes of FIR therapy three times weekly for 3 months postoperatively, N=37) and control group (without FIR therapy, N=38). Access flow of AV fistula was measured by Doppler ultrasonography at 4 timings, including 2 days, 1 month, 2 and 3 months after vascular surgery. Markers of Endothelial function, including asymmetric dimethyl arginine (ADMA) and L-arginine, were measured both immediately before and 3 months after the creation of AV fistula.

Results: Finally, 67 patients completed the study, including 33 in FIR group and 34 controls. In comparison with controls, patients in FIR group had lower values of incremental change of ADMA as well as higher values of Qa at all of the 4 timings and incremental change of L-arginine, thus leading to a higher incremental change in the ratio of L-arginine to ADMA 3 months later (as shown in table 1).

Comparison of the endothelial function and access flow of AVF between HD patients with and without FIR therapy for 3 months

Control group | FIR group | P value
--- | --- | ---
Case number completing study | 34 | 33 | ns
Qa (ml/min) | 259.1±85.5 | 337.9±111.3 | 0.002
Qa (ml/min) | 397.9±236.1 | 405.3±315.7 | 0.003
Qa (ml/min) | 881.2±275.2 | 925.8±354.6 | 0.002
Qa (ml/min) | 276.8±344.0 | 387.7±375.3 | 0.016
L-arginine (3.0 μM) | 2.87±1.2 | 2.67±1.5 | 0.007
ADMA 3.0 μM | 0.01±0.05 | 0.05±0.07 | <0.001
L-arginine/ADMA ×100 | 9.86±0.8 | 1.09±0.12 | <0.001
Qa/Qa | Qa | Qa | Qa measured at 2 days, 1, 2, or 3 months after AVF creation; ADMA/3 or L-arginine/3 indicates the concentration measured 1 day before/3 months after AVF creation.

Conclusions: In conclusion, 3 months of FIR therapy improves endothelial function and access flow of newly created AVF in patients with stage 5 CKD.

Funding: Government Support - Non-U.S.
TH-P0001
Response of the Human Kidney to Clamp Ischemia  
Dipen J. Parekh, 1 Barbara Ercole, 1 Claudia Schrimpf, 2 Manjeri A. Venkatachalam, 1 Joel M. Weinberg. 1UTHSC San Antonio; 2Harvard Institutes of Medicine; University of Michigan.

Background: Structural changes in tubule cells during clamp ischemia are well characterized for animal models, but their timing and extent in the human kidney has not been established and may differ significantly, depending on the clinical setting.

Methods: We biopsied and uninvolved areas of kidney in patients undergoing open partial nephrectomy for renal masses before renal artery clamping, then during periods ranging from 15 to 60 min. of warm and cold ischemia (80% > 30 min.), and then after 5 minutes of reflow for ultrastructure (N=39) and for immunofluorescence and rhodamine phalloidin staining (N=22).

Results: During the clamp period, apical membrane structure was remarkably well preserved with only patchy brush border clubbing, fragmentation, desquamation and blebbing and not in all patients. Mitochondria developed progressive swelling, which paradoxically was more prominent in distal than proximal tubule cells. This resided during the 5 minutes of reflow in most cells in most patients, but persistence of swelling and development of matrix condensation occurred occasionally. Using a composite 0-5 scale covering the full spectrum of ultrastructural changes, average scores were: Preclamp 1.02±0.07, End clamp 2.18±0.07, Post clamp 1.86±0.09. Consistent with the ultrastructure, staining for F-actin with rhodamine phalloidin was well preserved. Immunostaining for phosphothreonine, which reflects cellular ATP content, was decreased in 68.4% of the clamp biopsies and 52.6% of the post clamp biopsies with larger changes in proximal tubules, however αV integrin was decreased in only one post clamp biopsy. ICAM-1 expression in peritubular capillaries was increased in 46.7% of the clamp biopsies and 66.7% of post clamp biopsies. None of the patients developed acute kidney injury.

Conclusions: These data provide the first detailed analysis of the structural response of the human kidney to clamp ischemia and document many of the expected structural alterations based on prior animal work, but indicate a greater than expected resistance to injury in this commonly used clinical application of clamp ischemia.

Funding: NIDDK Support, Private Foundation Support

TH-P0002
MicroRNA-687 Targets PTEN To Regulate Hypoxic/Ischemic Kidney Injury  
Kirti Bhatt, Qingqing Wei, Zheng Dong. Department of Cellular Biology and Anatomy, Georgia Health Science University and Charlie Norwood VA Medical Center, Augusta, GA.

Background: MicroRNAs are small, endogenous, non-coding RNAs that are critical regulators of gene expression in various pathophysiological conditions. Recent studies have suggested an emerging role of microRNAs in both renal development and pathophysiology. Targeted deletion of Dicer (a key enzyme for microRNA biogenesis) from proximal tubules protects against renal ischemia-reperfusion injury (IRI), demonstrating a pathogenic role of microRNAs in renal IRI. Despite these findings, the specific microRNA species that contribute to renal IRI are not known.

Methods: To identify the pathogenic microRNAs, we analyzed microRNA expression by microRNA microarray.

Results: Out of 220 detectable microRNAs, 138 were decreased to varying degrees while 70 microRNAs were induced during renal IRI in C57BL/6 mice. Among these microRNAs, microRNA-687 (miR-687) was most dramatically up-regulated. miR-687 induction during hypoxia in cultured cells and renal ischemia-reperfusion in mice was followed by a concomitant decrease in PTEN expression. Notably, blockade of miR-687 with anti-miR-687 in renal IRI. Despite these findings, the specific microRNA species that contribute to renal IRI are not known.

Methods: To identify the pathogenic microRNAs, we analyzed microRNA expression by microRNA microarray.

Results: Out of 220 detectable microRNAs, 138 were decreased to varying degrees while 70 microRNAs were induced during renal IRI in C57BL/6 mice. Among these microRNAs, microRNA-687 (miR-687) was most dramatically up-regulated. miR-687 was also induced by hypoxia in cultured renal proximal tubular cells. Bioinformatic analysis suggested that the tumor suppressor PTEN is a top candidate target of miR-687. The 3' UTR of PTEN gene contains a miR-687 binding sequence, which was shown to be a target sequence of miR-687 in a luciferase reporter assay. Consistently, miR-687 induction in peritubular capillaries was increased in 46.7% of the clamp biopsies and 66.7% of post clamp biopsies. None of the patients developed acute kidney injury.

Conclusions: These data provide the first detailed analysis of the structural response of the human kidney to clamp ischemia and document many of the expected structural alterations based on prior animal work, but indicate a greater than expected resistance to injury in this commonly used clinical application of clamp ischemia.

Funding: NIDDK Support, Private Foundation Support

TH-P0003
Utility of Local Arterial Input Function in Dynamic Susceptibility Magnetic Resonance Imaging To Identify Regional Renal Perfusion Defect  
Andrew M. Siedlecki. Internal Medicine, Washington University in St. Louis, St. Louis, MO.

Background: Rapid acquisition of magnetic resonance signal in tissue may offer a highly sensitive platform for the study of renal blood flow. We hypothesize that dynamic susceptibility contrast-magnetic resonance imaging (DSC-MRI) can accurately measure regional renal blood flow in the kidney when the standard global arterial input function is replaced by per-vessel local arterial input function (LIAF).

Methods: We performed mild ischemic injury in mice and measured renal perfusion after injury by means of magnetic resonance imaging and Doppler flowmetry. For MR studies, a multi-dimensional integral was constructed to account for the properties of gadolinium contrast flow, within single vessels of space. Posterior probabilities were derived from conditional probabilities by applying Bayesian evidential theory. Invasive measurement of cortical and medullary blood flow was performed by Doppler flowmetry.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

TH-P0004
Over-Expression of GMP-Dependent Protein Kinase I (PKG-I) Attenuates Ischemia Reperfusion Induced Kidney Injury  
Shuxiao Wang. Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY.

Background: GMP-dependent protein kinase (PKG) is a multifunctional protein. Whether PKG plays a role in ischemia/reperfusion induced kidney injury (IRI) is unknown.

Methods: In this study, using an in vivo mouse model of renal IRI, we determined the effect of renal IRI on PKG-I levels and also evaluated whether overexpression of PKG-I attenuates renal IRI.

Results: Our studies demonstrated that PKG-I levels (mRNA and protein) were significantly decreased in the kidney from mice undergoing renal IRI. Moreover, PKG-I transgenic mice had less renal IRI, showing improved renal function and less tubular damage as compared to their wild type littermates. Transgenic mice in renal IRI group had decreased tubular cell apoptosis accompanied with decreased caspase 3 activity and increased expression of Bcl-2, Bag-1 and p-AKT. In addition, transgenic mice undergoing renal IRI demonstrated reduced macrophage infiltration into the kidney and reduced production of inflammatory cytokines. In vitro studied showed that peritonal macrophages isolated from transgenic mice had decreased migration as compared to control macrophages.

Conclusions: Taken together, these results suggest that PKG-I protects against renal IRI, at least in part through inhibiting inflammatory cell infiltration into the kidney, reducing kidney inflammation, and inhibiting tubular cell apoptosis.

Funding: NIDDK Support, Other NIH Support - NCCR P20

TH-P0005
Thrombin and PAR-1 Regulate Sphingosine Kinases 1 and 2 and Sphingosine-1-Phosphate Receptors Following Renal Ischaemia and Reperfusion Injury  
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Background: Recent studies have suggested sphingosine-1-phosphate signaling (SIP) via the SIP receptor-1 (SIP1r) contributes to protection following renal ischaemia reperfusion injury (IRI). SIP generation via expression and activation of sphingosine kinase 1 (SIPK1) has been suggested to be a potentially important downstream signaling pathway for protease activated receptor-1 (PAR-1).

Results: Invasive measurement of surface cortical blood flow by Doppler flowmetry returned to baseline within 35 minutes following mild (15 minute) ischemic injury (IRI, n=5; sham, n=5). In a separate cohort (IRI, n=5; sham, n=5), MR imaging showed a delayed transit in contrast through the kidney vasculature at a more distant time point, 45 minutes following ischemic injury. Perturbed blood flow in proceduralized animals compared to shams was manifest in a significantly prolonged contrast outflow (β, and mean transit time (MTT)(seconds) (p<0.01).

Conclusions: We apply for the first time the concept of convolution-free local arterial input function in the kidney using nuclear magnetic resonance technology. This study is an advancement in the analysis of renal blood flow and provides computational deconvolution and offers a non-invasive assessment of regional blood flow.
Methods: The TH-PO004 study was an established model of renal IRI using 25 min bilateral renal ischemia and 24 h reperfusion (2, 5 and 24 h). After clamping, we investigated the effect of PAR-1 inhibition on tubular injury. In addition, we determined the effect of thrombin inhibition on the expression of the organic cation transporters rOct1 and rOct2 (Slc22a1, Slc22a2). Although genetic deficiency, PAR-1 inhibition using hirudin and reduced thrombin generation using low-TF mice. As previously shown, all strategies that reduced PAR-1 signaling resulted in reduced renal injury. Results: In wild type (WT) mice, SPHK-1 mRNA was slightly induced at 2 and 5 h with just over a doubling by 24 h (2.31 ± 0.35 units). In contrast, SPHK-2 showed a similar level of induction at 2 h but decreased at 24 (0.76 ± 0.35 units) compared to sham control. PAR-1 deficient mice had mild induction of both SPHK-1 and -2 mRNA at 24 h. By 48 h, there was a 10-fold increase in SPHK-2 mRNA at 24 h in WT mice. PAR-1 deficient mice showed different patterns of SPHK receptor mRNA induction with WT showing early induction of SP1R1 and latter reductions compared to PAR-1 deficient mice that showed no induction of SP1 R1 and 2 at 24 h but dramatic up regulation at 5 and 24 h.

Conclusions: Taken together the data suggests that thrombin negatively regulates SPHK-2 and SP HKR expression via PAR-1 signaling. This suggests at least some of the protective effect of PAR-1 deficiency on renal IRI may be via increased SP1 signaling.

Funding: Clinical Revenue Support

TH-PO006
The Role of Fibrinogen in Ischemic Acute Kidney Injury
Inga Soerensen, Nathan D. Susnik, Hermann G. Haller, Roland Schmitt.
Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.

Background: Fibrinogen (Fib) has heterogeneous roles in tissue injury, inflammation and repair. We studied the role of Fib in ischemic acute kidney injury (AKI).

Methods: Intact renal Fib was analyzed in post-ischemic mouse kidneys using immunohistochemistry, immuno blot, PCR and in situ hybridization. Fibrin and Fib-/- mice that underwent unilateral or bilateral renal clamping were followed for survival and renal function. Post-ischemic kidneys were analyzed by quantification of damage markers, inflammatory infiltrates, apoptosis and cell proliferation. In transplant experiments kidneys from WT and Fib-/- mice were transplanted into recipient mice. In vitro, primary renal tubular epithelial cells and proximal tubular epithelial cells lines were stimulated with different conditions to test for Fib expression by qPCR.

Results: 27 min of transient renal ischemia was associated with more than a 10-fold increase in intrarenal Fib levels. While Fib signal was confined to the intravascular space in control kidneys, it was found throughout the tubulointerstitium in postischemic kidneys. In agreement with recent microarray data, Fib de novo expression was found in a subset of epithelial cells of postischemic kidneys by immunofluorescence and in situ hybridization. A significant renal mRNA up-regulation of Fib alpha, beta and gamma subunits was confirmed by qPCR. In vitro, Fib was induced in renal tubular epithelial cells by exposure to homogenate from ischemic kidneys, Oncostatin-M and hyper-IL-6. In vivo, Fib +/- mice subjected to renal ischemia showed reduced survival and worse renal function but levels of tubular injury markers Kim-1 and NGAL were significantly lower in Fib +/- kidneys. To address discrepancies between local and systemic effects of Fib deficiency, kidneys were transplanted from Fib +/- mice into Fib +/- recipients. Our results suggest that the deletion of intrarenal Fib expression may be protective against post-ischemic damage.

Funding: Government Support - Non-U.S.

TH-PO007
Tumor Necrosis Factor alpha Downregulates Tamm-Horsfall Protein (Uromodulin) Expression in Acute Kidney Injury
Tarek M. El-Achak, Ruth A. McCracken, Soline Bourgeois, Ziyad Al-Aly.1
St. Louis University and Saint Louis VA Medical Center;2 Saint Louis VA Medical Center; University of Zurich.

Background: Acute kidney injury (AKI) is a common disease with serious health implications. We recently described a protective role for Tamm-Horsfall protein (THP) against AKI. THP expression is exclusively in the thick ascending limbs (TAL) of the kidney, but the factors that regulate the expression of THP in AKI are still unknown. In this study, we investigated whether tumor necrosis factor alpha (TNF-α), a cytokine induced by pro-inflammatory signals in AKI, also regulates the expression of TNF-α. We found that TNF-α is a critical regulator of THP expression in AKI.

Methods: We used a mouse model of ischemia-reperfusion injury (IRI) achieved by bilateral pedicle clamping. We also performed in vitro IRI on a pure thick limb cell line (MKTAL) using an established model of mineral oil layering. Results: Using real-time PCR, we show that THP mRNA is transiently down-regulated in the kidney after IRI. Contrary to our observations in TNF mRNA levels after injury. Using immunofluorescence microscopy, we localized this TNF up-regulation to TAL and S3 segments of the outer medulla. Similarly, in MKTAL cell culture, IRI increased TNF expression while suppressing THP expression. Incubating MKTAL cells with TNFα suppressed WT RNR-dependent expression of THP. We also observed a suppression of TNF-α-dependent expression of THP in vitro.

Conclusions: In conclusion, our data suggest that the surge of TNFα at the onset of AKI upregulates THP expression. Because of the protective function of THP, modulation of THP expression by TNFα blockade could present a therapeutic opportunity to prevent and attenuate AKI.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO008
Nitric Oxide Induced Regulation of Renal Organic Cation Transport after Renal Ischemia/Reperfusion Injury
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Background: Renal organic cation transporters (Oct) are downregulated by inflammatory nitric oxide (NO) production in rat endotoxemia. NO generated by inducible NO synthase (iNOS) is substantially increased in renal cortex after renal ischemia/ reperfusion (I/R) injury. Therefore, we investigated effects of iNOS-specific NO inhibition on the expression of the organic cation transporters rOct1 and rOct2 (Slc22a1, Slc22a2) after I/R injury; both in vivo and in vitro.

Methods: In vivo, I/R injury was induced by bilateral clamping of the renal arteries for 45 min in SD-rats, while I/R injury was simulated in vitro in renal proximal tubular cells (NRK-52E) by a condition of oxygen depletion, aglycemia and acidosis for 120 min. N-(1-iminoethyl)-L-lysine (L-NIL) was applied as specific iNOS inhibitor.

Results: In vivo, L-NIL inhibited NO generation after I/R injury. Moreover, L-NILabolished I/R-induced downregulation of rOct1 and rOct2 as determined by qPCR and western blotting. Functional evidence was obtained by measuring fractional excretion (FE) of the endogenous organic cation serotonin. Concurrent with the expression of the rate-limiting Oct, the FO of serotonin decreased after I/R injury and was abolished by L-NIL. In vitro, I/R downregulated both rOct1 and rOct2, which were also abolished by L-NIL; the same was true for the uptake of the organic cation MPP.

Conclusions: We showed that renal I/R injury downregulates rOct1 and rOct2 expression as well as concomitant transport function; both most probably mediated via NO. In principle, this may be an autocrine effect of proximal tubular epithelial cells. Moreover, we conclude that rOct1, or rOct1 and rOct2 limit (also NO-dependent) the excretion rate of endogenous serotonin; this may have additional clinical impact due to its potential of inducing vascular damage.

Funding: Government Support - Non-U.S.

TH-PO009
Effect of Gender Differences on Renal Inflammatory Response after Renal Ischemia-Reperfusion Injury in Mice
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Background: Male gender is associated with more rapid progression in renal disease, while female gender is slowly. This gender disparity may be influenced by sex hormonal milieu. The purpose of this study is to investigate the effect of gender differences on the renal inflammatory responses after acute ischemia-reperfusion injury in mice.

Methods: Experiments were performed in male and female C57BL/6 by bilateral renal ischemia-reperfusion injury. In other study, orthocytome or ovariectomy was carried out 14 day before I/R injury. Different groups of animals were treated by testosterone and 17p'-estradiol. Histologic examination and qRT-PCR for TNF-α, IFN-γ, and MCP-1 were performed.

Results: In acute ischemia-reperfusion injury model, female gender was more resistance to the kidney injury compared to the male gender. The tubular injury score and the number of F4/80(+) macrophages were increased in male mice compared to the female mice. The expressions of TNF-α, IFN-γ, and MCP-1 were increased at 2 d of AKI in male mice compared to female. Orthocytome male mice were more resistance to renal injury compared to the normal male mice. But, treatment of testosterone to the orthocytome mice increased renal injury and infiltration of F4/80(+) macrophages. Ovariecimetic female mice were prone to renal injury and associated with increased infiltration of F4/80(+) macrophages. The expressions of TNF-α, IFN-γ, and MCP-1 were decreased in the orthocytome male mice, while increased in the ovariecimetic female mice compared to the normal male and female mice. Hormone replacement such as testosterone and estrogen reverse these cytokine expressions.

Conclusions: These results suggested that male gender is more susceptible to the ischemia-reperfusion renal injury. The testosterone may be one of causes of increased susceptibility, while estrogen may be protective in renal inflammatory response after ischemia-reperfusion injury.

Funding: Government Support - Non-U.S.
TH-PO010
ADAM-10 Is the Major Sheddase of Membrane-Associated Meprin A Release during Acute Kidney Injury
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Background: Meprin A, comprised of α and β subunits is a potent membrane-bound metalloproteinase highly expressed in proximal tubules of the kidney cortex where it is bound by its subunit to the brush-border membrane. In acute kidney injury (AKI) meprin A is released into the bloodstream and transported to the cytosol and may become deleterious to the kidney. The present study has identified the protease involved in the release of meprin A during AKI.

Methods: Meprin A localization was determined by double-immunofluorescence staining and Western blot of mouse kidneys exposed to cisplatin (CP) or ischemia-reperfusion injury. Stable transfectants of meprin β and transfectants containing CRIF1-α subunits in HEK cells served as an in vitro model to investigate the protease involved in meprin A shedding. Broad-spectrum inhibitors to serine-, metallo-, acidic-proteases and the ADAM family of proteases followed by highly specific inhibitors were examined for their ability to prevent shedding of meprin A in the presence/absence of the inflammatory agent phorbol myristate acetate (PMA).

Results: Meprin A localization was altered from the brush-border membrane towards the cytosol and the basolateral membrane in mice subjected to CP and IR injury. Broad-spectrum inhibitors of various classes of proteases indicated involvement of proteases of the ADAM family. Since ADAM-9, -10 and -17 are known for extracellodomain shedding we determined whether one or all of them are involved in the shedding. GI-254023X, a highly potent inhibitor of ADAM-10 and -9 to a lesser degree of ADAM-17 reduced meprin shedding significantly in HEK cells stably transfected with meprin β and α. CRIF1 to ADAM-10 reduced shedding to less than 50% whereas CRIF1 to ADAM-9 and -17 were much less effective (~30% or ~10%) and the expression of ADAM-10 by transient transfection + PMA led to a 2-fold increase in shedding in both the meprin β and α transfectant.

Conclusions: Our results demonstrate that ADAM-10 is the major sheddase for meprin A and both meprin A and ADAM-10 can therefore be valuable therapeutic targets in the prevention of AKI.

Funding: NIDDK Support, Veterans Administration Support

TH-PO011
SFks Regulate Both Pore and Leak Pathways of Paracellular Permeability in Renal Epithelial Cells
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Background: Regulation of renal epithelial cell paracellular permeability is critical for normal renal tubular function. Dysregulation is associated with many renal pathologic states, including renal ischemia/reperfusion injury, radiocontrast-induced nephropathy, and exposure to calcium oxalate crystals. We have begun investigating the role of src Family Kinases (SFks) in regulating steady state renal epithelial cell paracellular permeability.

Methods: Renal cells were grown on semipermeable membranes. Paracellular permeability was quantitated by measuring calcine flux and TER. Protein content was quantitated by Western blot.

Results: As reported previously, under steady state conditions renal epithelial cells (LLC-PK1, mIMCD3, and MDCK) maintain a barrier to the paracellular movement of both small ions (pore pathway; measured as TransEpithelial Resistance (TER)) and large molecules (leak pathway; measured as 0.5μm Dextran-FITC). In HEK293 cells expressing Src family kinases, phosphorylation of α-subunits is a potent membrane-bound src-family kinase substrate. We have previously shown that PMA treatment and treatment with src-family kinase inhibitors produced a concentration-dependent increase in both pore and leak pathway permeabilities without producing significant cell death (Trypan Blue-staining nuclei). This effect was observed within 1-2 hours. Neither basal nor the PMA-mediated increase in paracellular permeability was reversed by the selective src-family kinase inhibitor PP1/2.

Conclusion: These results demonstrate that AKI is a real target of miR-127 in NRK-52E cells, modulating proximal tubule paracellular function. These miRNAs is involved in cell-cell adhesion maintenance, playing a critical role in preservation of epithelial barrier function. Thus, miR-127 appears as a key regulator in pathologic manifestations in virtually all major organs.

Funding: Veterans Administration Support, Private Foundation Support, Government Support - Non-U.S.

TH-PO013
Collecting Duct Specific Mitochondrial Injury Aggravates Unilateral Ureteral Obstruction Renal Injury in Mice
Young Tai Shin,1 Jin Young Jeong,1 Bo Ra Lee,1 Won Ik Jang,1 Hyunjun Ju,1 Yoon-Kyung Chang,2 Seong Suk Kim,1 Dae Eun Choi,1 Kang Wook Lee.1

Background: There are few model of mitochondrial injury plays important role in renal injury. There are few model of mitochondrial injury, we generated collecting duct specific mitochondrial injury model. And, we evaluated the effects of mitochondrial dysfunction on collecting ducts in unilateral ureteral obstructed mice kidney.

Methods: For generation of collecting duct specific mitochondrial injury mice, CRIF1-knockout (CRIF1-KO) mice were used. CRIF1-knockout mice were generated by crossing CRIF1-KO mice with Hoxb7-Cre (Hoxb7-Cre) mice. For evaluation of mitochondrial injury in kidney. Also, there is few study for roles of mitochondria on cellular basis. We generated collecting duct specific mitochondrial injury model and, and, we evaluated the effects of mitochondrial dysfunction on collecting ducts in unilateral ureteral obstructed mice kidney.

Results: Exposure of cells to 10% AKI sera x 48 hrs caused, compared to incubation with PMA or sham sera, increased metabolic activity (CellTiter-Blue) but was not significantly different compared to control.

Conclusion: These data identify yet inadequately understood pathogenic signals that are generated by the injured kidney, and whose nature, once defined, should advance our understanding of renal pathophysiology.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO014
Collecting Duct Specific Mitochondrial Injury Aggravates Unilateral Ureteral Obstruction Renal Injury in Mice
Young Tai Shin,1 Jin Young Jeong,1 Bo Ra Lee,1 Won Ik Jang,1 Hyunjun Ju,1 Yoon-Kyung Chang,2 Seong Suk Kim,1 Dae Eun Choi,1 Kang Wook Lee.1

Background: There are few model of mitochondrial injury plays important role in renal injury. There are few model of mitochondrial injury, we generated collecting duct specific mitochondrial injury model. And, we evaluated the effects of mitochondrial dysfunction on collecting ducts in unilateral ureteral obstructed mice kidney.

Methods: For generation of collecting duct specific mitochondrial injury mice, CRIF1-knockout (CRIF1-KO) mice were used. CRIF1-knockout mice were generated by crossing CRIF1-KO mice with Hoxb7-Cre (Hoxb7-Cre) mice. For evaluation of mitochondrial injury in kidney. Also, there is few study for roles of mitochondria on cellular basis. We generated collecting duct specific mitochondrial injury model and, and, we evaluated the effects of mitochondrial dysfunction on collecting ducts in unilateral ureteral obstructed mice kidney.

Results: Exposure of cells to 10% AKI sera x 48 hrs caused, compared to incubation with PMA or sham sera, increased metabolic activity (CellTiter-Blue) but was not significantly different compared to control.

Conclusion: These data identify yet inadequately understood pathogenic signals that are generated by the injured kidney, and whose nature, once defined, should advance our understanding of renal pathophysiology.

Funding: Veterans Administration Support, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

Acute Kidney Injury: Basic Mechanisms, Therapy
Poster/Thursday
with WT UUO kidneys, Urinary 8-OHdG was increased in CRF1-KO-mice compared with WT. Also, CRF1-KO mice showed significantly increase of 8-OHdG-positive cell recruitment compared to WT mice. CRF1-KO-UUO-kidneys were shown more increase recruitment of 8-OHdG-positive cells compared to WT-UUO-kidneys.


Funding: Government Support - Non-U.S. Government

TH-PO015
Chronic Nicotine Exposure Induces p66Shc Expression in Renal Proximal Tubule Cells: Impact on Oxidative Stress and Injury
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Background: We reported that chronic NIC exposure exacerbates oxidative stress and consequent injury in a mice model of AKI. Since the adaptor protein p66Shc mediates ROS release via cytochrome c binding we tested the hypothesis that chronic NIC exposure aggravates oxidative stress-linked ROS production and injury through increase in cytochrome c-bound p66Shc in proximal tubule cells.

Methods: Wild type (w.t.), p66Shc knockdown (k.d.) or cytochrome c binding deficient (W134F-p66Shc) mouse renal proximal tubule cells were treated with 200 μM NIC for 24 hours prior to treatment with 200 μM H2O2 and ROS production as well as LDH release was determined. Immunoprecipitation studies were performed to determine mitochondrial as well as cytochrome c binding of p66Shc. The fluorescent dye JC-1 was used to determine mitochondrial depolarization.

Results: Chronic NIC treatment increased H2O2-induced mitochondrial and cytochrome c-binding of p66Shc and also exacerbated H2O2-induced ROS production, depolarization of the mitochondria and LDH release. Conversely, NIC+H2O2-induced ROS production and LDH release was attenuated in p66Shc k.d. or W134F cells compared to w.t. cells. Ser36 phosphorylation of p66Shc –which is necessary for its mitochondrial localization- was increased by NIC+H2O2 but not by NIC treatment. Surprisingly, chronic NIC treatment increased expression of p66Shc through dose-dependent induction of its promoter. Importantly, increased expression of p66Shc was detected in the kidneys of mice that were chronically exposed to NIC.

Conclusions: Chronic NIC treatment increases expression of p66Shc, which in turn, Ser36 phosphorylated via oxidative stress. The result is augmented mitochondrial/cytochrome c binding of p66Shc and consequent ROS production/injury through opening the mitochondrial permeability transition pore. This mechanism may be responsible for the augmented oxidative stress in the ischemic kidney of mice with chronic nicotine exposure.

Funding: NIDDK Support

TH-PO016
Role of Nonesterified Fatty Acids in Respiratory Impairment and Mitochondrial Deenergization of Proximal Tubule Secondary to Hypoxia/Reoxygenation
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Background: Hypoxia/reoxygenation (H/R) of proximal tubule leads to persistent ATP depletion due to decreased mitochondrial membrane potential (MMP) resulting from nonesterified fatty acid (NEFA)-mediated uncoupling that is paradoxically accompanied by respiratory inhibition rather than the stimulation expected for uncoupled states.

Methods: Since NEFA have been reported to directly inhibit electron transport in some settings we assessed respiratory function in tubules after H/R as a function of NEFA availability.

Results: Compared to respiration supported by the complex II-dependent substrate, succinate, which was highly uncoupled after H/R but relatively well preserved (ADP-stimulated respiration (S3) of permeabilized tubules 71.0±8.5% of normoxic control (NC)), respiration supported by complex I-dependent substrates that normally predominate in cells was also uncoupled, but S3 was reduced to 26.9±3.3% of NC, P < 0.001 vs. succinate. In contrast, the rate of mitochondrial membrane potential (MMP) was increased 50% at 4h and decreased to 48% of controls at 24h following TBHP injury. F0F1-ATPase activity and ATP content decreased to 59% and 60%, respectively, in TBHP injured RPTC. Oxidant exposure increased ROS production by 200% and induced 47% RPTC lysis. Blocking Pkoα activation by overexpressing inactive Pkoα mutants were overexpressed in primary cultures of RPTC prior to oxidant exposure (0.35 mM tert-butil hydroperoxide, TBHP; 45 min). Pkoα activation, mitochondrial functions associated with oxidative phosphorylation, production of reactive oxygen species (ROS), and cell viability were determined after TBHP treatment.

Conclusions: We conclude that: 1) activation of PKoα promotes recovery of mitochondrial function and active Na+ transport following oxidant injury in renal proximal tubular cells (RPTC). However, the specific PKc isozyme involved in the recovery of mitochondrial function is unknown. This study investigated the role of PKcα in decreases and recovery of mitochondrial functions and cell survival in RPTC injured by an oxidant.

Funding: NIDDK Support

TH-PO017
Activation of Protein Kinase Cα Promotes Mitochondrial Function and Cell Survival in Renal Proximal Tubular Cells Injured by Oxidant
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Background: Previously, we have shown that protein kinase C (PKc) activation promotes recovery of mitochondrial function and active Na+ transport following oxidant injury in renal proximal tubular cells (RPTC). However, the specific PKc isozyme involved in the recovery of mitochondrial function is unknown. This study investigated the role of PKcα in decreases and recovery of mitochondrial functions and cell survival in RPTC injured by an oxidant.

Methods: Wild-type PKcα and inactive PKcα mutants were overexpressed in primary cultures of RPTC prior to oxidant exposure (0.35 mM tert-butil hydroperoxide, TBHP; 45 min). PKcα activation, mitochondrial functions associated with oxidative phosphorylation, production of reactive oxygen species (ROS), and cell viability were determined after TBHP treatment.

Results: The studies indicate that selectively impaired utilization of complex I substrates to support respiration after H/R promotes NEFA-induced deenergization and is only minimally improved by acutely removing NEFA. In the presence of NEFA, the highest efficiency of complex I substrates to support electron transport does not mitigate the impact of the impaired respiration on MMP. However, lowering NEFA within cells for 60 min. allows strong recovery of MMP despite persistence of some respiratory impairment.

Funding: NIDDK Support, Private Foundation Support

Conclusions: We conclude that: 1) activation of PKcα during oxidant injury in RPTC promotes mitochondrial functions and decreases cell death and 2) blocking ROS production does not prevent oxidant-induced RPTC death.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

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TH-PO017
Transcription Factor Nrf2 Regulates CYP2E1 Mediated Rhabdomyolysis
Induced Acute Kidney Injury
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Background: CYP2E1 is a member of the Cytochrome P450 family that plays an important role in the rhabdomyolysis induced AKI (JASN 21: 289A, 2010). Nrf2 regulates the expression of anti-oxidative and other cytoprotective genes against variety of insults. The purpose of the current study was to 1. Evaluate the role of Nrf2 in the up regulation of anti-oxidative protective factors generated in response to CYP2E1 induced oxidative stress and 2. Examine the ability of Nrf2 to protect against the cytotoxic actions of CYP2E1.

Methods: Male SD rats were injected with 50% glycerol following an overnight deprivation of water. Specific CYP2E1 inhibitor Chloromethiazole (CMZ) was administered prior to glycerol injection. Both mRNA and protein expression of Nrf2, heme- oxygenase-1 (HO-1) including other Nrf2 regulated enzymes were studied invivo and in LLC-PK1 cells exposed to myoglobin. Cytotoxicity was measured by the LDH release.

Results: Increases in the Nrf2 mRNA and nuclear protein including upregulation of the HO-1 and other Nrf2 regulated genes were observed in the kidney in the glycerol treated rats with the breakdown of the CYP2E1 protein and prior to AKI as measured by Sct.

Administration of CMZ significantly decreased oxidative stress and blocked the increases in the Nrf2 mRNA and nuclear protein including HO-1. Pre-treatment of the LLC-PK1 cells with an activator/inhibitor of Nrf2 prior to myoglobin exposure significantly decreased/increased ROS generation and cytotoxicity.

Conclusions: Nrf2 plays a key role in the adaptive response against increased oxidative stress caused by CYP2E1. Inhibition of CYP2E1 coupled with prior activation of Nrf2 may be a valuable approach to reduce CYP2E1 mediated rhabdomyolysis induced AKI.

Funding: NIDDK Support
Carbonyl Stress Induced Protein Modification in Snake-Bite Mediated Acute Renal Failure – A Pathogenesis Link

Pinnati Mukhopadhyay, Debarati Mukherjee, Raghwendra Mishra, Monaj Kar

Background: To identify the role of damaged proteins in association with oxidative stress (OS) and carbonyl stress (CS) in the pathogenesis of snakebite mediated acute renal failure (SARF).

Methods: Oxidative stress index, MG (methylglyoxal), thiobarbituric acid reactive substances (TBARS), AOPP, AGE, pentosidine and dityrosine were measured in 41 CRF and 58 SARF patients and compared with 36 normal control subjects. One way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis was performed to check any statistical difference, significance level was considered at p<0.05. Pearson correlation coefficient (r) was evaluated between the studied parameters from the aggregate of patients and control. Receiver-operating characteristic (ROC) curve analyses were performed regarding protein damage markers, CRF and SARF groups.

Results: SARF had significantly elevated level of oxidative stress index (OSI) compared to normal control (p=0.020) and CRF (p=0.019). This may be attributed to increased total oxidative stress (TOS) level in SARF compared to control (p=0.007) and CRF (p=0.009). Both MG and TBARS level were found to be significantly raised (p<0.001) in SARF and CRF group compared to normal controls. AOPP level of SARF was higher than that of CRF (p=0.002) and control (p=0.001). SARF group has significantly higher level of AGE (p=0.002), dityrosine (p=0.001) and pentosidine (p=0.001) than control. CRF shows significantly higher level of all protein damage markers than control (p=0.001). The studied protein damage markers were found to be positively correlated with the surrogate renal failure marker, i.e. serum creatinine, oxidative cellular damage marker TBARS and the carbonyl stress marker MG.

Conclusions: The toxins of snake venom induce protein damage associated with carbonyl and oxidative stress. Although it is still uncertain that whether these damages are cause or effect of SARF, this study indicates that they might play a pivotal role in pathogenesis of SARF in a similar way which occurs in CRF with only difference in the carbonyl and oxidative stress. Although it is still uncertain that whether these damages are cause or effect of SARF, this study indicates that they might play a pivotal role in pathogenesis of SARF in a similar way which occurs in CRF with only difference in the carbonyl and oxidative stress. Although it is still uncertain that whether these damages are cause or effect of SARF, this study indicates that they might play a pivotal role in pathogenesis of SARF in a similar way which occurs in CRF with only difference in the carbonyl and oxidative stress. Although it is still uncertain that whether these damages are cause or effect of SARF, this study indicates that they might play a pivotal role in pathogenesis of SARF in a similar way which occurs in CRF with only difference in the carbonyl and oxidative stress.
Selective Stabilization of HIF-1c in Renal Tubular Cells by 2-Oxoglutarate Analogues

Gunnar Schley,¹ Bernd Klank,¹ Yohannes Hagos,³ Kerstin U. Amann,³ Birgitta C. Burckhardt,² Kai-Uwe Eckardt,¹ Carsten William,¹
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Background: 2-oxoglutarate dependent enzymes, which comprise e.g. the hypoxia inducible factor (HIF), represent promising therapeutic targets to prevent progressive renal fibrosis or to preserve renal function in acute kidney injury. Lipophilic 2-oxoglutarate analogues (2OGAs), which are widely taken up in many cells of most organs, have been explored as inhibitors of these enzymes. Given the selective expression of organic anion transporters (OAT) in renal tubular cells we hypothesized that hydrophilic 2OGAs could be used to specifically target these cells.

Methods: HIF stabilization and HIF target gene induction by hydrophilic and lipophilic 2OGAs were analyzed in different cell lines and in C57BL/6N mice. Mice were subjected to bilateral renal ischemia-reperfusion 6h after ip injection of 2OGAs and kidney functions and morphology were evaluated 3 days later.

Results: In vitro analyses showed that in contrast to the lipophilic 2OGA, HIF stabilization and target gene induction by the hydrophilic 2OGA was dependent on the expression of OAT1. In vivo the functional effects of hydrophilic 2OGAs are largely limited to the kidney, particularly in renal proximal tubular cells expressing OAT1. Lipophilic 2OGAs resulted in HIF accumulation in tubular and interstitial renal cells and extra-renal tissues. Both lipophilic and hydrophilic 2OGAs induced HIF-dependent genes expressed in tubular cells, but only lipophilic 2OGAs induced erythropoietin synthesis. Preconditioning application of the lipophilic 2OGA protected the kidney against ischemia-reperfusion injury, surprisingly the hydrophilic 2OGA did not, suggesting that HIF stabilization in proximal tubular cells is insufficient to achieve organ protection.

Conclusions: This study provides proof of concept for selective drug targeting of proximal tubular cells. This may expand the options for therapeutic use of 2OGAs by limiting undesired side effects.

Funding: Government Support - Non-U.S.

TH-PO025

Pharmacological Modulation of Soluble Epoxy Hydroxide Activity Attenuates Ischemia Reperfusion Injury in Kidney

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Background: Soluble epoxy hydroxide (sEH) in endothelial cells determines the plasma level of epoxyeicosatrienoic acids (EETs), which may control the vascular tone as one of the vasoactive materials. We hypothesized that the regulation of sEH activity may have a therapeutic value in the prevention of acute kidney injury by the control of EETs level.

Methods: Ischemia-reperfusion injury (IRI) was induced in C57BL/6 mice, and sEH activity was controlled by the intraperitoneal administration of 12-(3-adamantan-1-yl)propenoic acid (AUDA). The deterioration of kidney function induced by IRI was partially moderated and prevented by AUDA treatment. In addition, AUDA treatment significantly attenuated tubular necrosis induced by IRI, especially inner medulla area. sEH was expressed prominently in the intraglomerular capillary loops and periglomerular arterioles under normal condition. The expression was induced by down-regulation of sEH, while AUDA administration did not generate an impact on the expression pattern of sEH induced by IRI. In vivo activity of sEH was assessed by the measurement of substrate (EpOME) and its metabolite (DHOME) using LC/MS/MS. Ischemic injury did not change the plasma level of epoxyeicosatrienoic acids (EETs), which may control the vascular tone.

Results: The deterioration of kidney function induced by IRI was partially moderated and prevented by AUDA treatment. In addition, AUDA treatment significantly attenuated tubular necrosis induced by IRI, especially inner medulla area. sEH was expressed prominently in the intraglomerular capillary loops and periglomerular arterioles under normal condition. The expression was induced by down-regulation of sEH, while AUDA administration did not generate an impact on the expression pattern of sEH induced by IRI. In vivo activity of sEH was assessed by the measurement of substrate (EpOME) and its metabolite (DHOME) using LC/MS/MS. Ischemic injury did not change the plasma level of epoxyeicosatrienoic acids (EETs), which may control the vascular tone.

Conclusions: These results suggest that modulation of sEH activity by AUDA might be a feasible therapeutic strategy to prevent acute kidney injury.

TH-PO026

Preservation with Cardiotrophin-1 Prevents Cold Ischemia Injury and Inflammatory Response and Improves Survival and Graft Function in a Syngeneic Rat Kidney Transplant Model

Jose M. Lopez-Novoa,1,3 María Inflammatory Response and Improves Survival and Graft Function in a Syngeneic Rat Kidney Transplant Model

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Background: Reperfusion injury (RI) in grafted kidneys due to warm and cold ischemia (CI) is clinically manifested as a high incidence of delayed graft function. Cardiotrophin-1 (CT-1) is a member of the ciliary neurotrophic factor (CNTF) family, has been shown to have a protective effect on myocardial and liver damage induced by RI. The purpose of the present study has been to assess the effect of CT-1 added to perfusion and preservation fluid on renal ischemia-reperfusion injury induced by CI and in renal function in the Fisher-Fisher rat model of syngenic kidney transplantation (KTx).

Methods: Once extracted, kidneys were perfused with University of Wisconsin (UW) fluid at 4°C containing CI-1 (0.2 mg/L) and immersed in 15 ml of cold UW with or without CT-1 (0.2 mg/L). Orthotopic transplantation of the left donor kidney was performed after 24 h of cold storage as above reported. The receptor’s right kidney was removed at the time of KTx. At different time points after CI or KTx, kidneys were obtained, frozen and several markers of inflammation (superoxide anion (SOA) production, TNF-α release, iNOS expression, serum levels of soluble ICAM-1 and VCAM-1 and NFκB activation) were evaluated.

Results: 24 or 48 h after CI without CT-1, there was a marked increase in SOA and TNF-α production, iNOS, and VCAM-1 expression and NFκB activation. The presence of CI-1 in the UW fluid completely prevented these increases. When kidneys were grafted after 24 h preservation, the addition of CT-1 to the UW fluid reduced reperfusion injury (lower serum creatinine and higher creatinine clearance), improved early and long-term survival, and reduced renal SOA production, iNOS expression and NFκB activation, and serum levels of TNF-α, soluble ICAM-1, and VCAM-1.

Conclusions: Preservation with CT-1 of kidneys to be grafted improves short- and long-term outcomes and inflammatory response after CI and syngenic KTx in rats.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.
levels of both uremic toxins, i.e., 0.39- and 0.23-fold decline vs those levels in untreated rats (Table 5). PCS, respectively, accompanied with a significant alleviation of renal injury (Ser, 0.45-fold and BUN, 0.52-fold).

Conclusions: In conclusion, the sulfate-conjugated uremic toxins could be key metabolic factors influencing the development or progression of IR-AKI, therefore proposing pre-treatment of their serum accumulations might be a possible renoprotective treatment against derangements in AKI.

Funding: Government Support - Non-U.S.

**TH-PO029**

Therapeutic Potential of Anti-Transforming Growth Factor-β Antibody on Acute Tubulo-Interstitial Injury in Aristolochic Acid Nephropathy

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Background: Aristolochic acid nephropathy (AAN) characterized by rapidly progressing renal fibrosis of toxic origin is primed by acute injury of proximal tubular epithelial cells (PTEC). Anti-transforming growth factor-β (TGF-β) antibody has been shown to improve renal fibrosis in various models of glomerular diseases, but its roles in primary tubulo-interstitial nephropathies are not yet well known.

Methods: We studied the efficacy of a murine pan-specific anti-TGF-β monoclonal antibody (1D11) in an acute phase of AAN. Weight matched rats were daily sc. injected with AA (15 mg/kg/day) or vehicle (polyethylene glycol-PEG) from day 0 to day 5. Four groups (n=6/group) were randomly assessed: PEG+1D11; AA alone; AA+1D11 and AA+control isotype (13C4). The anti-TGF-β and control isotype antibodies (5 mg/kg) were administered intraperitoneally at days 0, 1, 2 and 4.

Results: After 5 days of treatment, renal function and morphology remained normal in the control group PEG+1D11. Anti-TGF-β antibody statistically attenuated AA induced acute kidney injury, as attested by less increased creatininemia and urinary excretion of N-acetyl-β-glucosaminidase, less severe necrosis of PTEC from S3 segment and reduced macrophages infiltration (ED-1 staining) in outer medulla. Intrarenal T cells (CD4+CD25+highFoxp3+) infiltration and neo-angiogenesis (FVIII staining) around the injured areas were also reduced by 1D11 as compared with 13C4. The proliferation of PTEC from S3 segments (neutral endopeptidase proliferating nuclear cell antigen double staining) did not seem to be affected by 1D11.

Conclusions: Our results demonstrate that anti-TGF-β antibody significantly attenuates acute PTEC injury, reduces macrophages infiltration and modulates the regulatory T cells immune response. Therefore, 1D11 could be a potential, new renoprotective therapy interfering with early fibrogenesis events after a toxic insult.

**TH-PO030**

Apolipoprotein A-I Mimetic Peptide 4F Attenuates Kidney/Heart Injury and Endothelial Dysfunction in Sepsis

Roberto De Souza Moreira,1 Antonio C. Seguro,1 Lucia Andrade,1 and Endothelial Dysfunction in Sepsis

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Background: Kidney, heart injury and cytokine-induced vascular hyperpermeability are associated with high morbidity and mortality in sepsis. Although the mechanism of organ injury is still not well understood, it is suggested that high endothelial permeability is involved in sepsis. Apolipoprotein A-I mimetic peptide 4F (4F) has been demonstrated to protect liver and heart from chemical and ischemic damage. The purpose of this study was to assess if 4F administration could protect the kidney function in an experimental model of CN in rats receiving previously a low doses of gentamicin as a predisposing agent.

Methods: The study has been performed in 6 groups of Wistar rats: Control group (C): rats receiving saline solution; Gentamicin group (G): rats receiving G (50mg/kg/day, i.p.) for 6 days; Contrast agent gastrographin (Gg) group: rats receiving a single dose of Gg (3.7mg/Kg i.v.); Cardiotrophin group (CT-1): rats receiving CT-1 (100 μg/kg/day i.v.) for 6 days; G + Gg group: rats receiving G for 6 days and then Gg as described. G + CT-1 + Gg group: rats receiving G and CT-1 for 6 days and then Gg (as described). CN severity was assessed by measuring plasma urea (pU) and creatinine (pCr), creatinine clearance (CrCl), glomerular filtration rate (GFR), renal excretion of tubular damage markers (NAG, KIM-1 and PAI-1), renal cell apoptosis and lipid peroxidation (TBARS) and by histological studies.

Results: pG vs. pC there was an increase in pCr and pU, a decrease in CrCl, a lower GFR and TBARS, a high urinary excretion of NAG, KIM-1 and PAI-1, and marked tubular necrosis. These increases were markedly lower in the group that also received CT-1 (G+CT-1+Gg). In the group G+CT-1+Gg there was a significant decrease in pCr and pU, an increase in CrCl, a higher GFR and TBARS, a lower urinary excretion of NAG, KIM-1 and PAI-1, and a lower tubular necrosis.

Conclusions: CT-1 administration prevents most of the alterations in kidney function and structure associated to this model of CN. Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

**TH-PO001**

Cardiotrophin-1 Protects the Kidney from Contrast Agent-Induced Nephropathy

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Background: Cardiotrophin-1 (CT-1) is a cytokine of the IL-6 family that protects live and heart from chemical damage. Contrast nephropathy (CN), although generally reversible, is often associated to longer hospitalization time, dialysis and higher incidence of cardiovascular events. The purpose of this study was to assess if CT-1 administration could protect the kidney function in an experimental model of CN in rats receiving previously a low doses of gentamicin as a predisposing agent.

Methods: The study has been performed in 6 groups of Wistar rats: Control group (C): rats receiving saline solution; Gentamicin group (G): rats receiving G (50mg/kg/day, i.p.) for 6 days; Contrast agent gastrographin (Gg) group: rats receiving a single dose of Gg (3.7mg/Kg i.v.); Cardiotrophin group (CT-1): rats receiving CT-1 (100 μg/kg/day i.v.) for 6 days; G + Gg group: rats receiving G for 6 days and then Gg as described. G + CT-1 + Gg group: rats receiving G and CT-1 for 6 days and then Gg (as described). CN severity was assessed by measuring plasma urea (pU) and creatinine (pCr), creatinine clearance (CrCl), glomerular filtration rate (GFR), renal excretion of tubular damage markers (NAG, KIM-1 and PAI-1), renal cell apoptosis and lipid peroxidation (TBARS) and by histological studies.

Results: pG vs. pC there was an increase in pCr and pU, a decrease in CrCl, a lower GFR and TBARS, a high urinary excretion of NAG, KIM-1 and PAI-1, and marked tubular necrosis. These increases were markedly lower in the group that also received CT-1 (G+CT-1+Gg). In the group G+CT-1+Gg there was a significant decrease in pCr and pU, an increase in CrCl, a higher GFR and TBARS, a lower urinary excretion of NAG, KIM-1 and PAI-1, and a lower tubular necrosis.

Conclusions: CT-1 administration prevents most of the alterations in kidney function and structure associated to this model of CN. Funding: Pharmaceutical Company Support, Government Support - Non-U.S.
**TH-PO033**

Probenecid Prevents Acute Tubular Necrosis and Interstitial Fibrosis in a Mouse Model of Aristolochic Acid Nephropathy

The mechanisms of both acute tubular necrosis (ATN) and interstitial fibrosis (IF) are still not well understood. There is evidence for the role of oxidative injury in ATN. We studied whether probenecid (PBN), an OATs inhibitor, prevents ATN and IF in a mouse model of acute renal ischemia-reperfusion injury (IRI).

Methods: Adult, male, Wistar rats (280–310 g) were divided into groups: SHAM (45 min), Isch 30, Isch 30+NAC, Isch 30+NAC+NAC, Isch 45, Isch 45+NAC, Isch 45+NAC+NAC. Rats were treated with vehicle only. We evaluated renal function and the expression of inflammatory cytokines such as IL-1β, TNF-α.

Results: In the 30 min RPC groups, only probenecid prevented acute tubular necrosis and interstitial fibrosis. In the 45 min groups, only probenecid decreased the expression of cytokines.

Conclusions: Our data show that probenecid reduces AA-DNA adduct formation and reduces tubule toxicity, possibly by attenuation of interstitial injuries. This study demonstrates for the first time the nephroprotective effect of PBN towards acute PTEC toxicity and interstitial fibrosis in a mouse model of AA-N.

**Funding:** Private Foundation Support

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**TH-PO034**

Sildenafil and N-Acetylcysteine Role in Renal Ischemia Reperfusion Syndrome Is Time Dependent

Sildenafil (SIL) has shown a role in vasodilation and scavenges reactive oxygen species (ROS). This study evaluated the effect of SIL and NAC in a time-dependent ischemia reperfusion (IRI) model.

Methods: Adult, male, Wistar rats (260–310 g) were divided into groups: SHAM (45 min before RPC), Isch 30, Isch 30 + NAC (45 min before and after RPC), Isch 45, Isch 45 + NAC, Isch 45 + NAC+NAC. Rats were treated with vehicle only. We evaluated renal function and the expression of inflammatory cytokines such as IL-1β, TNF-α, and IFN-γ.

Results: In the 30 min RPC groups, only probenecid prevented acute tubular necrosis and interstitial fibrosis. In the 45 min groups, only probenecid decreased the expression of cytokines.

Conclusions: Our data show that probenecid reduces AA-DNA adduct formation and reduces tubule toxicity, possibly by attenuation of interstitial injuries. This study demonstrates for the first time the nephroprotective effect of PBN towards acute PTEC toxicity and interstitial fibrosis in a mouse model of AA-N.

**Funding:** Private Foundation Support

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**TH-PO005**

Therapeutic Effects of GLP-1 Agonist in Gentamicin-Induced Kidney Injury in Rats

Gentamicin (GM) is known to cause renal injury by accumulating in the proximal convoluted tubule (PCT), leading to tubular cell death. GLP-1 agonists (exendin-4) have been shown to improve renal function and reduce inflammation in experimental models of kidney injury.

Methods: We randomized 3 groups of adult male SD rats (n=6). 1) Sham (S), 2) I/R-AKI Control (CT), 3) I/R-AKI + GLP-1 agonist (TP). Rats were treated with vehicle only. We evaluated renal function and the expression of inflammatory cytokines such as IL-1β, TNF-α.

Results: In the 30 min RPC groups, only probenecid prevented acute tubular necrosis and interstitial fibrosis. In the 45 min groups, only probenecid decreased the expression of cytokines.

Conclusions: Our data show that probenecid reduces AA-DNA adduct formation and reduces tubule toxicity, possibly by attenuation of interstitial injuries. This study demonstrates for the first time the nephroprotective effect of PBN towards acute PTEC toxicity and interstitial fibrosis in a mouse model of AA-N.

**Funding:** Private Foundation Support

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**TH-PO036**

Acute Testosterone Supplementation Improves Renal Ischemia-Reperfusion-Induced Acute Kidney Injury (I/R-AKI)

Testosterone (T) has various extra-pancreatic actions. In the kidney, T improves renal function, reduces inflammation, and increases renal blood flow. This study investigated the effect of acute testosterone supplementation on renal function and inflammation in a murine model of I/R-AKI.

Methods: We randomized 3 groups of adult male SD rats (n=6). 1) Sham (S), 2) I/R-AKI Control (CT), 3) I/R-AKI + testosterone propionate (TP). Rats were treated with vehicle only. We evaluated renal function and the expression of inflammatory cytokines such as IL-1β, TNF-α.

Results: In the 30 min RPC groups, only probenecid prevented acute tubular necrosis and interstitial fibrosis. In the 45 min groups, only probenecid decreased the expression of cytokines.

Conclusions: Our data show that probenecid reduces AA-DNA adduct formation and reduces tubule toxicity, possibly by attenuation of interstitial injuries. This study demonstrates for the first time the nephroprotective effect of PBN towards acute PTEC toxicity and interstitial fibrosis in a mouse model of AA-N.

**Funding:** Private Foundation Support

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**Underline:** represents presenting author.
Conclusions: Our data suggest that TST improves AKI as demonstrated by: 1) reversing of OM/REB of injured rats, 2) attenuation of renal injury and inflammation (Proteinuria, Kim-1, TNFα), 3) blunting of AKI-induced VEGF reduction. In conclusion, acute TST infusion improves renal hemodynamics, function, injury, and VEGF expression after I/R-induced AKI.

Funding: Other NIH Support - This work was supported by NIH DK073401

TH-PO037
Preconditioning Effects of Rituximab on Subsequent Ischemia-Reperfusion Injury in Mouse Kidney
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Background: The adaptive immune system contributes to robust inflammatory response after ischemia-reperfusion (IR) renal injury. There is growing evidence of the roles of B-cell in postischemic kidney, and we investigated preconditioning effects with rituximab against subsequent I/R injury in mouse kidney.

Methods: Male C57BL-6 mice were used. Rituximab (5, 10 and 40 mg/kg) was administered 7 days before I/R injury by clamping both renal pediciles for 22 min. The effect of rituximab pretreatment was evaluated in terms of renal function, tubular necrosis score, serum immunoglobulin (Ig) G and IgM levels.

Results: Pretreatment with 10 and 40 mg/kg rituximab decreased blood urea nitrogen and serum creatinine at 1, 3, and 10 days postischemia. Renal function was not improved in mice treated with 5 mg/kg rituximab. Tubular necrosis scores were lower in mice with 10 mg/kg rituximab treatment than in control mice. The serum IgG level was not different between control and rituximab-treated mice, but serum IgM level was tended to be reduced in rituximab-treated mice.

Conclusions: Our study demonstrates that preconditioning with more than 10 mg/kg rituximab exerts a protective effect against subsequent renal I/R injury.

Funding: Private Foundation Support

TH-PO038
Gamma-Tocotrienol Protects Against Oxidative Damage in Renal Proximal Tubule Cells
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Background: Alpha, beta, gamma, and delta tocopherols and tocotrienols are well known for their antioxidant properties. Oxidative stress is a major mechanism of acute kidney injury, diabetic nephropathy (50%) and chronic kidney disease. This study tested in gamma-tocotrienol (GT3) protects against oxidative stress and mitochondrial dysfunction in renal cells.

Methods: Primary cultures of renal proximal tubular cells (RPTC) were treated with tert-butyl hydroperoxide (0.35 mM TBHP, 45 min), a model oxidant. Some RPTC were pre-treated with 5 μM GT3 prior to TBHP exposure. Production of reactive oxygen species (ROS), mitochondrial functions, and cell viability were measured after TBHP exposure.

Results: ROS production in injured RPTC increased 200% and was followed by dose-dependent decrease in ATP (31%) and respiratory control ratio (47%). Membrane potential decreased 39% and 82% at 4h and 24h, respectively, in TBHP-injured RPTC. Oligomycin-sensitive respiration declined 63%, F,ATPase activity decreased 31%, and ATP content decreased 65% in TBHP-injured RPTC. Cell death increased to 22% in 4h and 88% in 24h with increased TBHP exposure. Pretreatment of RPTC with GT3 blocked production of ROS, prevented the decreases in state 3 respiration, and maintained the respiratory control ratio in TBHP-injured RPTC. TBHP-induced decreases in oligomycin-sensitive respiration and F,ATPase activity in mitochondria were prevented by GT3. Further, GT3 ameliorated decreases in ATP content after TBHP exposure. Finally, pretreatment with GT3 blocked cellular lysis at 4h and reduced it from 52% to 13% at 24h after injury.

Conclusions: This is the first report demonstrating the protective properties of GT3 in RPTC. GT3 protected against RPTC injury by: 1) decreasing production of ROS, 2) improving mitochondrial respiration and coupling, 3) maintaining mitochondrial membrane potential, 4) blocking decreases in F,ATPase function, 5) maintaining intracellular ATP levels, and 6) preventing RPTC lysis and death. This study suggests that GT3 could be used as a potential treatment to prevent renal injury associated with oxidative stress.

Funding: NIDDK Support

TH-PO039
Role of Cilastatin Against Cisplatin-Induced Nephrotoxicity and Inflammation in Rats
Alberto Tejedor Jorge.1 Blanca Humanes Sanchez,1 Sonia Camañofournier,1 José Antonio Lázaro Manero,1 Jose Manuel Lara Martinez,2 and Syed M. Ali1, Shenyang Li,1 Didier Portilla,1 Nermant Gokden,1 and Shenyang Li1

Background: Cisplatin is a major antineoplastic drug for the treatment of solid tumors, but its nephrotoxicity is a major complication and a dose limiting factor for antancer therapy. Several evidences have shown that inflammation contribute to the pathogenesis of cisplatin-induced acute renal failure. In this study, we have investigated the potential use of cilastatin, a renal dehydroepiandrosterone S inhibitor as nephroprotective on cisplatin-induced renal injury and inflammation in rats.

Methods: Male Wistar rats were divided into 4 groups: control rats, cisplatin-control rats, cisplatin-pretreated rats, and cisplatin-cilastatin-pretreated rats. Nephrotoxicity was assessed 5 days after cisplatin treatment, by measuring serum creatinine, blood urea nitrogen (BUN), glomerular filtration rate, proteinuria and renal morphology. Inflammation was measured by electrophoretic mobility assay (EMSA) and immunohistochemical studies. Levels of proteinuria, inflammation and oxidative stress markers were measured in cisplatin-pretreated and saline control rats. Proteinuria, cisplatin-induced oxidative stress markers, proteinuria and renal morphology were assessed.

Results: Cisplatin-pretreated rats showed significant elevations in BUN, creatinine, and proteinuria and decreased the glomerular filtration rate when compared with control rats. Cisplatin rats also exhibited severe morphological changes such as necrosis and extensive vacuolization of the proximal tubule and inflammatory mediators were increased. Cisplatin-induced oxidative stress markers were prevented or totally these changes in renal cells, which suggests that the protective effect of cilastatin in cisplatin-treated animals. Cilastatin also reduced serum tumor necrosis factor-alpha (TNF-α) levels, nuclear factor Kβ activation and ED1 (monocytes/macrophages) positive cells. Conclusion: This study provides evidence that cilastatin reduces renal injury and inflammation by preventing inflammation and may represent a novel strategy in the prevention of cisplatin-induced acute renal injury.

Funding: Government Support - Non-U.S.

TH-PO040
CD73-Deficiency Protects in Kidney Ischemia Reperfusion Injury: The Role of Adenosine A2B Receptors
Sonia Camaño Paez,1 José Antonio Lázaro Manero,1 Jose Manuel Lara Martinez,2 and Monika M. Nowak.

Background: Adenosine, acting at A1, A2A, A2B and A3 receptors, is protective in numerous models of ischemia reperfusion injury (IRI). Extra-cellular adenosine is generated by the successive hydrolysis of nucleotides by the ecto-enzymes CD39 and CD73. CD39 over-expression promotes in kidney IRI while CD73 deficiency and inhibition also confer protection. Given CD73-deficiency results in reduced adenosine generation, the mechanism by which this protection is ascribed remains unclear.

Methods: Wild-type (WT) and CD73-deficient (CD7KIO) mice underwent right nephrectomy and 18 minutes left renal ischemia. After 24-hour reperfusion, renal function (serum creatinine, urate) and histological renal injury (score S:1-9) were assessed. Mice were pre-treated with specific A1, A2A, A2B or A3 receptor antagonists.

Results: Compared to WT mice [Cr=96.3±6.5, p=0.5], CD7KIO mice are protected [Cr=45.5±8.8, p=0.05]. A1-antagonist treatment has no significant effect on WT mice (Cr=96.3±6.5, p=0.5) and protection is maintained in CD7KIO mice [Cr=59.0±4.9, p=0.5]. A2A-antagonist treatment worsens renal function and histological damage in both WT [Cr=145.8±4.9, p=0.05] and CD7KIO mice [Cr=122.8±5.8, p=0.01]. A2B-antagonist treatment has minimal effect on WT mice [Cr=45.5±8.8, p=0.3] but significantly worsens renal dysfunction in CD7KIO mice [Cr=146.5±5.6, p=0.01]. A3-antagonist treatment abrogates injury in WT mice [Cr=49.8±1.5, p=0.05] and protection in CD7KIO mice is maintained [Cr=43.0±5.5, p=0.05].

Conclusions: CD73-deficiency and A3 receptor inhibition protect in kidney IRI. Protection mediated by CD73-deficiency is not dependent on A1, A2A or A3 receptor signaling and is dependent on A2B receptor signaling. The A2B receptor plays a protective role in states of lower adenosine generation.

Funding: Government Support - Non-U.S.

TH-PO041
Increased Angiopoietin-like Protein 4 (Angptl4) Expression and Hyperlipidemia in Acute Kidney Injury
Sved M. Ali,1 Shenyang Li,1 Nermant Gokden,1 and Didier Portilla,1,2

Background: In the present study we examined the mechanisms by which cisplatin causes hyperlipidemia and the potential role of hyperlipidemia in nephrotoxicity. We studied whether changes on Liproprotein Lipase (LPL) activity, the enzyme responsible for the hydrolysis of triglycerides(TG), and changes in the expression of Angptl4, a potent inhibitor of LPL account for CP-mediated hyperlipidemia.

Methods: C57BL/6 wild type mice, receiving peroxisome proliferator activated receptor-alpha ligand WY received a single intraperitoneal injection of saline or CP (25 mg/kg BW). Serum, white epididymal adipose tissue, liver and kidney tissue were collected after CP exposure.

Results: CP caused a time-dependent reduction in white epididymal adipose tissue mass by 40% at day 3, that was accompanied by focal necrosis and increased lymphocytic infiltration. LPL activities measured in serum and adipose tissue were inhibited by 70% when compared to saline treated mice. CP also inhibited kidney tissue LPL activity by 40%. Angptl4 mRNA levels were increased by 2- and 4-fold in liver and kidney tissue and by 2-fold in adipose tissue of CP-treated mice. Treatment of adipose tissue cells in culture with 25 micromolar CP for 24 hrs led to 70% reduction in LPL activity and a two-fold increased expression of Angptl4 mRNA levels. CP also increased the accumulation of TG in serum and kidney tissue. Renal function, CP-mediated inhibition of LPL activity, increased expression of Angptl4, and accumulation of TG in kidney tissue were ameliorated by the use of a PPARalpha ligand.

Conclusions: Cisplatin mediated hyperlipidemia is caused by direct effects of CP on LPL activity, 2) Inhibition of LPL activity and increased Angptl4 gene expression in adipose, liver, and kidney tissue represent a common biochemical mechanism

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involved in CP-mediated hypertiglycemia, 3) Fibrates ameliorate renal function and prevent the accumulation of TG in kidney tissue by preventing CP-mediated inhibition of LPL activity via reduced expression of Ang124 in the proximal tubule.

**Funding:** NIDDK Support, Veterans Administration Support

**TH-PO042**

**Regulation of Nrf2 Mediated Signaling during Acute and Chronic Hypoxic Stimulation of Human Proximal Tubular Epithelial Cells**

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**Background:** Acute kidney injury (AKI) caused by ischemia reperfusion (IR) is a major clinical problem in both native and transplanted kidney, but mechanisms by which IR contributes to AKI are largely undefined. We have previously shown that gene deletion of Nrf2 enhances susceptibility to ischemic AKI in mice. Because Nrf2-dependent transcriptional response is essential for mitigating cellular stress, we investigated whether hypoxic reoxygenation impairs activation of Nrf2 signaling in kidney epithelial cells, thereby worsening tubular injury in AKI.

**Methods:** Human renal proximal tubular epithelial cells, HK2, were exposed to acute (2 h) and chronic (12 h) hypoxic conditions with and without re-oxygenation for up to 6 h. The expression levels of Nrf2, Keap1 (an endogenous inhibitor of Nrf2), and several antioxidant genes, as well as Nrf2 recruitment to the endogenous antioxidative genes were evaluated.

**Results:** Gene expression analysis revealed a distinct and dynamic regulation of expression levels of Nrf2 and Keap1 in response to acute and chronic hypoxic-reoxygenation conditions. Hypoxia alone stimulated significantly Nrf2 mRNA expression, whereas re-oxygenation led to a modest decrease. However, hypoxia and re-oxygenation altered the expression level of Keap1. There was persistently elevated level of Hmox1 expression during reoxygenation, whereas Nqo1 expression was transiently induced. The analysis of Nrf2 recruitment to the antioxidant gene promoters using chromatin immunoprecipitation (ChIP) assays revealed an increased Nrf2 binding to the antioxidant response elements (AREs) of Hmox1 and Nqo1 promoters during acute hypoxic-reoxygenation condition, but not in the chronic exposure.

**Conclusions:** Our data demonstrate a dynamic and distinct regulation of Nrf2 activation by hypoxia in kidney epithelial cells. Modulation of Keap1 expression may play a role in regulating ARE-mediated gene expression during hypoxic-reoxygenation in AKI.

**Funding:** NIDDK Support

**TH-PO043**

**Renal Dysfunction Associated with Increased Intra-Absdominal Pressure in Experimental Heart Failure: Nephroprotective Effects of Phosphodiesterase-5 Inhibition**

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**Background:** Rats with CHF are vulnerable to the adverse renal effects of elevated IAP in rats with CHF. While IAP of 7 mmHg in sham-controls did not affect V, UNaV, GFR and RPF, IAPs of 10 and 14 mmHg produced dose-dependent reductions in these parameters. Basal GFR, RPF and increased NGAL excretion compared to sham controls. Pretreatment with Tadalafil abolished renal dysfunction and AKI induced by high IAP, supporting a therapeutic role for PDE5 inhibition in laparoscopic surgery in CHF states.

**Funding:** Government Support - Non-U.S.

**TH-PO044**

**Role of Autophagy for Tubular Maintenance, Aging and Stress Adaptation**

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**Background:** Autophagy is a major pathway that delivers damaged proteins and organelles to lysosomes to maintain cellular homeostasis. Recent studies indicate an upregulation of autophagy in tubular cells under stress conditions. However, the precise function of tubular autophagy in vivo remained unclear. To determine the role of autophagy in tubular cells, we generated Atg5 flox/flox: Pdx1Cre mice and Atg5 flox/flox: Ksp-Cre mice where Atg5 is deleted in all tubular segments or only the distal tubular compartment, respectively. Atg5 deletion in the complete tubular system resulted in accumulation of p62-positive protein aggregates and increased creatinine excretion 4 months after doxycycline administration. Under pathophysiological condition such as ischemia/reperfusion injury, proximal tubules displayed upregulated autophagic activity and autophagy-deficient mice exhibited significantly increased signs of tubular injury.

**Funding:** Government Support - Non-U.S.

**TH-PO045**

**The Impact of Protein Kinase Cα/λ Deficiency in Proximal Tubule Epithelial Cells**

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**Background:** Podocyte specific deletion of atypical protein kinase C α/λ (PKCα/λ), in mice, results in a severe glomerulosclerosis phenotype with severe mesangial and glomerular hypertrophy, cell-cell junctions and proteinuria. Because of the great effect of PKCα/λ on podocyte polarity we wanted to investigate the role of PKCα/λ in the highly polarized kidney tubules using a transgenic mouse model.

**Methods:** We specifically deleted PKCα/λ in proximal tubule epithelial cells by breeding transgenic Sgl2-Cre mice with PKCα/λ floxed mice. Offspring of these mice were tested for renal function before and after treatment with aristolochic acid to induce the murine model of acute kidney injury aristolochic acid nephropathy (AAN). Sections of kidneys were examined for proliferation by Ki67 staining. Furthermore, cDNA of WT and KO kidneys was tested via real-time PCR for the gene expression of acute kidney injury markers, NGAL and Kim-1. To investigate the PKCα/λ deficiency in vitro we isolated proximal tubule epithelial cells from WT and KO kidneys and analyzed them in a Ca2+ switch assay.

**Results:** The histology of the PKCα/λ Sgl2-Cre mouse revealed no obvious renal phenotype up to 6 month after birth. After injection of aristolochic acid, both WT and KO mice developed acute renal injury marked by an increase of serum creatinine and serum urea at day 3-6. On day 14 we sacrificed the animals, and compared the histology of WT and KO by HE stainings. We detected a different amount of tubular damage by a semiquantitative damage scoring. Realtime PCR analysis confirmed the damage score with the expression measurements of NGAL and Kim-1. In stainings we found an increase of Ki67 positive cells in the KO mice.

In isolated PKCα/λ deficient proximal tubule epithelial cells we could demonstrate a disturbed expression pattern of the junction protein E-cadherin, especially in the Ca2+ switch assay.

**Conclusions:** In summary, we found that atypical PKCα/λ is an important factor for the reorganization of proximal tubular epithelial cells after acute kidney injury.

**Funding:** Government Support - Non-U.S.

**TH-PO046**

**TWEAK Modulates Proliferation, Matrix Remodeling and Migration in Renal and Embryonic Fibroblasts through Ras Signaling**

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**Background:** There is scarce information about the role of TWEAK in tissue repair after injury. Renal tubular cells. TWEAK blockade ameliorates renal damage in mice AKI models, but there is scarce information about the role of TWEAK signaling in proximal tubular epithelial cells.

**Methods:** While TWEAK induced proliferation in cultured renal fibroblasts (TFB cells) through ERK pathway. Looking for the link between TWEAK signaling and ERK, we tested whether TWEAK signaling and ERK activity are blocked in proximal tubular epithelial cells by Ras inhibition.

**Results:** TWEAK induced proliferation in cultured renal fibroblasts (TFB cells) through ERK pathway. Looking for the link between TWEAK signaling and ERK, we tested whether TWEAK signaling and ERK activity are blocked in proximal tubular epithelial cells by Ras inhibition. When treated with Ras, TWEAK induced proliferation and an inflammatory phenotype in renal tubular cells. TWEAK blockade ameliorates renal damage in mice AKI models, but there is scarce information about the role of TWEAK in tissue injury after renal injury. Renal fibroblasts and their matrix-related properties are key players in tissue remodeling after injury and in renal fibrosis.

**Funding:** Government Support - Non-U.S.
Results: Renal fibroblasts are characterized by an active role in extracellular matrix remodeling and the ability to secrete TWEAK, which directly stimulates MMP-9 and PAI-1 production. Similar effects over collagen type I and fibronectin were observed in MEFs and were seen to be Ras-dependent. These results suggest that TWEAK modulates tissue remodeling via Rac-1/RhoA-Rac-1 signaling pathways, so that the potential functional activity of this pathway in a wound healing assay. Wound-closure time was smaller in MEF with active Ras in the presence of TWEAK. This effect disappeared in Ras-deficient MEFs.

Conclusions: TWEAK regulates fibroblast proliferation, matrix proteins production and wound closure capacities depending on Ras. These results suggest that TWEAK/Ras pathway may contribute to tissue remodeling during tissue repair.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO047

Signalling Pathways Responsible for Proximal Tubule Cell Adhesion Promotion during I/R by Darbepoetin α: New Therapeutic Targets for a New Use of Darbepoetin α Ignacio Blanco Sanchez,1 Marina Arranz,2 Elisa Conde,3 Elia Aguado Fraile,4 Edurne Ramos,5 Nuria Villegas,5 Rafael Selgas,5 Jose-Antonio Sanchez-Tomero,5 Laura Garcia-Bermejo.6

Methods: We used a mice model of IR and an in vitro model of hypoxia/reoxygenation (H/R) in mouse proximal tubule cells (MCT) where DPO is administered after ischemia insult. In vivo, LIMK phosphorylation was studied by WB, Rac1, RhoA and PKCa traslocation was observed by immunofluorescence and cell adhesion was estimated by cell impedance studies.

Results: DPO promotes LIMK phosphorylation in vitro, in a dose dependent manner. LIMK can be regulated by Rho-GTPases, we thus determined localization of RhoA and Rac1 in cells submitted to H/R and treated with DPO. Our results indicated that Rac-1 was translocated to the cell membrane during reoxygenation, indicating Rac1 activation, and this translocation is promoted by DPO treatment. In contrast, RhoA traslocation is not increased by DPO. Finally, we checked PKCa activity in vitro and in vivo finding that DPO does not exert any effect on its traslocation. Moreover, in vitro use of GO9760, a specific PKCa inhibitor, did not affect cell adhesion.

Conclusions: DPO exhibited a beneficial effect in experimental acute kidney injury activating Rac-1/LIMK pathways, avoiding cytoskeleton reorganizations without PKCa contribution. New targets for DPO in cytoprotection have been identified and they may be relevant for a new therapeutic use of DPO.

Funding: Government Support - Non-U.S.

TH-PO048

Renal Ischaemia Reperfusion Injury Promotes Mid-Term Tubular Senescence Yoichiro Ikeda, Reiko Inagi, Toshiro Fujita, Masami Nangaku. Division of Nephrology and Endocrinology, University of Tokyo School of Medicine, Japan.

Background: Renal ischaemia reperfusion injury (IRI) leads to acute kidney injury, but the changes have not been studied so much after mid-to-long term of this injury. Levels of serum creatinine and urine nitrogen in this model increased within a day, but recovered some days after injury. And morphologically, most tubular damages repaired after a month or later, but fibrosis was observed depending on the magnitude of ischemia. It is unknown that tubulointerstitial areas other than the fibrotic areas remain normal or not. Here we revealed the enhanced senescence status in renal tubules in outer medulla 15-30 days after IRI. We conducted a prospective, multicenter observational study to compare the role of HIF in the regulation of miR-21 expression in primary cultures of human renal epithelial cells (HRE). The regulation of miR-21 expression in primary cultures of human renal epithelial cells (HRE).

Methods: HRE cells were treated with cobalt chloride (300µM for 4h) or hypoxia (2% O2, for 24h). HIF decoy oligodeoxynucleotides (40mM) were used to block the activity of miR-21.

Results: Treatment of HRE cells with cobalt chloride or hypoxia, which are classical inducers of HIF activation, caused significant up-regulation of miR-21 by 183% ± 9% (vs. control, n=6, P<0.05) and 168% ± 22% (vs. normoxia, n=6, P<0.05), respectively. Both hypoxia and cobalt chloride activity with a decoy significantly reduced miR-21 levels in HRE cells treated with cobalt chloride by 42% ± 13%, compared to scrambled oligonucleotides (n=6, P<0.05). The up-regulation of miR-21 expression by cobalt chloride was correlated with down-regulation (by 57% ± 8%, P<0.05) of miRNA levels of programmed cell death protein 4 (PDCD4), a pro-apoptotic target gene of miR-21. The HIF decay significantly blunted the down-regulation of PDCD4 (P<0.05).

Conclusions: These findings suggest a new mechanism in which HIF activation mediates up-regulation of miR-21. The mechanism may be relevant to renal protection against ischemic injury.

Funding: Other NIH Support - HL085267, DK084405, HL082798, HL029587, and a CTSG grant, Government Support - Non-U.S.

TH-PO051

Predictive Performance of Biomarkers in Acute Kidney Injury (AKI) Using AKI Network (AKIN) Serum Creatinine or Urine Output Criteria Josec Bouchard,1 Rakesh Malhotra,2 Ashita J. Tolvani,2 Ravindra L. Mehta,2 1Université de Montreal, Canada; 2University of California San Diego; 3University of Alabama at Birmingham.

Background: There is limited information on the value of biomarkers to diagnose acute kidney injury (AKI) according to AKIN serum creatinine compared to urine output criteria.

Methods: We conducted a prospective, multicenter observational study to compare the usefulness of urine neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C, osteopontin, interleukin-18 (IL-18) and N-acetyl-beta-D-glucosaminidase (NAG) levels to diagnose AKI in 51 critically ill patients using the AKIN urine output criteria. Biomarker samples were collected every 12 hours after intensive care unit (ICU) admission for a 48 hours and up to 10 days.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.

121A
Results: The Area Under the Curve (AUC) of the different biomarkers to predict AKI are included in the table:

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>AUC (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKIN serum creatinine</td>
<td>0.748 (0.664-0.826)</td>
</tr>
<tr>
<td>AKIN urine output</td>
<td>0.702 (0.622-0.781)</td>
</tr>
<tr>
<td>AKIN urine output and/or serum creatinine</td>
<td>0.847 (0.787-0.905)</td>
</tr>
<tr>
<td>Urine NGAL</td>
<td>0.710 (0.630-0.789)</td>
</tr>
<tr>
<td>Urine KIM-1</td>
<td>0.702 (0.622-0.781)</td>
</tr>
<tr>
<td>Urine cystatin C</td>
<td>0.684 (0.604-0.764)</td>
</tr>
<tr>
<td>Urine osteopontin</td>
<td>0.702 (0.622-0.781)</td>
</tr>
<tr>
<td>Urine IL-18</td>
<td>0.702 (0.622-0.781)</td>
</tr>
<tr>
<td>Urine NGAG</td>
<td>0.702 (0.622-0.781)</td>
</tr>
</tbody>
</table>

Conclusion: Biomarkers of AKI at the time of clinical criterion rise improve risk stratification and identify patients with newly diagnosed AKI who are at the highest risk for the most severe forms of AKI and the worst patient outcomes.

Funding: NIDDK Support, Other NIH Support - R01HL-085757

TH-PO054

Multiple Nephrotoxic Events and Associations with Acute Kidney Injury and Mortality

Background: Acute kidney injury (AKI) occurs in 15% of hospitalized patients and is associated with significant morbidity and mortality. We hypothesized that patients would encounter multiple potentially nephrotoxic events, and that those experiencing more events would be at higher risk of AKI and mortality.

Methods: We investigated all patients greater than 18 years old admitted between 2009 and 2010 at Montefiore Medical Center, a tertiary medical center in the Bronx, NY. We examined 16 potentially nephrotoxic exposures including systolic blood pressure < 90 mm Hg, ICD-9 diagnoses of sepsis, and exposure to different medications (e.g. NSAIDs, iv contrast). AKI was defined as a 50% increase in serum creatinine over a pre-admission value. Mortality data was collected via linkage to the Social Security Death Index.

Results: There were 46,754 admissions meeting our eligibility criteria. 2,111 patients developed AKI (5%). Those developing AKI were more likely to have higher Charlson scores (2.3 versus 2.0, p=0.002), diabatic complications (5.9% versus 4.4%, p=0.001) or an eGFR that was < 60 ml/min/1.73 m(2) (37% versus 22% p=0.001).

Conclusions: In a multicentered study, NSAID use was associated with a higher risk of AKI (OR 1.5, 95% CI: 1.40, 1.78), and statin use was associated with a lower risk (OR 0.86, 95% CI: 0.77, 0.96). The median number of potential nephrotoxic events was 2 (IQR 1 to 3). Each additional event resulted in the increased the multivariable-adjusted odds risk of AKI by 4% (95% CI 1%, 7%, p=0.006).

A total of 4788 patients died. Of those that died, 22% had a prior episode of AKI compared to 11% mortality without previous AKI (p=0.001). AKI was associated with a multi-variable adjusted higher risk of mortality (OR 1.5, 95% CI 1.3, 1.8). Each additional potential nephrotoxic event was associated with a 21% higher odds of mortality (95% CI 19%, 24%) even after adjusting for AKI.

Conclusions: In hospital acquired AKI, a greater number of potential nephrotoxic events was associated with a higher risk of AKI and mortality. Future research should focus on trying to minimize the number of nephrotoxic events in hospitalized patients.

TH-PO055

Acute Kidney Injury Time as a Predictor of Adverse Clinical Outcomes in Critically Ill Hospitalized Patients

Background: Acute kidney injury (AKI) is a complex and frequent complication of many diseases with a high mortality rate. Recent evidence shows that in addition to AKIN staging of severity, the duration of kidney failure is associated to poor outcomes. Prognostic classification systems such as RIFLE and AKIN do not take into account the etiology, duration or recovery time of AKI. Studies in diabetic patients have suggested that the time resolution of AKI is an independent prognostic factor for mortality. We report a similar finding in a population of critically ill hospitalized patients.

Methods: To determine the association between length of AKI and adverse clinical prognosis, we performed a cohort study in a hospitalized patients at the Hospital Civil de Guadalajara, from January 2009 to January 2010 that met the criteria for AKI by AKIN. Association was determined by Pearson’s X2 or t-test depending on the nature of the variable. Correlation of data was analyzed using Pearson’s correlation coefficient. Survival was determined by Kaplan-Meier method. A p <0.05 value was considered statistically significant.

Results: Length of AKI and outcomes

<table>
<thead>
<tr>
<th>Variable</th>
<th>&lt;2 days n=47</th>
<th>&gt;2 days n=66</th>
<th>RR (IC 95%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>47.16±12.32</td>
<td>66.24±17.6</td>
<td>0.01†</td>
<td></td>
</tr>
<tr>
<td>Mechanical Ventilation, %</td>
<td>4</td>
<td>27</td>
<td>4.82 (12.17-8.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>Vasopressors, %</td>
<td>11</td>
<td>41</td>
<td>3.31 (1.44-7.62)</td>
<td>0.01</td>
</tr>
<tr>
<td>Death, %</td>
<td>9.48 (2.38-36.95)</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Final S creatinine &gt;1.5 mg/dl</td>
<td>2</td>
<td>52</td>
<td>9.48 (2.36-39.47)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Conclusions: The time resolution of AKI is an independent prognostic factor for mortality. We report a similar finding in a population of critically ill hospitalized patients.

TH-PO053

Biomarkers of Kidney Injury and Risk of AKI Progression Following Cardiac Surgery

Background: Acute kidney injury (AKI) following cardiac surgery is associated with poor patient outcomes.

Methods: Using samples from the Translational Research Investigating Biomarker Endpoints in AKI (TRIBE AKI) study, we evaluated whether biomarkers of kidney function and injury could forecast the severity of AKI at the time of a clinical diagnosis of AKI. Among 426 individuals who developed at least Acute Kidney Injury Network (AKIN) Stage 1 AKI, serum creatinine (Scr), urinary IL-18, albumin, and plasma NGAL were measured on the day of AKI diagnosis. The primary end point was progression of AKI, defined by worsening of AKIN Stage (e.g. from Stage 1 to Stage 2) prior to hospital discharge. We categorized each biomarker into quintiles (Q) and grouped them [Low (Q1 & Q2), Intermediate (Q3 & Q4) and High (Q5)]. Using multivariable logistic regression, we determined the adjusted odds of AKI progression.

Results: The 50 subjects (11.7%) who progressed beyond their original AKIN Stage were more likely to have higher Scr (p=0.03), IL-18 (p=0.02), and plasma NGAL (p=0.03) compared to those who did not progress. After adjustment for clinical predictors, when compared to Q1 and Q2, the highest quintile of each biomarker remained associated with AKI progression; [Odds ratio (95% CI)] percent change Scr 3.6 (1.6-8.2), IL-18 3.5 (1.5-8.0), ACR 4.6 (1.8-11.1), plasma NGAL 2.2 (0.99-4.7), and plasma NGAL 11.64 (4.2-32.4). After including potential confounders changes in SCr in the clinical model, IL-18 4.0 (1.5-10.4) and plasma NGAL 9.7 (3.4-27.5) were all independently associated with AKI progression. Each biomarker provided improvement in risk classification over the clinical model alone with plasma NGAL performing the best: Net Reclassification Index 0.53 (p=0.0001) and Integrated Discrimination Improvement 0.11 (p=0.0001).

Conclusions: Biomarkers of AKI at the time of clinical criterion rise improve risk stratification and identify patients with newly diagnosed AKI who are at the highest risk for the most severe forms of AKI and the worst patient outcomes.

Funding: NIDDK Support, Other NIH Support - R01HL-085757
Conclusions: AKI duration regardless of stratification by AKIN, is independently associated with poor outcomes. It could provide additional prognostic information than the rise of serum creatinine alone. These data needs to be validated by prospective studies of AKI. Finally, the longer duration of renal failure is associated with a risk of a high final serum creatinine. The latter could be a risk factor for CKD in the long term.

TH-PO056
Plasma Neutrophil Gelatinase-Associated Lipocalin Predicts Acute-on-Chronic Kidney Injury after Adult Cardiac Surgery: A Multicenter Prospective Study
Kent Doi,1 Masahiro Urata,2 Daisuke Katagiri,1 Kousuke Negishi,1 Toshiro Fujita,1 Seiichiro Murata,3 Motoyuki Hisagi,1 Minoru Ono,1 Eisie Noiri,1 1Nephrology and Endocrinology, University of Tokyo, Japan; 2Cardiovascular Surgery, Itabashi Central General Hospital, Japan; 3Cardiothoracic Surgery, University of Tokyo, Japan.

Background: Plasma NGAL is a recently developed new AKI biomarker and previous clinical evaluations for plasma NGAL focused on only AKI occurred in patients without CKD. It is unclear whether plasma NGAL can predict acute-on-chronic kidney injury after cardiac surgery, because CKD significantly increases plasma NGAL levels in stable condition and may hamper the prediction of AKI by plasma NGAL.

Methods: The present study prospectively evaluated 143 adult patients who had cardiac surgery at two general hospitals. Plasma NGAL was measured before surgery, at ICU arrival (0 hr), and 2, 4, 12, 24, 36 hr after. AKI was diagnosed by the AKIN criteria. 67 patients (46.9%) were complicated with CKD (eGFR<60) before surgery.

Results: Of 143 patients, 54 patients (37.8%) developed AKI after surgery and a multiple logistic regression analysis revealed that complication of CKD was significantly associated with AKIN occurrence. Plasma NGAL before surgery, at 0, 2, 4, 12, 24, and 36 hr after ICU arrival in AKI patients were significantly higher than non-AKI both in CKD and non-CKD populations (Fig). ROC analysis showed the highest area under the ROC curve with plasma NGAL at 4 hr in CKD and non-CKD populations (CKD 0.738, non-CKD 0.809). The cutoff values of AKI prediction were 158 ng/ml in CKD and 97 ng/ml in non-CKD.

Conclusions: Plasma NGAL measured at pre-surgery and early time points in the post-surgery (4 hr) will predict AKI not only in non-CKD patients but CKD patients when the cutoff values properly determined.

Funding: Government Support - Non-U.S.

TH-PO057
Pre-Operative Serum Brain Natriuretic Peptide and Risk of Acute Kidney Injury after Cardiac Surgery

Background: Acute kidney injury (AKI) following cardiac surgery is associated with poor outcomes and is difficult to predict. We conducted a prospective study to evaluate whether pre-operative brain natriuretic peptide (BNP) levels predict postoperative AKI among patients undergoing cardiac surgery.

Methods: The TRIBE-AKI Consortium enrolled 1,139 adults undergoing cardiac surgery at six hospitals from 2007-2009, who were selected for high AKI risk. Pre-operative BNP was categorized into quintiles (Fig).

Results: AKI was common using AKI Network definitions; at least mild AKI was a ≥0.3mg/dL or 50% rise in creatinine, n=407 (36%), and severe AKI was either a doubling of creatinine or the requirement of acute renal replacement therapy, n=58 (5.1%). In analyses adjusted for pre-operative characteristics, pre-operative BNP was a strong and independent predictor of mild and severe AKI. Compared with the lowest BNP quintile the highest quintile had significantly higher risk of mild AKI (risk ratio [RR] 1.87, 1.40-2.49) and severe AKI (RR 3.17; 1.06-9.48). After adjustment for clinical predictors, addition of BNP improved the area under the curve to predict mild (p=0.02) and severe AKI (0.73 to 0.75, p=0.11). Compared with clinical parameters alone, BNP also improved risk prediction of AKI cases into lower and higher risk [net reclassification index was 22.8% (p=0.0003) for mild AKI and 38.0% (p=0.0049) for severe AKI].

Conclusions: Pre-operative BNP level is a strong independent predictor of post-operative AKI in high-risk patients undergoing cardiac surgery. If confirmed in other types of patients and surgeries, pre-operative BNP levels may improve risk stratification and discrimination among surgical candidates.

Funding: Other NIH Support - The research reported in this article was supported by the American Heart Association Clinical Development award, the grant R01HL-085757 from the National Heart, Lung, and Blood Institute. The study was also supported by CTSA Grant Number UL1 RR024139 from the National Center for Research Resources (NCRR). The plasma BNP assay was donated by Biosite. The granting agencies and Biosite, Inc. did not participate in the protocol development, analysis and interpretation of the results.

TH-PO058
P-Creatinine Production Is Reduced in Septic Patients on Continuous Renal Replacement Therapy
Pei-Chen Wu,1 Ravindra L. Mehta.2

Background: Doi et al. reported that sepsis resulted in reduced creatinine production (Pc) in a cecal ligation and puncture of necrophatized mouse model (J Am Soc Nephrol. 2009; 20(6): 1217-21). Reduced Pc may hinder the elevation of serum creatinine (SCr) and thus delay the diagnosis of acute kidney injury (AKI). This finding has not yet been validated in humans.

Methods: This is a retrospective study of patients treated with CVVHDF from June 2008 to August 2010 at an academic medical center. Sepsis was defined as ≥ 2 SIRS signs suspected or known to be caused by a bacterial or fungal infection. All patients had serum and effluent samples analyzed for urea nitrogen and creatinine every 12 hours during therapy. Once patients were in steady state, usually at least 48 hours after CVVHDF initiation, we computed daily Pc using an equation published previously (Am J Kidney Dis. 2009; 53: 444-453). Pc of the first 4 days in steady state was recorded and analyzed. Every SCr was computed daily Pc using an equation published previously (Am J Kidney Dis. 2009; 53: 444-453).

Results: There were total 221 treatment-days in steady-state in 65 patients (mean age ± SD was 55.7 ± 14.9 years, 81.5% was male). Pc was lower in septic patients.

Conclusions: Plasma NGAL measured at pre-surgery and early time points in the post-surgery (4 hr) will predict AKI not only in non-CKD patients but CKD patients when the cutoff values properly determined.

Funding: Government Support - Non-U.S.
Conclusions: Our results show that creatinine production is altered in septic patients requiring CVVHD. Additional studies are required to ascertain the mechanisms for reduced creatinine production. Serum creatinine levels may understate the severity of renal dysfunction in septic patients requiring dialysis and should be used with caution as a marker of AKI in sepsis.

Funding: Government Support - Non-U.S.

TH-PO059

The Utility of Fractional Excretion of Sodium and Urea in Advanced Chronic Kidney Disease

Tarak Noureideen, Joel Topf

Nephrology, St John Hospital and Medical Center, Detroit, MI.

Background: The most common etiologies of acute renal failure are prerenal azotemia and acute tubular necrosis (ATN). The fractional excretion of sodium (Fe Na) and urea (Fe urea) are often used to differentiate between these two entities. Little is known regarding the effect of advanced chronic kidney disease on the validity of these measurements.

Methods: We retrospectively analyzed 33 patients with acute renal failure. Data elements included Fe Na, Fe urea, baseline creatinine, estimated glomerular filtration rate, age, diuretic use and time from diagnosis to measurement of acute renal failure indices. Patients were classified to be ATN or pre-renal azotemia based on response to therapy, judgment of the treating nephrologist and objective data including ultrasound, microscopic urine analysis and biochemical findings. Data were analyzed using $\chi^2$ and ROC analysis.

Results: Fe urea did not assist in differentiating pre-renal azotemia from ATN. The data shows, however, that as kidney function deteriorates the fractional excretion of sodium increases independent of diuretic use. Using ROC analysis we found that a cut point of 2.4% rather than 1% increased the accuracy of Fe Na in discriminating pre-renal azotemia vs. ATN. In our population of patients with advanced chronic kidney with a mean glomerular filtration rate of 39 ml/min.

Conclusions: Fe urea was not a reliable indicator of pre-renal azotemia vs. ATN. However, Fe Na was a useful test using a cut point of 2.4% in our population of patients with advanced kidney disease presenting with acute renal failure.

TH-PO60

Plasma and Urine Neutrophil Gelatinase-Associated Lipocalin (pNGAL) and uNGAL) as a Novel Indicator for Early Identifying Critically Ill Acute Kidney Injury (AKI) Patients Who Subsequently Require Replacement Therapy

Tarik Noureldeen, Chulalongkorn University, Bangkok, Thailand.

Background: The high mortality in critically ill AKI patients still persisted despite the advancement in renal replacement therapy (RRT). This might be explained by a delay in initiating RRT caused by using current traditional indicators. The levels of pNGAL and uNGAL which were correlated with the degree of tubular cell injury might be utilized as the novel indicator for early initiation of RRT. This prospective cohort study was conducted to determine the accuracy of using pNGAL as a predictor in early identifying the AKI patients who subsequently needed RRT.

Methods: Forty seven critically ill AKI patients with RIFLE stage II-III who did not reach the diagnosis of renal failure and to predict mortality. Measurement of uNGAL can be useful in assessing acute renal failure in cirrhosis.

Results: Of forty-seven critically ill AKI patients (31 males) with mean age 63.0±18.1 years had APACHE II score 18.8±7.7. The serum creatinine level at enrollment was 2.35±0.93 mg/dL. pNGAL could predict further RRT requirement with area under ROC 0.813 (p<0.001, 95%CI 0.86-0.90). At cut-point of 960 ng/mL provided specificity and sensitivity of 72.2 and 89.6% as well as positive and negative predictive of 81.25 and 83.8%. The uNGAL which could be obtained from thirty-three non-anuric patients were provided slightly lower area under ROC 0.806 (p=0.005, 95%CI 0.63-0.98). At cut-point of 2600 ng/mL provided sensitivity and specificity of 34.5 and 90.9% as well as positive and negative predictive of 75.0 and 80.0%.

Conclusions: The pNGAL is an excellent early biomarker for RRT-initiation in critically ill AKI patients. Moreover, the cut-point 960 ng/mL might be used as the early new indicator for early initiation of RRT that might improve patient survival.

TH-PO601

Evaluation of the Effect of Renin Angiotensin Aldosterone System (RAAS) Blockade on Glomerular Hemodynamic and Renal Function in Patients Undergoing Intravascular Contrast Studies

Shiti Arora, Prachi Aggarwal, Anubhav Kaul, Anmar Husan, Shivtej Kaushal, Manu Bansal, Ajay Gupta, Ejaz H. Chokhavatla, Julian M. Dupont, Internal Medicine, Wyckoff Heights Medical Center, Brooklyn, NY.

Background: Contrast induced nephropathy (CIN) is the third leading cause of acute renal failure. Along with other preventative measures, there are studies suggestive of improvement in renal hemodynamics and function with the use of RAAS blockade. Current recommendations are to withhold ACEI and ARB 48 hours prior to administration of contrast. Our study aims to evaluate the effect of RAAS blockade on renal function in patients undergoing intravenous contrast exposure.

Methods: In a retrospective study, 239 patients who had received IV contrast and hydratation as per protocol were selected. Group 1 (study group) included patients on RAAS blockade and group II (control group) patients were without RAAS blockade. Creatinine clearance was calculated using the Cockcroft-Gault (C-G) formula before and 48-72 hours after contrast exposure. CIN was defined as 25% or 0.5mg/dl increase in creatinine from baseline.

Results: The prevalence of CIN was 14.6% in patients within the control group versus 17% in the study group, demonstrating no significant difference. Patients in the study group had a mean change in GFR of 2.77±2.11 ml/min compared to 8.24±2.70 ml/min in the control group, demonstrating neither significant difference between the two groups (p=0.112) nor any decline in renal function post IV contrast administration. Linear regression analysis showed no major differences in variables predicting change in GFR; however, the presence of hypertension had a partial correlation of -0.227 (p=0.35) with change in GFR in the study group; whereas age had a partial correlation of -0.180 (p=0.036) with change in GFR in the control group.

Conclusions: Our study demonstrates that there was no significant difference in the serum creatinine and GFR post contrast administration with or without RAAS blockade. Therefore, we theorize the fact there is no beneficial effect in withholding RAAS therapy prior to contrast exposure.

TH-PO062

Evaluation of 4 Urinary Biomarkers in Assessing Renal Failure in Cirrhosis. Role for Urinary Neutrophil Gelatinase-Associated Lipocalin (uNGAL)


Hospital Clinic, Barcelona, Spain.

Background: Urinary biomarkers are useful in assessing acute renal failure and outcome prediction in various clinical settings. There is little information on their relevance in cirrhosis.

Methods: Aim: to evaluate the usefulness of 4 urinary biomarkers in the differential diagnosis of renal failure and evaluate prognosis in cirrhotic patients. Urinary neutrophil gelatinase-associated lipocalin (uNGAL), Kidney injury molecule (KIM)-2 microglobulin, and N-Acetyl glucosaminidase (NAG) were measured in 241 patients.

Results: 71 without ascites, 88 with ascites without renal failure, and 82 with ascites and renal failure (defined by serum creatinine > 1.5mg/dL) due to different causes. All biomarker levels, except KIM-2 microglobulin, were higher in patients with renal failure compared to those without renal failure. Of all biomarkers, uNGAL was the best to discriminate among the different causes of renal failure. Patients with acute tubular necrosis (ATN, n=11) and infection-associated renal failure (n=12) had higher values (324 (195-1018) and 284 (73-486) ng/mg creatinine, respectively) median (IQR), while patients with hypovolemia-related renal failure (n=16) and hepatorenal syndrome (n=22) had lower values (30 (20-59) and 74 (43-147) ng/mg creatinine, respectively). Neither KIM-1 nor NAG were able to discriminate between causes of renal failure. KIM-2 microglobulin was similar in all causes except for higher levels in patients with ATN. Patients with high uNGAL (≥44 mg/mg creatinine) had lower 30th survival compared to patients with low uNGAL (76% vs 85%, respectively; p=0.0038). A logistic model performed to estimate the effect of biomarkers on 30th survival showed that only uNGAL and the presence of renal failure had independent prognostic value.

Conclusions: In conclusion, only uNGAL was capable of distinguishing between causes of renal failure and to predict mortality. Measurement of uNGAL could be useful in assessing renal failure in cirrhosis.

Funding: Government Support - Non-U.S.

TH-PO063

Comparison of Clinical, Biochemical and Biomarker Predictors of Acute Kidney Injury in Cardiac Surgery

Ganesh Kambhampati, Mourad Alssabgh, Gurjit Dhatt, Abdo Asmar, Uma Krishna Pukkaventana, A. Ahsan Ejaz.

Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL.

Background: We investigated the utility of clinical, biochemical and novel biomarkers to predict acute kidney injury in cardiac surgery.

Methods: Data from a prospective observational study were analyzed to compare the utility of positive fluid balance, serum MCP-1, TNF-alpha, IL-8, urine NGAL and urine IL-18 to predict acute kidney injury as measured by creatinine.

Results: 100 patients were analyzed. Receiver operating characteristic (ROC) curves were analyzed to determine each test’s overall accuracy, as measured by the area under the curve.

The diagnostic performance of the various tests as measured from the AUC (95%CI) is as follows- AUC for 24 hr FB was 0.6695 (0.5434-0.7955, p = 0.004), sIL-18 was 0.6540 (0.5247-0.7833, p = 0.0100), uNGAL was 0.6238 (0.4952-0.7523, p = 0.0214), sIL-8 was 0.6788 (0.5554-0.8023, p = 0.0092), sMCP1 was 0.6603 (0.5339-0.7867, p = 0.0195) and sTNF-alpha was 0.7664 (0.6556-0.8771, p = 0.0004). At 24hrs, positive FB provided similar information as urine NGAL and urine IL-18 to predict acute kidney injury. The positive predictive power of sIL-8, sMCP-1 and sTNF-alpha was better compared to 24hr fluid balance.

Conclusions: None of the tested biomarkers were better than urine NGAL and urine IL-18 to predict acute kidney injury as measured by creatinine.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author. 124A
CONCLUSIONS: Positive FB at 24-hours following cardiac surgery may be an early and simple diagnostic tool to predict AKI. It is as good as the novel biomarkers such as urine NGAL and urine IL-8, sILR, sILP-1 and STNF-alpha were better predictors of AKI compared to positive FB.

TH-PO064

Iodine/eGFR Is a Simple, Useful Indicator for Determining the Safe Contrast Media Dosage To Avoid Contrast-Induced Nephropathy during Coronary Angiography  Moon-Jae Kim.1  1Division of Nephrology & Hypertension, Inha University Hospital, Incheon City, Korea; 2Division of Nephrology, Department of Internal Medicine, Soonchunhyang University Bucheon Hospital, Bucheon-Si, Korea.

Background: Contrast-induced nephropathy (CIN) has been commonly defined as a sudden, rapid deterioration in renal status after study of iodinated contrast medium (CM) in the absence of any other causes. To avoid the risk of developing contrast-induced nephropathy (CIN), it has been suggested that patients be subjected to a minimal necessary dose of contrast medium (CM-dose).

Methods: The ratio of CM-dose to eGFR in predicting the risks of CIN was assessed and sought to determine the safe level of CM-dose/eGFR in patients undergoing coronary angiography (CA). We enrolled a total of 226 patients and calculated the ratio of CM-dose to eGFR.

Results: Overall, there were 16 cases (7.1%) of CIN. On univariate and multivariate regression analysis, g-I/eGFR alone was found to be an independent predictor for CIN. The ratio of CM-dose to eGFR in predicting the risks of CIN was assessed and sought to determine the safe level of CM-dose/eGFR in patients undergoing coronary angiography (CA). We enrolled a total of 226 patients and calculated the ratio of CM-dose to eGFR.

Conclusions: Iodine/eGFR is a simple, useful indicator for determining the safe CM-dose.

TH-PO065

A Biomarker Panel of Plasma Neutrophil Gelatinase-Associated Lipocalin and Endotoxin Activity Assay in Septic Acute Kidney Injury  Daisuke Katagiri,1 Kent Doi,1 Kousuke Negishi,1 Takehiro Matsubara,2 Naoki Yahagi,2 Toshiro Fujita,1 Eisei Noiri.1  1Nephrology and Endocrinology, University of Tokyo, Japan; 2Critical Care Medicine, University of Tokyo, Japan.

Background: Plasma NGAL and endotoxin activity assay (EAA), which is a rapid ex vivo diagnostic test using the biological response of patient neutrophils to an immunological complex of endotoxin, are predictive for sepsis. This study evaluated a biomarker panel consisting of these two markers in AKI.

Methods: We evaluated two cohorts of 55 AKI patients who needed CRRT and 40 AKI patients who did not receive CRRT in ICU. Plasma NGAL and endotoxin activity (EA) in whole blood were measured using the Triage NGAL Device (Alere, USA) and the EAA system (Spectral Diagnostics, Canada).

Results: Plasma NGAL and EA values in septic AKI patients were significantly higher than in the other AKI patients (Table). A scatter diagram of plasma NGAL and EAA demonstrated the combination of these biomarkers enhanced the accuracy over each biomarker (Figure). The combination can detect sepsis in AKI with high AUC-ROC values both in the CRRT and the non-CRRT group (AUC-ROC 0.92 and 0.98, respectively).

Conclusions: Sepsis and septic AKI are extremely complex and have multiple pathophysiological mechanisms. Therefore, combinations of biomarkers reflecting different pathways might be necessary for these complicated conditions. The present report describes a new biomarker panel of septic AKI with plasma NGAL (AKI and neutrophil activation) and EAA (endotoxemia), which is a useful diagnostic tool for septic AKI.

Funding: Government Support - Non-U.S.

TH-PO066

Plasmapheresis Rescue Therapy in Progressive ANCA-Associated Systemic Vasculitis  Anoek A.E. Joode, de, Johannes S. Sanders, Coen A. Stegeman. Internal Medicine/Nephrology, University Medical Center Groningen, Groningen, Netherlands.

Background: We evaluated outcome of 25 patients with ANCA-associated vasculitis (AAV) with progressive disease despite treatment with cyclophosphamide and high dose steroids treated with additional plasmapheresis (PPh). Outcome was compared with 47 matched-controls (C).

Methods: Patients newly diagnosed with AAV from January 1990 until December 2009 were evaluated (n=72). Patients were included when PPh was not started at diagnosis but was added for progressive disease during the course of initial standard therapy (n=25). We selected controls matched for BVAS and creatinine at diagnosis. Primary endpoint was eGFR or death.

Results: Plasmapheresis was added in 25 patients at 22 (range 2 to 51) days after start of therapy. In 20 of 25 patients a rise in serum creatinine > 30% despite induction therapy led to PPh. In 14, renal biopsy showed ongoing disease-activity, in 6 the disease was...
made on clinical ground. Progressive pulmonary disease and progressive necrotic lesions were seen on lung biopsies.

Renal involvement was present in all patients; in the PPh- group, 5 patients temporarily needed dialysis, 1 patient needed dialysis in the C-group. At 6 months and 5 years, 2 and 9 patients had died in both groups (RR 1.29; 95% CI 0.37-4.75).

At baseline mean eGFR was 46 ml/min/1.73m2 in PPh-group versus 43 in C-group, while at start of PPh eGFR was 25 ml/min/1.73 m2. At 6 months mean eGFR in the PPh- group had significantly improved (p=0.0001) and was 49 and 50 respectively in both groups. During long-term follow-up there was no difference in renal function between the groups at 12 months and 5 years.

Relapses occurred in 13 patients in PPh-group and in 20 in C-group (p=0.44). However, time to first relapse differed significantly between the PPh- and C- group (p=0.0084).

Conclusions: Patients with progressive disease despite induction therapy with cyclophosphamide and steroids in whom plasmapheresis was added, had significant improvement in renal function and similar long-term outcome as matched controls. Research is needed to determine factors that identify patients early in the course of therapy in whom plasmapheresis should be instituted.

TH-PO067
Proteinuria Independently Associate with the Development of Acute Kidney Injury in Intensive Care Unit
Fen Jiang, Xinling Liang, Wei Shi, Yuan Han Chen, Wenjian Wang, Penghua Hu. Division of Nephrology, Guangdong General Hospital, Guangdong Academy of Medical Sciences, Guangzhou, Guangdong Province, China.

Background: Proteinuria as a very important factor for the increasing mortality of chronic kidney injury(CKD), but the role of proteinuria for acute kidney injury(AKI) is still unclear. The purpose of the study is to analyze the relationship between proteinuria and AKI in adult Intensive care unit(ICU).

Methods: From October 2009 to July 2010 patients≥18 years who in ICU were enrolled and retrospectively evaluated. All patients were free of chronic disease for three months before admission and both of the levels of serum creatinine and urinary protein in the first 48 hours after admitted in ICU were recorded. Both RIFLE and AKIN criteria were employed for the diagnosis and staging of AKI in ICU. Dip-stick was used to detect proteinuria, and over the reference category: no proteinuria, mild-proteinuria and heavy-proteinuria by the level of urine protein.

Results: Five hundred and twenty-four patients were included in this study. Ninety-nine patients were diagnosed as AKI by RIFLE criteria, while the number increased to one hundred and five according to AKIN criteria. One hundred and fifty-eight patients were diagnosed as AKI by RIFLE criteria and the dip sticks maybe an effective method for early diagnosis of AKI in ICU, and the dpi sticks may be an effective method for early diagnosis of AKI.

Funding: Government Support - Non-U.S.

TH-PO068
Utility of Urinary HGF for Differential Diagnosis of Acute Kidney Injury in Cirrhotic Patients
Won K. Han, John R. Fontanilla. Medicine/Nephrology, Thomas Jefferson University Hospital, Philadelphia, PA.

Background: To test the utility of a single measurement of urinary biomarkers in distinguishing acute tubular injury from prerenal azotemia as AKI, a prospective study was conducted in 90 cirrhotic patients with or without acute kidney injury (AKI) at a single institution.

Methods: Urine samples were collected within 48 hours from initial admission and/or at the time of renal consultation during hospitalization (63 AKI and 27 non-AKI). Urine concentrations of hepatocyte growth factor (HGF), kidney injury molecule-1 (KIM-1), and N-acetyl-b-D-glucoaminidase (NAG) were assessed. AKI was defined as a greater than 50% increase in serum creatinine from baseline. Patients were followed during their hospital course through clinical and laboratory data to determine etiology of AKI.

Results: Patients with acute tubular injury (n=21) had a significantly elevated median urinary HGF level compared with PRA and non-AKI groups (48) (1.81 ng/mg urine creatinine, p=0.0001). A cut-off value of 0.88 ng/mg in PRA group, sensitivity and specificity of HGF for detecting acute tubular injury were 0.86 (95% CI, 0.64 to 0.97) and 0.83 (95% CI, 0.70 to 0.93), respectively. These values were superior to those for KIM-1 and NAG. Urima KIM-1 was not a sensitive biomarker for distinguishing AKI in this study.

Conclusions: In conclusion, our results demonstrate that a single measurement of urinary HGF helps to distinguish acute tubular injury from normal function and PRA in patients with cirrhosis.

TH-PO069
Urinary Cystatin C as a Marker for Tubular Dysfunction Associated with Sepsis in Patients with Severe Sepsis and Septic Shock
Castro Ortuño Anderiz,1 Alberto Barrientos,2 Antonio Cruceyra,2 Gonzalo Navarro,1 Marta Cubells,1 Carmen Gijon,1 Enrique Morales,1 Antonio Blesa,2 Miguel Angel Gonzalez,2 Fernando Martinez,1 Sonia Vazquez,1 Miguel Sanchez Garcia,1 Critical Care, Hospital Clinico San Carlos, Madrid, Spain; 2Nephrology, Hospital Clinico San Carlos, Madrid, Spain; 3Biochemistry, Hospital Clinico San Carlos, Madrid, Spain.

Background: Preliminary data in heterogeneous patient groups suggest that urinary cystatin C (UCysC) increases as a function of renal tubular injury, independent of glomerular filtration rate. We prospectively studied the correlation of UCysC levels with renal function and the need for renal replacement therapy (RRT) in patients presenting to our ICU with severe sepsis or septic shock (SS/SSS).

Methods: Consecutive patients with SS/SSS were included if plasma creatinine (Pcr) < 2 mg/dl. We also studied a control group of simultaneous patients admitted without SS/SSS. Cases were followed for 5 days in vivo acute kidney injury (AKI) ensued and until recovery of renal function or for a maximum of 5 days. We used univariate analysis with Student’s t-test or non-parametric tests as appropriate.

Results: We enrolled 53 cases, 50 (84.7%) in septic shock, and 44 controls, 9 (20.5%) in non-septic shock, over 20 months. Age, sex and admission group were similar between groups. CRP levels were 1.0±0.3 mg/L in PPh-group versus 1.0±0.4 mg/L in C-group (p=0.177, OR 1.89; 95%CI 0.90-3.94; p=0.121) or AKIN criteria (OR 3.478; 95%CI 1.544-7.839; p=0.003) or AKI patients had a different level of urinary protein compared with the no-AKI patients [OR 3.478; 95%CI 1.544-7.839; p=0.003] or AKIN criteria(OR 3.589; 95%CI 1.679-7.668; p=0.003) while at start of PPh eGFR was 25 ml/min/1.73 m2. At 6 months mean eGFR in the PPh-group had significantly improved (p<0.0001) and was 49 and 50 respectively in both groups.

In conclusion, our results demonstrate that a single measurement of urinary biomarkers in distinguishing acute tubular injury from prerenal azotemia (PRA), a prospective study was conducted in 90 cirrhotic patients with or without acute kidney injury (AKI) at a single institution.

Methiods: Urine samples were collected within 48 hours from initial admission and/or at the time of renal consultation during hospitalization (63 AKI and 27 non-AKI). Urine concentrations of hepatocyte growth factor (HGF), kidney injury molecule-1 (KIM-1), and N-acetyl-b-D-glucoaminidase (NAG) were measured. AKI was defined as a greater than 50% increase in serum creatinine from baseline. Patients were followed during their hospital course through clinical and laboratory data to determine etiology of AKI.

Results: Patients with acute tubular injury (n=21) had a significantly elevated median urinary HGF level compared with PRA and non-AKI groups (48) (1.81 ng/mg urine creatinine, p=0.0001). A cut-off value of 0.88 ng/mg in PRA group, sensitivity and specificity of HGF for detecting acute tubular injury were 0.86 (95% CI, 0.64 to 0.97) and 0.83 (95% CI, 0.70 to 0.93), respectively. These values were superior to those for KIM-1 and NAG. Urima KIM-1 was not a sensitive biomarker for distinguishing AKI in this study.

Conclusions: In conclusion, our results demonstrate that a single measurement of urinary HGF helps to distinguish acute tubular injury from normal function and PRA in patients with cirrhosis.
The Value of Urine L-FABP Level as a New Biomarker after Cardiac Surgery in Adults

Liu Shang, MiaoLin Che, Renhua Lu, Zhaohui Ni, Jia Qiu Tian, Yucheng Yan. Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Background: Acute kidney injury (AKI) is one of the most common and serious complications of cardiac surgery associated with substantial morbidity and mortality. A good biomarker can help early detection of AKI and perhaps, interventions. This study is to determine the value of uL-FABP of predicting the development and the prognosis of AKI following cardiac surgery.

Methods: By examining serum creatinine and uL-FABP corrected by urine creatinine at preoperative, 6h and 2h postoperative time points, we compared the differences between AKI and non-AKI groups, complete and incomplete renal recovery groups, patient survival and death groups. ROC curves were used to evaluate their predictive accuracy.

Results: As a Result, We found the levels of urine L-FABP were both significantly higher in AKI patients compared to non-AKI at 6h and 2h after cardiac surgery (7247.68 vs 174.43 ng/ml and 3474.99 vs 118.71 mg/ml, both P<0.001) and the AUCs at 6h and 2h postoperative to predict AKI and AKI stage II-III were 0.844, 0.818 and 0.832, 0.805 respectively. Patients with incomplete renal recovery at discharge had higher levels of urine L-FABP at 2h postoperative (12051.9 vs 2871.10 ng/ml, P=0.05) and it showed good predictive values for detecting renal prognosis with the AUCs of 0.775. Similar results were found the level of uL-FABP at the two time points postoperation might be useful in predicting hospital mortality (14093.98 vs 292.11ng/ml and 7528.33 vs 200.68ng/ml, all P<0.05), with the AUCs of 0.893 and 0.873.

Conclusions: In conclusion, urine L-FABP which increased at the early stage after cardiac surgery might be an alternative biomarker for the early detection of AKI and comosite end points.

Funding: Government Support - Non-U.S.

TH-PO072

A Comparison of Clinical Outcomes between the Selective Cytopheretic Device and Case-Matched Historical Controls from the PICARD Database

Ravindra L. Mehta,1 Alexander S. Yevzlin,2 James A. Tumlin,3 Lakhmir S. Singal,1 Ashita J. Tolwani,5 John J. Dillon,6 Kevin W. Finkel,7 J. Ricardo Da Silva,8 Ana Maria Perez,9 Elisa Mulay,9 Joseph H. Tommaso,9 William L. Mentzer,10 J. Ricardo Da Silva,11 Department of Medicine; 2University of Wisconsin; 3SERRI; 4George Washington University; 5University of Alabama; 6University of Texas; 7Cytopherx, Inc.; 8University of Michigan.

Background: Dialysis-dependent acute kidney injury (AKI) is a significant complication in ICU patients and is associated with hospital mortality rates between 50-60%. A recent pilot study of the Selective Cytopheretic Device (SCD), a novel cartridge that is able to selectively bind and deactivate neutrophils as an adjunct to CRRT therapy, showed improved in kidney function and mortality rate.

Methods: We conducted a retrospective analysis of the PICARD database and the SCD pilot study, comparing mortality and dialysis dependence in both groups. 35 subjects from PICARD were matched to 35 subjects from the SCD pilot study using the diagnosis of AKI, CRRT therapy, and SOFA score. Logistic regression was used to compare mortality as the dependent variable and age, SOFA score, and presence of SCD therapy as independent variables.

Results: Mean age did not differ significantly between the two groups: 60.2 vs. 56.9 in PICARD and SCD respectively (t-test, p=0.12). Mean SOFA score did not differ significantly between the two groups: 12.38 ± 11.3 in PICARD vs. SCD, respectively (t-test, p=0.80). The in-hospital mortality rate was 56.9% in PICARD vs. 31.4% 60 day mortality in SCD (chi square, p=0.08). Logistic regression revealed odds ratio of death to be 1.02 (CI 0.1598 to 1.0355, p=0.38) for age, 1.22 (1.025 to 1.4635, p=0.03) for SOFA score, and 0.28 (0.09797 to 0.7971, p=0.02) for SCD therapy. 7.7% (1/13) of survivors from PICARD were matched to 35 subjects from the SCD pilot study, comparing mortality and dialysis dependence in both groups. 35 subjects underwent interventions. One of pts was start on dialyses and exclude from analyses. Factors influencing CysC were analyzed performing a linear mixed model to take account of the repeated measures.

Conclusions: In our 20 pts, CysC decreases rapidly in the first month (M) (16.2%) p<0.001), slower between 1 M and 1 year (y) (3.9% per month, p<0.001) and stabilizes after 1 y (0.2% per month, p=0.83). CysC was significantly increased in pts with bilateral KM (p=0.02) and in TCF2 pts (p=0.002). The decrease of the CysC over time was less pronounced in pts with unilateral KM (p=0.04) and in TCF2 pts (p<0.001), these pts therefore presenting a worse prognosis in RF. Creatinine decrease with age, rapidly the first M (p<0.001) and then stabilized.

Conclusions: Renal function follow-up in pts diagnosed with CAKUT, using CysC showed a worse prognosis over time in pts with bilateral kidney malformation or TCF2 mutation.

Funding: NIDDK Support

TH-PO074

Early Urinary Levels of Cell Death Markers Predict AKI after Cardiopulmonary Bypass

Michael R. Bennett, Nunnatau PYianneh, Catherine D. Krawczeski, Prasad Devarajan. Cincinnati Children’s Hospital Medical Center.

Background: Acute kidney injury (AKI) occurs commonly after cardiopulmonary bypass (CPB), often as a result of ischemia reperfusion injury (IRI). Serum creatinine (Scr) is an inadequate marker for AKI. Studies of animal models of IRI have demonstrated that the apoptotic pathway plays a significant role in the development of AKI. We set out to determine if apoptotic markers could be detected in the urine of patients undergoing CPB to determine if they could predict AKI, and to help pinpoint the timing of kidney cell death in these patients.

Methods: Urine samples were obtained prospectively before and at intervals after CPB in 391 patients. AKI was defined as a ≥50% Scr rise within 48h of CPB. For this pilot - we selected 35 patients, 18 of which developed AKI, and measured caspase-cleaved cytokeratin 18 (M-30, a marker of apoptosis), as well as total cytokeratin (M-65, total cell death) using commercially available ELISAs. Measurements were made at 0, 4 and 12 hours post CPB. These markers have recently been found to be elevated in urine of patients with chronic kidney disease.

Results: Defined by pRIFLE criteria, the AKI group consisted of R (n=8), I (n=8) and F (n=2). AKI and non-AKI groups did not differ significantly by race, sex or length of CPB. The median age of the AKI group (0.5 yrs IQR=0.4-1.6) was significantly lower than the non-AKI group (p=0.001; 7 yrs IQR=4.7-13.6). M30 levels were not significantly different between AKI and non-AKI groups at any time point. Markers of total cell death, however, were markedly increased at 4 hours post CPB in the AKI group (medium 1467 U/L, IQR=429-3060) vs the non-AKI group (p=0.003; 158 U/L, IQR=62-507). Total cell death levels did not differ at 0 or 12 hours post CPB.

Conclusions: While urinary levels of the apoptotic marker M30 were not different between AKI and non-AKI groups, total cell death was greatly increased at as little as 4 hours post CPB. This indicates that significant cellular damage occurs very early after CPB and makes early diagnosis and intervention imperative. This data also indicates that urinary markers of cell death may be valuable early biomarkers of AKI.

Funding: NIDDK Support

TH-PO075

Efficacy of Urine Neutrophil Gelatinase-Associated Lipocalin as a Predictive Marker for Acute Kidney Injury in Different Patient Populations

Kianoush Banaei-Kashani,1 Laura N. Hanson,2 Otto Stephan Schwarz Vignolo,3 John C. Lieske.1 1Nephrology and Hypertension, Mayo Clinic, Rochester, MN; 2Mayo Validation Support Services, Mayo Clinic, Rochester, MN; 3Pulmonary Critical Care, Atlanta Pulmonary Group/Critical Care of Atlanta, Atlanta, GA.

Background: Increased urine uNGAL (uNGAL) excretion is a promising biomarker to predict subsequent Acute Kidney Injury (AKI). In this study we evaluated its utility among specific patient populations within a single center prospective cohort at a tertiary medical center.

Methods: All ICU admissions were screened and high risk patients with at least 30 minutes of hypotension were evaluated for possible inclusion. All patients who met RIFLE criteria for AKI were enrolled. All subjects provided consent, 204 patients were followed for seven days or until hospital discharge for AKI as defined using RIFLE criteria (I or greater). ROC curves were prepared to assess uNGAL at ICU admission as a predictor of subsequent AKI, stratified by patient characteristics.

Results: Baseline serum creatinine was an overall poor predictor of AKI. uNGAL performed reasonably well in medical but not surgical ICU patients (AUC 0.71 vs 0.53, Table 1). When stratified by diagnosis (Table 2), in a univariate analysis uNGAL performed better in patients with congestive heart failure and sepsis (AUC 0.70 and 0.66) compared to those with diabetes (AUC 0.53).

Conclusions: uNGAL performance as a biomarker of AKI varies between patient groups. When assessing biomarkers for detection of AKI, it is important to take into account the diagnoses, type of patients and comorbidities of the patients being evaluated.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

127A
Compared to Delayed AKI in Hospitalised Patients

Hannah R. Wilson, Jennifer Black, Amy Irvine, Scott R. Henderson, Hannah E. Wilkinson, Bernard Freudenthal, Deepaaree Bangaru-Raju, Thomas Sanctuary, Mark T. Kiniron, Maria Ostermann.

Comparison of the Recognition and Management of Early Acute Kidney Injury Compared to Delayed AKI in Hospitalised Patients

Methods: During a 7 day period in May 2011, electronic patient records of all level 1 medical and surgical patients in a London teaching hospital were screened. Medical notes of patients with AKI as defined by the KDIGO criteria were reviewed by doctors independent from the treating team to assess compliance with NCEPOD recommendations.

Results: 50 patients had AKI on admission (52% male; mean age 73 years; 66% medical, 34% surgical). 94% had risk factors for AKI (median 2 [range 0-5]). 24 patients were classified as stage 1 AKI, 13 as stage 2, and 13 as stage 3.

Conclusions: Despite increasing publicity, AKI is poorly recognised both when present on admission and when developed in an inpatient. Even when recognised, adherence with NCEPOD recommendations is poor. More awareness and better tools are necessary to recognise AKI early and guide management.

TH-PO077
In Acute Kidney Injury; NGAL in Contrast to Procalcitonin Is a Biomarker of Injury Irrespective of Sepsis; Whereas the Latter of Sepsis Regardless of Injury


Epidemiology of Acute Kidney Injury in Newborns Born with Perinatal Depression

Methods: Between February 2010 and May 2011, we screened 146 infants of which 70 consented to the study. Serum creatinine (SCr) was measured daily from birth to day 7. Consent was obtained from a single parent or substitute legal guardian.

Results: 74 infants were included over 6 months. NGAL and Procalcitonin beside serum creatinine were measured.

Conclusions: NGAL predicted AKI 24 hours before RIFLE criteria with a concordance statistic (C-stat) of 0.72, which was the best among known AKI biomarkers. However, by multivariate logistic regression analysis identified a panel of 2 urine biomarkers that performed even better (C-stat of 0.8). The addition of uNGAL to the 2 biomarker panel did not improve the model.

Conclusions: We have identified two novel biomarkers that together predict AKI stages I and F 24 hours before RIFLE criteria. These biomarkers need to be validated in a second prospective cohort, and in other patient populations.

Multivariable logistic regression analysis

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Funding: Private Foundation Support

TH-PO080
Impact of Radiocontrast-Induced Nephropathy Following Coronary Angiography in Hospitalized Patients


Results: There was no statistically significant difference in the level of uNGAL in the different groups of diagnosis. There was no statistically significant difference between the level of uNGAL in the different groups of prognosis. Serum Procalcitonin was significantly higher in the septic patients than that in the non septic patients. The serum Procalcitonin level was the highest in the group of septic patients who died.

Conclusions: The correlation between serum creatinine and serum procalcitonin (r = 0.108, p = 0.649) is weak and insignificant preserving the diagnostic capacity of procalcitonin as a marker of sepsis even in the setting of AKI, also the correlation between uNGAL and serum procalcitonin (r = 0.094, p = 0.655) is weak and of no statistical significance. From the fact that procalcitonin is a well established marker for sepsis and the weak correlation between urinary uNGAL and serum Procalcitonin we can conclude that uNGAL is indeed unique for AKI regardless of sepsis.

TH-PO078
Novel Biomarkers of AKI: Multicenter Prospective Cohort Study

Methods: All ICU admissions in three tertiary medical centers were screened, and high risk patients with at least 30 minutes of hypotension, or sepsis were evaluated for possible inclusion. All patients who met RIFLE criteria at enrollment were excluded. After consent, 342 patients were followed for seven days or until hospital discharge for AKI as defined using RIFLE criteria (I or F). Blood and urine samples were collected at enrollment, 12 and 24 hour and then daily up to 7 days to measure serum creatinine, other known AKI biomarkers, and more than 140 novel biomarker candidates.

Results: Urinary NGAL predicted AKI 24 hours before RIFLE criteria with a concordance statistic (C-stat) of 0.72, which was the best among known AKI biomarkers. However, by multivariable logistic regression analysis identified a panel of 2 urine biomarkers that performed even better (C-stat of 0.8). The addition of uNGAL to the 2 biomarker panel did not improve the model.

Conclusions: We have identified two novel biomarkers that together predict AKI stages I and F 24 hours before RIFLE criteria. These biomarkers need to be validated in a second prospective cohort, and in other patient populations.

Multivariable logistic regression analysis

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Funding: Private Foundation Support
Methods: We retrospectively utilized a population-based linked administrative database of hospitalized patients who underwent coronary angiography from January 2008 through December 2009 to analyze in-hospital and long-term outcomes associated with RCIN. RCIN was defined as an increase in serum creatinine > 0.5 mg/dL, absolute increase in serum creatinine ≥ 0.5 mg/dL, or decrease in estimated glomerular filtration rate of ≥ 25% within 72 hours after contrast exposure. Patients with end-stage kidney disease and those exposed to other contrast medium or acute dialysis before the procedure were excluded.

Results: A total of 1,168 patients were included in the study. RCIN occurred in 82 of 382 patients (21.7%) with eGFR ≤ 60 mL/min/1.73m2 and in 108 of 596 patients (15.3%) with eGFR ≥ 60 mL/min/1.73m2. In the group with lower eGFR, the in-hospital mortality for those who developed RCIN was 12.2% (10/82) vs. 2.9% (11/382) for those without RCIN (P = 0.0002) but the 1-year mortality was not significantly different (6.9% [5/72] vs. 6.2% [23/371], P = 0.812). Additionally, patients with lower eGFR had more frequent irreversible increase in serum creatinine (> 0.5 mg/dL) and outpatient dialysis initiation when they developed RCIN (23.6% [17/72] vs. 10.8% [40/371], P = 0.0029 and 5.6% [4/72] vs. 1.1% [4/371], P = 0.009, mean follow up of 16 months, respectively). In the group with higher eGFR, both in-hospital and 1-year mortality were significantly higher in those who developed RCIN (5.6% [6/108] vs. 0.5% [3/596], P = 0.0001 and 8.6% [9/92] vs. 2.5% [15/593], P = 0.0013, respectively).

Conclusions: The occurrence of RCIN following coronary angiography in hospitalized patients is associated with higher in-hospital mortality independently of baseline eGFR. Patients with impaired baseline eGFR who develop RCIN tend to have more frequent long-term kidney function deterioration and subsequent dialysis initiation.

TH-PO081
Quality of Care and Outcomes in Hospitalized Patients with Acute Kidney Injury (AKI)
Emmett D. Ratigan, Ravindra L. Melta. 1Medicine, University of California San Diego, San Diego, CA; 2Medicine, University of California San Diego, San Diego, CA.

Background: AKI is common in hospitalized patients and is associated with adverse outcomes including in-hospital mortality, development of chronic kidney disease (CKD), progression to end-stage renal disease (ESRD), and long-term mortality. A recent cohort from the UK (NCEPOD; 2009) demonstrated significant lapses in quality of care (QC) for hospitalized patients with AKI. We assessed the QC in a consecutive cohort of 100 hospitalized patients discharged with an ICD-9 coded diagnosis of AKI. We hypothesized that the recognition and management of AKI would be variable and influenced by time of development of AKI.

Methods: We reviewed charts and extracted data on timing of AKI, rapidity of diagnosis, nephrotoxin administration, management of AKI complications, and in-hospital outcome. All survivors were reviewed to one year post-discharge for incidence of nephrotoxic exposures, re-hospitalizations, repeat AKI, development of ESRD, and all-cause mortality. Patients were characterized having AKI at admission (AKIDMIT, n=75) and those developing it during the hospitalization (AKIPOST, n=25).

Results: All patients were verified to have AKI by the acute AKIN and RIFLE serum creatinine criteria. Amongst the cohort, the mean age was 59, 65% were male, 47% had hypertension, 43% CKD, and 35% diabetes. QC parameters were different for AKIADMIT vs AKIPOST.

Conclusions: Despite advances in the understanding of AKI, in-hospital management for AKI has room for improvement. The timing and quality of follow-up care of AKI survivors is highly variable and may contribute to the lack of recognition and development of CKD post AKI.

TH-PO082
The Risk Factors and Impact of AKI Following Stroke
Rupesh Raina, Charbel A. Salem, Martin J. Schreiber, Sevag Demirjian. Nephrology, Cleveland Clinic Foundation, Cleveland, OH.

Background: Acute Kidney Injury (AKI) has been shown to be an independent predictor of long-term mortality and cardiovascular outcomes following acute stroke. However, these studies were not based on strict definitions of AKI and the risk factors of AKI on admission were not assessed before.

Methods: We ran a retrospective analysis on 628 patients who were admitted with their first ever acute stroke. AKI was defined as a rise in Cr of ≥0.3 mg/dL or a percent increase of ≥25% from baseline or a reduction in Urine output as defined by AKIN criteria. Hospital and in-hospital mortality and the hospital was closed or patient was discharged to poor outcome facility (vascular disease, ventilator or dialysis facility) or good (acute rehabilitation or home). Multivariable models were used to assess the correlation between outcomes (AKI, persistence of AKI) and the risk factors (age, gender, race, HTN, CKD, DM, CAD, HPL). Using chi-square test, outcomes were compared for patients with AKI and those with no AKI.

Results: The mean age was 63, and 50.6% were females. 90 patients developed AKI and seventy-one patients had persistent AKI. The results of the regression analysis for AKI on admission are shown in the table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR (p-value)</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.05 (1.04-1.07)</td>
<td>1.01 (2.51-3.63)</td>
</tr>
<tr>
<td>AKI</td>
<td>2.24 (1.56-3.20)</td>
<td>1.20 (1.75-5.84)</td>
</tr>
<tr>
<td>CAD</td>
<td>2.61 (1.21-5.25)</td>
<td>1.51 (1.03-2.22)</td>
</tr>
<tr>
<td>DM</td>
<td>1.34 (1.01-1.76)</td>
<td>1.88 (1.29-2.74)</td>
</tr>
<tr>
<td>HPL</td>
<td>0.00 (0.00-1.00)</td>
<td>0.84 (1.29-2.74)</td>
</tr>
</tbody>
</table>

The risk factors for AKI persistence were very similar with significant CKD being the leading risk factor. 32 patients with AKI (36%) were discharged to acute rehab, while 32 (36%) were discharged to home, and 9 (10%) died. Patients with persistent AKI on discharge were 2.4 times more likely to have a poor discharge disposition than those without AKI (p=0.011).

Conclusions: Using a strict definition of AKI following stroke, we identified the risk factors behind the development and persistence of AKI. We also showed a significant impact of AKI on short-term outcomes. CKD was the most significant risk factor for both AKI admission and its persistence on discharge, followed by HTN and age. Patients with these risk factors and acute stroke should be monitored closely and renal protective measures should be applied.

TH-PO083
Acute Kidney Injury Is Associated with Increased Odds of CKD and Death Among Patients Who Undergo Revascularization for Peripheral Vascular Disease
Pradeep Arora, Pooja Mahajan, Nauman Tahir, James W. Lohr, Nader Naderi, Hassan H. Dosluoglu. 1Department of Medicine, SUNY at Buffalo, Buffalo, NY; 2Division of Nephrology, VAMC, Buffalo, NY; 3Department of Anesthesia, VAMC, Buffalo, NY; 4Department of Surgery, VAMC, Buffalo, NY.

Background: Several databases, sub-analyses of existing studies and single center studies have shown that acute kidney injury (AKI) is associated with development of chronic kidney disease (CKD) in long term follow up. However, the impact of AKI on long term survival has not been studied. We determined the impact of AKI on survival in peripheral vascular disease patients undergoing vascular procedures/surgery.

Methods: Study population included 740 peripheral vascular disease patients undergoing lower extremity revascularization as the primary procedure at VA Western New York Healthcare System between January 1, 2001 and December 31, 2009. All data were collected prospectively. Kaplan Meier and Cox proportional analyses were performed to study the impact of AKI on survival. Logistic regression was performed to evaluate the impact of AKI on development of CKD.

Results: 70 patients developed AKI as defined by AKIN criteria. 39 of these patients died by the last follow up date. 188 of 670 patients died among those who did not have AKI. Risk of Cox regression are shown in the table. 149 patients developed CKD by the last follow up date (analysis was done after excluding pre procedure CKD patients). 50% of those who had AKI subsequently developed CKD, compared to 22% who did not have AKI. Logistic regression results are shown in the table.

Conclusions: AKI following vascular interventions in peripheral vascular disease patients are associated with a significant increase in CKD and mortality.

Long-Term Risk of Mortality for Acute Kidney Injury in HIV-Infected Patients
Mário Raimundo, Maria Joao Melo, Antonio Gomes da Costa, Jose António Lopes. Nephrology and Renal Transplantation, Hospital de Santa Maria-CHLN, Lisbon, Portugal.

Background: Acute kidney injury (AKI) is a common complication in human immunodeficiency virus (HIV)-infected patients and it is associated with increased in-hospital mortality. The impact of AKI on long-term mortality of hospitalized HIV-infected patients has however not been extensively addressed. The aim of the present study was therefore to evaluate the influence of AKI on long-term mortality of HIV-infected patients who are hospitalized and survive to acute episode.

Methods: Three hundred ninety nine HIV-infected patients (mean age: 41 years; 265 male; 268 caucasian) who were hospitalized in the Department of Infectious Diseases of our Hospital (Hospital de Santa Maria, Lisbon, Portugal) between January 2005 and December 2007 and were discharged alive were studied retrospectively. Acute kidney injury was defined according to Risk Injury Failure Loss End-stage kidney disease (RIFLE) classification based on serum creatinine. The outcome measure was mortality at 2 years of follow-up. Kaplan-Meier method was used to determine survival curves and log-rank test was employed to evaluate statistical differences between the survival curves. Cox regression method was used to determine independent predictors of 2-year mortality. Risk factors were assessed with univariate analysis, and variables that were statistically significant (P<0.05) in the univariate analysis were included in the multivariate analysis.

Results: Patients who had AKI with the hospitalization (N=59) had higher 2-year mortality than those patients who did not develop AKI (N=340) (22% versus 10.9%; log-rank P=0.008; unadjusted hazard ratio 2.3, 95% confidence interval 1.2 to 4.3, P=0.01). After
adjusting for other covariates with prognostic importance, AKI still remained associated with increased 2-year mortality (adjusted hazard ratio 2.1, 95% confidence interval 1.1 to 3.9, P=0.024).

Conclusions: Acute kidney injury was independently associated with increased 2-year mortality of hospitalized HIV-infected patients who were discharged alive.

TH-PO085
Validation of the Acute Kidney Injury Risk Index for Patients Undergoing General Surgery at the Philippine General Hospital Rommel P. Batacan, Section of Nephrology, Dept. of Medicine, Philippine General Hospital, Manila, Philippines.

Background: Post-operative Acute Kidney Injury (AKI) is one of the most common cause of hospital morbidities, and an independent risk factor for in-hospital mortality. A General Surgery AKI Risk Index can be used to stratify pre-operatively, patients at high risk to develop AKI post-operatively.

Methods: This is a validation study of an AKI Risk Index Score. All adult patients who underwent general surgery at the Philippine General Hospital from June 1, 2010 to May 31, 2011 will be included. They will grouped into a particular Risk Group depending on the presence of certain risk factors. It will then be determined if AKI happened in the patient using the RIFLE Criteria. Based on the incidence of AKI in the Risk Groups, Sensitivity, Specificity, Positive & Negative Predictive Values will be computed. A Receiver Operating Curve (ROC) will also be constructed.

Results: In 2013 patients were included. Frequency of AKI among patients who underwent general surgery was 3.4% (n=28) and the mortality rate of patients with AKI was 17% (n=5), with overall mortality of 0.6%. Based on the scoring system, patients under Class I (presence of 0-2 risk factors), Class II (3 risk factors), Class III (4 risk factors), Class IV (5 risk factors) and Class V (6-8 risk factors) and were associated with risk of developing AKI of 0.05%, 0.10%, 2.91%, 3.88% and 6.15% respectively. Overall, sensitivity and specificity were 82.2% and 51.1% respectively. Discrimination test by receiving operating characteristics (ROC) curve showed an area under the curve (AUC) of 0.731.

Conclusion: The General Surgery Acute Kidney Injury Risk Index can be used in our local setting to predict AKI among stable chronic kidney disease patients after general surgery.

TH-PO086
Risk Factors and Outcomes of Acute Kidney Injury (AKI) Associated with Outpatient Parenteral Anti-Infective Therapy (OPAT) Charuhas V. Thakar, 1 Sashi Armeneni, 1 Loretta Simbhart, 1 Stephen Kralovic, 1 George Smulian, 1 1Internal Medicine, University of Cincinnati, OH; 2Medical Service, Cincinnati VA, Cincinnati, OH.

Background: Treatment of infections in hospitalized patients frequently requires OPAT. Incidence, risk factors and outcomes of AKI associated with OPAT are not well studied.

Methods: We studied 244 acute care discharges between 2006 and 2009 requiring OPAT in a VA healthcare system. We examined the association of patient characteristics and drug exposure with AKI (defined by AKIN criteria). In patients receiving vancomycin (Vanc), average trough levels before meeting AKI criteria were used for analysis. Chi-square, t-tests and logistic regression was used for analysis.

Results: Sample was 99% male, with mean age of 61 [standard deviation (SD) 10.9], and mean baseline creatinine of 1.1 mg/dl (SD 0.5). 71% of cases received Vanc based regimen. 111/244 (45%) patients developed AKI (Stages 1 - 63%, II - 12%, III - 25%); of whom 10 required dialysis. Of the AKI cases 63% met AKI criteria after discharge. Older age (p = 0.014), high Vanc trough (p = 0.002), high baseline creatinine (p = 0.005), diabetes (p = 0.001), ICU admission (p < 0.0001), proteinuria (p = 0.004) diuretic use (p = 0.02), and amphotericin B use (p = 0.004) were associated with AKI by univariate analysis. In a multivariate model, ICU stay [odds ratio (OR): 4.1, 95% confidence interval (CI), 1.1 - 14.1], proteinuria (OR: 3.3, 95% CI, 1.1 - 10.2), and Vanc trough levels (OR per unit increase: 1.08, 95% CI, 1.01 - 1.15) were associated with AKI. In a separate model that included patients receiving Vanc based regimens, an average trough of ≥ 20 vs < 20 was associated with AKI (OR 3.8, 95% CI, 1.6 - 8.9).

Re-admission at 90-days was 57% in AKI vs 38% in no AKI group (p = 0.003). Creatinine at 2-years was higher in AKI versus no AKI group [1.54 (SD, 0.92) versus 1.09 (SD, 0.72) respectively]. In 7/10 patients dialysis was deemed permanent.

Conclusion: AKI is a significant and under-recognized complication associated with OPAT, frequently occurs after discharge, and may result in irreversible damage. Risk stratification and monitoring of these patients is needed to reduce kidney toxicity.

Funding: Veterans Administration Support

TH-PO087
Determination of Renal Function Improves Significantly the Prediction of Survival in Palliative Care Patients Scott O. Grebe, 1 Oliver Schmalz, 1 Ruediger Trebst, 1 Thomas F. Mueller, 2 Manfred F. Weber. 1 Medizinische Klinik I, HELIOS-Klinikum Wuppertal, Univ. of Witten/Herdecke, Wuppertal, Germany; 2 Div. of Nephrology and Transpl. Immunology, Univ. of Alberta, Edmonton, AB, Canada; 3Medizinische Klinik I, Klinikum Köln-Merheim, Univ. of Witten/Herdecke, Cologne, Germany.

Background: One of the most important challenges in palliative care medicine is an exact estimation of patient survival. For this purpose various clinical scoring systems such as the palliative prognostic score (PPS) are used. Despite impaired renal function is a known risk factor for patient outcome in general, its influence on the survival of palliative care patients has not yet been studied.

Methods: All patients admitted to our palliative care unit in 2008 were included in the study. The relation between renal function and survival was prospectively analyzed and compared to currently used scores. Besides clinical routine measurements serum creatinine (S-Crea), the eGFR using the simplified MDRD-formula, diastolic blood pressure (D-BP), mean systolic blood pressure (S-BP), Karnofsky-Index (KI) and the PPS were determined at admission. In addition to descriptive statistics the influence of these parameters on survival was tested using Kaplan-Meier estimates and Wilcoxon scores. In order to identify the most important prognostic factors a multiple regression analysis based on the Cox proportional hazards model was performed.

Results: Overall 308 patients (mean age=70±12.9 years, 58.4% male, mean survival 45±56 days) were included. The mean values of S-Crea and eGFR were x=1.5±1.6 mg/dl and 78±60 ml/min, resp. The percentage of patients with significant U-Protein was 35.2%. The median KI and PPS were 40% (range 10-90%) and 30-50% (range 10-90%), resp. In the univariate survival analysis only age (p=0.0016), KI (p=0.001), PPS (p=0.0001), S-Crea (p=0.0074) and eGFR (p=0.0213) showed a significant association with patient outcome. The strongest predictors in the age-adjusted stepwise multiple regression model were PPS (p=0.0001), KI (p=0.002) and eGFR (p=0.0053). None of the other parameters met the 0.05 significance level.

Conclusion: The determination of the eGFR adds significantly to a reliable prediction of survival in palliative care patients.

TH-PO088
Identification of Potentially Controllable Riskfactors for AKI after Cardiac Surgery Sanne Vellinga, Gert A. Verpooten, Karin Janssen van Doorn. Nephrology-Hypertension, University Hospital Antwerp, Edegem, Antwerp, Belgium.

Background: Acute kidney injury (AKI) in the immediate postoperative period after successful cardiac surgery was proven to be of major influence on morbidity and mortality. Our study investigates the potential prognostic value of periperaoperative packed-cell transfusion on the development of AKI.

Methods: Retrospectively, we reviewed 565 patients who underwent coronary artery bypass grafting (CABG). In the perioperative period, following parameters were examined: demographic characteristics, diabetes, history of congestive heartfailure, urgency of surgery, lowest perioperative hematocrit, intra- and postoperative packed cell transfusion, serumcreatinine (at admission, every 24h minimum 48h during 7 days and after 8 weeks), eGFR (MDRD-formula), postoperative administration of furosemide, duration of cardiopulmonary bypass and crossclamping and need for renal replacement therapy. AKI was defined by the AKIN-criteria.

Results: A total of 83 patients (14,7%) evolved to AKI in the post-operative period. Two patients needed renal replacement therapy in the first seven days and both died. AKI was associated with age (mean 66.5±4.9 vs 69.6±10.2 AKI p=0.05), pre-existing renal function (mean eGFR 86.7±51.8 ml/min/1.73m² vs 70.6±24.0 ml/min/1.73m², p<0.01), urgency of surgery (5.3% vs 13.4%, p<0.03), both intra- and postoperative packed cell transfusion (9.9% intra-operative transfusion vs 25.3%, p<0.01 and 16.3% postoperative transfusion vs 42.2%, p<0.01) and postoperative administration of furosemide (60.0% vs 84.3%, p<0.01). In multivariate analysis pre-operative creatinine (p<0.01), postoperative packed cell transfusion (p<0.01) and postoperative administration of furosemide (p<0.01) remained significant.

Conclusion: Our study clearly shows that pre-existing renal disease, postoperative packed cell transfusion and postoperative administration of furosemide are important risk factors for the development of AKI after CABG. As two of these factors are easily controllable our results advocate further exploration of these factors and their potential to reduce AKI related morbidity.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author. 130A
TH-PO089

Improved Survival in Patients Requiring Dialysis after Pediatric Hematopoietic Cell Transplantation (HCT) Julian S. Rajaia, Rachel I. Vogel, Clifford E. Kashthan, Angela R. Smith. University of Minnesota, Minneapolis, MN.

Background: HCT, a critical therapy for many children with life threatening illnesses, is associated with a substantial risk of acute kidney injury (AKI) requiring dialysis. The purpose of this study is to compare survival in these patients in two decades, 1990-1999 and 2000-2009.

Methods: 1427 patients <21 years old who had their first HCT at the University of Minnesota between January 1990 and December 2009 were reviewed using the center database. Kaplan-Meier estimates and 95% confidence intervals for 100 days and 1 year post-HCT overall survival (OS) are reported by dialysis group and treatment decade (1990-1999 vs. 2000-2009) and compared using the log-rank test.

Results: The incidence of AKI requiring dialysis was not significantly different between the two cohorts (1990-99: n=65,778, 8.35% vs. 2000-09: n=62,649, 9.55%, p = 0.42).

Conclusion: Over the last two decades, survival has significantly improved at both 100 days and 1 year post-transplant, regardless of dialysis status. Despite a recent reduction in mortality for those who require dialysis, mortality remains significantly higher than for patients who do not need dialysis.

TH-PO090


Background: Drug-induced kidney injury is not an uncommon adverse event in drug development. The greatest issue is the late identification of Acute Kidney Injury due to the current standards (i.e. serum creatinine (sCr) and blood urea nitrogen (BUN)) which are delayed indicators of injury and may not be significantly changed until 2/3 of the kidneys function has already been lost. Over the last three years there has been progress with preclinical qualification processes for kidney biomarkers (PSTC and HESI qualification function has already been lost. Over the last three years there has been progress with preclinical qualification processes for kidney biomarkers (PSTC and HESI qualification).

Methods: AKI requiring dialysis is an important complication of pediatric HCT that has shown no change in incidence over the last two decades. Survival has significantly improved at both 100 days and 1 year post-transplant, regardless of dialysis status. Despite a recent reduction in mortality for those who require dialysis, mortality remains significantly higher than for patients who do not need dialysis.

Results: Patients requiring dialysis in the 2000-2009 cohort had significantly higher survival than those in the 1990-1999 cohort at both +100 days and at 1 year (+100 days: 42% vs. 18%, p = 0.0009; 1 year: 25% vs. 11%, p = 0.0010).

Conclusion: In this FIM Phase I dose escalation study, there were no reported DLTs or evidence of a dose-dependent change in the number or severity of AEs. The observed AE profile appeared consistent with that expected for cardiac surgical patients post-operatively. The safety data support continued evaluation of QPI-1002 for prophylaxis of AKI in pts undergoing cardiac surgery.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Proteinuria in Community-Acquired Pneumonia as a Prognostic Marker for Outcome

Simone Spoorenberg,1 Sabine Meijvis,1 Gerjan Navis,1 Jan Grutters,1 Simone Spoorenberg,1 Sabine Meijvis,1 Gerjan Navis,2 Jan Grutters,1 ST Antonius Hospital;2 University Medical Centre Groningen.

Background: Acute Kidney Injury (AKI), classified according to the Risk, Injury, Failure, Loss and End-Stage kidney disease (RIFLE) criteria, is occasionally seen in patients with Community Acquired Pneumonia (CAP). It is known that RIFLE criteria may underestimate the occurrence of AKI. We investigated the incidence and predictive value of proteinuria in patients with CAP, and compared the results with the RIFLE criteria.

Methods: We retrospectively investigated RIFLE criteria and proteinuria in patients hospitalized with CAP. Proteinuria, defined as total-protein/creatinine ratio > 23 mg/mmol, was measured in a urine sample from the day of admission. Primary outcome was length of hospital stay (LOS). Secondary outcomes were in-hospital mortality and one-year mortality.

Results: In 319/496 patients (64%) a urine sample was available. 198 patients (62%) had proteinuria. Patients with proteinuria had a significantly longer LOS and an increased in-hospital mortality. RIFLE class was a predictor for LOS, in-hospital mortality and one-year mortality. In multivariate analysis, proteinuria only was an independent predictor for LOS.

Proteinuria and RIFLE 1-3

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>OR or HR</th>
<th>95% CI</th>
<th>OR or HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Length of stay</td>
<td>Unadjusted</td>
<td>1.63</td>
<td>1.28-2.06</td>
<td>1.55</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>1.58</td>
<td>1.08-1.75</td>
<td>1.21</td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>Unadjusted</td>
<td>5.59</td>
<td>4.27-24.63</td>
<td>8.81</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>3.27</td>
<td>0.69-15.45</td>
<td>1.81</td>
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<tr>
<td>One-year mortality</td>
<td>Unadjusted</td>
<td>1.74</td>
<td>0.94-3.22</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>Adjusted</td>
<td>1.09</td>
<td>0.56-2.10</td>
<td>1.81</td>
</tr>
</tbody>
</table>

OR:1 Odds ratio and HR:2 Hazard ratio

Conclusions: The incidence of proteinuria during CAP is high. Proteinuria and RIFLE criteria are both associated with adverse outcome in CAP. Only proteinuria is an independent predictor for LOS. Proteinuria can be a marker for outcome and might be used to assess the severity of CAP.

Proteinuria in Acute Tubular Necrosis

Kevin K. Pandya, Kavitha Potluri, David J. Leechy, Alexander R. Chang, Holly J. Kramer. Division of Nephrology, Loyola University Medical Center, Maywood, IL.

Background: Acute kidney injury (AKI) as defined by well-established criteria is observed in about 5-7 percent of hospitalized patients and 66% of intensive care patients. AKI accounts for nearly 50% of AKI cases in hospitalized patients. Because of tubular damage in ATN, the normal mechanism of protein reabsorption is likely to be altered, with receptor-mediated endocytosis of albumin expected to decrease, leading to overt proteinuria. However, data on the incidence of proteinuria in the setting of ATN are sparse. We examined the changes in urine protein excretion after an episode of ATN in adults without proteinuria.

Proteinuria in Acute Tubular Necrosis

<table>
<thead>
<tr>
<th>Phenomenon</th>
<th>OR or HR</th>
<th>95% CI</th>
<th>OR or HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number at risk</td>
<td>Proteinuria</td>
<td>0.81</td>
<td>0.53-1.26</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>No Proteinuria</td>
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<td>1.00-1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Length of stay (days)</td>
<td>Proteinuria</td>
<td>0.81</td>
<td>0.53-1.26</td>
<td>0.90</td>
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<tr>
<td></td>
<td>No Proteinuria</td>
<td>1.00</td>
<td>1.00-1.00</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Conclusions: The majority of patients with ATN in this study had a Pr/Cr > 0.3 g/g and these patients had higher mortality and need for RRT. Urine Pr/Cr may be a simple and readily available biomarker to predict outcome in ATN.

Proteinuria

TH-PO093

Proteinuria in Acute Tubular Necrosis

Poster/Thursday

Acute Kidney Injury: Clinical - I

Proteinuria

TH-PO095

The Capacity of Nephrology and Critical Care Services in England and Wales To Manage Acute Kidney Injury

Ben Bray.1 Beverley Matthews,2 Donal O'Donoghue.1,NHS Greater Glasgow and Clyde, Glasgow, United Kingdom;2 NHS Kidney Care, London, United Kingdom;3 Salford Royal Foundation NHS Trust, Salford, United Kingdom.

Background: Acute Kidney Injury is increasingly recognised as an important quality and safety challenge in all health economies. In England, coded admissions for AKI are rising by over 10% per annum (Department of Health) and a national audit of AKI deaths (NCEPOD 2009, Adding Insult to Injury) highlighted access to specialist nephrology and critical care specialist services as a factor in many deaths from AKI. Here we report the result of a survey of the capacity of nephrology and critical care units in England and Wales to meet the demand for management of AKI.

Methods: A web based questionnaire was sent to clinicians representing all hospitals in England and Wales offering inpatient nephrology and critical care services. The questionnaire included items relating to staffing, facilities, use of guidelines and AKI related bed occupancy on World Kidney Day 2011. The AKIN classification of AKI was used.

Results: Complete results were obtained from 41/59 (69%) nephrology centres and 45/175 (26%) critical care centres in England and Wales. There was variation in the model of AKI management between centres – only 29% of nephrology centres had dedicated high dependency facilities. Bed occupancy in nephrology centres was 97%. 23% of nephrology centres had dedicated high dependency facilities. Bed occupancy in nephrology centres was 97%. 23% of nephrology centres had dedicated high dependency facilities. Bed occupancy in nephrology centres was 97%. 23% of nephrology centres had dedicated high dependency facilities. Bed occupancy in nephrology centres was 97%. 23% of nephrology centres had dedicated high dependency facilities.

Conclusions: Delays in access to specialist nephrology and critical care services may lead to worse outcomes for patients with AKI. High bed occupancy in nephrology services in England and Wales suggests that there is little scope for increasing access to these services without increasing efficiency or increasing capacity. One suggested solution would be better use of protocols to define planned pathways of care for patients with AKI.

TH-PO096

Stress-Induced Hyperglycemia and Acute Kidney Injury in Critically Ill Children

Roberto Cordella.1 Robert Woroniecki.2 Pediatrics, University of Illinois College of Medicine at Peoria, IL;1 Pediatrics, Albert Einstein College of Medicine, Bronx, NY.

Background: Stress-induced hyperglycemia (SIH) is common in critically ill patients and has been associated with increased mortality and morbidity. However, it is not clear if SIH is associated with acute kidney injury (AKI) in children. We hypothesized that SIH is associated with intensity of AKI.

Methods: This is an observational study focused on patients age > 18 years who experienced an episode of AKI as defined by AKIN/RIFLE criteria. Criteria to select patients with ATN included FENa > 2%, serum urea nitrogen-to-creatinine ratio < 20, urine-to-plasma creatinine ratio < 0.2, as well as the nephrologist’s clinical determination based on available data. Patients with known proteinuria (UA showing >1+ proteinuria) were excluded from the study. Proteinuria (based on Pr/Cr ratio) was measured at time of diagnosis of ATN. All study patients were followed during the hospitalization for secondary endpoints: mortality and need for dialysis.

Results: A total of 32 patients with absence of proteinuria prior to ATN episode were identified over an approximately 7 months time period. 83% of the patients (24/32) had urine Pr/Cr < 0.3 (g/g). Of the patients who required dialysis, 100% (13/13) were noted to have Pr/Cr > 0.3. Lastly, of the patients who died within the selected cohort, 91% (10/11) had Pr/Cr ratio > 0.3.
Methods: We analyzed the records of children with SIH admitted between 01/2005-01/2010 to the pediatric intensive care unit (PICU). Subjects with prior kidney disease or diabetes were excluded. Intensity of AKI was defined by pediatric RIFLE criteria. Serum glucose (SG) and estimated glomerular filtration rate (eGFR) on admission, 48 hours, and at 7 days after admission were obtained. Delta glycemia (DG) was defined as the difference between peak and lowest SG during hospital stay.

Results: Of 37 subjects, 13 (35%) had AKI. Results are shown in Table. We found no correlation between SG and eGFR at 0hr, 48hr and 7days. Subjects with SIH and AKI stayed longer in PICU and hospital, and we found a positive correlation between DG and length of stay in PICU (P=0.0003, r=0.3) and length of hospital stay (P=0.0005, r=0.34).

Conclusions: In children with SIH, SG and DG is not associated with intensity of AKI, but it does correlate with length of hospital and PICU stay.

TH-PO097

Natriuretic Peptide and Loop Diuretic Agents in Cardiac Surgery Associated Acute Kidney Injury Prevention

Sagar U. Nigwekar, Khuloud Shukra, Sushrut S. Waikat. Brigham and Women's Hospital.

Background: In animal studies, natriuretic peptide (NP) and loop diuretics (LD) are shown to prevent cardiac surgery associated acute kidney injury (CSAKI). However, randomized controlled trials (RCTs) in humans have shown inconsistent results and are underpowered for evaluation of hard outcomes such as renal replacement therapy requirement (CSAKI-K) and mortality. There also remains a question regarding their safety. Our study aimed to systematically review these RCTs to ascertain efficacy and safety of these agents in CSAKI prevention.

Methods: We searched MEDLINE, EMBASE, and Google Scholar for RCTs evaluating NP or LD for CSAKI prevention. Outcomes analyzed were CSAKI (defined per the individual RCT), CSAKI-K, mortality and durations of intensive care unit (ICU) and hospital stay. Statistical analyses were performed using the random effects model and results expressed as relative risks (RR) for dichotomous outcomes and weighted mean difference (WMD) for continuous outcomes, with 95% confidence intervals (CI). Methodological quality assessment was performed by Jadad score.

Results: Fourteen RCTs (12 NP, 2 LD) involving 1,599 patients met the inclusion criteria. NP dose administered in these RCTs was noted to be lower than that administered in Auricul Anaridate Acute Renal Failure Study. Pooling analysis of RCTs evaluating NP showed reduction in CSAKI (RR 0.45, [0.23 to 0.96]), CSAKI-K (RR 0.23, [0.08 to 0.70]), mortality (RR 0.50, [0.27 to 0.93]), ICU length of stay (WMD -0.44 days, [-0.82 to -0.06 days]) and hospital length of stay (WMD -4.08 days, [-6.32 to -1.83 days]) in the NP group. There was no statistical heterogeneity in these analyses. Statistical analysis could not be performed due to limited number of RCTs evaluating LD. Both NP and LD agents were well tolerated. Methodological quality of the RCTs was variable; however, sensitivity analysis of high quality studies did not change the findings.

Conclusions: NP agents, in low doses, are well tolerated and may prevent CSAKI and its associated complications. Appropriately powered RCT is urgently needed to confirm these results. Role of LD agents is not adequately studied in this population.

TH-PO098

Regional Citrate Anticoagulation Decreases the Degranulation of Neutrophils in Continuous Venovenous Hemofiltration in Critically Ill Patients

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Background: During continuous venovenous hemofiltration in critically ill patients, anticoagulation is administered to prevent clotting of the hemofilter. In case of a high bleeding tendency, regional anticoagulation using citrate is an option. Citrate creates an almost calcium-free environment in the extracorporeal circuit, not only preventing coagulation, but potentially also downregulating the degranulation of neutrophils, as these results. Role of LD agents is not adequately studied in this population.

Methods: We activated A2BR with the selective A2BR-agonist BAY 66-6583 during GN to determine its effects on kidney injury, expression of cytokines/chemokines, and recruitment of inflammatory infiltrates.

Results: Normal kidney had little A2BR mRNA expression; however, its expression was increased in nephritic kidneys. Activation of A2BR in the acute inflammatory phase of GN did not attenuate CD8 T-cells but increased ED1 macrophage accumulation compared with the control group. However, necrotizing lesions and glomerular hypercellularity were more pronounced in the A2BR agonist-treated group. As a result of the attenuation of kidney damage, reduced proteinuria and serum creatinine were observed in the A2BR agonist-treated group. The expression of CXCL1, CCL2, CCL4, CCL5, CCL19, and CCL22 chemokines, was not different between both groups. In contrast, the expression of anti-inflammatory cytokine, TGF-β, was significantly induced in the A2BR agonist-treated group. In addition, activation of A2BR reduced the expression of osteopontin-1 a pro-inflammatory and anti-angiogenic mediator. Notably, in both groups macrophages were predominantly M1 phenotype, however, in the control group 71% of macrophages were M1 phenotype compared with 51% in the A2BR agonist-treated group.

Conclusions: These findings suggest that A2BR could protect from kidney injury by modulating pro- and anti-inflammatory mediators. Similarly, markedly reduced in necrotizing lesion by A2BR activation suggests a potential angiogenic capillary repair for this adenosine receptor.

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TH-PO100

EP2-Receptor Deficiency Ameliorates the Course of Crescentic Glomerulonephritis in Mice

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Background: Prostaglandin E2 (PGE2) has important immune modulatory roles in various diseases in different organs. PGE2 mediates its effects through four different EP-receptors, named EP1 to EP4. Different intracellular signals of the receptor-subtypes and different combinations of subtypes on the cell surface may partly be responsible for the diverse effects of PGE2 in inflammation.

Methods: Clinical parameters: measurement of BUN and albuminuria. Histological assessment of renal damage on PAS-stained tissue sections and immunohistochemistry against CD3 and F4/80. Quantitative PCR of Cox-2, CCL2, IL-6 and TNFalpha. FACS-based intracellular staining of IL-17 and IFN gamma.

Results: In order to elucidate the potential role of the EP2 receptor in glomerulonephritis, the nephototoxic-serum-nephritis (NTN) was induced in EP2 deficient (EP2−/−) and in wildtype (WT) mice (C57BL6 background). Successful onset of the disease was evaluated functionally at day 4 after NTN-injection. No differences between EP2−/− and WT mice was detected in this early phase of the disease. 10 days after induction of the nephritis (during the T-cell dependent autologous phase), EP2−/− mice developed less severe nephritis in terms of significantly better preserved kidney function, less glomerular damage, reduced renal infiltration of CD3 T-cells and F4/80 positive macrophages/dendritic cells and less RNA-expression of the pro-inflammatory mediators Cox-2, CCL2, IL-6 and TNFalpha when compared with nephritic WT-mice. Interestingly, renal Th-17 immune response seemed to be impaired in nephritic EP2−/− mice.

Conclusions: Thus, the EP2 receptor seems to play an important role in the development of NTN.

Funding: Government Support - Non-U.S.
Suppression of Hyaluronan Synthesis with 4-Methylumbelliferone in NZB/W F1 Mice Is Associated with Reduced Renal Inflammation and Renal Function Improvement

**Background:** We previously demonstrated that glomerular hyaluronan (HA) expression is increased in lupus nephritis, but its role in pathogenesis remains to be determined. This study investigated the effect of 4-methylumbelliferone, a specific inhibitor of HA synthesis, on disease manifestations in lupus prone mice used in a series of pilot studies using the model of nephrotic nephritis in Wistar Kyoto rats. The NZB/W F1 mouse is chosen for this purpose due to the increased expression of proinflammatory cytokines and subsequent onset of proteinuria (day 4) to assess the antibody in a clinically relevant model. Rats were randomized into three treatment groups (isotype control, 20 mg/kg and 40 mg/kg anti-MIF mAb) and treated on day 0 and day 4.

**Results:** The severity of glomerular injury was assessed on day 8. Proteinuria, glomerular macrophage infiltration and glomerular crescents were reduced dose dependently following treatment with anti-MIF mAb [Table 1]. In addition, urinary levels of proinflammatory cytokines like TNFα and IL-1β were reduced.

<table>
<thead>
<tr>
<th>mAb</th>
<th>isotype control</th>
<th>BaxB01 (20 mg/kg)</th>
<th>BaxB01 (40 mg kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteinuria (mg/day)</td>
<td>105.6 ± 5.3</td>
<td>93.9 ± 0.7</td>
<td>78.3 ± 3*</td>
</tr>
</tbody>
</table>
| Microscopic glomerular
  cross section 10.5 ± 1.5 | 22.0 ± 1.4* | 19.3 ± 1.5 *** |
| TAM infiltration (%) | 30.5 ± 1.5 | 86.3 ± 1.4* | 78.3 ± 3*** |
| *p<0.05, **p<0.005, ***p<0.001 |

**Conclusion:** A fully human anti-MIF mAb was able to neutralize the proinflammatory effects of MIF in vitro and was effective for treating antibody-mediated experimental glomerulonephritis in even advanced stages of lupus nephritis. This antibody has important potential for clinical use in nephritis and other MIF-related diseases. **Funding:** Pharmaceutical Company Support

**TH-PO104**

The Cross-Talk between NKT Cell and Th17 Response in the Experimental Autoimmune Glomerulonephritis

**Background:** Th17 cells are emerging as a major player in several autoimmune diseases such as multiple sclerosis and rheumatoid arthritis which were known as Th1 mediated diseases. NKT cells, which have a role of linkage between the innate and adaptive immune response, are also associated with autoimmune diseases. But the cross-talk between NKT cell and Th17 has not been evaluated in the context of autoimmune disease. In present study, we examined the role of NKT cell/Th17 response in experimental autoimmune glomerulonephritis (AGN) utilizing a murine model of chronic graft-versus-host disease.

**Methods:** AGN was induced by the adoptive transfer of lymphocytes from C57BL/6 to F1 hybrids into wild C57BL/6, type I NKT cell deficient, and type I NKT cell deficient mice. The transferred lymphocytes infiltrated into the glomeruli inducing cell infiltration.

**Results:** The severity of AGN, confirmed by deterioration of kidney function, proteinuria, and renal pathology, was attenuated with the absence of NKT cells compared to wild type mice. Complement 3 deposition and T cell infiltration were consistent with the severity of AGN. NKT cells were recruited into glomeruli with the induction of AGN. The systemic immune responses as measured by splenic T cell activation, intracellular IL-17, and inflammatory cytokines, are enhanced with the induction of AGN. But the absence of NKT cells, especially both of type I and II NKT cells, reduced the systemic immune responses. The intranaral immune response induced by AGN was parallelized with systemic responses. Moreover, intrarenal STAT3 phosphorylation, which is the major transcription factor for Th17 response, was significantly attenuated in NKT cell deficient hosts. NKT cells secreted IL-17 as well as inflammatory cytokines (TNF-α, IL-6, IL-12, IFN-γ) when activated. The intranaral immune response induced by AGN was parallelized with systemic responses. Moreover, intrarenal STAT3 phosphorylation, which is the major transcription factor for Th17 response, was significantly attenuated in NKT cell deficient hosts. NKT cells secreted IL-17 as well as inflammatory cytokines (TNF-α, IL-6, IL-12, IFN-γ) when activated.

**Conclusion:** The cross-talk between NKT cell and Th17 response might be a pivotal linkage for the development of AGN.
as a strong inducer of CXCL5. IL-17A expression in NTN peaked at day 7-10 preceding renal CXCL5 expression. In line, CXCL5 expression was downregulated in nephritic IL-17/- mice.

Conclusions: In conclusion, our study shows that the CXCL5/CXCL1-CXCR2 axis orchestrates renal neutrophil recruitment in a time and compartment specific manner. CXCL5 mediated neutrophil recruitment is driven by IL-17 and contributes to renal tissue injury in crescentic glomerulonephritis.

TH-PO106
IFN-α/β and IFN-κβ Specifically Affect Renal Progenitors and Podocytes In-Vitro and In-Vivo

Conclusions: The data propose that IFN-κβ stimulates progenitor differentiation in vitro mostly impairs progenitor differentiation in vitro. IFN-α/β and IFN-κβ specifically suppressed the proliferation of human renal progenitors, while contrast IFN-κβ inhibited differentiation of renal progenitors towards podocytes as determined by induced nephrin expression. Next we compared the impact of recombinant IFNα and IFNβ injections on adriamycin-induced nephropathy in SCID mice which allowed us to exclude IFN-dependent effects on adaptive immunity. Both IFNs increased proteinuria as compared to control mice injected with adriamycin only. Quantitative morphometry by confocal microscopy revealed that IFNβ injections had specifically reduced the number of WT1+/nephrin+ podocytes while IFNα injections specifically reduced the number of proliferating parietal epithelial cells, respectively. In summary, both type I IFNs can aggravate glomerular pathology, albeit in different ways. IFNβ impairs the proliferation of podocyte progenitors, while IFNα mostly impairs progenitor differentiation in vitro.

Conclusions: We propose that IFN-α/β and IFN-κβ contribute to glomerulonephrosis and proteinuria by specifically affecting homeostasis of podocytes and their potential repair by local progenitors, a mechanism that may contribute to in vivo infection-associated glomerular pathology.

Funding: Government Support - Non-U.S.

TH-PO107
Human Bone Marrow Derived Mesenchymal Stromal/Stamp Cells Attenuate Tubular Inflammation upon Albumin Challenge

Results: Real-time qPCR revealed that co-culture with BM-MSC significantly reduced the up-regulation of pro-inflammatory genes including IL6, CCL2, CCL5, IL8, TNF-α, IL-1β, and ICAM-1 in PTEC exposed to HSA. The suppression of pro-inflammatory genes translated into reduced secretion of IL-6, CCL2, CCL5, IL8 and TNF-α proteins as detected by ELISA. In addition, the reduction of these proinflammatory cytokines and chemokines by BM-MSC was associated with attenuation of HSA induced i kB phosphorylation in PTEC. To dissect the potential mechanism, we detected that the anti-inflammatory genes, HGF and IL1RN (IL1 receptor antagonist), were significantly induced in BM-MSC during co-culture with PTEC under protein overload condition.

Conclusions: Our in vitro data suggest an anti-inflammatory role of BM-MSC in HSA-exposed PTEC inflammation, probably through paracrine effects of HGF and IL1RN via NF-kB signaling.

This research is supported by Seed Funding from the University of Hong Kong and Hong Kong Society of Nephrology Research Grant 2010

Funding: Government Support - Non-U.S.

TH-PO108
Macrophage-Specific Thymosin β4 Knockout Protects Against LPS-Induced Inflammation

Background: Macrophages are a rich source of thymosin β4 (Tβ4), but little is known about the role of Tβ4 in macrophage function. We investigated whether Tβ4 modulates macrophage function.

Methods: Floxed Tβ4 short hairpin RNA (Tβ4shRNAbox) mice were crossed with lysMCre mice to generate macrophage-specific Tβ4 KO (Mac-Tβ4 KO) mice. Peritoneal thioglycollate-elicited macrophages from wild type (WT, n=3) and Mac-Tβ4 KO (n=3) mice were isolated and exposed to LPS (50 ng/ml) or buffer for 48 hrs to induce inflammation. Expression of Tβ4 protein, phospho-c-Jun and total cJun in macrophages were examined by Western Blot. Unilateral urolateral obstruction (UUO) was performed in adult male WT (n=5) and Mac-Tβ4 KO (n=4) mice. Macrophage infiltration was assessed at day 7 after UUO. Data are expressed as mean±SE.

Results: Macrophage Tβ4 protein level was markedly diminished in Mac-Tβ4 KO vs WT by 87%. c-Jun expression was robustly induced by LPS in WT macrophages, but was significantly reduced by 52±8% in macrophages from Mac-Tβ4 KO mice. UUO-induced kidney macrophage infiltration was significantly inhibited in Mac-Tβ4 KO vs WT by 28.5%.

Conclusions: Macrophage Tβ4 deletion protects against LPS-induced inflammation, and decreases macrophage infiltration in obstructed kidneys. Our data suggests that targeting macrophage Tβ4 may offer novel treatment of inflammation.

Funding: NIDDK Support

TH-PO109
Mice Fed Adenine-Containing Diet Is Optimal Model of Tubulointerstitial Damage with Renal Dysfunction

Results: These datum showed that mice fed 0.25%Ad with short term exposure of 7 days showed increased TID and renal dysfunction. These datum showed that mice fed 0.25%Ad with short term exposure of 7 days showed increased TID and renal dysfunction.

Conclusions: The aim of this study is to evaluate the protocol for the optimal mouse model of tubulointerstitial damage (TID) with renal dysfunction induced by adenine feeding. Inbred mouse C57BL/6 mice (9 weeks old) were fed a standard laboratory powder diet or a powder diet containing 0.25% adenine (0.25%Ad). Mice fed 0.25%Ad were euthanized on days 3, 7, and 14 (Group 1,3,5), and mice fed the control diet were euthanized on days 3, 7, 14 (Group 2,4,6). Additionally, we analysed mice fed 0.25%Ad for 7 days with subsequent control diet euthanized on days 14 (Group 7).

Results: Mice fed 0.25%Ad showed nephrolithiasis, extensive tubular dilation, inflammation, necrosis and fibrosis (Mason’s Trichrome stain) with the elevation of serum creatinine and TID area. (Table 1) These data suggest that dietary adenine leads to a decrease in renal parenchyma and urea possibly due to dehydration observed in Group 3 were ameliorated in Group 7. The observation of Group 3 showed that once 0.25%Ad stopped, renal dysfunction recovered and TID did not advance.

Conclusions: These datum showed that mice fed 0.25%Ad with short term exposure is one of the candidate of optimal models of tubulointerstitial damage and repair with renal dysfunction.

Funding: Government Support - Non-U.S.

TH-PO110
Reduction of Renal Fibrosis and TGF-β1 Production by Late Treatment with a Spleen Tyrosine Kinase Inhibitor in Experimental Glomerulonephritis

Background: Spleen tyrosine kinase (Syk) is important in antibody mediated inflammation. Treatment with fostamatinib, a selective Syk inhibitor, is effective in reducing inflammation in experimental glomerulonephritis (GN). However, the effect on fibrosis has not been investigated. The aim of this study is to investigate whether inhibition of Syk at the fibrotic stage will reduce renal fibrosis and glomerular synthesis of profibrotic factors.

Methods: Fostamatinib (400 mg/kg) or vehicle was given daily to Wistar Kyoto rats from day 14 to 28 after nephrotoxic serum. Renal injury was assessed by creatinine clearance

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underlines represents presenting author.

135A
C(Ci), proteinuria and histology. Effect of Syk inhibition on TGF-β production was investigated by isolating glomeruli ex vivo in the presence of R406 (active metabolite of fasudil) or control medium. **Results:** On day 14, all 20 rats had GN with proteinuria. By day 28, rats treated with fasudil had better renal function (C(Ci) and less glomerular α-smooth muscle actin (SMA), interstitial fibrosis, infiltrating macrophages and crescents (table) than those treated with vehicle only. One rat in the vehicle group died. Proteinuria was unaffected in untreated animals. Incubation of nephritic glomeruli ex vivo with R406 reduced the glomerular production of TGF-β by 68% (p<0.01).

**CrCl (mlday)**

**Proteinuria (mg/day)**

**Intercellular collagen (%)**

**Glomerular aSMA (IU)**

**Glomerular collagen (IU)**

**Intestinal Mao (IU)**

**Glomerular Mo (GCS)**

**Vehicle (n=9)**

0.33±0.07

165±18

1.72±0.2

0.26±0.02

99.6±3.1

1.05±0.12

3.4±0.45

**Fasudil (n=10)**

0.92±0.04***

175±7.6**

0.9±0.00*

0.08±0.01***

34.4±1.3***

0.59±0.40***

0.7±0.25***

*p<0.05, **p<0.01, ***p<0.001, ns not significant; IU imaging analysis unit; Mo macrophage; GCS glomerular cross section

**Vehicle vs fasudil: 0.33±0.07 vs. 0.92±0.04*** (p<0.01).

**Conclusions:** Our results is the first report showing that Syk inhibition reduces glomerular TGF-β synthesis and renal fibrosis. Syk is a potential therapeutic target in prevention of antibody mediated renal fibrosis.

**Funding:** Clinical Revenue Support, Government Support - Non-U.S.

**TH-PO111**

**The Blockade of IL-17 Ameliorates the Hypertension and the Upregulation of Profibrotic and Proinflammatory Mediators in Kidney of DOCA-Salt Rats**


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**Background:** Inadequate levels of aldosterone cause hypertension, inflammation, fibrosis and kidney disease. Recent findings have implicated the Interleukin-17 (IL-17) pathway in hypertension, and we showed that aldosterone promotes autoimmune damage and Th17 inflammatory response in experimental Autoimmune Encephalomyelitis. We hypothesized that hypertension and interstitial fibrosis in nephritic kidneys are dependent on IL-17. We studied if fibrotic/inflammatory damage in kidney of DOCA-salt rats is dependent on IL-17. We treated rats with DOCA-salt for the last 12 days of treatment. Anti-IL-17 ameliorated hypertension (112±3mmHg vs. DOCA-salt 138±6mmHg or IgG groups p<0.05). Moreover, Anti-IL-17 reduced renal fibrosis, namely collagen deposition, and the effect of CCR2 inhibition on cytokine production.

**Results:** Anti-IL-17 ameliorated hypertension (112±3mmHg vs. DOCA-salt 138±6mmHg or IgG groups p<0.05). Moreover, Anti-IL-17 reduced renal fibrosis, namely collagen deposition, and the effect of CCR2 inhibition on cytokine production.

**Conclusions:** Our results suggest that the macrophage population may be responsive to CCR2 signaling and involved in the pathogenesis of diabetic nephropathy. Studies are now underway to determine whether these macrophages are concentrated in the glomeruli of diabetic kidneys, the cytokines these cells produce that may have nephritic potential, and the effect of CCR2 inhibition on cytokine production.

**Funding:** Pharmaceutical Company Support

**TH-PO114**

**Adrenocorticotropin (ACTH) Gel Suppresses Renal Tubulointerstitial Inflammation and Injury by Direct Stimulation of the Melanocortin 1 Receptor (MC1R)**

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**Background:** ACTH is a pituitary neuro-immuno-endocrine hormone and a prototype agonist of the melanocortin system. Clinical and experimental evidence demonstrates that ACTH has beneficial actions in chronic kidney disease; however, the mechanism(s) remain uncertain. This study examined the effect of ACTH gel (Acthar Gel, Questcor Pharmaceuticals, Inc.) on progressive renal tubulointerstitial injury.

**Methods:** Following unilateral ureteral obstruction (UO) surgery with or without simultaneous adrenalectomy, rats were administrated ACTH gel (10 IU/kg) or saline (CON) every other day for 2 weeks. Renal histology was assessed. Expression of cognate receptors of ACTH was evaluated in vivo and in cultured murine renal tubular epithelial cells (TEC).

**Results:** Compared to CON, ACTH gel prevented enlargement of the obstructed kidney as assessed by the increase in kidney to body weight ratio (1.3±0.14 vs. 1.6±0.99 g/100g, p=0.02). Morphologically, renal interstitial fibrosis and tubular atrophy were significantly ameliorated by ACTH gel. In addition, renal inflammation, marked by ED-1 positive macrophage infiltration, and UUO induced expression of chemokines MCP-1 and RANTES in tubules, were also attenuated by ACTH gel treatment. Although somewhat diminished, the beneficial effects of ACTH gel were still evident in UUO rats that underwent adrenalectomy, suggesting a steroid independent mechanism. Consistently, abundant expression of MC1R was observed in renal tubules of rat kidneys and in cultured TEC. In vitro, ACTH gel markedly suppressed TNF-α induced proinflammatory events in cultured TEC, including NFκB activation and downstream target gene expression. This effect was significantly diminished in TEC in which MC1R expression was silenced by RNAi, demonstrating that ACTH gel induced anti-inflammatory signaling in TEC requires MC1R.

**Conclusions:** ACTH gel markedly suppresses tubulointerstitial inflammation, tubular atrophy and fibrosis in progressive chronic kidney disease due, at least in part, to direct, anti-inflammatory effects on TEC. By silencing MC1R expression for the first time in kidney cells, we have also shown that these effects are mediated via the MC1R.
TH-PO115

Effects of Rho Kinase Inhibitor, Fasudil on Spontaneously Hypercholesterolemic Rats
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Background: Recent reports indicate the renoprotective effects of rho kinase inhibitor, fasudil by the inhibition of macrophage infiltration. Therefore we examine the long term therapeutic effects of fasudil on the spontaneously hypercholesterolemic (SHC) rat, paying special attention to the phenotypic changes in macrophages.

Methods: Eight-week-old male SHC rats were randomly assigned to 6 groups (n=7 each): 1) the vehicle-treated group (Ve), 2) the low dose fasudil-treated group (D0, 30mg/kg/day), 3) the high dose fasudil-treated group (f100, 100mg/kg/day), 4) the OL-treated group (OL, 5mg/kg/day), 5) the combination of low dose of fasudil and OL treated group (c30), 6) the combination of high dose of fasudil and OL treated group (c100). As a healthy control group, SD rats were treated with vehicle alone (n=7). Rats were killed after treating for 24 weeks.

Results: Urinary protein excretion in f100, OL and c30 groups was significantly less than that in Ve group. Surprisingly urinary protein excretion level in c100 decreased to the same level as control group. The total collagen content was significantly decreased in the c100 group compared with Ve group. Prominent interstitial ED-1 positive cells were observed in the Ve group were significantly attenuated in all the treatment groups. The CD80 and CCL3 (M1 macrophage marker) mRNA level relative to the GAPDH mRNA level was significantly decreased in c100 group. The relative expressions of CD206 (M2 macrophages marker), as assessed by the ratio of the CD206 mRNA to CD68 mRNA levels, was significantly greater in c100 than other treatment groups.

Conclusions: Fasudil showed significant beneficial effects on the interstitial fibrosis of SHC rats, the mechanism of which may be associated with antiproteinuric effects and increment in M2 macrophages.

TH-PO116

Human Prevents Intra-Renal Microvascular Remodeling, Inflammation, and Fibrosis in ApoE Knockout Mice
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Background: Atherosclerosis promotes chronic kidney disease. Human (HN) is an endogenous mitochondria-derived cytoprotective peptide, but its role in atherosclerosis-induced kidney damage is unknown.

Methods: Ten C57BL/6 and 12 ApoE KO mice were fed 16 wks of normal or high cholesterol (HC) diet, respectively, supplemented daily with Intraperitoneal Injection of saline (Control, n=4, HC, n=6) or HN (4 mg/kg/d, Control-HN, n=6, HC-HN, n=6). Microvascular remodeling was assessed ex-vivo with micro-CT and alpha-SMA staining. Angiogenesis, inflammation and apoptosis were evaluated in kidney tissue by Western-blots, and fibrosis by trichrome staining.

Results: ApoE KO mice had elevated serum cholesterol but normal creatinine levels. Cortical microvascular spatial density and media/lumen ratio were significantly increased in HC and restored in HC-HN.

Conclusions: HN attenuates murine HC-induced renal microvascular proliferation and remodeling by upregulating anti-angiogenic factors, and mitigates inflammation, apoptosis, and fibrosis. These findings suggest HN as a novel therapeutic target in atherosclerosis.

Funding: Private Foundation Support

TH-PO117

Role of mTOR Signaling in Interstitial Inflammation and Kidney Fibrosis
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Background: Progressive inflammation and fibrosis are responsible for chronic kidney failure, however, the molecular mechanism remains obscure. Recent studies have highlighted the mammalian target of rapamycin (mTOR) as a regulator of inflammatory reaction and collagen expression. In this study, we revealed a role of mTOR signaling in the progression of interstitial inflammation and fibrosis after kidney injuries.

Methods: Ischemia-reperfusion injury (IRI) or unilateral ureteral obstruction (UUO) mouse models were used in the experiment. Daily administration of Rapamycin was performed to inhibit mTOR signaling in designated groups. Immunohistochemical staining and western blot were performed to evaluate the activation of mTOR signaling.

Results: Ischemia-reperfusion signaling was significantly up-regulated 24-48 hours post-injuries in rodent models of either IRI or UUO. Confocal imaging confirmed that pS6K, a downstream target of mTOR signaling, was expressed in de-differentiated epithelial cells, myofibroblasts and macrophages in mouse models of severe IRI and UUO. Up-regulation of SMA, vimentin and collagen-I correlated well with the expression of pS6K in fibrotic kidneys. Interestingly, although high expression of pS6K was observed in macrophages, little could be detected in migrated T cells or neutrophils, which suggested macrophages may be primary effectors of mTOR signaling that triggered the inflammatory cascade at the onset of kidney fibrosis. Administration of rapamycin, a specific mTOR complex inhibitor, significantly reduced renal fibrotic production (SMA and collagen I) and interstitial inflammation in a mouse UUO model, including chemokines production (MCP-1, TNF-a, IL-1b and CXCL-1) and cells infiltration.

Conclusions: Taken together, our data suggest that activation of mTOR signaling mediates persistent tubulointerstitial inflammation and fibrosis after kidney injuries. Inhibition of mTOR signaling may be potential of anti-fibrosis in chronic kidney diseases.

Funding: Government Support - Non-U.S.

TH-PO118

Ischemic Preconditioning Lowers Renal Reperfusion Injury and Inflammatory Stress
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Background: Acute kidney injury is associated with major in-hospital morbidity and mortality. In addition to ischemia, inflammation linked to reperfusion injury amplifies tissue damage. The aim of the study was to evaluate the effect of ischemic preconditioning (IP) on a kidney ischemic-reperfusion injury model.

Methods: Male Wistar rats were randomly distributed in four groups (n=8 each). 1) Sham, 2)ISCHEMIA, both renal pedicles occluded for 40 minutes; 3)IP-10 minutes of ischemic preconditioning, 10 minutes of reperfusion and 40 minutes of ischemia; 4)IP-15 fifteen minutes of ischemic preconditioning, 15 minutes of reperfusion and 40 minutes of ischemia. Under topiical ip, laparotomy was performed and renal pedicles were occluded using Aescula Braun clamps. Ischemia and reperfusion were confirmed visually. A blood sample was obtained (tail vein) prior to surgery and 24 hs later. 48 hs after procedure, animals urine and blood samples were taken. Left kidney tissue sections were obtained for immune-histochemistry using anti-nitrotyrosine antibody. On right kidney tissue we measured CD40, IL5, IL10, TNF-a, TGF-b, TRL 3 and TRL 4 by RT-PCR. Values are reported as mean ± SD and statistical analysis was performed by ANOVA test.

Results: IP vs ISCHEMIA groups had significantly lower plasma urea levels at 48 hs (37.6 vs 112.5; P<0.0002) and creatinine levels at 24 hs (0.76 vs 2.06; P=0.004) and at 48 hs (0.57 vs 1.17; P<0.002). PI groups showed lower expression of pro-inflammatory molecules CD40, TNF-a and TLR3 (P<0.05) but not TLR4 (P=0.06). IL6, IL10 and TGF-b expression was no significantly different. IP groups showed less anti-nitrotyrosine staining than ISCHEMIA group (IP-10: 0.29±0.19; IP-15: 0.52±0.14; ISCHEMIA: 1±0.13; P=0.001).

Conclusions: Ischemic preconditioning, either 10 or 15 minutes, showed a protective effect from a further prolonged ischemic injury. This could be explained, at least in part, due to a lower inflammatory stress.

Funding: Government Support - Non-U.S.
TH-PO119
Activation of Type II NKT Cells Mediates Kidney Ischemia/Reperfusion Injury
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Background: CD1d-restricted type I and type II natural killer T (NKT) cells bridge innate and adaptive immune responses in kidney ischemia-reperfusion injury (IRI). Although type II NKT cells have not been as extensively studied as type I NKT cells, these cells have been shown to play a critical role in cancer and autoimmune diseases. We hypothesized that type II NKT cell activation is also involved in the immune response of kidney IRI.

Methods: We subjected mice to bilateral renal ischemia for 24 min followed by 24 hrs reperfusion, and plasma were collected for creatinine measurement.

Results: Compared to WT mice, CD1dKO mice (both type I and type II NKT cell deficient) were markedly protected, while CD1dAKO mice (type II NKT cell deficient) showed an intermediate degree of protection (P<0.05). Plasma creatinine was (mg/dl): 1.38±0.19, 0.78±0.02, 0.93±0.02, and 1.07±0.02 respectively. An optimal dose of sulfadiazine (1 g/kg) significantly and specifically activates type II NKT cells and promoted mild kidney IRI; plasma creatinine was 1.11±0.10 vs. 0.47±0.04, respectively (P<0.01). IL-12/IL23p40 and IL-23 pathways are required for sulfadiazine-induced inflammation in kidney IRI as a protective effect was shown in sulfadiazine treated IL-12/125KO, IL-23p40KO and IL-23p19KO mice (P<0.01).

Conclusions: Understanding the pathogenic role of NKT cells in the initiation of the immune response will provide new information for designing novel therapeutic strategies for AKI.

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TH-PO120
Mannose-Binding Lectin Mediates Renal Ischemia Reperfusion Injury Independent of Complement Activation
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Background: Ischemia/reperfusion injury (IRI) is an inevitable event in kidney transplantation and has a major impact on short- and long-term graft survival. Complement activation, a central component of innate immunity, is one of the hallmarks of renal IRI. Generally, high serum levels of Mannose Binding Lectin (MBL) have been associated with improved renal allograft survival.

Methods: We used an experimental rat model of IRI by 45 minute ischemia of uni-nephrectomized Lewis rats and investigated the role of MBL-mediated complement activation using blocking antibodies against MBL or C5, followed by functional and histological analysis. In vitro cultured human proximal tubular epithelial cells (PTEC) were used to investigate direct effects of MBL on tubular integrity

Results: Transient inhibition of MBL completely preserves renal function and tubular integrity and prevents influx of neutrophils and macrophages following experimental renal IRI in rats. Although complement deposition (C3 and C5b-9) is observed from 24 hrs onwards in non-treated rats, inhibition of the down stream complement mediators C3 and C5 was not protective. Histological signs of tubular injury were already observed within hours after reperfusion, accompanied by vascular leakage of MBL into the interstitium, reaching tubular epithelial cells in ischemic but not control kidneys. Exposure of in vitro cultured human PTEC to purified MBL resulted in specific binding and internalization of MBL, followed by a dose- and time-dependent induction of cell death. We demonstrated that we dependently activates NF-κB for TNF production as well as the base level of NF-κB expression. The activation of NF-κB and tissue production of HO-1 was significantly increased in MBL deficient kidneys subjected to hypoxia in vitro and in MBL-/- mice after ischemia/reperfusion in vivo. However, compared with the normal HK-2 cells and sham operated mice, the production of IL-12p35 was significantly lower in HK-2 and HK-1 was still low in MBL deficient HK-2 cells in vivo and in vitro. We observed severe histological changes in MBL deficient kidneys and in MBL-/- mice subjected to ischemic/hypoxic injury. By using pharmacological and genetic analyses, we found that activation of NF-κB in response to hypoxia/ischemia is dependent on MBL for the production of pro-inflammatory cytokines.

Conclusions: Our results show that MBL/MF2-related factor 2 (Nrf2), a basic leucine zipper transcription factor that regulates inflammation, leading to expression of Nrf2-regulated genes including heme oxygenase-1 (HO-1).

Funding: Other U.S. Government Support

TH-PO122
Acute Kidney Injury in Cisplatin Nephrotoxicity Is Dependent on Mast Cell Degranulation and TNF Release
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Background: Mast Cells (MCs) are pluripotent immune cells, which contain a large number of vasoactive and inflammatory molecules, which are released after degranulation. Previously we demonstrated that compared to C57BL/6 wild type (WT) mice, mast cell deficient, KitWsh/Wsh mice, are protected from cisplatin nephrotoxicity (CN). In these studies we sought to define the role of MCs in CN.

Methods: Initially we assessed the role of MC degranulation after cisplatin administration. To determine the role of MCs in AKI we administered cisplatin to WT and KitWsh/Wsh mice. We assessed kidney TNF mRNA expression and serum TNF production as well as pro-inflammatory chemokines. Subsequently we reconstituted KitWsh/Wsh mice with MCs from WT and TNF-/- mice, and then administered cisplatin. Renal injury and leukocyte recruitment was assessed 4 days later.

Results: Administration of cisplatin resulted in an increase in serum (control 33.4±2.7 vs. cisplatin treated 53.2±7.1 ng/ml, P<0.05) and kidney (control 1.7±0.7 vs. cisplatin treated 7.6±1.5 ng/ml, P<0.01) MC degranulation. Serum TNF levels (84.4±7.4 vs. 60.5±4.9pg/ml) were lower in KitWsh/Wsh mice. Intrarenal TNF mRNA expression, as well as mRNA for key T cell (RANTES/CCL5, IP-10/CXCL10), and neutrophil chemokines (KC/CXCL1 and MIP2/CXCL2) were also decreased in KitWsh/Wsh mice. To define the mechanism of MC-mediated AKI, KitWsh/Wsh mice, reconstituted with MCs derived from WT or TNF-/- mice, were given cisplatin. Compared to KitWsh/Wsh mice reconstituted with WT MCs, renal injury was attenuated in KitWsh/Wsh mice reconstituted with TNF-/- MCs (BUN 100.3±16.2 vs. 41.6±15.9mmol/l, P<0.01; histological injury assessed semiquantitatively 3.3±0.1 vs. 2.2±0.2, P<0.001). Compared to mice reconstituted with WT MCs there was a decrease in serum TNF production (WT MCs 104±6.3±7 vs. TNF-/- MCs 50±9.8 pg/ml, P<0.001) and kidney neutrophil recruitment.

Conclusions: Acute kidney injury induced by cisplatin is dependent on MC degranulation and TNF release.

Funding: Government Support - Non-U.S.

TH-PO123
Autoantibodies to LAMP-2 in ANCA Negative Pauci-Immunofocal Necrotizing Glomerulonephritis (pIFN GN) Andrew J. Rees, Renate Kain.

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Background: pIFN GN occurs characteristically in people with ANCA-associated vasculitis. However, ANCA assays are negative in up to 10% of patients; antibodies to MPO and PR3 cannot be detected thus leaving the cause of injury obscure. We reported a large number of patients with pIFN GN who had antibodies to pIFN GN and showed they could induce pIFN GN in rats. The purpose of this study was to ascertain the frequency of antibodies to LAMP-2 in ANCA negative pIFN GN more generally and to determine why the antibodies concerned fail to bind to LAMP-2 in neutrophils by determining indirect immunofluorescence.

Methods: Eleven ANCA negative patients were studied (10 with isolated pIFN GN and 1 with renal, lung and skin involvement). We standard confirmed fluorescent ANCA assays and ELISA for MPO and PR3 were negative in all 11. All 8 patients’ autoantibodies cross-reacted with LAMP-2. 6/8 patients with active pIFN GN (1 ANCA negative individual) and showed they could induce pIFN GN in rats. The purpose of this study was to determine the frequency of antibodies to LAMP-2 in ANCA negative pIFN GN more generally and to determine why the antibodies concerned fail to bind to LAMP-2 in neutrophils by determining indirect immunofluorescence.

Results: Antibodies to LAMP-2 in ANCA negative patients bound native LAMP-2 purified from glomeruli.

Methods: In the present study, we found that the expression of human proximal tubule epithelial cells (HK-2) and kidney-microdissected tubules in patients with active pIFN GN are significantly increased in renal biopsy LAMP-2-related factor 2 (Nrf2), a basic leucine zipper transcription factor that regulates inflammation, leading to expression of Nrf2-regulated genes including heme oxygenase-1 (HO-1).

Results: Suppression of MyD88 by specific shRNA transfection in HK-2 cells and MyD88-/- mouse renal microdissected tubules in patients with active pIFN GN are significantly increased in renal biopsy LAMP-2 related factor 2 (Nrf2), a basic leucine zipper transcription factor that regulates inflammation, leading to expression of Nrf2-regulated genes including heme oxygenase-1 (HO-1).

Conclusions: Our results show that MdB2-dependent mechanisms in the coordination of innate immune responses to ischemic/hypoxic acute renal tubular injury

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.

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Conclusions: In summary, we demonstrated that some ANCA negative patients with pIFNGN have antibodies to LAMP-2 that bind glomerular LAMP-2 extracellular domain in our standard ELISA and by immunoblot.

Funding: Government Support - Non-U.S.

TH-PO124
Expression of the PTPN22 Gain-of-Function Variant Results in ERK Inhibition Leading to Down-Regulation of IL-10 Expression Yali Cao, 1,2 Jia Jin Yang, 1 Susan L. Hogan, 1 Yichun Hu, 1 Caroline E. Jennette, 1 Elisabeth Berg, 1 J. Charles Jennette, 1 Ronald J. Falk, 1 Gloria A. Preston, 1 1 UNC Kidney Center, UNC-CH, Chapel Hill, NC; 2 Nephrology, China-Japan Friendship Hospital, Beijing, China.

Background: We predicted that cellular signaling dynamics would be disrupted by expression of the disease-associated allele of the protein tyrosine phosphatase PTPN22 (R620W), conferring a gain-of-function phenotype. This change negatively affects SRC activation due to loss of CSK sequestration. Signaling through RAAS is also affected through GRB2-PTPN22 (R620W) interactions.

Methods: Phosphatase activity was measured in leukocytes from patients with anti-neutrophil cytoplasmatic autoantibody (ANCA) disease, comparing patients with the gain-of-function allele to patients with the normal allele. PTPN22 protein was captured using an anti-PTPN22 antibody on two separate microtiter plates. One was analyzed for total protein captured and the other for activity status of the captured protein, reported as an activity/protein ratio. Activation of downstream signaling pathways was determined by western blot analysis. IL-10 transcription was quantitated by TaqMan PCR.

Results: Basal activity of PTPN22(R620W) variant was persistently elevated in leukocytes (p<0.0001), neutrophils (p=0.0004) and lymphocytes (p=0.0003), while undetectable in leukocytes expressing normal PTPN22. Inappropriate activity of the gain-of-function phosphatase resulted in downregulation of ERK, opposite to controls. Instead, p38 MAPK was up-regulated. IL-10 transcription, which is reliant on the ERK pathway, was negatively affected by expression of the variant with reduced levels compared to controls (p=0.0001). Over the course of disease, patients expressing variant PTPN22(R620W) did not show a spike in IL-10 transcription as they entered remission in contrast to controls, implying that environmentally triggered signals were blunted (p<0.0001).

Conclusions: Sustained activity of PTPN22, due to the gain-of-function mutation, acts as a dominant negative regulator of ERK activity leading to blunted cellular responsiveness to environmental stimuli and expression of protective cytokines.

Funding: NIDDK Support

TH-PO125
Spleen Tyrosine Kinase Is Involved in the Production of Pro-Inflammatory Cytokines by Human Mesangial Cells Following Stimulation with Polymeric IgA1 Isolated from IgA Nephropathy Patients Min Jeong Kim, 1 Jonathan Barratt, 2 Karen Molyneux, 2 Esteban S. Masuda, 2 Charles D. Pusey, 1 Frederick W.K. Tam, 1 Imperial College Kidney and Transplant Institute, London, United Kingdom; 2Dept. of Infection, Immunity & Inflammation, Univ. of Leicester, United Kingdom; 3Rigel Pharmaceuticals, South San Francisco.

Background: Polymeric IgA1 (pIgA) isolated from the serum of IgAN patients stimulates human mesangial cells (HMC) to produce pro-inflammatory cytokines. The aim of our study was to determine if spleen tyrosine kinase (Syk) is involved in HMC in the downstream signaling pathway of IgA receptors, leading to the production of pro-inflammatory cytokines.

Methods: HMC were incubated with pIgA, control IgA from healthy human (cIgA), or heat aggregated IgA (aIgA). We measured MCP-1 by ELISA and additional 26 cytokines by multiplex cytokine assay from the culture supernatant after 24h. We then incubated HMC with a Syk inhibitor, R406, 1h before stimulation with pIgA (50μg/mL) and performed real-time RT-PCR and ELISA. HMC were then transfected with Syk siRNA or negative control siRNA, 72h before stimulation with pIgA. Transfection efficiency was proved by Western blot.

Results: MCP-1 was significantly higher upon stimulation with pIgA than cIgA or aIgA in a dose dependent manner, and this was inhibited significantly by R406 (Fig 1g). The same was true for other cytokines (IL-6, IL-8, IL-9, IFN-γ and RANTES). The MCP-1 mRNA was also reduced significantly. The Syk expression was effectively suppressed and MCP-1 protein level after stimulation with pIgA was significantly reduced upon transfection with Syk siRNA (Fig 3).

Conclusions: Our data strongly suggest the involvement of Syk in HMC in the production of pro-inflammatory cytokines upon stimulation with pIgA, and its role in the pathogenesis of IgAN. Syk may be considered as a potential target in the treatment of IgAN.

TH-PO126
HIV Induces Activation of Renin Angiotensin System in Lymphocytes through Downregulation of Vitamin D Receptor Nirupama Chandel, Hersh Groel, Shabina Rehman, Ashwani Malhotra, Mohammad Hussain, Pravin C. Singhal. Medicine, North Shore LIJ Hofstra medical School, Great Neck, NY.

Background: Interstitial lymphocytes aggregation is frequently associated with renal fibrosis. Activation of the renin-angiotensin system (RAS) has been demonstrated to play a key role in the progression of HIV-associated nephropathy (HIVAN). Interstitial lymphocytes serve as a source for profibrotic cytokines and vasoactive agents such as Ang II. Vitamin D receptor (VDR) has been reported to be a negatively regulator of renin transcription. In the present study, we evaluated the effect of HIV infection on lymphocyte VDR expression and activation of the RAS.

Methods: Lymphocytes (LY) were isolated from human blood obtained from the New York Blood Bank (n=5). LYs were incubated with V4 virus or buffer for two hours and then washed and re-incubated in media for 24 hours. RNA and proteins were extracted from lymphocytes. Immunoblotting and real time PCR studies were performed for expression of VDR, angiotensinogen (Agt) and renin. Ang II ELISA was carried out on lymphocytes prepared under similar conditions. To establish a causal relationship between VDR and the RAS activation, lymphocytes with or without silenced VDR (siRNA-VDR/LY) were evaluated for the expression of Agt and renin and production of Ang II. To confirm the relationship between HIV and VDR, Lys and HIV/LY were treated with Vitamin D2 analogue for 24 hours; subsequently, VDR, renin, and Agt expression was determined by immunoelectron microscopy.

Results: HIV/LY showed attenuated expression (P<0.001) of VDR but displayed enhanced expression of Agt and renin (P<0.01). Moreover, HIV/LY showed 2.5 fold increased Ang II production (P<0.01) when compared to control lymphocytes. On the other hand, Vit D treated HIV/LY not only showed upregulation of VDR but also displayed attenuated expression of renin and diminished (P<0.05) production of Ang II.

Conclusions: These findings indicate that HIV enhances lymphocyte RAS activation through VDR downregulation. The present study provides mechanistic insight into the role of lymphocytes in the activation of the RAS in HIVAN.

Funding: NIDDK Support

TH-PO127
Angiotensin II Stimulates S100A12 Production in Macrophages Fiko Matsouka, Yasukiyo Morii, 1 Yaoi Shiotani, 1 Atsushi Kosaki, 2 Department of Cardiology and Nephrology, Kyoto Prefectural University of Medicine, Kyoto, Japan; 2Department of Medicine II, Kansai Medical University, Osaka, Japan.

Background: S100A12, formerly called EN-RAGE, is an endogenous ligand for the receptor for advanced glycation end products (RAGE). Our working hypothesis is that S100A12 protein might contribute to the development of atherosclerosis. We recently found that plasma S100A12 level was an independent factor associated with the prevalence of cardiovascular disease in hemodialysis patients (Shiotsu Y and Mori Y et al. Clin J Am Soc Nephrol 2011; 6: 718-723). S100A12 is reported to be abundantly expressed and secreted from neutrophils and monocytes/macrophages in human. However, the detail of regulatory mechanism of S100A12, especially in chronic kidney disease, remains unknown. In this study, we evaluate the effect of Angiotensin II (Ang II) on S100A12 transgenic mice (S100A12tg).

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Methods: We exploited the fact that S100A12 is not present in mice and generated the \( \text{TH}-\text{PO128} \) expressing human S100A12 in myeloid cells under the control of CD11b promoter. RT-PCR using the specific primer for human S100A12 and ELISA for human S100A12 protein, which was developed by our laboratory, were used to evaluate the S100A12 transcripts and its protein. Macrophages were collected from peritoneal cavities of S100A12tg. Because we found the expression of S100A12 in HepG2, the cell line from human hepatic carcinoma, in the preliminary experiment, HepG2 was used as a reference.

Results: In HepG2, Ang II increased the level of S100A12 mRNA in a time-dependent manner (2-fold; p<0.01). The expression of S100A12 was significantly increased in the supernatant of S100A12tg after stimulation with deSial/deGal IgA1 (1.44 ng/mL) after the treatment with Ang II.

Conclusions: These findings suggest that Ang II stimulates S100A12 production in macrophages.

TH-PO130

Stimulation of Wnt/beta-Catenin Signaling by Paricalcitol Prevents oxLDL-Induced Tregs Apoptosis and Restores Their Function

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Background: Ox-LDL induces Tregs cell cycle arrest and apoptosis affecting their suppression capacity and finally promote a constant micro-inflammatory state in patients with ESKD. This is partly due to the down-regulation of the Wnt-beta-catenin signaling that promotes phosphorylation of beta-catenin leading this latter to its ubiquitination and degradation by the proteasome. Because vitamin D analogs can modulate beta-catenin in other tissues, we tested whether the vitamin D analog paricalcitol could ameliorate Tregs response by stimulating Wnt-beta-catenin signaling in presence of oxLDL.

Methods: Tregs from healthy blood donors were incubated for up to 72 h in RPMI alone with oxLDL (100 μg/mL) before washing and then treated with paricalcitol (1.5 ng/mL). To better analyze the effects of paricalcitol, Tregs were stimulated with Abs to CD3/CD28 (10 μg/mL). RT-PCR and Western blot analyses were performed. Cell viability was measured using a MTT assay and apoptosis indirectly assessed by Fas staining (flow cytometry) and confirmed by DNA fragmentation. To address their suppressive capacity, activated Tregs were analyzed in co-culture with CD4+/CD25+ T cells \( (T_{\text{reg}}) \) in presence of APC.

Results: Compared with vehicle-treated Tregs, paricalcitol significantly upregulated beta-catenin in activated Tregs after oxLDL treatment. Quantitative determination showed a more than 150-fold and 80-fold reduction of beta-catenin protein over the controls and vehicle-treated Tregs, respectively. Immunohistochemical staining confirmed the beta-catenin upregulation in cytoplasm and nucleus. The expression of the anti-apoptotic protein, Bcl-\( x_\text{s} \), an upstream mediator of the Wnt/beta-catenin signaling in Tregs was examined. Bcl-\( x_\text{s} \), was markedly down regulated in oxLDL-treated Tregs; and administration of paricalcitol largely stimulated nuclear Bcl-\( x_\text{s} \), synthesis in parallel with the lower expression of cell-surface apoptotic marker Fas and less DNA fragmentation.

Conclusions: Thus paricalcitol improves the cytotoxic and nuclear expression of beta-catenin in that it turn favors Bcl-\( x_\text{s} \), transcription protecting Tregs to enter into apoptosis; and finally, significantly ameliorates oxLDL-treated Tregs function.

Funding: Clinical Revenue Support

TH-PO131

Neutrophil Granulocytes Modulate T Lymphocyte Response in Peritonitis in Mice and Humans

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Background: In patients undergoing peritoneal dialysis, peritonitis is a common complication and can limit this treatment. Under resting conditions most peritoneal leukocytes are T lymphocytes, but neutrophilic granulocytes invade the peritoneal cavity in large numbers during peritonitis. Here we investigated how neutrophilic granulocyte accumulation alters cellular antigen-specific host response by glycolipid-specific invariant natural killer T (iNKT) cell response.

Methods: Leukocytes were recovered from peritoneal fluid from stable patients on peritoneal dialysis and patients with acute peritonitis. Neutrophilic peritonitis was induced in mice by thygocollate injection. T lymphocytes were co-incubated with granulocytes in vitro. iNKT cells were stimulated with the specific antigen alpha-Galactosyl-ceramide. Multi-color flow cytometry and cytokine ELISA were used for analysis.

Results: Neutrophilic inflammation in experimental peritonitis in mice decreased iNKT cell function by decreased cell cycle arrest and apoptosis affecting their suppression capacity and finally promote a constant micro-inflammatory state in patients with ESKD. This is partly due to the down-regulation of the Wnt-beta-catenin signaling that promotes phosphorylation of beta-catenin leading this latter to its ubiquitination and degradation by the proteasome. Because vitamin D analogs can modulate beta-catenin in other tissues, we tested whether the vitamin D analog paricalcitol could ameliorate Tregs response by stimulating Wnt-beta-catenin signaling in presence of oxLDL.

Funding: Other NIH Support - K. Ley and M. Kronenberg were supported by NIH R01A145053, R37AI71922 (M.K.) and HL58108 (K.L.), Government Support - Non-U.S.

TH-PO132

Lipoxyein A4 Programmers CD16+ Mononuclear Cells

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Background: The diversity of macrophage influence on kidney disease is related to their phenotypic and functional heterogeneity. CD16+ macrophages (M1) are pro-inflammatory, contributing to renal fibrosis and progression of CKD, whereas CD16- cells (M2) are anti-inflammatory and pro-resolving. Monocytes exhibit similar non-uniformity,
with upregulation of a CD16 subset occurring in haemodialysis, and contributing to increased monocyte/macrophage recruitment. L-FABPs are endogenously produced transcription factors, with established anti-inflammatory and pro-resolving properties. The purpose of this study was to determine whether lipoxin A4 (LXA4) would prove effective in reducing pro-inflammatory mononuclear cell populations.

Overexpression of Human Liver-Fatty Acid-Binding Protein (L-FABP) Enzymatically Enhances Peroxisome Proliferator-Activated Receptor (PPAR)-α Activity Possibly Via Inducing Hepatocyte Nuclear Factor (HNF)-4α in Immortalized Mouse Proximal Renal Tubular Cells (mProx)

Results: Treatment of both monocytes and macrophages with LXA4 resulted in a significant (p < 0.05) decrease in CD16 expression, as compared with IFN-γ treated cells, suggesting that the monocyte and macrophage inflammatory subsets, and promotes the macrophage M2 phenotype. Analysis by ELISA of supernatants revealed significantly reduced macrophage production of the pro-inflammatory cytokines IL-6 and TNF-α (p < 0.05), following treatment with LXA4, again suggesting a role for this agent in directing macrophage phenotype.

Conclusions: Lipoxin A4 may have therapeutic potential in reprogramming pro-inflammatory monocytes and macrophages, thereby inducing a diminishment of their detrimental influence, but a beneficial promotion of the anti-inflammatory and pro-resolving activities of M2 macrophages.

Funding: Government Support - Non-U.S.

TH-PO113

Interactions between Complement and Kidney Cells: Implications in Renal Diseases Yasuhiro Tsuchiya,1 Satoshi F. Zipfel,2 and Gunter B. Wolf.2 1Research Institute for Natural Product Research and Infection Biology, Hans Knöll Institute, Jena, Germany; 2Internal Medicine III, University of Jena, Jena, Germany.

Background: The complement system is an enzyme cascade of more than 60 proteins and activation products which are involved in innate immune activation and regulation that leads to effector functions including the deposition of the C3b, release of the anaphylatoxin C3a, and formation of the terminal complement complex (TCC). Recently, complement has been implicated in the pathogenesis of primary renal disease and renal transplant rejection. There are caused by complement gene mutations of components, changes in complement levels in the circulation, or presence of complement components in renal tissues. This study focuses on the expression and activity of complement components and regulators of renal cells and their modification during lysis.

Methods: Since kidney cells were damaged when exposed to normal human serum (NHS) in most kidney diseases, human renal proximal tubular cells (HK-2) were challenged with NHS and viability, complement activation, TCC deposition, and presence of complement membrane regulators were assessed using flow cytometry, real time RT-PCR and viability assays.

Results: Exposure to increasing amounts of NHS caused damage revealed by C3b deposition, C3a generation, surface deposition of TCC and decreased viability of the renal cells. However, complement regulators CD46, CD55 and CD59 were detected on the surface of the cells. The complement regulator CD59 was also upregulated in HK-2 cells upon NHS incubation as shown in real time PCR. Moreover, HK-2 cells produced C3 which could be cleaved by Kallikrein and Thrombin, two members of the coagulation system.

Conclusions: The generated C3a acts as an effector molecule and as a cytokine and initiates the induction of pro- and anti-inflammatory factors. Thus the induction of anti-inflammatory response of lysed kidney cells. These results show that exposure to NHS affects viability of renal cells due to activation of the complement system. However, renal cells respond by expressing membrane complement regulators like CD59. Thus, the complement system plays an essential role in the pathogenesis of renal diseases.

Funding: Government Support - Non-U.S.

TH-PO134

Overexpression of Human Liver-Fatty Acid-Binding Protein (L-FABP) Endogenously Enhances Peroxisome Proliferator-Activated Receptor (PPAR)-α Activity Possibly Via Inducing Hepatocyte Nuclear Factor (HNF)-4α in Immortalized Mouse Proximal Renal Tubular Cells (mProx) Hideki Kimura,1 Daisuke Mikami,1 Kazuko Kamiyama,1 Kenji Kasuno,1 Naoki Takahashi,1 Takeshi Sugaya,1 Haruyoshi Yoshida.2 1Div of Nephrol, Dept of General Med, Sch of Med, Univ of Fukui, Fukui, Fukui, Japan; 2CMIC Co, Ltd, Tokyo, Japan; 3Dept of Med, Obama Municipal Hosp, Obama, Fukui, Japan.

Background: PPARα reportedly exerts anti-inflammatory and anti-fibrotic actions in proximal renal tubular cells (PRTC) under inflammation and hypoxia. L-FABP abundantly expressed in PRTCs of human kidney may modulate PPAR activity via their molecular interaction as well as serves as an anti-oxidant. However, no information has been known about the effects of L-FABP induction on PPAR activity in PRTCs.

Methods: Human immortalized proximal tubular epithelial cell line mProx (hL-FABP-mProx) was generated by transfection of the genomic DNA, and analyzed for specific factors involved in PPAR pathway and inflammatory effector molecules, because mouse L-FABP is scarcely expressed in mProx.

Results: In hL-FABP-mProx, hL-FABP expression was up-regulated by over 300-fold as compared with mProx. PPAR-α expression was increased by 10-fold, while PPAR-γ was down-regulated by 5-fold. Palmitate-induced PPRE-luciferase activity (PPAR-α activity) was increased by 2-fold, while PPAR-γ activity was unchanged. mRNA levels of mouse L-FABP, a target gene of PPAR-α, were 40-fold greater in hL-FABP-mProx than in mProx, which was not further augmented by an exogenous PPAR-α activator (fenofibrate), while hL-FABP-mProx PPAR-γ were augmented by the exogenous PPRE activators for PPAR-α expression, hL-FABP overexpression enhanced HNF-4α expression by over 100-fold, but had no affect on Lipin 1 expression. Hypoxia decreased HNF-4α and PPAR-α expression by 40-50% in hL-FABP-mProx. Finally, hL-FABP-mProx showed not only reduced basal expression of MCP-1, PAI-1 and CTGF, but also reduced TNF-α-stimulated expression of MCP-1 by 40-70% as compared with mProx.

Conclusions: hL-FABP overexpression in mProx endogenously enhances PPAR-α activation possibly via inducing HNF-4α expression. PPAR-α and HNF-4α induction may cause the reduced inflammatory response in hL-FABP-mProx.

Funding: Other NIH Support - NICHD Child Health Research Career Development Award

TH-PO115

Uremic Priming of a Macrophage Pro-Inflammatory Response Neal B. Blatt,1 Patricia L. Christopherson,1 Timothy Cornell,1 Thomas P. Shanley.2 1Pediatrics - Nephrology, University of Michigan, Ann Arbor, MI; 2Pediatrics - Critical Care, University of Michigan, Ann Arbor, MI.

Background: Monocytes from ESRD patients demonstrate increased cellular activation including increased pro-inflammatory cytokine production. These findings implicate these cells as a key player in the development of ESRD-mediated chronic inflammatory state and excess cardiovascular disease. However, the majority of monocyte samples have been obtained from patients on dialysis (with bioincompatible cuprophane membranes), making it difficult, if not impossible to distinguish the impact of dialysis from the impact of ESRD itself on monocyte function. Our goal is to use a mouse model of chronic renal failure to determine the impact of uremia on macrophage function.

Methods: 129SvJ mice underwent subcutaneous nephrectomy (SNx) or a sham procedure. Bone marrow-derived macrophages (BMDM) were isolated from sham and SNx mice and then stimulated with the Toll-like receptor (TLR) ligands lipopolysaccharide (LPS) or lipopolysaccharide (LPS).

Results: SNx mice developed an approximate doubling in BUN values within 4 weeks post-surgery that remained stable for at least 6 months (SNx: 47.9 ± 9 vs Sham: 24.3 ± 3 mg/dL, P < 0.001). Inducing the development of uremia, BMDM from SNx mice showed increased TNF-α production in tissue culture supernatants following stimulation with both LPS (SNx: 661.3 ± 74 vs sham: 394.4 ± 42 mg/mL, P < 0.05) and LTA (SNx: 2248 ± 100 vs sham: 1808.6 ± 62, P < 0.05). At the mRNA level, SNx BMDM showed 50% less TNF-α transcript in unstimulated conditions (SNx: 0.5 ± 0.1 vs sham: 1.0 ± 0.1 fold-induction, P < 0.05), however upon LPS stimulation, the TNF-α transcript increases in parallel to what is seen at the protein level (SNx: 40.5 ± 8.7 vs sham: 5.9 ± 6.7-fold-induction, P < 0.05).

Conclusions: We have utilized a well-established mouse model of chronic renal failure to demonstrate that macrophages from uremic mice are primed to respond to TLR ligands. These findings suggest that uremia by itself is able to reprogram the bone marrow to foster the development of a chronic inflammatory state. Additional experiments are in progress to look at epigenetic regulation of these pro-inflammatory macrophage cytokine responses.

Funding: Other NIH Support - NICHD Child Health Research Career Development Award

TH-PO135

Thrombin Stimulates Production of Colony Stimulating Factors in Proximal Tubular Epithelial Cells – Possible Role for Tubulointerstitial Injury Via Chemokine Regulations Michiko Shimada,1 Yuko Shimaya,1 Hideaki Yamabe,2 Yoshiko Shuto,3 Takeshi Fujita,4 Norio Nakamura,4 Ken Okumara.5 1Department of Nephrology, Hirosaki University, Hirosaki, Japan.

Background: Colony-stimulating factors (CSFs) are well-known hematopoietic growth factors. However, recent studies revealed that CSFs are involved in many inflammatory conditions. In the experimental anti-glomerular membrane glomerulonephritis, both glomerular lesions and tubulointerstitial lesions were attenuated in the granulocyte-macrophage CSF (GM-CSF) knockout mice. Whereas, it is suggested that Granulocyte-CSCF (G-CSF) may attenuate cisplatin-induced acute kidney injury by accelerated re-generation and decreased apoptosis of tubular cells. However, the local productions of CSFs and its regulation in the kidney is not well elucidated.

Methods: We used primary human proximal tubular epithelial cells (PTEC) to test the effect of thrombin for CSFs production, since thrombin is suggested to induce tubulointerstitial injury. PTEC were incubated with thrombin (0.5–5.0 Units/ml) and the effect on the production of GM-CSF, G-CSF, macrophage-CSF (M-CSF), interleukin-8 (IL-8) and monocyte chemotactic protein-1 (MCP-1) were measured in the cell supernatant by enzyme-linked immunosorbent assay (ELISA) and mRNA expressions were analyzed by quantitative RT-PCR. Also, we used direct thrombin inhibitor argatroban to verify the regulation of thrombin. Furthermore, we use mouse CSFs for the chemokine expressions, using transient blockade of CSFs by sIRNA technique.

Results: Thrombin stimulated the production of CSFs (GM-CSF, G-CSF, M-CSF) and chemokines (MCP-1 and IL-8) demonstrated by ELISA for protein expression and by quantitative RT-PCR for mRNA expression. These effects of thrombin were significantly known reduced by argatroban demonstrating specific effects of thrombin. Besides, induction of MCP-1 by thrombin was attenuated by M-CSF knockdown, and IL-8 induction was attenuated by G-CSF knockdown, showing chemokine expressions in PTEC were at least in part regulated by CSFs produced locally by PTEC.

Conclusions: Thrombin stimulates the production of CSFs by PTEC. Locally produced CSFs at least in part regulates chemokine expressions in PTEC.

Funding: Other NIH Support - NICHD Child Health Research Career Development Award
PGA-Peptoid I Inhibits Apoptosis and Inflammation in Renal Cells

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Background: Tissue injury is an unwanted adverse effect of inflammation. Inflammatory stimuli may induce cell injury and death and, in turn, injured cells may promote inflammation. Thus, a therapeutic agent that targets both cell death and inflammation may be of particular interest.

Results: A family of Paf-1 inhibitor nanomedicines that mediate apoptosis includes PGA-peptoid (QM56). In a pilot report that QM56 inhibits apoptosis in renal tubular cells co-stimulated by the lethal cytokine cocktail TWEAK/TNFα/INFγ. In addition, QM56 inhibited MCP-1 and Rantes chemokine expression in response to this stimulus. The anti-inflammatory effect of QM56 was independent from protection of apoptosis, since it also inhibited the inflammatory response to TWEAK (a cytokine that alone promotes tubular cell proliferation but not apoptosis) in tubular cells as well as renal fibroblasts. In addition, the anti-inflammatory effect showed by QM56 was independent from Paf-1, since it was observed in MEF-Paf-1-/- cells. TWEAK-induced MCP-1 and Rantes synthesis is NF-κB-dependent and is prevented by the NF-κB inhibitor parthenolide. TWEAK also activated the JAK2/STAT pathway. JAK2/STAT activation was required for the full expression of TWEAK-induced chemokine mRNA, but not for p65 nuclear translocation. In this regard, QM56 also failed to inhibit p65 nuclear translocation and DNA binding in response to TWEAK while it was inhibiting the JAK2/STAT3 phosphorylation/activation and hence chemokine expression elicited by the cytokine. Along with the inhibition of chemokine synthesis, blockade of the JAK2/STAT3 pathway by QM56 was also corroborated in MEF-Paf-1-/- treated with TWEAK.

Conclusions: We conclude that the anti-inflammatory activity of QM56 was independent of its role as inhibitor of apoptosis and that it could have potential therapeutic relevance.

Funding: Private Foundation Support, Government Support - Non-U.S.

Enhanced VASP Expression during Human Glomerulonephritis and Diabetic Nephropathy

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Background: Vasodilator stimulated Phosphoprotein (VASP) is a central cytoskeletal protein that has been involved in platelet adhesion, migration and endothelial barrier function and integrity in experimental disease models including passive crescentic nephritis by our group (JASN 2005 Apr;16(4)) but not in human kidney disease such as glomerulonephritis (GN) or diabetic nephopathy (DN).

Methods: We performed immunohistochemical staining in 120 human renal biopsies with diabetes, chronic GN, IgAN, lupus nephritis, vasculitis, Schönlein-Henoch, endocardial GN, MPGN or crescentic nephritis and 50 biopsies with DN. We investigated VASP in glomerular and tubulointerstitial (TI) compartments regarding expression and distribution and compared data to biopsies without apparent renal disease (GR). We compared the number of progressors and non-progressors with DN with regard to VASP expression.

Results: Compared to controls where VASP expression was low, biopsies from most GN demonstrated enhanced VASP expression in glomeruli (p<0.05 for IgAN, vasculitis, MPGN, lupus N, crescentic nephritis, endocardial GN) and also in the TI (p<0.05 for controls) and in patients with lupus, vasculitis, MGN, MPGN. In most biopsies there was a prominent endothelial expression pattern of VASP in glomerular as well as peritubular capillaries. Immunostaining of inflammatory cells also showed relevant amounts of VASP. Endothelial VASP was prominent in mild diabetic lesions but demonstrated no further increase with ongoing injury. In GN, TI VASP expression correlated with the decline in GFR as estimated by the MDRD equation (P<0.0001, r=0.341).

Conclusions: VASP as a known important regulator of EC barrier and function shows a prominent endothelial expression pattern in human GR and DN, where EC function is markedly disturbed. The correlation of TI VASP and falling GFR also indicates this functional link.

Uregulation of Serum Amyloid A in ESRD Patients Is Associated with Presence of a Unique Biomarker Identified by Protein Chip Array Analysis

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Background: Serum amyloid A (SAA) is an acute phase reactant which is regulated by IL1, IL-6 and TNFα. The circulating levels of serum amyloid A are markedly increased in inflammatory conditions. SAA is a 104 amino acid polypeptide with a molecular mass of 12-14 KDa. Protein Chip Array profiling has been used to identify unique biomarkers in ESRD patients. The purpose of this study was to determine the relationship of a previously reported biomarker in ESRD with circulating levels of SAA.

Methods: Plasma samples from 117 ESRD patients, over the age of 18 on maintenance hemodialysis and 50 age matched controls (healthy volunteers) were included in this study. SAA levels were measured by using a sandwich ELISA method (Abayzne, Needham, MA). Protein Chip Array profiling was carried out utilizing surface enriched laser desorption (SELDI), BioRad Corp, Hercules, CA utilizing gold chips.

Results: The plasma SAA levels were markedly elevated in ESRD (41.8±12.8; range 4.6-98.1 µg/ml) in contrast to the normals (4.5±1.2; range 1.1-9.8 µg/ml). SELDI analysis revealed a unique biomarker signal in 78 out of 117 (67%) in the ESRD patients and 2 out of 50 (4%) in the normal individuals. The presence of the 11.6 KDa biomarker correlated with the high SAA levels.

Conclusions: These studies suggest that SAA level upregulation is associated with the presence of the 11.6 KDa biomarker. Protein Chip Array profiling may be useful in the risk stratification of ESRD patients.

Serum CRP and IgA1 O-Glycosylation as Predictors of Disease Progression in IgA Nephropathy

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Background: IgA nephropathy (IgAN) is an inflammatory glomerulonephritis which is progressive in some patients. We have shown that abnormal O-glycosylation of serum IgA1 early in the disease is associated with risk of progression. C-reactive protein (CRP) may also be associated with progression but the relationship between CRP and IgA1 O-glycosylation has not been investigated.

Methods: We studied sera from 70 patients with biopsy-proven IgAN obtained early in the course of their disease while renal function was normal, and 70 healthy controls. The patients were classified as progressors (serum creatinine increased by >100% during follow up, n=32), or non-progressors (serum creatinine remained normal after a minimum ten years follow up, n=38). CRP was measured by ELISA. IgA1 O-glycosylation was previously measured.

Results: In our study, the mean serum CRP was higher in patients than controls (IgAN 2.8±0.49µg/ml, controls 1.78±0.27µg/ml, p=0.012). Furthermore, the progressors had higher CRP than non-progressors (progressors 3.18±0.52µg/ml, non-progressors 2.59±0.79µg/ml, p=0.023). However, there was no correlation between IgA1-HA and CRP in progressors, non-progressors or controls. We compared the number of progressors and non-progressors with high or low CRP and IgA1-HA (defined as above or below median respectively). Combining the two markers, 12 progressors and 6 non-progressors had both high IgA1-HA and high CRP compared to 4 progressors and 15 non-progressors with both low IgA1-HA and low CRP (p=0.008).

Conclusions: Our results confirm that both high IgA1-HA and high CRP at an early stage of IgAN are associated with subsequent development of progressive renal disease. However, neither could individually account for progression. We found no correlation between IgA1-HA binding and CRP, indicating that they are independently associated with progression in IgAN. Combining them gave a better indication of a subsequent course of the disease.
The Activation of Renin-Angiotensin System Is Involved in Hyperlipidemia Mediated Renal Injuries in Apolipoprotein E Knockout Mice

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Background: The activation of renin-angiotensin system (RAS) and hyperlipidemia play crucial roles in the progression of chronic kidney disease (CKD). Whether there is the interaction of hyperlipidemia with RAS activation in the accelerating process of CKD remains unknown. This study was to investigate the role of RAS activation in hyperlipidemia mediated renal injuries using an atherosclerotic mouse model from apolipoprotein E knockout mice (apoE-/- mice).

Methods: Male apoE-/- mice were respectively fed with high fat diet (HF group, containing 40% fat and 0.15% cholesterol, n=8) and normal chow diet (control, n=8) for 8 weeks. Lipid profile in the plasma was analysed by clinical biochemistry assay. The plasma levels of prorenin, renin, and angiotensin II were checked by radioimmunoassay. The protein expressions of angiotensinogen, angiotensin II, renin, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2) in kidneys of apoE-/- mice were checked by immunohistochemical staining and Western Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Results: It was found that mice fed with high fat diet developed hyperlipidemia with significant extracellular matrix deposition in renal tubular interstitium of kidneys compared to the controls. The plasma levels of renin and angiotensin II didn’t present significant changes between groups except that plasma prorenin level was significantly reduced in HF group. The protein expressions of angiotensinogen, angiotensin II, renin, ACE, AT1 and AT2 in the kidneys of HF group were significantly increased, which was significantly associated with the extracellular matrix deposition.

Conclusions: Our findings suggest that the activation of intra-renal RAS might be involved in hyperlipidemia mediated renal injuries, suggesting a new potential mechanism for the synergistic effect between hyperlipidemia and RAS activation in accelerating the progression of CKD.

Funding: Government Support - Non-U.S.

Role of Mineralocorticoid Receptor and Rac1 in Inflammatory Cytokine Production in Rat Peritoneal Macrophages

Yuan Huang, 1 Gerda A. Noordmans, 1 Jan-Luuk Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Methods: Male apoE-/- mice were respectively fed with high fat diet (HF group, containing 40% fat and 0.15% cholesterol, n=8) and normal chow diet (control, n=8) for 8 weeks. Lipid profile in the plasma was analysed by clinical biochemistry assay. The plasma levels of prorenin, renin, and angiotensin II were checked by radioimmunoassay. The protein expressions of angiotensinogen, angiotensin II, renin, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2) in kidneys of apoE-/- mice were checked by immunohistochemical staining and Western Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Results: It was found that mice fed with high fat diet developed hyperlipidemia with significant extracellular matrix deposition in renal tubular interstitium of kidneys compared to the controls. The plasma levels of renin and angiotensin II didn’t present significant changes between groups except that plasma prorenin level was significantly reduced in HF group. The protein expressions of angiotensinogen, angiotensin II, renin, ACE, AT1 and AT2 in the kidneys of HF group were significantly increased, which was significantly associated with the extracellular matrix deposition.

Conclusions: Our findings suggest that the activation of intra-renal RAS might be involved in hyperlipidemia mediated renal injuries, suggesting a new potential mechanism for the synergistic effect between hyperlipidemia and RAS activation in accelerating the progression of CKD.

Funding: Government Support - Non-U.S.

Continuous Monitoring of Hemodialysis with Pulse-Wave Analysis

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Background: Blood pressure (BP) and pulse (HR) monitoring can’t always predict symptomatic circulatory stress during hemodialysis (HD), which doesn’t always indicate simple hypovolemia. In pulse-wave analysis (PWA), pulse stress from a pulse-oximetry waveform analyzes the combined hemodynamic effects of cardiac ejection and arterial distensibility, expressed as effective circulatory flow (eCF). Comparing eCF to HR estimates hemodynamic stress and provides a real-time estimate of autoregulatory reserve capacity.

Methods: We studied 24 subjects with known heart disease or frequent symptoms with the CV Insight™, a novel PDA device, during 33 HD. PDA data was compared to continuous HR and BP monitoring, with clinical observation to detect dialysis symptoms such as cramps and nausea. We analyzed observations post-hoc to develop algorithms to predict events/symptoms.

Results: 16/33 HD had PDA patterns resembling volume depletion in lower body negative pressure experiments. eCF fell progressively with volume removal. HR typically increased when eCF had decreased to <60% of initial values; hypotension or diastolic symptoms often followed within 25-45 min. Such patients typically felt cold during stress. In 6 HD starting with marked volume expansion, eCF increased and HR decreased during the first 1-2 hours, before a typical pattern emerged. In 7 HD, eCF was consistently high, even with increased HR and symptoms; these patients typically felt uncomfortably hot under stress, suggesting inappropriate peripheral vasodilation. In 2 HD, arrhythmia interfered with interpretation of PWA.

Conclusions: PWA is a novel non-invasive technique yielding valuable information about circulatory stress and autoregulatory capacity during HD. This pilot study shows distinct patterns of compartment hydratuics which may explain patient variability in ultrafiltration tolerance. PWA profiling may assist clinicians in assessing optimal target weight and minimizing the risk of volume overload.

Funding: Private Foundation Support

Tests with Novel Adsorbents In Vitro

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Background: As part of the Dutch/European consortia iNephron iNephron+ we are involved in the development of an adsorbent-based wearable artificial kidney device (WAKD). With this WAKD we aim to replace renal function continuously 24h. Currently we tested adsorbance by a zeolite targeting K+, a ferric hydroxide targeting PO4-, and a modified carbon targeting protein-bound substances, such as PAH.

Methods: Plasmapheresis plasma of Goodpasture and anti-GBM nephritis patients (N=3) was exposed to 100mg of each matrix for 1 to 3 hours.

Results: After one hour K+ decreased from 4.11±0.30 to 2.51±0.30 mM (P<0.001), and relative PAH concentration from 100% to 14±10% (P<0.001), and the relative PAH concentration from 100% to 14±10% (P<0.001).

Conclusions: Our study identified strain differences with respect to the presence and number of perivascular immune cell clusters in aged kidneys. A genetic analysis identified novel candidate genes that might participate in renal aging and provide novel means to prevent aging-related diseases.

Funding: Private Foundation Support

Inflammatory Cytokine Infiltration in Aging Kidneys and Identification of Associated Genes

Yuan Huang, 1 Gerda A. Noordmans, 1 Jan-Luuk Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Methods: Male apoE-/- mice were respectively fed with high fat diet (HF group, containing 40% fat and 0.15% cholesterol, n=8) and normal chow diet (control, n=8) for 8 weeks. Lipid profile in the plasma was analysed by clinical biochemistry assay. The plasma levels of prorenin, renin, and angiotensin II were checked by radioimmunoassay. The protein expressions of angiotensinogen, angiotensin II, renin, angiotensin converting enzyme (ACE), angiotensin II type 1 receptor (AT1) and angiotensin II type 2 receptor (AT2) in kidneys of apoE-/- mice were checked by immunohistochemical staining and Western Blot. The extracellular matrix deposition in kidneys was evaluated by Masson-staining.

Results: It was found that mice fed with high fat diet developed hyperlipidemia with significant extracellular matrix deposition in renal tubular interstitium of kidneys compared to the controls. The plasma levels of renin and angiotensin II didn’t present significant changes between groups except that plasma prorenin level was significantly reduced in HF group. The protein expressions of angiotensinogen, angiotensin II, renin, ACE, AT1 and AT2 in the kidneys of HF group were significantly increased, which was significantly associated with the extracellular matrix deposition.

Conclusions: Our findings suggest that MR participates in the inflammatory cytokine secretion in peritoneal macrophages, implicating the cross-talk between Rac1 and MR cascades.

Funding: Government Support - Non-U.S.
better estimates of bone Ca buffering. Although prediction errors were small on average, the
by taking into account individual pre-HD serum citrate levels, pH levels and shifts, and
directed towards further explaining and reducing the spread of the prediction error, e.g.
was underestimated by 0.046±0.074 mmol/L on bicarb dialysate and overestimated by
dialysates. This was fairly consistent across the spectrum of iCa levels. End-HD iCa
were adapted to measured values to assess the intra-HD simulation quality.
model and compared to measured values. For post-HD simulations, starting iCa levels in
model of CD (Thijssen S et al., Blood Purif 2010). The goal of this study was to validate a
by these adsorbents. Preclinical in vivo experiments will be performed in goats.
Funding: Private Foundation Support

**TH-PO147**

Independent Validation of a Versatile Citrate Dialysis Model  
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**Background:** Citrate dialysis (CD) offers many advantages over heparin dialysis,
but calcium kinetics in CD are complex. We recently introduced a versatile mathematical
model of CD (Thijssen S et al., Blood Purif 2010). The goal of this study was to validate a
refinement of this model in a new setting using data from an independent clinical trial.

**Methods:** Data from a recently completed trial on the effects of citrate-containing
dialysate on heparin requirements (Sands et al., ASN 2010) were used. Briefly, patients
were studied prospectively on standard bicarb dialysate as well as on Citrasate® dialysate
(citrates, 2.4 mEq/L; Ca, 2.5 mEq/L). Pre- and post-HD serum iCa was simulated with our
model and compared to measured values. For post-HD simulations, starting iCa levels in
the model were adapted to measured values to assess the intra-HD simulation quality.

**Results:** Pre-HD iCa was underestimated by about 0.12±0.06 mmol/L with both
dialysates. This was fairly consistent across the spectrum of iCa levels. End-HD iCa
was underestimated by 0.046±0.074 mmol/L on bicarb dialysate and overestimated by
0.077±0.046 mmol/L on Citrasate® (Fig. 1). The prediction errors with both dialysate types
were less variable with this refined model than our previously published model.

**Conclusions:** Average prediction quality for serum iCa was good. Efforts have to be
directed towards further explaining and reducing the spread of the prediction error, e.g.
by taking into account individual pre-HD serum citrate levels, pH levels and shifts,
and better estimates of bone Ca buffering. Although prediction errors were small on average,
the model should be adjusted to avoid underestimation of end-HD serum iCa in regular bicarb
dialysate treatments and overestimation with use of citrate-containing dialysate.

**TH-PO148**

*p-Cresol Sulfate and Indoxyl Sulfate Induce Similar Cellular Inflammation and Immune Response in Cultured Proximal Renal Tubular Cells*  
Chiyo-Yin Sun. Nephrology, Chang Gung Memorial Hospital, Keelung, Taiwan.

**Background:** Uremic toxins, *p*-cresol sulfate (PCS) and indoxyl sulfate (IS), have
important roles in the progression of CKD. Previous study has shown that IS induces
cellular oxidative stress. The aim of this study is to find the cellular inflammation responses
to PCS and IS.

**Methods:** Cultured mouse proximal renal tubular cells (PRTK-1) were treated with
PCS or IS at concentrations of 0, 1, and 5 mg/L were analyzed by PCR array with inflammation
and immune panel. The gene-annotation enrichment analysis and functional annotation
clustering were analyzed with the DAVID v6.7. The functional networks of target genes
were analyzed by the GeneMANIA.

**Results:** There were 12 and 30 genes up-regulated by PCS at concentrations of 1 and
5 mg/L greater than 1.5 × respectively. There were 13 and 32 genes up-regulated by IS at
concentrations of 1 and 5 mg/L, greater than 1.5 × vs. control respectively. Ccr2, Csf2 and
Ptrcr were down-regulated by PCS and IS. Sixteen up-regulated functional annotation
clusters of cells treated with PCS or IS at a concentration of 5 mg/L were noted by
functional annotation clustering analysis. Eleven common functional annotation clusters
of up-regulated genes were noted in cells treated with PCS or IS. The calculated function
networks were similar between the PCS and IS and IS, Csf1/3, Cxcl10, Fast1, Stat1 and Ikbkb
were the major cytokines in the functional networks of PCS and IS. Stats, Smads, Nfkβ2,
Bcl2 and Bax were the major intracellular signals for PCS and IS. Real-time PCR
results showed that PKSV cells treated with PCS or IS had significantly increased Tgbf1
expression. The molecular functional networks with the highlight of Tgbf1 for the cells
treated with PCS and IS at a concentrations of 5 mg/L were analyzed. In both PCS and
IS, Csf2, Cxcl10, Fast1, Stat1 and Ikbkb were the target genes in the predicted molecular
functional networks with the Tgbf1.

**Conclusions:** PCS and IS stimulated significant cellular inflammation reactions. The
cellular inflammation and immune response induced by PCS and IS were similar in cultured
proximal renal tubular cells.  
Funding: Government Support - Non-U.S.

**TH-PO149**

Distribution of Hydrogen Sulfide (H₂S)-Producing Enzymes and the Roles of H₂S in Diabetic Nephropathy  
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Medicine, Toyoake, Aichi-Ken, Japan.

**Background:** Hydrogen sulfide (H₂S) has recently been found to play beneficial
roles in ameliorating several diseases. Cystathionine β-synthase (CBS) and cystathionine
γ-lyase (CSE), the main enzymes in the transsulfuration pathway, catalyze H₂S production
in mammalian tissues. However, the distributions and precise roles of these enzymes in
the kidney have not yet been identified. In this study, we examined the localization of CSE
and CBS in kidney and the effect of H₂S donor, NaHS in renal peritubular capillary (PTC)
blood flow velocity, diameter and blood flow.

**Methods:** We performed immunohistochemical studies of CSE and CBS in kidney
and NaHS in renal peritubular capillary (PTC) blood flow velocity, diameter and blood flow.
We examined the expression of CSE and CBS in nTg and β-cell-specific calmodulin-
overexpressing transgenic mice (CaMTg) as a model of diabetes at 3 month old used.
Immunohistochemical studies examined localization of CSE and CBS in kidney
Expression of nTg and CaMTg by Western blot analysis. Then we examined effect of NaHS on PTC blood flow
velocity, diameter and blood flow.

**Results:** In the nTg kidney, we detected expression of both CBS and CSE in the brush
border and cytoplasm of the proximal tubules, but not in the glomeruli or distal tubules.
expression was markedly reduced under diabetic conditions, whereas CBS expression was unchanged. In contrast, OAT1 and OAT3 increased. Cysteinylglycine and blood flow. Progress in diabetic nephropathy showed vasoconstriction and a loss of blood flow in PTCs that was ameliorated by NFN HS treatment.

**Conclusions:** The biological profile of CSE resembles that of endothelial nitric oxide synthase (eNOS) and the subunit of NOS. CSE expression in the proximal tubules of the kidney also regulates tubulointerstitial microcirculation via H2S production. H2S could represent a target of treatment to prevent progression of ischemic injury in diabetic nephropathy.

**TH-PO150**

**In Vitro and In Vivo Characterization of New Pumping System in a New Hemodialysis Device**

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**Background:** Hemodialysis devices typically use peristaltic pumping technology. A new hemodialysis device under development uses a pneumatically controlled diaphragm pump (PCDP). The pump contains a fluid and air compartments with flexible diaphragm separating the two sides. Positive or negative pressure is applied to the air compartments to move fluid into and out of the fluid compartment of the pump.

**Methods:** In a study (n=12) were performed during simulated in vitro treatments (4 h, blood flow Qb 400 ml/min) with bovine blood and donated human blood in the new device and hemodialysis device with peristaltic roller pump (PRP). For bovine blood, PCDPs were heated for 3-year shelf life (70°C, 40 days, 65±5% humidity) per American Society for Testing and Materials standards (ASTM F1984). Hemolysis was evaluated by modified index of hemolysis (MIH) per ASTM F1841-97. In vivo studies in sheep (n=6) were done with the PCDP device during simulated 4 h dialysis at Qb 400 ml/min. Hemolysis was assessed by mean cell volume (MCV), mean cell hemoglobin concentration (MCHC), mean cell hemoglobin (MCH), plasma free hemoglobin, and lactate dehydrogenase (LDH) levels.

**Results:** The MIH in the heat aged PCDP was 0.68±1.32 and 1.86±0.53 for the PRP (p<0.01). When human blood was used, the MIH was 0.55±0.09 for PCDP in contrast to 1.16±0.36 for PRP (p<0.0007). Using the sheep model during simulated dialysis the PCDP device showed stable levels of MCV, MCH and MCHC during the 4 h treatment. The LDH levels and free hemoglobin levels were maintained at baseline levels throughout the 4 h session.

**Conclusions:** The new device using PCDP demonstrated significantly reduced hemolysis as compared to the device with PRP in vitro using bovine and human blood. Data from sheep dialysis showed stable markers of hemolysis. Therefore, the PCDP in the new hemodialysis device may provide an alternative option for hemolysis. Additional testing will be performed in human clinical trials.

**Funding:** Pharmaceutical Company Support

**TH-PO151**

**Autologous Adipose Tissue Depots To Inhibit Arteriovenous Graft (AVG) Stenosis**

Christi M. Terry, William G. Sanders, Huan Li, Yuxia He, Alford K. Cheung, Medicine, Univ. of UT, SLC; Pharmacaceutics, Univ. of UT, SLC; Medicine, VASLHCS, SLC, UT.

**Background:** AVG often fail due to underlying neointimal hyperplasia (NH). Adipose tissues (AT) produce adiponectin (ADPN), that inhibits smooth muscle cell (SMC) proliferation, a contributor to NH. The anti-diabetic vasculoprotective glitazones induce ADPN from AT. This research describes an autologous AT raphe polymer wrap that provides unidirectional diffusion of sunitinib towards the graft and vessel wall into the lumen.

**Methods:** Two formulations were created: In “A”, sunitinib was dissolved in a non-porous poly(lactic-co-glycolic acid) (PLGA) wrap. In “B”, dry sunitinib was mixed into a hyaluronic acid (HA) hydrogel which was then infused into a porous PLGA network. A pliable non-porous PLGA backing without drug was incorporated onto both formulations to promote unidirectional diffusion. In the in vitro drug release from the formulations into the media was assayed by HPLC tandem mass spectrometry. Unidirectional drug release was assessed at a modified co-culture chamber with the non-porous backing as the separating insert. Drug traffic from “A” into tissue was tested with an explanted porcine artery in an ex vivo flow chamber.

**Results:** Formulation “A” yielded a slow release rate, with drug exhausted after 40 days. Formulation “B” produced a high initial burst with almost complete release by 14 days. The non-porous PLGA backing effectively prevented drug diffusion into the lower co-culture chamber. The sunitinib concentration within the artery wall tissue at 24 h after wrap placement was 17.8 nM, corresponding to three times the in vitro EC50 for SMC inhibition and 0.4% of the total drug load. Concentrations in the circulating media were 0.72 nM (0.014% of total load) after 24 h, suggesting some trafficking of the drug through the vessel wall into the lumen.

**Conclusions:** Our biodegradable formulation “A” polymer wrap provided a directed, sustained release of sunitinib that rapidly achieved high vascular wall tissue drug concentrations. This data supports the further characterization of the formulation in vivo in a porcine model of AVG stenosis.

**Funding:** Other NIH Support - R21 HD67646, Veterans Administration Support, Private Foundation Support
TH-PO154
Protoyping a Peritoneal Dialysis (PD)-Based Automated Wearable Artificial Kidney (AWAK): A Progress Report
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Background: AWAK is designed to provide round-the-clock, dialysis-on-the-go for patients with end-stage renal disease (ESRD). Based on sorbent technology, and tidal peritoneal dialysis (TPD), AWAK continuously regenerates spent dialysate and recycles fresh dialysate at rates up to 96 L/day. This is an update on our prior reported prototype.

Methods: The first prototype consists of a disposable and a non-disposable assemblies. TPD is conducted by first draining the tidal volume (TV) out of the peritoneal cavity (PC) into the disposable assembly (outflow mode, OM). Following regeneration of the spent dialysate, the fresh dialysate is returned into the PC (inflow mode, IM). Cycles of OM-IM continued until the time for sorbent cartridge (SC) replacement. The PC is then completely drained into an UF receptacle. A volume equal to TV plus RV is returned to the PC and the remainder fluid is discarded as UF. Several improvements have been made on this prototype and using the new set up we have validated the regeneration and reconstitution of spent peritoneal dialysate.

Results: In the updated design all wetted components are completely housed in the disposable assembly, which is replaced with each cartridge exchange, i.e. every 7 or 12h, depending of the type of cartridge used. A common non-disposable assembly controls all fluid movements. It runs no contamination risk, given its never wetted by the dialysate. The configuration using the “regular”, 7h SC weighs less than 1kg and is easily wearable, e.g., in a shoulder bag, without attracting undue attention. It regenerates and reconstitutes fresh dialysate (similar in composition to the “2 bag bicarbonate dialysate”) from both a synthetic spent peritoneal dialysate and spent dialysate from PD patients. The intermittent UF phase with complete drainage of the PC makes the “overfill” syndrome unlikely.

Conclusions: Further safety measures and miniaturization have been successfully engineered into the AWAK prototype for animal and clinical trials.

Funding: Private Foundation Support

TH-PO155
Hydrodynamic-Mediated Transgene Expression of Baculovirus Vectors in Live Mammalian Kidneys
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Background: Viral vectors have been investigated for their use in mammalian gene transfer for decades. While gene transfer has been difficult to accomplish in the kidney, reports have indicated that hydrodynamic caviation may be used to facilitate renal gene transfer.

Methods: We present a method that utilizes tissue cavitation to deliver baculovirus vectors for relatively rapid renal transfection in live animals. In devising this approach we hypothesized that the hydrodynamic forces generated from pressured injections were sufficient to facilitate both the passage of baculoviral particles across epithelial and endothelial layers, and ultimately their endocytic cellular uptake. We tested this hypothesis using Green fluorescent protein (GFP) and GPF-actin chimeric baculovirus vectors.

Results: GFP and GFP tagged actin expressing baculovirus vectors were introduced into 30 rodent kidneys using renal vein guided, retrograde pressurized injections. Transgene incorporation and expression was examined using intravitral multiphoton fluorescence microscopy. This method produced detectable levels of protein expression in various tubular, glomerular and vascular segments, throughout a period of 3 weeks. Figure 1 shows intravitral multiphoton microscopy images taken from kidney proximal tubules (PT) and distal tubules (DT) from Sprague-Dawley rats that received GFP-actin baculovirus vectors. The images were taken across a 3 week time frame, post viral delivery: (A) tissue autofluorescence, (B) Day 1, (C) Day 2, (D) Day 3 (E) Day 4, (F) Day 5, (G) Day 6 and (H) Day 21. Arrows are used to indicate regions with transgene expression. Moreover, we found that transgene expression was stable for 21 days.

Conclusions: These results outline a potential approach for renal gene transfer in live animals employing a simple, reproducible delivery system.

Funding: NIH/NIH Support

TH-PO156
Physiologic Assessment of Arteries for Arterio-Venous Fistula (AVF) Creation for Hemodialysis Access
Rosa M. Marticorena,1 Cesar Ginocchio,1 Arash Jaberi,2 Niki Dacouris,3 Vern Malcolm Campbell,1 Sandra A. Donnelly,3 1Nephrology, Keenan Research Centre Li Ka Shing Knowledge Institute, St. Michael’s Hospital, University of Toronto, Toronto, ON, Canada; 2Radiology, Ottawa Hospital, Ottawa, ON, Canada.

Background: Achieving adequate flow for hemodialysis treatment is the primary goal of fistula creation. Arterial dilatation is essential to increase radial artery flows from 20-30 mL/min to 600-1200 mL/min. Arterial size is insufficient to determine if fistula maturation will occur as evident by a 40% rate of failure to mature in spite of having the requisite 2mm diameter and the successful creation of fistula in children. The increased blood flow provides the necessary hemodynamic change in venous wall shear stress that is a key determinant of outflow vein dilatation. The objective of this work was to determine if healthy arteries defined by normal physiologic parameters of pulse wave velocity (PWV), augmentation index (AIX) and elasticity was associated with adequate vascular remodeling to generate successful AVF maturation.

Methods: End stage renal disease patients of a tertiary care academic hemodialysis program were studied between June 2009 and Dec 2010. Patients who had functioning fistulas (Group I n=28) were compared to patients who had a failed fistula (Group II n=10).

Blood pressures and radial artery waveform were recorded with HDI/Pulsewave CR-2000 Research CardioVascular Profiling Systems (Hypertension Diagnostics, Inc.,Minneapolis, USA) and large and small artery elasticity were estimated by a modified Windkessel model. Carotid, radial and femoral pulse wave velocity and waveform were evaluated with SphygmoCor® (AtCor Medical, Inc., IL, USA).

Results:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>52±1.4</td>
<td>59±1.1</td>
</tr>
<tr>
<td>AIX</td>
<td>26.0±10.2</td>
<td>35.2±11.3</td>
</tr>
<tr>
<td>PWV (M/sec)</td>
<td>8.6±1.3*</td>
<td>7.2±1.3</td>
</tr>
</tbody>
</table>

Mean±SD *p<0.05 Group I vs Group II

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In multivariate analysis, successful AVF maturation was negatively correlated with AIX and DM and positively correlated with PWV and male sex. Measures of large artery stiffness and small artery elasticity were not associated with successful AVF maturation.

Conclusions: These preliminary observations suggest that physiologic assessment of arteries may enhance vessel selection for AVF creation.

TH-PO157

Generation of Infusible Dialysate through a New Hemodialysis System

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Background: A new hemodialysis system under development generates online infusible dialysate for priming, bolus and rinse back eliminating the need for bagged solutions. To meet the American Association for Medical Instrumentation (AAMI) standards for infusible dialysate, the system is required to achieve ≥9-log reduction in bacteria and at least ≥2-log reduction in endotoxin. The system uses sequential ultrafiltration via an endotoxin and avenous blood line samples. Results: Dialysate fluid post ultrafilter, at AAMI ultrapure dialysate standards with bacteria levels of <0.1 CFU/ml and endotoxin level of <0.03 IU/ml. The quality of the post-dialyzer venous blood line samples (for priming, bolus and rinse back) met the AAMI standards for infusible dialysate with no bacteria being cultured (0 CFU/L) from all samples representing ≥9 log reduction and endotoxin levels of <0.03 EU/mI, representing greater than 2 log reduction in endotoxin.

Conclusions: A new hemodialysis system using sequential ultrafiltration via endotoxin and bacterial retentive filter and PES dialysate is capable of generating infusible dialysate when challenged with markedly high levels of bacteria and endotoxin. Therefore, generation of infusible quality dialysate has been shown using a robust in vitro testing procedure.

Funding: Pharmaceutical Company Support

TH-PO158

Effect of Serum Thyroid Stimulating Hormone Levels on Glomerular Hemodynamics Examined by Inulin and Para-Amino-Hippuric Acid Clearance

Akhiro Tsuda, Eiji Ishimura, Yoshiteru Ohno, Hideaki Shima, Shinsuke Yamada, Toshiki Nagasaki, Katsuhito Mori, Hideaki Shima, Akihiro Tsuda, Eiji Ishimura, Yoshiteru Ohno, Hideaki Shima, Shinsuke Yamada, Toshiki Nagasaki, Katsuhito Mori, Hideaki Shima, Masaaki Inaba. Osaka City University Graduate School of Medicine, Osaka, Japan.

Background: Glomerular hemodynamics can be examined by the method of Gomez’s formula, in which both inulin and para-amino-hippuric acid clearance (PAH) clearance are simultaneously measured. Although the effect of drugs is examined, few have examined the effect of intrinsic hormones on glomerular hemodynamics. Hypothyroidism is reported to reduce glomerular filtration rate (GFR), although its mechanism is unknown. In the present study, we examined the effect of serum levels of thyroid stimulating hormone (TSH), which has been demonstrated to affect peripheral arterial resistance (J Clin Endocrinol Metab 89:3455, 2004), on glomerular hemodynamics.

Methods: Twenty-eight patients (60.6 ± 12.8 years, 9 males and 19 females, serum creatinine < 1.0 mg/dl) with mild IgA glomerulonephritis (n = 5) or type 2 diabetes (n = 23), who agreed with the study, were examined. All patients were of euthyroidism. Inulin and PAH clearance were simultaneously measured according to the methods of Horio et al (Clin Exp Nephrol 13:50, 2009). Resistance of afferent arteriole (R-a) and efferent arteriole (R-e) was calculated with use of Gomez’s formula.

Results: We identified 11 markers at a threshold of p<10^-5, two of which (rs1411766, rs10868025) overlapped with SNPs identified using LR (Pezzolesi et al, and a third, rs10868025 was in compete linkage disequilibrium with rs1411766; we also noted that p-values were roughly similar, using both approaches. Higher p-value thresholds using larger feature spaces, improved the sensitivity and specificity of SVM prediction over the LR approach.

Conclusion: We utilized the PLINK whole genome association analysis toolset, for performing quality control of the GoKinD dataset. We used WEKA, an open source data mining package that provides multiple classifiers, to run both logistic regression and SVM with the Radial Base Function (RBF) kernel model. We identified 11 markers at a threshold of p<10^-5, of which (rs1411766, rs10868025) overlapped with SNPs identified using LR (Pezzolesi et al, and a third, rs10868025 was in compete linkage disequilibrium with rs1411766; we also noted that p-values were roughly similar, using both approaches. Higher p-value thresholds using larger feature spaces, improved the sensitivity and specificity of SVM prediction over the LR approach.

Comparison of p-values generated between our results and Pezzolesi et al. (2009)

SNP  P value Pezzolesi et al. (2009)  Our P value
rs39659  5.0 x 10^-6  0.9 x 10^-4
rs10868025  5.0 x 10^-7  1.2 x 10^-7
rs739041  6.4 x 10^-6  6.3 x 10^-5
rs1101401  3.1 x 10^-6  3.6 x 10^-5
rs1014146  3.1 x 10^-6  1.5 x 10^-5
rs1411766  1.8 x 10^-6  4.0 x 10^-6
rs64922258  8.1 x 10^-6  1.3 x 10^-5
rs7989848  7.0 x 10^-6  2.3 x 10^-5
rs9521445  2.9 x 10^-6  4.0 x 10^-5

Conclusion: Using SVMs, we were able to identify several novel SNPs correlated to type 1 diabetic nephropathy. Taking a wide net approach and testing all highly correlated SNPs will generate more accurate models. We intend to validate the models on an external data set (DCCT-EDIC).

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TH-PO161

In Vivo siRNA Targeting to the Podocyte

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Background: Development of methods for rapid in vivo knockdown of gene function by siRNA would greatly aid in the validation of therapeutic targets for drug discovery. Because of the central role of the podocyte in glomerular disease, siRNA targeting to these cells is of particular interest. Here, we test the ability of polymer-bound siRNA to reduce gene expression in podocytes, focusing on the slit diaphragm protein nephrin. In the kidney, nephrin is expressed solely in the podocyte, and loss of nephrin function leads to rapid increase in urinary protein excretion.

Methods: Nephrin-specific siRNA was complexed with a poly(ethylene glycol)-poly(L-lysine) block copolymer and the complex was administered intraperitoneally. Results: After 72 hours, quantitative real-time PCR showed a 70% decrease in nephrin mRNA and a 68% decrease in nephrin protein levels when compared to animals injected with a non-targeting siRNA. In addition, we observed a doubling of urinary albumin levels after nephrin siRNA administration.

Conclusions: We are currently testing whether multiple doses of nephrin siRNA can further knock down gene expression and induce more severe proteinuria, as well as evaluating the targeting of siRNA to other tissues through this method. Taken together, these data show a rapid and effective method to target gene expression in the podocyte which may drastically reduce the time and cost of validating new therapeutic targets for glomerular disease.

Funding: Pharmaceutical Company Support

TH-PO162

Quantification of Interstitial Fibrosis by Second Harmonic Imaging in Rat Kidney Tissue Sections

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Background: Interstitial fibrosis is a powerful indicator of nephropathy in chronic kidney diseases. However, accurate quantitative analysis of fibrosis by Sirius Red morphology (SRM) remains a challenge. Our aim was to establish an imaging technique that would enable us to easily and accurately quantitate fibroblastic collagen in rat kidney tissues.

Methods: We used a Multiphoton System to develop the novel methodology of Second Harmonic Generation (SHG) for imaging renal fibrosis. 3mm unstained sections of rat kidney at day 5 after unilateral ureteral obstruction (UUO) were assessed for cortical fibrosis. The Two Photon Excitation Fluorescence (TPEF) allowed for the visualization of kidney background and tubular organization, while SHG was used for the display of the architecture of fibroblastic collagen.

Results: To validate the specificity of the fibroblastic components detected by the SHG in renal fibrosis, the relationship between SHG signal and immunohistochemistry for collagen Types I, III, and IV was analyzed. Our results showed that the SHG signal strongly correlated with fibroblastic collagens I and III, but not with collagen IV. To examine relationship between SHG and traditional SRM analysis of global fibrisc, kidneys from rats at day 5 after UUO treated with ACRE (n=6/group) were also assessed by both methods with similar trends of quantitative fibrisc area. However, SRM showed 23.2% to 38.9% reduction of fibrisc area by ACRE, while SHG showed 36.1% to 50.2% reduction of fibrisc. Our results demonstrated that SHG imaging can be used for specific detection of the main types of fibroblastic collagens involved in renal fibrosis, and can offer more sensitive and a greater dynamic range for evaluation of the therapeutic effects on interstitial fibrosis.

Conclusions: We conclude that combination of SHG and TPEF imaging of unstrained kidney tissues provide a novel alternative to traditional SRM and is a powerful tool for the quick visualization and quantitative assessment of renal fibrosis.

TH-PO163

Assessment of Renal TSPO Expression Using Micro PET Imaging in Murine Models

Mohammed Noor Tantawy,1 H. Charles Manning,1 Keiko Takahashi,2 Hiroki Fujita,3 Christopher Chad Quarles,1 Raymond C. Harris,2 John C. Gore,1 1Vanderbilt University Institute of Imaging Science, Nashville, TN; 2Vanderbilt O’Brien Mouse Kidney Physiology and Disease Center, Nashville, TN; 3Akita University Graduate School of Medicine, Akita, Japan.

Background: Translocator protein18-kDa (TSPO) is a crucial mitochondrial protein involved in various cellular functions, including cholesterol metabolism, steroidogenesis, and apoptosis. TSPO is abundantly expressed in kidney tissue; however, its involvement in renal pathology is poorly understood. To effectively characterize the TSPO expression in mouse models of kidney diseases, here we evaluated a TSPO PET imaging assay for mouse kidney using a novel radioligand, [18F]PBR06.

Methods: Mice were injected with a radioligand, [18F]PBR06. Bioluminescence was recorded using a microPET system for 90 min in a dynamic sequence. The time-activity curves (TAC) of the kidney were recorded over the duration of the scans. Specificity of binding was evaluated by displacement of [18F]PBR06 with excess PBR06. Adding to wild type mice, 20-wks old db/db or non-diabetic db/m mice were subjected to the imaging assay. TSPO presence in kidney tissues was assessed by Western blot.

Results: The TACs plateaued in kidney at 50-80 min, indicating a state of equilibrium, and the cold ligand displaced ~75% of the radiotracer. The probe was not present in bladder at this time, indicating that the kidney signals resulted from binding of [18F]PBR06 to renal TSPO but not renal blood flow. Interestingly, [18F]PBR06 binding was remarkably reduced in db/db kidney compared to db/m kidney, while no difference was observed in liver. % injection dose per kidney; db/m 10.5 ± 0.2% vs. db/db 6.2 ± 0.5%. Similar reduction in renal TSPO protein was also observed in db/db mice by Western blot.

Conclusions: Renal TSPO expression can be assessed by in vivo PET imaging using [18F]PBR06. Reduced expression in db/db mice suggests a significant role of TSPO in diabetic nephropathy.

Funding: NIDDK Support

TH-PO164

α3 Chain Type IV Collagen as an Non-Invasive Optical Biomarker for Chronic Glomerular Diseases

Kapil Chaudhary, Nino Kvirkvelia, Maggie McMenamin, Michael P. Madaio. Department of Medicine, George Health Science University, Augusta, GA.

Background: Type IV collagen, a major structural component of the GBM, with relatively limited expression including the kidney. Expression of α3 chain of type IV collagen, α3(IV) is altered during the course of chronic kidney disease. Hence, irrespective of the underlying etiology, quantification of α3(IV) expression has a potential as a biomarker of kidney disease progression. For this purpose, we hypothesized that ex vivo quantification of human anti-α3(V)NC1 antibody (Ab) binding, in real time, could serve this role.

Methods: Human mAb F1.1 was conjugated with Alexa Fluor 488 and Alexa Fluor 750 fluorophores is well known to produce a strong near infrared fluorophore (Dylight 750 or 800). Effective conjugation was determined by both 20% SDS PAGE with antigenic specificity of the conjugate maintained. Following injection of conjugated mAb F1.1 into normal mice, live animal imaging was performed by XenogenTM camera; under anesthesia. Kidney specific fluorescence was quantified by software provided with the live imaging system. Pharmacokinetics of fluorophore-mAb F1.1 was studied by imaging the mice at 6,12,24,36 & 72 hrs after injection in the presence of suitable positive and negative controls. Tissue specificity of fluorophore-mAb F1.1 was evaluated by analysis of formalin fixed kidney, liver, lung, spleen and heart under XenogenTM camera. Glomerular specificity of human mAb F1.1- fluorophore conjugate was further evaluated by IF on 4 μm kidney tissue sections from same experiment.

Results: Human mAb F1.1 was effectively conjugated with fluorophore Dylight 750 and 800. After injection of conjugated F1.1, kidney specific fluorescence was highest at 24-36 hrs and was detectable after even 72hrs. mAb F1.1-fluorophore conjugate is able to provide non-invasive quantification of α3 chain, type IV collagen in kidney.

Conclusions: The results provide the potential to pursue human mAb F1.1 as an optical biomarker of progressive glomerular disease in experimental kidney disease models by providing non-invasive quantification of type IV collagen. If successful, this approach and reagents/antibody will have a potential application to patients with many forms of chronic kidney disease.

Funding: NIDDK Support, Private Foundation Support

TH-PO165

Optimization of High Field MRI Methods for the Mouse Kidney Imaging

Feng Wang,1 Rosie T. Jiang,2 Mohammed Noor Tantawy,1 Keiko Takahashi,2 Raymond C. Harris,2 Christopher Chad Quarles,1 Takamune Takahashi,3 1Vanderbilt University Institute of Imaging Science, Nashville, TN; 2Vanderbilt O’Brien Mouse Kidney Physiology and Disease Center, Nashville, TN.

Background: MRI can provide non-invasive and non- radiation imaging of the kidney, nephrin is expressed solely in the podocyte, and loss of nephrin function leads to rapid increase in urinary protein excretion.

Methods: T1W, T2W and MTC imaging protocols were optimized on 7T MRI system to enhance the structural delineation, signal to noise, and image contrast. Rapid acquisition MRI methods were tested to minimize motion artifacts, including coherent parallel imaging, multishot and multi-slice-excitation/relaxation fast gradient echo. Respiratory gating and fat saturation were also considered.

Results: We demonstrated that a coherent GE sequence is superior to IR fast GE for T1W, T1 contrast was optimized with a ±35° flip angle at TR 45ms. A fast spin echo sequence yielded reliable T2W and optimal contrast was achieved with a TE of ∼35° flip angle at TR 2000ms. The MT images were optimized using the GE sequence similar to that used for T1W and the greatest improvement was observed when fat saturation was employed. High-field T1W, T2W and MTC images enabled the qualitative and quantitative evaluation of kidney size, shape, contrast, thickness, and mTOR rescue in UUO induced diabetic kidney disease.

Conclusions: We have developed the optimal MRI protocols to allow the visualization of fine renal structural details, achieving resolutions of 0.1 x 0.1 x 0.5 mm. These methods should be effectively used for evaluating renal structural integrity in mouse models of kidney diseases.

Funding: NIDDK Support

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Underlines represent presenting author.
TH-PO166
MRI Assessment of Mouse Kidney Blood Volume at 7T; Feng Wang; Rosie T. Jiang; Mohammed Noor Tantawy; Keiko Takahashi; Raymond C. Harris; Christopher Chad Quarles; Takamune Takahashi. Vanderbilt University Institute of Imaging Science, Nashville, TN; Vanderbilt O’Brian Mouse Kidney Physiology and Disease Center, Nashville, TN.

Background: MRI mapping of relative blood volume (RBV) is widely used to assess pathological differences in tissue vascular density. However, this method has not been applied to renal disease. In this study, we optimized MRI acquisition methods for RBV mapping in mouse kidney and assessed the vascular property in UUO model.

Methods: Multi-slice T2-weighted fast spin echo images (TR = 2000ms, TE = 48ms, RARE-factor = 8, 256 matrix, 25.6 mm FOV, 0.5 mm slice thickness) of the mouse kidneys were acquired before and after the injection of an iron oxide contrast agent (CA) at 7T MRI. Navigator and respiratory gating was employed to minimize motion artifacts. RBV was calculated pixel by pixel from the steady-state T2-weighted images using a standard formula. The dose of CA was optimized based on the CNS value. Reproducibility of the RBV data was assessed by measuring RBV (n=10) on consecutive days. Renal RBV maps were evaluated in UUO mice (n=6) at 3hrs, 1, 3, 6, and 10 days.

Results: The dose of CA was optimized at 6 mg/kg for RBV mapping in mouse kidney. The mean kidney RBV measured on consecutive days was 19.97 ± 1.50 and 19.86 ±1.62, yielding a concordance correlation coefficient of 0.94, indicating that this approach is highly reproducible. The RBV values of medulla were rapidly reduced in UUO kidney (~10% at day 6). While those of contra-lateral kidney was gradually increased (~10% at day 6).

Conclusions: MRI measurements of renal RBV provide a valuable assay to characterize vascular density in renal disease.

Funding: NIDDK Support

TH-PO167
Reproducibility of Renal Artery Blood Flow and Intrarenal Oxygenation Measurements Using Magnetic Resonance Imaging in Patients with Chronic Kidney Disease and Healthy Controls; Dinah Sherzad Khattar; Michael Pedersen; Steffen Ringgaard; Bente Jespersen; Niels Henrik Bius.

Department of Renal Medicine, Aarhus University Hospital, Skejby, Aarhus N, Denmark; 2MR Research Center, Aarhus University Hospital, Skejby, Aarhus N, Denmark.

Background: To evaluate the reproducibility of measurements using magnetic resonance imaging (MRI) in patients with chronic kidney disease (CKD) and healthy controls.

Methods: RBF and oxygenation in both kidneys were determined on two occasions with 1-2 weeks interval in 7 CKD patients (mean 65 years, eGFR = 30 ml/min) and 8 healthy volunteers (mean 43 years, eGFR >90 ml/min). All investigations were performed in a 1.5 Tesla Siemens Avanto MRI system. RBF was measured by phase-contrast sequence with velocity gradients applied orthogonally to the renal artery and data were acquired with ECG gating. RBF was calculated as the product of the cumulative vessel blood velocity and the diagonal vessel area. Regions of interest were drawn according to the vessel, and automatic segmentation was done with manual correction. Blood Oxygen Level Dependent (BOLD) MRI using R2*-sensitive echo planar imaging sequence was applied to renal disease. In this study, we optimized MRI acquisition methods for RBV mapping in mouse kidney and assessed the vascular property in UUO model.

Results: Both scan procedures were successful in all subjects. RBF (ml/min/ kidney) for patients were 189.5 ±153.2 and 190.4 ±158.1 and for controls 356.1 ±119.3 and 348.4 ±107.3 (P=0.05 vs. patients) for first and second scans (fig1). In both patients and controls RBF measurements were highly reproducible with a linear correlation coefficient of r2=0.96. Renal medulla R2* (ms-1) for patients were 29.9 ± 4.4 and 29.8 ± 4.4, and for controls 25.9 ± 2.5 and 26.1 ± 2.2 (P=0.05 vs. patients) for first and second scans (r2=0.44).

Conclusions: MRI-based determinations of RBF and R2* are reproducible in CKD patients and healthy controls.

TH-PO168
The Usefulness of Contrast-Enhanced Ultrasound (CEUS) for Diagnosis of Renal Cell Carcinoma by Employing a Time-Intensity Curve (TIC): The Characteristics of Microcirculation in Renal Cell Tumor Conformed by Renal Tumor Biopsy under CEUS; Tokunori Yamamoto, Hideki Mizuno, Yasushi Yoshino, Momokazu Gotoh.

Urology, Graduate School of Medical Sciences, Nagoya University, Nagoya, Aichi, Japan.

Background: To evaluate the usefulness of contrast-enhanced ultrasound (CEUS) for diagnosis of renal cell carcinoma by employing a time-intensity curve (TIC).

Methods: From May 2008 to October 2009, CEUS was performed prior to surgery in 30 patients with renal masses. 10 of the 30 patients had cystic renal masses. The final diagnoses of all patients were pathologically confirmed. Contrast enhancement as a function of time was measured in two (tumor or solid component of cystic lesions and normal parenchyma) regions of interest (ROI) and TICs were obtained. The time to the contrast enhancement peak (TTP), intensity change from the baseline to peak (ΔI), and ΔI / TTP of the tumor and the normal parenchyma were measured from the TIC. In addition, we performed renal tumor biopsy under CEUS to confirm the characteristics of the TIC.

Results: Pathological diagnoses were renal cell carcinoma in 30 patients. The TTP of the cancer was shorter than that of the normal parenchyma in all cases (6.0 ± 2.0 s vs. 10.4 ±3.0 s; p < 0.0001). The ΔI did not differ between the cancer and normal parenchyma (21.3 ±5.9 db vs. 20.9 ± 7.0 db; p = 0.68); the ΔI / TTP of the cancer was significantly higher than that of the normal parenchyma (3.9 ± 1.4 db/s vs. 2.2 ± 0.94 db/s; p < 0.0001). TIC patterns of solid cancer and cystic cancer were very similar. TIC of the normal tissue and the cancer in pathology conformed by renal biopsy for the tumor under CEUS also demonstrated similar to the characteristics of the TIC.

Conclusions: An objective and quantitative diagnosis of renal cell carcinoma by CEUS using a second-generation ultrasound contrast agent can be made by employing a TIC. The TIC patterns of solid and cystic cancers were very similar, despite their morphological and vascular differences. CEUS using a TIC is a promising tool in the diagnosis of cystic renal cancer.

TH-PO169
The Usefulness of Contrast-Enhanced Ultrasound (CEUS) for Evaluation of Transient Time from Lobular Artery Via Glomerular Capillary to Outer Medulla by Employing a Time-Intensity Curve (TIC); Tokunori Yamamoto, Hideki Mizuno, Yasushi Yoshino, Momokazu Gotoh.

Urology, Graduate School of Medical Sciences, Nagoya University, Nagoya, Japan.

Background: The usefulness of contrast-enhanced ultrasound (CEUS) for evaluation of the graft function in transient time from lobular artery via glomerular capillary to outer medulla by employing a time-intensity curve (TIC).

Methods: From May 2008 to October 2009, CEUS was performed prior to operation in 12 patients with cadaveric(n=6) and living renal transplantation(n=6) (Figure1). Contrast enhancement as a function of time was measured in two (lobular artery lesions and outer medulla) regions of interest (ROI) and TICs were obtained, respectively. The rise time of difference between lobular artery lesions and outer medulla lesion were measured from the TIC.

Results: In living renal transplantsations, initial urine appeared immediately after the operation in all cases. The transient time are less than 10 sec. In cadaveric renal transplantsations with early ATN, initial urine appeared postoperative days (POD) from 7 to 10 days in all cases. The transient time are less than 10 sec since 10 POD.

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Results: Both in vivo and in vitro cortical AT1R binding were increased in ischemia but not in normal perfusion. The finding was further supported by immunofluorescence staining of the chronic stenotic kidney tissue, which revealed increased AT1R expression in some glomeruli. There was also a correlation between the distribution of AT1R and NGAL expression.

Conclusions: Increased AT1R binding measured with PET and autoradiography correlates with increased glomerular AT1R in the renal cortex. Thus, Immunofluorescence imaging corroborates well with the PET imaging results and supports the role of AT1R PET as an imaging biomarker of renal ischemic injury.

Funding: NIDDK Support, Other NIH Support - Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grant Number R01 DK50183) and shared instrumentation grant (NIH S10RR022528).

TH-POI172
Blood Oxygenation Level Dependent MRI in Reversible Unilateral Ureteral Obstruction Model
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Background: Reversible unilateral ureteral obstruction (rUUO) provides a useful model of chronic kidney disease (CKD) amenable for longitudinal monitoring of structural and functional changes during development of CKD (Punj T, AJP Renal Physiol 2010). We have reported strain-dependent susceptibility to development of CKD after rUUO in C57Bl/6 (susceptible) and Balb/C (resistant) mice. In this preliminary study, renal BOLD MRI was used to detect any early changes in oxygenation status in this model.

Methods: A total of 31 mice (19 C57Bl/6 and 12 Balb/C) underwent rUUO. Blood samples were obtained via retro-orbital bleeding for BUN measurements at three time points (baseline, -2days and -28 days post release of UUO). BOLD MRI was performed on a 4.7 T Bruker Biospec scanner using a 35 mm mouse body coil at the same time points.

Results: BUN showed enhanced values in both strains at day 28 but to a significantly higher value in C57Bl/6 mice compared to baseline (25.6±5.0 to 48.6±13.0 mg/dL, p<0.01 in C57Bl/6 and 27.5±3.2 to 36.4±6.0 mg/dL, p<0.01 in Balb/C). As shown in figure1, BOLD MRI demonstrated differential response at day 2 probably related to the difference in susceptibility to developing fibrosis.

Conclusions: BUN measurements were consistent with previous findings in this model. BOLD MRI measurements at -2 day time point in Balb/C are consistent with previous reports in kidneys with ureteral obstruction in humans [Thoeny H, Radiology 2008]. While it is not yet clear why C57Bl/6 do not exhibit similar reduction in R2*, it may be suggestive of increased level of hypoxia which in turn can explain higher functional deficit. Future studies should evaluate water content changes at this time point in order to better characterize renal oxygenation changes.

Funding: Other NIH Support - Clinical and Translational Science Award, Private Foundation Support.

TH-POI173
The Utility of Electronic Screening for Enrollment in Clinical Studies: A Descriptive Study
Kamosh Banaei-Kashani,1,2,* Laura N. Hanson,3 Man Li,2 Vitaly herpeschke.1 Nephrology and Hypertension, Mayo Clinic, Rochester, MN;1Pediatric and Children's Clinical, Rochester, MN;2Mayo Validation Support Services, Mayo Clinic, Rochester, MN;3Mayo Foundation Support Services, Mayo Clinic, Rochester, MN.

Background: Enrollment of patients to clinical studies with time sensitive conditions in the critical care field consumes significant portions of study coordinators time. They need to continuously evaluate patient’s medical records for eligibility or rely on the notification from bedside providers.

Methods: METRIC (Multidisciplinary Epidemiological and Translational Research in Intensive Care) Dataset which is a Microsoft SQL-based integrative near-real-time (15min to 1 hour delay) database was served as the main data source for rule based alert utility (sniffer). The sniffer engine periodically scans the data under the datamart and sends the alert to the study coordinator. The alerts were screened for eligibility or rely on the notification from bedside providers.

Results: Both in vivo and in vitro cortical AT1R binding were increased in ischemia but not in normal perfusion. The finding was further supported by immunofluorescence staining of the chronic stenotic kidney tissue, which revealed increased AT1R expression in some glomeruli. There was also a correlation between the distribution of AT1R and NGAL expression.

Conclusions: Increased AT1R binding measured with PET and autoradiography correlates with increased glomerular AT1R in the renal cortex. Thus, Immunofluorescence imaging corroborates well with the PET imaging results and supports the role of AT1R PET as an imaging biomarker of renal ischemic injury.

Funding: NIDDK Support, Other NIH Support - Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grant Number R01 DK50183) and shared instrumentation grant (NIH S10RR022528).

TH-POI171
Positron Emission Tomography and Immunofluorescence Microscopy of Angiotensin II Subtype 1 Receptor in Renal Ischemia
Ali Gholanareanzechad, Jinsong Xia, Majid Chalian, Kelvin Hong, Zsolt Szabo. Nuclear Medicine/ Radiology, Johns Hopkins Medical institutions, Baltimore, MD.

Background: Recent in vivo evidence from Positron Emission Tomography (PET) has demonstrated increased cortical radioactivity binding at the Angiotensin II Subtype 1 Receptor (AT1R) in animal models of renal ischemia. However, the histopathological distribution and cellular components showing upregulation of AT1R remain elusive. We investigated tissue distribution of AT1R expression in experimental models of pig renal ischemia, in order to clarify the significance of AT1R PET as a renal injury imaging biomarker.

Methods: Using domestic pigs, two animal models were developed: 1) chronic renal artery stenosis; and 2) renal artery reperfusion-revascularization by stent treatment. In vivo PET imaging of the AT1R was performed with [15O]water PET. Renal perfusion was estimated with [15O]water PET. Animals were then euthanized and kidneys were removed. The tissues were frozen, and were cut at ≤10-μm thickness. Tissue level distribution of AT1R, podocin and NGAL (Neutrophil Gelatinase-Associated Lipocalin) were imaged by indirect immunofluorescence staining and laser scanning confocal microscopy using cell nuclei were different colors. The sniffer engine periodically scans the data under the datamart and sends the alert to the study coordinator. The alerts were screened for eligibility or rely on the notification from bedside providers.

Results: Both in vivo and in vitro cortical AT1R binding were increased in ischemia but not in normal perfusion. The finding was further supported by immunofluorescence staining of the chronic stenotic kidney tissue, which revealed increased AT1R expression in some glomeruli. There was also a correlation between the distribution of AT1R and NGAL expression.

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Funding: NIDDK Support, Other NIH Support - Supported by the National Institute of Diabetes and Digestive and Kidney Diseases (Grant Number R01 DK50183) and shared instrumentation grant (NIH S10RR022528).

TH-POI172
Blood Oxygenation Level Dependent MRI in Reversible Unilateral Ureteral Obstruction Model
Tina S. Punj,1 Muhammad Haque,2 Tammy Franklin,3 Libby Mathew,1 Pottamurthy V. Prasad.1 1Medicine/Nephrology, University of Chicago, IL;2Radiology, Northshore University HealthSystem, Evanston, IL, Ukraine.

Background: Reversible unilateral ureteral obstruction (rUUO) provides a useful model of chronic kidney disease (CKD) amenable for longitudinal monitoring of structural and functional changes during development of CKD (Punj T, AJP Renal Physiol 2010). We have reported strain-dependent susceptibility to development of CKD after rUUO in C57Bl/6 (susceptible) and Balb/C (resistant) mice. In this preliminary study, renal BOLD MRI was used to detect any early changes in oxygenation status in this model.

Methods: A total of 31 mice (19 C57Bl/6 and 12 Balb/C) underwent rUUO. Blood samples were obtained via retro-orbital bleeding for BUN measurements at three time points (baseline, -2days and -28 days post release of UUO). BOLD MRI was performed on a 4.7 T Bruker Biospec scanner using a 35 mm mouse body coil at the same time points.

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Conclusions: BUN measurements were consistent with previous findings in this model. BOLD MRI measurements at -2 day time point in Balb/C are consistent with previous reports in kidneys with ureteral obstruction in humans [Thoeny H, Radiology 2008]. While it is not yet clear why C57Bl/6 do not exhibit similar reduction in R2*, it may be suggestive of increased level of hypoxia which in turn can explain higher functional deficit. Future studies should evaluate water content changes at this time point in order to better characterize renal oxygenation changes.

Funding: Other NIH Support - Clinical and Translational Science Award, Private Foundation Support.

TH-POI173
The Utility of Electronic Screening for Enrollment in Clinical Studies: A Descriptive Study
Kamosh Banaei-Kashani,1,2,* Laura N. Hanson,3 Man Li,2 Vitaly herpeschke.1 Nephrology and Hypertension, Mayo Clinic, Rochester, MN;1Pediatric and Children's Clinical, Rochester, MN;2Mayo Validation Support Services, Mayo Clinic, Rochester, MN;3Mayo Foundation Support Services, Mayo Clinic, Rochester, MN.

Background: Enrollment of patients to clinical studies with time sensitive conditions in the critical care field consumes significant portions of study coordinators time. They need to continuously evaluate patient’s medical records for eligibility or rely on the notification from bedside providers.

Methods: METRIC (Multidisciplinary Epidemiological and Translational Research in Intensive Care) Dataset which is a Microsoft SQL-based integrative near-real-time (15min to 1 hour delay) database was served as the main data source for rule based alert utility (sniffer). The sniffer engine periodically scans the data under the datamart and sends the alert to the study coordinator. The alerts were screened for eligibility or rely on the notification from bedside providers.
Results: After excluding patients who declined research authorization the number of admissions were 3,456 in 2008 and February 2009 was 3,779. During the same time period the alert system sent 1044 notifications for patients who met shock state criteria. The distribution of the alerts between work hours (7am - 5pm, Mon-Fri) and off-hours were 345 and 699, respectively. After evaluation for eligibility criteria 121 patients were recruited. Coordinators needed to screen only 27% of total ICU admissions (1044/3779) by using shock state sniffer.

Conclusions: Electronic screening (“shock state sniffer”) facilitated the enrollment into clinical study of AKI and potentially saves significant amount of study coordinator’s time for electronic medical records screening.

Funding: Private Foundation Support

TH-PO174

Delivery of Recombinant Erythropoietin (rEPO) in Conjunction with Renal Cell Therapy during Continuous Peritoneal Dialysis


Innovative BioTherapies, Inc., Ann Arbor, MI; University of Michigan, Ann Arbor, MI.

Background: Anemia is a common side effect of chronic kidney disease (CKD). It is postulated that impaired kidney function leads to hypo-responsive EPO release, resulting in reduced red blood cell maturation. Erythropoietic stimulating agents (ESA), including rEPO, and intravenous injections of polycarbonate-conjugated iron have been the corner stone for treating anemia associated with CKD by raising the hemoglobin concentration to 11-13 g/dL. To attempt to improve the treatment of anemia in CKD, a method to deliver rEPO during continuous ambulatory peritoneal dialysis (CAPD) is being tested. As a proof of concept, HEK-293 cells were stably transfected with a human EPO expression cassette and seeded as the surrogate cellular component of a BRECS. Once confluent, the BRECS unit was circulated in 250 ml of serum-containing media for 24 hours, prior to the infusion of this conditioned media into the dry peritoneum of a uremic sheep. Serum was monitored for human EPO every two hours for a 24 hour period using a human-specific EPO ELISA. The EPO concentration peaked between 4 and 8 hours, well within the expected range of human EPO levels (3.3-16.6 mU/ml), at ~12 mU/ml, and decayed in a near-linear fashion to 2.5 mU/ml at 24 hours. As a comparison, a similar experiment was constructed, with the exception that 250 ml of conditioned BRECS media was infused into the peritoneum in the presence of 2 liters of peritoneal dialysis fluid, prior to monitoring serum for the presence of human EPO. In this instance, the serum concentration of EPO peaked between 4 and 8 hours at ~4.5 mU/ml, and decayed in a near-linear fashion to a concentration of 2 mU/ml at 24 hours. These experiments demonstrate that this approach has the potential to provide hormonal stimulation for erythropoiesis in a manner much more consistent with normal physiology.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO175

Evaluation of Adult Human Renal Epithelial Progenitor Cells in Cell Therapy Devices

D. Buffington, D. Humes.

Innovative BioTherapies, Inc., Ann Arbor, MI; University of Michigan, Ann Arbor, MI.

Background: Renal epithelial cell (REC) therapy promises to improve the morbidity of patients with renal disease. To this end, therapeutic devices addressing the neglected biologic component of renal replacement therapy are being advanced. Because donor human tissue is limited, an enhanced propagation method to expand REC progenitors from available adult human kidney transplant discards has been established to provide the biomass for REC-based therapeutic products.

Methods: REC progenitor cell isolation has been successful using kidneys from donors including those previously considered suboptimal (n=19). Donor profiles include long-term diabetes, hypertension, greater than 70 years old, donation post cardiac death with prolonged warm ischemia, and one donor with end stage renal disease (ESRD) on dialysis for >2 years.

Results: Cell yields are consistently higher than 10^11 cells/gram cortex allowing for the manufacture of over 100,000 devices per donor kidney, indicating that autologous therapy may be possible using cells derived from a wedge biopsy taken early in the renal failure cascade. Kidneys from healthy donors (obtained due to anatomical flaw or procedural error) yielded over 10^10 cells/gram cortex or potentially over 1,000,000 devices per donor kidney. Greater than 50% of these cells were isolated from the cortex.

Conclusions: Differentiated REC progenitors, integrated into trabeculated carbon disk based bioartificial REC systems (BRECS), continue to be evaluated in previously established large animal models of ovine ESRD and porcine septic shock.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO176

Immuno-Modulatory Effect of the Bioartificial Renal Epithelial Cell System (BRECS) in an Ovine Model of Uremia


Innovative BioTherapies, Inc.; Univ. of Mi.; Huashan Hospital.

Background: Studies were performed to assess BRECS impact on the pro-inflammatory state that develops during chronic uremia. The BRECS is a compact, cryopreservable renal epithelial cell therapy device under development for treatment of renal and inflammatory disease states. The ovine uremic model employs a continuous-flow peritoneal dialysis (CFPD) circuit for uremic control and recycling PD from the host sheep through the BRECS.

Methods: Sequential nephrectomies were performed at the time of PD catheter placement and one day before initiation of CFPD. BRECS containing 10^7 cells were incorporated into the PD circuits. BRECS O₂ consumption and glutathione (GSH) degradation rates were assessed to monitor cell viability and functionality. Inflammatory indices were measured to assess BRECS impact to modulate the pro-inflamed uremic state. CD11b expression on the neutrophil (NE) cell surface was used as an indicator of the “activated state” of the NE population. Oxidative burst, as a measure of leukocyte function, was monitored and compared to acellular sham devices. Systemic NE apoptotic potential was assessed via Annexin V staining assay. BRECS therapy continued up to seven days, followed by an additional post-therapy evaluation period of 48 hours.

Results: BRECS remained viable with detectable O₂ consumption and GSH degradation rates for the entire therapeutic time course. CD11b mean fluorescent intensity was lower beginning after 5 days of BRECS therapy when compared to acellular control sheep. Enhancement in oxidative burst was also seen in the BRECS treatment group. Importantly, these two effects continued during the 48 hour post-BRECS therapy period. BRECS therapy caused a shift in the percent NEs that maintain apoptotic progression, indicating a return to steady state with respect to NE activation and function, as seen in normal, non-uremic sheep.

Conclusions: BRECS therapy positively modulates the inflammatory state of the uremic sheep, indicated by a decrease in the circulating NE activated state, increase in oxidative burst and improved NE apoptotic potential.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO177

Modeling Ultrasound (US)-Induced Heating of Hemodialysis Grafts

Yan-Feng E. Shih, Mark R. Britton, Alfred K. Cheung, Russell Stewart Stewart, Per Christian Christensen, Medicine, Univ. of Utah, SLC, UT; Bioengineering, Univ. of Utah, SLC, UT; Electrical and Computer Engineering, Univ. of Utah, SLC, UT; Medical Service, VASLCHCS, SLC, UT.

Background: Expanded polytetrafluoroethylene (ePTFE) grafts fail at high rates due to stenosis caused by neointimal hyperplasia (NH). Currently there is no effective method to prevent or treat NH. We explored a novel approach to reduce NH in ePTFE grafts using focused US-induced mild hyperthermia. We have previously reported a higher sensitivity to hyperthermia-induced death for cells cultured on ePTFE than on a surrogate tissue surface. We identified the temperature range (45-47°C) necessary to induce significant apoptosis among cells cultured on ePTFE but not on surrogate tissues. The present study addresses the feasibility of selectively heating ePTFE to the optimal temperature by US.

Methods: The acoustic finite-difference time-domain method was used to calculate the beam propagations from 1.5- and 3.25-MHz transducers through a simplified 3-dimensional model containing fat, muscle, blood flow and ePTFE graft. Next, the spatial pattern of the heat derived from the US power was simulated using the COMSOL Multiphysics heat-transfer module. The simulation included skin surface cooling to 20°C, laminar blood flow through the graft lumen, and the effect of blood perfusion on heat transfer in the tissue regions. Cyclic 30-sec heating and cooling periods were used to mimic a pulsed ultrasound cycle.

Results: The greater US attenuation by ePTFE than by tissues caused about 5 times more power deposited in ePTFE than in adjacent tissues. The transducer with a higher frequency produced a more confined power deposition pattern than the transducer with lower frequency. Importantly, high temperatures (48-49°C) were found only on ePTFE, while blood and most tissues remained at 37°C.

Conclusions: Our approach exploits the differential acoustic properties between ePTFE and tissues, and their differential sensitivity to hyperthermia-induced cell death. Our results show promise for the use of focused US as a safe and effective strategy to prevent and treat NH on the ePTFE surface through hyperthermia-induced apoptosis.

Funding: NIDDK Support, Other U.S. Government Support - NHLBI, Veterans Administration Support, Private Foundation Support

TH-PO178

ER-Stress Induced Mega-Cluster microRNAs in Mouse Models of Diabetic Nephropathy

Mitsuo Kato, Mei Wang, Zhuo Chen, Xiwei Wu, Sumanth Putta, Innovative BioTherapies, Inc., Ann Arbor, MI; 2University of Michigan, Ann Arbor, MI.

Background: microRNAs (miRNAs) have been shown to play major roles in renal diseases. However, the comprehensive study of miRNAs has not completed in diabetic nephropathy.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: In this study, we profiled miRNAs in RNA extracted from glomeruli from diabetic and control mouse kidney scaffolds using deep sequencing (solexa, Illumina Inc.). Expression levels of candidate miRNAs were validated by qPCRs.

Results: Some of confirmed miRNAs are miR-34 family (miR-34a, miR-34b, miR-34c), miR-125a, miR-125b, miR-126, miR-379, miR-379-3p. UCs are involved in renal hypertrophy. miR-486 is also involved in renal hypertrophy by targeting PTEN and activating Akt kinase under diabetic conditions. miR-379 is a member of miR-495-495 mega cluster miRNAs (~40 miRNAs). Other members (miR-495, miR-380, miR-376b, miR-379, miR-379-3p) are contained to be highly expressed in glomeruli from STZ-injected type1 diabetic mice. Interestingly, miRNAs in this cluster were regulated by ER stress induced transcription factor CHOP upregulated by high glucose conditions (HG) or TGF-β in kidney cells. The potential targets of this mega cluster are Tnrc6b (15 sites by 12 miRs), Cugbp2 (14 target sites by 12 miRs) and Pten (13 sites by 10 miRs). Decrease of these potential targets was confirmed in glomeruli from diabetic mice and in kidney cells treated with HG or TGF-β. Tnrc6b is a mammalian homologue of GW182 which is located in P-bodies and regulates protein translation. Cugbp2 is a regulator of insulin RNA splicing but also a negative translational regulator of Cox2 which is increased in diabetic glomeruli. PTEN inhibition by the mega cluster may activate Akt to enhance protein synthesis and hypertrophy.

Conclusions: These results demonstrate that diabetic condition induces renal hypertrophy through down regulation of negative translational regulators targeted by the mega cluster miRNAs upregulated by ER stress induced CHOP. ER stress induced mega cluster miRNAs may be therapeutic targets of diabetic nephropathy.

Funding: NIDDK Support

TH-PO1179

Basement Membrane Remodeling and Endothelial Differentiation When Mouse Stem Cells Are Seeded into Acellular Rat Kidney Scaffolds

Edward A. Ross,1 Dale R. Abrahamson,2 Matthew James Williams,1 Gary W. Ellison,1 Patricia St. John,1 Chris Batch.1

1University of Florida; 2Kansas University Medical Center.

Background: Due to transplant organ shortage we have pursued tissue regeneration using decellularized kidneys seeded with tubulopotent precursor cells. We hypothesize that these scaffolds retain matrix signals that can induce progenitor cells to differentiate and recapitulate native structures: matrix-to-cell signaling followed by cell-to-cell and then cell-to-matrix interactions that would gradually remodel and replace the original scaffold matrix. This would reduce scaffold antigenicity and enable xenografts.

Methods: We previously showed that when arterially seeded into acellular rat whole kidney scaffolds mouse embryonic stem cells (mESCs) attach, multiply and demonstrate cell-to-matrix interactions that would gradually remodel and replace the original scaffold matrix (‘‘mimurization’’), mESCs were infused arterially into rat kidney scaffolds and incubated for 10 days. Endothelialization of vascular cells was tested by endothelial specific BsLB4 lectin for differentiation. We now tested for more specific evidence of differentiation into endothelial membrane and remodeling of the matrix basement membranes from rat to mouse.

Results: As evidenced by co-localized staining with DAPI nuclear label, cells in arterially seeded scaffolds demonstrated expression of α-smooth muscle actin (α-SMA), α-actin, von Willebrand factor (vWF), and factor VIII related antigen (FVIII/RAg).

Conclusions: We have provided new evidence for matrix-to-cell signaling in acellular whole organ scaffolds that induces differentiation of pluripotent precursor cells to endothelial lineage. Production of mouse basement membrane supports remodeling of host (rat)-derived scaffolds and thereby warrants further investigation as a promising approach for xenotransplantation.

TH-PO1180

Bio-Mechanical Properties of the Mammalian Urothelium

Mark L. Zeidel,1 Enhua H. Zhou,2 Weiquan Yu,2 John Matha.1

1Medicine, Beth Israel Deaconess Medical Center, Boston, MA; 2Physiology and Bioengineering, Harvard School of Public Health, Boston, MA.

Background: Successful bladder filling, urine storage and voiding requires an intact urothelium because urothelial surface cell layers (UCs) maintain an exceptionally tight barrier between urine and blood. This barrier renders the urothelium impermeable to large molecules such as electrolyte and protein. The urothelium resists straining and subsequent relaxation, during the processes of bladder filling and voiding respectively. Specialized proteins called uropodins form plaques on the surface of umbrella cell and cover up to 90% of the urothelial surface, and is considered to be rigid. It is not clear how a rigid surface can be flexible as the bladder undergoes stretch and relaxation cycles. The bio-mechanical properties of UCs, including deformability and spontaneous dynamic remodeling, likely play a critical role in both barrier function and mechanotransduction.

Methods: To develop an understanding of the mechanical properties of the urothelial surface we used and magnetically coated with magnetic beads to the apical membranes of intact UCs on intact urothelium and measured their stiffness G, in response to oscillating magnetic fields (optical magnetic tracking cytometry, OMTC) as we varied the frequency of oscillation between 0.1 to 1000 Hz.

Results: UCs exhibited G values which tracked closely with highly deformable red blood cells. Removal of surface UCs with protease exposed underlying intermediate cells, which exhibited G values 12-15 fold higher than UC's, and similar to cultured MDCK.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Results: The final version of the terminology index has a total of 1104 items grouped in 14 pathologic categories: Symptoms, Signs and Syndromes, referral, Water and Electrolyte Disorders, Inherited Kidney Diseases, Glomerular Disorders, Multisystem Diseases, Diabetic Nephropathy, Infectious Diseases, Vascular Kidney Diseases, Acquired Chronic Tubulointerstitial Nephritis, Urinary Tract Infection, Nephrolithiasis, Hypertension, Renal Neoplasia and Acute Renal Failure. Each item has its corresponding CIE-9 MC pre-attached code. The global agreement observed between both coding technicians was 95%, 100% in most pathologies except 94% in Water and Electrolyte Disorders and 74% in Vasculitis.

Conclusions: A Standard Terminology Index by the Spanish Society of Nephrology with CIE9-MC system pre-coded will be a useful tool for Clinical Nephrology Outpatient care that will be available at the Society web site. A preliminary implementation study is needed to validate this classification. It would be desirable to complete the list with dialysis and transplantation terms.

Funding: Private Foundation Support

TH-PO185

A Novel Anemia Management Protocol (AMP)  Yossi Chait,1 Michael J. Germain,9 Joseph Horowitz,1 Christopher V. Hollot,2 Rajiv P. Shrestha,1
1University of Massachusetts; 2Western New England Renal & Transplant Associates.

Background: We report observations from a pilot clinical study on the feasibility of AMP designs based on mathematical modeling of erythropoiesis and on feedback control. This is in contrast to current AMPs, which are largely rule-based, expert systems formulated on the basis of experience and clinical studies.

Methods: A pharmacokinetic/pharmacodynamic (PK-PD) erythropoiesis model was employed and a new AMP was designed using robust control principles, based on retrospective 3x/week hemoglobin (Hgb) data. The dosing protocols for four ESRD patients were switched to the new AMP designs.

Results: For parsimonious parameter identification, we modeled the erythropoiesis stages using an Epo-stimulated saturable production function with a time delay to model the maturation of the progenitor cells into red blood cells (RBCs). Epo PK was modeled using linear plus nonlinear clearances. The RBC pool was modeled with a 2nd-order gamma distribution describing mean RBC lifespan. Quantitative Feedback Theory was used to design the AMP which used past and current Epo doses and Hgb data to compute the upcoming week’s Epo dose. Patient-specific model parameters and corresponding algorithm parameters were periodically updated based on newer Hgb data. Preliminary results support the feasibility of the new AMP in maintaining Hgb within the desired range (10-12 g/dL), with reduced variability and decreased Epo usage; see Figure 1. We also deliberate the hypothesis that variability in Hgb due to iron kinetics or fluid volume cannot be eliminated with an AMP based only on Hgb data.

Conclusions: Patient-specific AMPs based on PK-PD models and robust control principles can offer improved Hgb regulation.

Funding: Other U.S. Government Support

TH-PO184

Development of a Clinical Terminology Index by the Spanish Society of Nephrology: Standardization Process  Katia Lopez-Reveultas,1 Elena Corchete,1 Alberto Ortiz,1 1Nephrology, Hospital Universitario Fundación Alcorcón, Madrid, Spain; 2Nephrology, Fundación Jiménez Díaz, Madrid, Spain.

Background: Achieving a common medical terminology is a main concern for the implementation of electronic medical record. The use of standardized clinical terminology facilitates electronic health record function for multiple purposes: clinical, research, registries and health policy planning. Coding systems, fully implemented in hospitalization are lacking in outpatient care. PURPOSE: To develop a standard defined clinical terminology index validated by the Spanish Society of Nephrology, pre-coded by CIE9-MC system to apply in Clinical Nephrology Outpatient Care.

Methods: A systematic review of pathologies was carried out by the research team. Nutritional status and body composition were determined by body mass index (BMI), waist circumference (WC), hip circumference (WH), ratio (r) WH/HC, and fat and lean mass (FM and FL) by DEXA-absorptiometry. Obesity index WheiR was calculated as waist circumference (WC/cm) divided by (BMI cm2), waist-to-hip (r) ratio and (r) WH/HC. Patients were divided according to WheiR: Group 1: WheiR<0.55; n=63; Group 2: WheiR>0.55; n=71. Gender, age, BMI, WC, WH, DEXA total and central body fat (p<0.0001). WheiR index presented a better correlation with DEXA trunk fat (p<0.0001) than with DEXA total fat and WHR. The CRP, leptin, and HOMA-IR were higher in group 1, but HMW adiponectin was lower. The Spearman’s coefficient was significant between WheiR and IFN-γ and IL-10. In the stepwise multiple regression analysis, WheiR was positively associated with HOMA-IR and leptin and, negatively associated with HMW adiponectin.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: THERF is very easy to be measured, presented a significant correlation with C4d in Ckd patients, and can be used to evaluate C4d. In Ckd patients, high C4d was followed by inflammation. In conclusion present data suggest that body adiposity is an independent risk factor for CVD in patients with CKD and should be a target in the nutritional treatment of this population.

TH-PO187
Longitudinal Relationship between Blood Pressure and Left Ventricular Mass in Children with Chronic Kidney Disease Juan C. Kupferman,1,2 Dereck Ng,2 Joseph T. Flynn,2 Susan L. Furth,2 Bradley A. Warady,3 Mark Mitsnefel,2 1Maimonides Medical Center, Brooklyn, NY; 2CKID investigators.

Background: The longitudinal association between casual blood pressure (BP) and left ventricular mass index (LVMi) has not been previously studied in children with chronic kidney disease (CKD).

Methods: In a prospective cohort study, BP was assessed annually. Initial echocardiogram was performed 1 year after study entry (V2) and 2 years later (V4). A linear mixed model with a random subject effect assessed the effect of change in BP on LVMi. The main exposure was average BP z-score, defined as the average of 2 BPs spaced 1-year apart prior to each LVMi measurement. Models for systolic and diastolic BP (SBP, DBP) were adjusted for age, sex, race, height, CKD diagnosis, and years with CKD. Each model included a time effect, allowing LVMi to be different at V2-V4. We also allowed the effect (slope) of average BP to be different.

Results: There were 260 subjects with complete follow-up data; age (median [IQR]) 11 [8, 15], 61% male; 21% black; median iohexol GFR 42 ml/min/1.73m2. Over 2 year follow up, median SBP z-score decreased from 0.34 to 0.07 while DBP z-score decreased from 0.47 to 0.23; average LVMi decreased from 33.8 to 31.1 g/m2.

Effects of BP and Time of VTE:

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Estimated change (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (V2)</td>
<td>-1.9% (4.5%, -6.2%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>SBP (V4)</td>
<td>6.5% (3.3%, 9.4%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Effect of Time (2 yrs)</td>
<td>-32% (-44%, -17%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>DBP (V2)</td>
<td>3.4% (0.8%, 6.1%)</td>
<td>.01</td>
</tr>
<tr>
<td>DBP (V4)</td>
<td>5.8% (2.3%, 9.3%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Effect of Time (2 yrs)</td>
<td>-29% (-41%, -14%)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

LVMi had a 2-year average decrease of 32% when adjusting for SBP and confounders. For every one standard deviation increase in SBP at V2, LVMi increased by 3.9% and this effect increased to 6.5% at V4. The linear association between DBP z-score and LVMi was significant and the effect was higher for V4 compared to V2 (5.8% vs. 3.4%).

Conclusions: BP and LVMi improved in children with CKD over time. Elevated SBP and DBP are associated with increased LVMi. While the effect of BP on LVMi was not significantly modified by time, these results suggest a clinically meaningful effect of long-term elevated BP on LVMi.

Funding: NIDDK Support, Other NHLBI Support - NHLBI, NICHD

TH-PO188
Elevated Albuminuria Increases the Risk of Recurrent Venous Thromboembolism: Results from a Population Based Cohort Study Inge van Schouwenburg,1 B. Khan Mahmoodi,1,2 Nic Veeger,1,3 Hanneke C. Kluin-Nelemans,1,4 T. T. J. van der Meer,2 Karina Meijer,2 1Division of Hemostasis and Thrombosis, Department of Hematology, University Medical Center Groningen (UMCG); 2Department of Nephrology, UMCG; 3Department of Epidemiology, UMCG, Netherlands.

Background: Microalbuminuria has been identified as risk factor for first venous thromboembolism (VTE). Whether it is also a risk factor for recurrence is unknown. Knowledge on this issue is relevant because it may help in assessing the duration of anticoagulant therapy after a first VTE. We therefore investigated the risk of recurrent VTE in patients with elevated albuminuria.

Methods: Data of a prospective population based cohort study (PREVENT), that started in 1997 and included 40,856 subjects aged 28-75 yrs, were used. In all participants, albuminuria was measured and VTE occurrence was monitored. Patients with first VTE between study entry and January 2009 were identified by database record linkage with the national registries of hospital discharge diagnoses and death certificates and the regional anticoagulation clinic. Of identified patients medical records were utilized for verification and for obtaining additional information.

Results: Of 351 subjects with first venous thromboembolism (49% male; median age at first event 64 years, unprovoked 43% and provoked [external risk factor present] 48%) 37 subjects developed a recurrence during a median follow-up period of 3.3 (interquartile range, 1.1-6.4) years. Annual incidence of recurrence in subjects with elevated albuminuria (≥0.2 mg/100 ml) was 5.00 per 100 person years (95% confidence interval [CI]; 2.16-9.85) compared to 2.38 (95%CI; 1.59-3.41) in subjects with normal albuminuria (<2 mg/L).

Hazard ratio for recurrence was 1.95 (95%CI; 0.89-4.30) after adjustment for age and sex. This hazard ratio was 3.35 (95%CI; 1.18-9.47) in patients with first unprovoked, and 1.12 (95%CI; 0.25-5.0) in those with a first provoked event. Subjects with elevated albuminuria who experience an unprovoked VTE are at an increased risk of recurrence, independent of age and sex. These results implicate that such patients may benefit from long-term anticoagulant therapy.

Conclusions: THERF is very easy to be measured, presented a significant correlation with C4d in Ckd patients, and can be used to evaluate C4d. In Ckd patients, high C4d was followed by inflammation. In conclusion present data suggest that body adiposity is an independent risk factor for CVD in patients with CKD and should be a target in the nutritional treatment of this population.

TH-PO189

Background: Patients with chronic kidney disease (CKD) have an increased risk of developing peripheral arterial disease (PAD) compared to those with normal kidney function. The underlying etiology for this increased risk is not fully understood.

Methods: We studied the effects of novel risk factors on the development of PAD among 3,090 participants in the Chronic Renal Insufficiency Cohort (CRIC) study. Patients aged 21 to 74 years old with an estimated glomerular filtration rate (eGFR) of 20-70 mL/min/1.73 m2 were recruited from 7 clinical centers in the US. Incident PAD was defined as a new onset ankle-brachial index (ABI) of <0.9 or clinical PAD confirmed by an endpoint assessment committee during the follow-up period among participants with ABI>0.9 and no PAD history at baseline examination.

Results: After adjustment for age, race, gender, and clinical sites, the following novel risk factors were associated with an increased risk of incident PAD (hazard ratio and 95% confidence interval for a one standard deviation higher level): HOMA-insulin resistance (IR) (1.12; 1.05-1.19; p<0.001), hemoglobin (Hb) A1C (1.25; 1.13-1.39; p<0.001), total parathyroid hormone (1.12; 1.01-1.24; p<0.001), C-reactive protein (CRP) (1.11; 1.05-1.18; p<0.001), white blood cell count (1.18; 1.06-1.31; p=0.003), fibrinogen (1.29, 1.15-1.45, p<0.001), alkaline phosphatase (1.18; 1.09-1.29; p<0.001), cystatin C (1.19; 1.06-1.34; p=0.003), and 24-hour urine albumin (1.16; 1.04-1.29; p=0.006). After further adjustment for additional cardiovascular risk factors including eGFR, the following remained significantly associated with increased risk of PAD: HOMA-IR, HbA1C, CRP, fibrinogen, alkaline phosphatase, and cystatin C.

Conclusions: These data indicate that insulin resistance, inflammation, prothrombotic state, bone metabolism and kidney function are associated with risk of PAD independent from traditional risk factors among patients with CKD.

Funding: NIDDK Support

TH-PO190
Increasing Levels of Novel Kidney Function Markers Predict Outcomes in the General Population: The Atherosclerosis Risk in Communities (ARIC) Study Brad C. Astor,1 Tariq Shafi,2 Ron C. Hoogeveen,2 Christie Ballantyne,2 Josef Coresh.3 1Johns Hopkins University; 2Baylor College of Medicine.

Background: Decreasing kidney function is associated with higher risk of death, CVD and ESRD. Several novel markers of kidney function have been proposed as adjuncts to serum creatinine (Scr). The risk of outcomes associated with changes in these markers is unknown.

Methods: We examined the association between a ≥25% increase over 6 years in Scr, cystatin C (CysC), beta trace protein (BTP) and beta-2 microglobulin (B2M) and subsequent outcomes (death, coronary heart disease [CHD], hospitalized heart failure [HF], acute kidney injury [AKI] and ESRD) over the following 11 years among 2,186 selected participants in the ARIC Study with an estimated GFR (eGFR) ≥60 mL/min/1.73m2 at baseline.

Results: An increase in 1 or more markers was associated with death and heart failure after adjustment. Increases in 2 ≥ markers was strongly associated with all outcomes. Results were similar among participants without a ≥25% increase in Scr (n=1,821) or CysC (n=1,621).

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
154A
Endogenous Anti-Atherosclerotic Property Is Decreased in Patients with Renal Dysfunction: Evaluation Form the Circulating Solute Fms-Like Tyrosine Kinase-1 (sFlt-1) Level after Heparin Injection

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Background: CKD is usually associated with atherosclerosis. Placental growth factor (PIGF), a family of VEGF, plays important roles in the development of atherosclerosis, which is antagonized by sFlt-1, a soluble form of PIGF receptor. sFlt-1 is circulating and is also bound to extracellular matrices of vascular bed by its binding protein motifs. Given that heparin injection increases plasma sFlt-1 levels, we hypothesized that the heparin-induced increment of plasma sFlt-1 level would indicate the antagonizing capacity, but baseline circulating sFlt-1 level wouldn’t. Here we verify the hypothesis and investigate significance of PIGF/sFlt-1 system in CKD-related atherosclerosis.

Methods: Five minutes after bolus injection of heparin (0.4unit/kg), plasma sFlt-1 levels were increased from 111.8±52.5pg/ml to 291.6±145.7pg/ml (p=0.001) in 251 CKD patients. The increment was increased with elevating eGFR (r=0.746, p<0.001). Consequently, baseline sFlt-1 levels were negatively correlated with eGFR (r=-0.456, p<0.001), but those after heparin injection were positively correlated (r=0.549, p<0.001). Plasma PIGF levels were not changed by heparin, and the ratio of PIGF to sFlt-1 was more strongly correlated with eGFR (r=0.688, p<0.001). The ratio was also significantly associated with severe coronary artery disease in 329 CKD patients, who underwent coronary angiography with (0.4-0.5 unit/kg) heparin injection (p=0.001). In mice with 5/6 nephrectomy, sFlt-1 mRNA expression in organs examined (kidney, lung, and heart) was lower by about 20-50% than that in normal mice. In the pharmacokinetic analysis of PIGF, plasma level of human PIGF immediately after injection of recombinant human PIGF (3.0mg/rabbit) was higher in rabbits with 3/4 nephrectomy than in normal rabbits. These findings support the reduction of antagonizing capacity of sFlt-1 for PIGF in CKD.

Conclusions: PIGF and sFlt-1 at least partly play roles in development of atherosclerosis in CKD.

Atherosprotective Function of High Density Lipoprotein (HDL) is Defective in End Stage Renal Disease Patients on Hemodialysis (ESRD-HD)

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Background: Traditional risk factors do not account for increased cardiovascular disease (CVD) in CKD, particularly in ESRD-HD, who are also resistant to traditional lipid lowering therapies. Since HDL provides important anti-atherogenic benefits through cholesterol efflux and anti-inflammatory capacities, we examined HDL functionality in ESRD-HD.

Methods: Cellular cholesterol efflux and inflammation were assessed in human macrophage THP-1 cells exposed to HDL isolated from ESRD-HD (n=29) and matched normal controls (n=28). HDL was isolated by sequential density ultracentrifugation, and cellular lipid content assessed by gas chromatography measured cholesterol efflux. Inflammatory markers were determined by cytokine mRNA. HDL was isolated by sequential density ultracentrifugation, and cellular lipid content assessed by gas chromatography measured cholesterol efflux. Inflammatory markers were determined by cytokine mRNA.

Results: There were no differences in plasma lipids, whereas hsCRP was elevated in ESRD-HD. HDL from ESRD-HD was dramatically impaired in facilitating efflux from cholesterol-loaded THP-1 (p=0.001). Efflux impairment was also observed in subgroups of diabetes (ESRD-HD vs diabetic 8.1±6.1 vs 13.6±6.1%, p<0.04) and was not improved in subgroups on statin therapy. HDL of ESRD-HD had less effective anti-chemotactic function and heightened inflammatory capacity. Statins do not improve cholesterol efflux and circulating hsCRP or cellular inflammation.

Conclusions: HDL of ESRD-HD has profoundly impaired cholesterol acceptor function and heightened inflammatory capacity. Statins do not improve cholesterol efflux but quell inflammatory response. Findings predict increased foam cell formation and suggest that dysregulated cellular lipid metabolism is a key driver for excess CVD that may explain dissociation of CVD with plasma lipids and resistance to lipid lowering therapies in ESRD-HD.

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CHRONIC KIDNEY DISEASE, PULMONARY HYPERTENSION AND ALL-CAUSE MORTALITY

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Background: Pulmonary hypertension is associated with high mortality rates. Chronic kidney disease (CKD) is widely prevalent in patients with pulmonary hypertension. We examined the associations of non-diagnosis dependent CKD and all-cause mortality in patients with pulmonary hypertension (PH).

Results: Out of 1278 patients, 482 (37.7%) had stage 3 CKD (eGFR 30-59 ml/min/1.73m2) and 70 (5.4%) had stage 4 CKD (eGFR <30 ml/min/1.73 m2). On multivariable analysis, older age, presence of hypertension, higher pulmonary artery systolic pressure and pulmonary capillary wedge pressure were independently associated with CKD. Kaplan-Meier survival plots indicated significant differences in all-cause mortality for patients with and without CKD (log rank p=0.001) (Figure 1). After adjusting for relevant covariates, presence of stage 4 CKD was associated with all-cause mortality ( Hazard ratio[HR] 1.59, 95% CI 1.02, 2.40), while stage 3 CKD was not associated with a statistically significant increased hazard for death (HR 1.19, 95% CI 0.94, 1.52). When GFR was examined as a continuous measure, every 5 ml/min decline in GFR was associated with a 3% (95% CI 1.02-1.06) increased hazard for death.

Conclusions: CKD is common in patients with PH. Stage 4 CKD is associated with all-cause mortality in patients with PH. Future studies should explore the mechanisms that may underlie these associations.

Identification of Differentially Expressed Genes in Arteries from Patients with Renal Failure

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Background: The molecular pathology behind arterial disease in uremia is only partially known. Our aim was to identify differentially expressed genes in human arteries from uremic patients by microarray and pathway analysis.

Methods: Tissue samples were obtained from 16 iliac arteries from uremic patients undergoing kidney transplantation and from 19 renal arteries from living kidney donors. In addition, mammary artery samples from 10 patients undergoing coronary by-pass operations were used (5 patients with plasma creatinine above 140 µM and 5 age- and gender-matched individuals with no known kidney disease). Gene expression profiles of these samples were generated using Affymetrix HG-U133A 2.0 microarrays.

Results: We found that 17 gene transcripts differed significantly in iliac samples from patients with uremia with a false discovery rate (FDR) <0.003, p<1.0*10-5. Using GenMAPP, we found that 13 pathways were significantly regulated. Both the apoptosis pathway and the TNF-alpha-NFkB pathway were up-regulated, while the smooth muscle contraction- and glycogen metabolism pathways were down-regulated. Verhoef-van Giesen stained artery samples showed no obvious compositional differences between the 2 groups. To compensate for bias because of different artery location between the uremic and non-uremic groups, we extended our findings with microarray data obtained from the mammmary artery samples from the coronary by-pass patients. We then found that 23 gene transcripts were congruently downregulated and 8 gene transcripts upregulated in both comparisons when the setting FDR <0.05 in the iliac/renal artery study and the p-value <0.2 in the mammmary artery study were used. This gene transcript group contains vimentin, CD9, hypoxia inducible factor 3A, matrix molecules, and smooth muscle differentiation factors.

Conclusions: The identified genes are likely to be associated with the development of cardiovascular disease in patients with renal failure.

Funding: Private Foundation Support
TH-PO195

Brain White Matter Abnormalities in Children with Chronic Kidney Disease
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Background: There is increasing evidence that children with chronic kidney disease (CKD) have neurocognitive deficits. We hypothesized that these deficits result from subtle brain injury and abnormal brain white matter microstructure. Our objectives were: (1) to determine the prevalence of brain injury or abnormalities and (2) to compare white matter integrity in children with CKD relative to healthy controls.

Methods: Magnetic resonance imaging (MRI) studies were obtained in a prospective cohort of 29 children with CKD (mean age of 14.4 ± 2.7 years (range 9–18)), and 20 healthy age matched controls (mean age of 13.6± 2.9 years (range 9 – 18)) at two pediatric renal centres. Conventional MRIs were reviewed by a single blinded neuroradiologist to determine the prevalence of brain injury. Fractional anisotropy (FA) maps calculated from diffusion tensor imaging scans were generated to compare white matter microstructure in both groups using tract-based spatial statistics.

Results: Focal or multifocal white matter injury was seen on conventional MRI in 6 children with CKD (21%) and in 1 control. Relative to controls, reduced white matter FA was seen in the anterior limb of the internal capsule of the CKD subjects, a region that contains white matter pathways involved in cognitive functions. The FA reduction in CKD children was greater in the non-transplant CKD group relative to the transplant group.

Conclusions: In this preliminary study, white matter injuries are observed in children with CKD. Abnormal white matter microstructure, reflected in reduced FA, is also observed in children with CKD relative to healthy controls. The potential difference observed between children with renal transplant vs. CKD requires further investigation.

Funding: NIDDK Support, Private Foundation Support

TH-PO196

Polyclonal Immunoglobulin Free Light Chain Concentrations and Survival in Patients with Renal Disease
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Background: Chronic kidney disease (CKD) affects around 10% of adults, with associated high morbidity and mortality. Enhanced risk stratification to identify those with CKD at highest risk of poor outcomes is required to optimise the management of CKD. Polyclonal immunoglobulin free light chains (FLC) are both markers of kidney function and of systemic inflammation. The purpose of this study was to evaluate the utility of polyclonal serum FLCs for mortality risk in people with CKD.

Methods: Serum concentrations of polyclonal FLCs were measured by immunoassay in 1275 individuals with CKD, these comprised: (i) 848 with CKD stages 1-5; (ii) 382 renal transplant recipients; and (iii) 45 dialysis patients (CKD 5d). The cohort was prospectively followed up for a median of 45 months. Cox regression analysis was undertaken to determine variables associated with survival.

Results: Serum FLC concentrations closely correlated with kidney function. The linearity between the two FLC isotypes (κ and λ) and total FLC levels was high, therefore in the survival analysis total levels were used. In the overall cohort, high serum polyclonal FLC levels were an independent risk factor for death: HR 2.06 (1.55-2.75), P<0.001.

Conclusions: Serum concentrations of polyclonal FLCs independently predicted survival in patients with CKD and ESRF but not renal transplant recipients. Strategies now need to be devised to apply this observation to clinical practice.

TH-PO197

Rapide Decline of eGFR Predicts Long Term Improvement of Renal Function after Revascularization of Renal Artery Stenosis
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Background: to determine if decline of renal function prior to the time of stenting impacts on renal outcome after stenting in a cohort of patients with Atherosclerotic Renal Artery Stenosis (ARAS)

Methods: 30 patients with CKD stages 3 to 4 and ARAS underwent renal stenting. Mean follow up: 33 (SD 21) months; the change of eGFR before and after stenting was expressed as negative or positive value in mL/min (ΔGFR).

We indentified 2 types of subgroups: the first on the basis of decline of renal function (cut point -0,25 mL/min) by assessing ΔGFR in a pre-stenting period of 10 months (Slow or Fast Progressor) N=24 and the second on the basis of stenosis type: 1 (unilateral) N= 13; 2 (7 bilateral; 2 single kidney ; 8 prevalent kidney ) N= 17. No difference at baseline was noted between subgroups for the following variables : age, eGFR, renal length, resistive index, proteinuria, diabetes and vascular diseases.

Results: 37 stents were placed successfully. No periprocedural death occurred. At latest follow up renal function improved in 14 patients (46,6%), stabilized in 6 patients (20%) and worsened in 10 patients (33%). 7 of these 10 patients reached ESRD which required hemodialysis.

Being in Fast Progressor subgroup was associated with improved renal function after stenting (8 of 13 patients ; p=0,013; Fisher’s test).

Belonging to the subgroup 2 was predictor for improvement of renal function (11 of 17 patients ; p=0,032; Fisher’s test )

After stenting median ΔGFR was significantly greater in the Fast Progressor subgroup compared to the Slow Progressor ( 0,10 vs -0,14 ; p=0,04; Mann Whitney test)

Conclusions: Predictable benefit from renal stenting may be most likely in patients presenting with a rapid decline of GFR associated with ARAS affecting the whole renal mass that is both kidneys or single functioning kidney.
Conclusions: In the largest European study of its kind to date we found significant attenuation of neurological outcome following thrombolysis for acute stroke in patients with renal impairment but no excess in hemorrhagic complications. This may relate to greater subclinical cerebrovascular burden and uremic endothelial dysfunction and requires comprehensive study.

TH-PO201

Serum Phosphate Qualify as Atherosclerotic Risk Factor, Particularly in the Setting of Older Chronic Disease Patients

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Background: Translational studies demonstrate that hyperphosphatemia promotes atherosclerotic vascular calcification. However, the association of serum phosphorus (P) with parameters of atherosclerosis (AT) remains controversial in Chronic Kidney Disease (CKD) patients.

Methods: This multicenter observational study included 1279 CKD patients from the spanish multicenter study NEFRONA (54, 33 and 14% at CKD stages III, IV and V, respectively), 60±12 years, 63±12 months, 28±2% diabetics. We applied a standardized protocol including carotid intima-media thickness, ankle-brachial index and presence/absence of carotid plaques to determine the AT score (Coll B et al, NDT 2010). The influence of study variables was evaluated applying uni- and multivariate logistic regression model, and using AT score as the dependent variable.

Results: Patients were classified in 2 groups: 1) no or mild AT (44%); 2) moderate to severe AT (56%). The presence and severity of AT was significantly associated with older age (HR 1.087 [C95% 1.073-1.102], p<0.001), male sex (1.361 [1.007-1.841], p=0.045), higher P (1.354 [1.135-1.616], p<0.001), systolic blood pressure (1.010 [1.004-1.016], p=0.002), smoking (2.056 [1.555-2.754], p<0.001), and diabetes (1.554 [1.148-2.102], p=0.004) in the multivariate model. The level of renal failure, serum calcium, PTH, and dislipidemia were not associated with AT score. In subsequent analysis, the risk for moderate to severe AT is multiplied by a factor of 2.8 for patients >63 years with P=4.2 mg/dl (2.761 [1.639-4.649], p<0.001).

Conclusions: We verified that high P represent a risk for AT in CKD patients with GFR<60 ml/min. This association is more consistent in older patients even with P levels within or close to normal range.

TH-PO202

Carotid Plaques Predict Presence and Severity of Coronary Artery Calcium in Chronic Kidney Disease

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Background: Coronary artery calcium (CAC) measured by multi slice computer tomography (MSCT) is predictive of mortality even in patients with renal failure. The presence and severity of carotid plaques may represent additional cardiac risk and can be used to stratify the risk of patients with CKD.

Methods: Ninety-one CKD-5D patients (mean vintage 31±24 months, 51% women, 71% Black, 68% diabetic) underwent CT for CAC imaging. CAC scoring was performed with the Agatston method and stratified as < 10 (minimal), 10-99 (mild), 100-399 (moderate), and ≥ 400 (severe). Patients were also grouped according to presence of carotid plaques and classified as follows: group 1 (no plaques), group 2 (unilateral plaque/s), and group 3 (bilateral plaque/s).

Results: Carotid plaques were visualized in 74 (81%) patients. CAC score was <10 in 35 (38%), mild in 18 (20%) and moderate to severe in 38 (42%) patients. The mean CAC was 55.6 ± 103 for group 1, 114 ± 150 for group 2 and 586 ± 951 for group 3 (P=0.02 for trend).

Conclusions: There was a significant association between groups 1 vs. 2 (unilateral plaque/s) and 1 vs. 3 (bilateral plaque/s) for CAC score. The presence and severity of carotid plaques may represent additional cardiac risk and can be used to stratify the risk of patients with CKD.
Background: Atorvastatin may slow eGFR decline in CKD patients with inflammation.

Methods: Atorvastatin slowed progression of CKD-5D patients. These results suggest that CUI may be used to predict the presence of coronary vasculopathy without need to perform radiation based imaging.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Hyperleptinemia Is Associated with the Presence and Severity of Myocardial Ischemia in Non-Dialysis Dependent Chronic Kidney Disease  
Antonio C. Cordeiro, 1,2 Marco A.C. Oliveira, 1 Paola Smanio, 1 Celso Amoddeo, 1 Fernanda C. Amparo, 1 Amanda G.M. Sousa, 1 Bengt Lindholm, 1 Juan J. Carrero, 1 Dante Pazzanese Institute of Cardiology, Brazil; 2 Baxter Novum and Renal Medicine, Karolinska Institute, Sweden.

Background: Experimental data suggest that leptin receptors are expressed in coronary arteries and that hyperleptinemia causes significant coronary endothelial dysfunction. It is presently unknown if uricemic hyperleptinemia is linked to myocardial ischemia (MI) in CKD patients (pts).

Methods: We cross-sectionally evaluated 118 non-dialysis dependent CKD stages 3-5 pts (median age: 59 years [52–67], 80 men). MI (presence and extension) was screened by 99mTc-sestamibi myocardial perfusion scintigraphy (MPS). All analyses were performed by the same, blinded, physician.

Results: Fifty five pts (33 men) presented an ischemic MPS. MI extension was positively correlated with BMI (rho = 0.25, P < 0.01), leptin (rho = 0.26, P < 0.01) and leptin/MI (rho = 0.24, P < 0.01). Pts who did not present MI were divided in three groups (no-MI [n=63; 64% men], MI < 10% [n=28; 68% men], and MI ≥ 10% of cardiac area [n=27; 78% men]). Across these groups, and as MI became more severe, BMI (27.8 ± 5.7 vs. 31.7 ± 6.6 vs. 30.8 ± 5.6 Kg/m²; P < 0.01), Leptin (11.9 [4.0 – 25.4] vs. 19.1 [4.9 – 44.2] vs. 26.0 [10.2 – 42.0] ng/mL; P < 0.02), as well as the Leptin/MI BMI (0.17 [0.85] vs. 0.64 [0.18 – 1.15] vs. 0.74 [0.48 – 1.36]; P < 0.03) were increasingly higher. In crude and adjusted multivariate logistic regressions (considering pts without MI as the reference category), leptin/MI was associated with higher odds of increased severity in MI (Table 1).

Conclusions: Excess leptinemia (as depicted by an increased Leptin/MI) is associated to the presence and severity of MI in non-dialysis dependent CKD pts. Our data suggests further links between hyperleptinemia and cardiovascular risk in CKD.

Funding: Government Support - Non-U.S.
TH-PO211
Glimertral Filtration Rate and Cardiovascular Outcomes after Acute Myocardial Infarction: Results from Korea Acute Myocardial Infarction Registry
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Background: Renal dysfunction is associated with one of the highest risks, but relation between chronic kidney disease (CKD) stage and cardiovascular outcomes after acute myocardial infarction (AMI) is not well defined.

Methods: As a part of the Korea Acute Myocardial Infarction Registry (KAMIR), we identified 12,636 patients with acute myocardial infarction between November 2005 and January 2008. The glomerular filtration rate (GFR) was estimated by means of the four-component Modification of Diet in Renal Disease equation, and the patients were grouped according to GFR. Primary end points were death and complication in hospital courses. Secondary end points were major adverse cardiac event (MACE) during follow-up.

Results: The mean age was 64 ± 13 years, and 70.4% of the percent of the group were men. A graded association was observed between GFR and clinical outcomes. The group III, IV and V independently predicted short-term and long-term MACE. Use of beta-blocker, angiotensin converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARB) and statin was associated with reduced risk for MACE.

Conclusions: Renal dysfunction was an independent risk factor for the mortality, and complications of AMI, while degree of GFR did not affect the short-term MACE in patients with GFR ≤ 30 mL/min/1.73 m². The use of beta-blockers, ACEi or ARB and statin reduced risk for short-term and long-term MACE.

TH-PO212
Relationship between Use of AST-120 and Abdominal Aortic Calcification in Chronic Kidney Disease Patients
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Background: Although several experimental papers have reported indoxyl sulfate has progressed vascular calcification, little is known about the association between indoxyl sulfate and vascular calcification in human. The aim of the current study was to investigate the relationship between abdominal aortic calcification and use of AST-120, which has adsorption ability for indoxyl sulfate, in pre-dialysis chronic kidney disease (CKD) patients.

Methods: We conducted a retrospective analysis for all the pre-dialysis CKD patients (stage 4 and 5) who underwent abdominal plain computed tomography in our institution between 2005 and 2010. Abdominal aortic calcification was assessed by aortic calcification index (ACI). We divided these patients into two groups by whether AST-120 was taken for at least 6 months or not and compared their ACI between the two groups.

Results: Two hundred patients were enrolled in the present study. ACI was significantly lower in patients taking AST-120 (12.2 [2.5-30.3] vs. 25.8 [13.5-45.3] %, p < 0.05). By multivariate analysis, use of AST-120 was independently and significantly associated with ACI after adjustment for age, sex, diabetes, hypertension, total cholesterol, estimated glomerular filtration rate, hemoglobin, albumin, calcium, phosphorus, smoking, and use of statin.

Conclusions: It is suggested that AST-120 suppresses the progression of aortic calcification in pre-dialysis CKD patients.

TH-PO213
Vitamin D Status and the Cardiac Autonomic Response to Angiotensin II in Healthy Humans
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Background: Cardiovascular disease (CVD) remains the leading cause of death in chronic kidney disease (CKD) patients. Increased cardiac sympathetic activity [low-frequency (LF) heart rate variability (HRV)] and vagal (high-frequency (HF) HRV) withdrawal, are novel risk factors for CVD in CKD. CKD patients also exhibit upregulation of the renin-angiotensin system (RAS) and often low vitamin D (VD) levels, both of which are associated with CVD, thus we sought to evaluate the role of VD status in modulating HRV in response to angiotensin (Ang) II in healthy humans.

Methods: 36 subjects (21 women, 15 men, age 38±2 yrs) were studied in high-salt balance. Subjects were categorized according to serum 25(OH) D levels (VLD < 25, MD 25-60, HD > 60 ng/mL). Cardiac power spectral analysis (LF, HF, and LF/HF), was recorded at baseline and in response to AngII (2ng/kg/min x30min, 6ng/kg/min x30min). The primary outcome was the association between VD status and the total and HRV response at 60 minutes.

Results: Baseline cardiac autonomic function differed according to VD status. In response to AngII, increasing VD status was associated with a blunting of an unfavourable increase in sympathetic activity (LF p=0.17 for trend), decrease in vagal activity (HF p=0.23 for trend) and over-shift in sympathovagal balance (LF/HF, p=0.75 for trend).

Conclusions: Poor VD status may be associated with unfavourable cardiac autonomic function. In response to AngII, VD sufficient healthy adults largely maintain sympathovagal balance. This cardioprotective response appears to be impaired in VD insufficient/deficient humans, suggesting that low VD status coupled with RAS activation may augment the risk of CVD in CKD.

TH-PO214
Effect of Elevated Blood Pressure on Quality of Life in Children with Chronic Kidney Disease
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Background: Hypertension (HTN), which is common in children with chronic kidney disease (CKD), has known adverse impact on health-related quality of life (HRQoL) in adults, yet similar pediatric data are lacking.

Methods: Study design and Participants: Cross-sectional and longitudinal analysis of blood pressure (BP), antihypertensive medication use, and HRQoL of children with CKD enrolled in the prospective, multicenter Chronic Kidney Disease in Children Follow-up Study (CKiD). BP was measured standardly by auscultation with normal diastolic BP, and sphygmomanometer at annual study visits and HRQoL was assessed using the PedsQL questionnaire. Data analysis: Cross-sectional analysis of BP and PedsQL domain scores at baseline, and longitudinal analysis using logistic regression to assess whether higher baseline score of antihypertensive medications was associated with decline in QOL scores over time.

Results: Univariate baseline comparisons of PedsQL scores across BP groups showed a significant difference (p=0.02) in the child reported physical PedsQL median score for children with diastolic HTN (median score: 78) compared to those with normal diastolic BP (median score: 84). Logistic regression analysis showed children who were taking one or more antihypertensive medication were twice as likely to have decline in physical functioning QOL over time regardless of blood pressure, OR 2.043 (p=0.0042) in model adjusted for systolic BP, and OR 2.069 (p=0.0039) in model adjusted for diastolic BP.

Conclusions: Children with diastolic HTN have significantly lower median physical PedsQL scores at baseline as compared to normotensive children. In this analysis, elevated BP was not associated with a decline in HRQoL over time. However, children on antihypertensive medication are twice as likely to show decline in self-reported physical QOL, regardless of BP status.

Funding: NIDDK Support, Other NIH Support - Division of Kidney, Urologic, and Hematologic Diseases of the NIDDK, National Institute of Neurological Disorders and Stroke, National Institute of Child Health and Human Development, and National Heart, Lung, and Blood Institute

TH-PO215
The Association between Arterial Stiffness and Mildly Kidney Damage among a Chinese Population-Based Population
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Background: Previous study revealed increased arterial stiffness among patients with advanced chronic kidney disease (CKD), while studies among patients with mildly kidney damage were limited.

Methods: Eight hundred and eight-two community-based participants in Beijing, China were included in the present study. Arterial stiffness was assessed by brachial-ankle Pulse Wave Velocity (PWV). Indicators of kidney damage included estimated glomerular filtration rate (eGFR) and urinary albumin-creatinine ratio (ACR) were tested. All participants had a random early morning urine sample (eGFR >30ml/min/1.73m²) and none of them had ACR meet the definition of macroalbuminuria. Multivariable linear regression models were used to evaluate the associations between kidney damage and PWV.

Results: The average age was 65.4±9.2 years and 45.1% were males. Altogether 105 (9.9%) participants had microalbuminuria, and 28 (3.2%) had eGFR <60 ml/min/1.73m². PWV was significantly higher among participants with microalbuminuria (1995.4±575.1 cm/s vs 1738.7±357.1 cm/s, P<0.001) and among participants with eGFR <60 ml/min/1.73m².

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

160A
<60 ml/min/1.73m² (1950:1.416.6 cm/s vs 1763.4³2.396.0 cm/s, p=0.01). After adjusting for potential confounders, microalbuminuria, but not eGFR, was still positively associated with PWV (β=±2.27,p=0.001).

Conclusions: Microalbuminuria was independently associated with arterial stiffness.

TH-PO216
Outcome of Hyperkalemia in the Emergency Department: Impact of Hyperkalemic Severity, Renal Function and CHF on Survival
\[ Vineeta Velagapudi,^1 Bruce Barton,^2 Jeffrey S. Stoff.\]^1

Background: Hyperkalemia is common and lethal electrolyte disorder with little known long-term consequences. This was retrospective, observational study of hospitalized patients with initial serum K⁺ > 5.3 mEq/L. 143 consecutive episodes of hyperkalemia were analyzed in 133 patients. Survival was analyzed by parameters of renal dysfunction (admit eGFR), CHF, admit K and EKG abnormalities.

Methods: Hazard ratios (HR) for mortality were computed by Cox proportional hazards multivariate regression. Primary end point, all-cause mortality determined by Social Security Death Index and medical record review.

Results: Admit eGFR was the most powerful predictor of mortality. The effect of renal function was nonlinear.

Highest mortality is eGFR group of 15-59 HR 6.92. More severe renal impairment with eGFR <15 HR 4.10 and AKI requiring hemodialysis (HD) HR 3.67. ESRD had lower mortality HR 1.33.

Hyperkalemic severity had a modest effect. Compared to patients Admit K 5.3-5.9 mEq/L, patients with K 6-7, HR 2.21 (p=0.0210) and K >7.0, HR 2.62 (p=0.0521). History of CHF, increased mortality by univariate analysis (p=0.0001) but CHF had no independent effect in HD patients. In non-HD patients, CHF had an independent effect when both admit eGFR and K were added to the model. Patients with EKG abnormalities had higher K (p=0.003), but these changes did not impact mortality (p=0.126).

Conclusions: Survival in hyperkalemic patients is predicted by lower admit eGFR in a non-linear fashion. ESRD patients exhibited lower mortality perhaps reflecting adaptation to chronic hyperkalemia. CHF has an additive effect on mortality in non HD patients. We emphasize that 86% of the mortality was after discharge. This extraordinary mortality necessitates the need to develop risk stratification strategies in the long-term care of the hyperkalemic patients.

Funding: Private Foundation Support, Clinical Revenue Support

TH-PO217
An Unbiased Proteomic Screen Is Linked to Clinical Outcomes Elucidating the Mechanism of Cardiovascular Disease in Patients with Kidney Disease
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Background: Biomarker discovery is a major goal of proteomic analysis. We hypothesized that certain serum proteins contribute to cardiac disease in patients with chronic kidney disease which can be targeted for therapeutics.

Methods: Each patient was followed prospectively over a 42 month period from October 2007 – March 2011. Participants were monitored for either heart failure exacerbation requiring hospitalization or death. Proteomic analysis was performed on each blood specimen. Relative levels of oligopeptides in each sample were derived from 182 proteins identified in each patient. 7 unique proteins were identified in each patient. 7 patients had a cardiac event, 3 of which were diagnosed with chronic kidney disease that did not require dialysis (GFR 20.3 ± 5 ml/min) and 3 of which received intermittent hemodialysis. 182 proteins were identified in each patient. 7 unique proteins were elevated in the cohort of patients that achieved the primary endpoint of heart failure or death.

Conclusions: Proteomic analysis can identify novel biomarkers that contribute to the renal-cardiac syndrome. The veracity of these markers is confirmed by outcomes analysis which increases the likelihood of clinical impact in diagnosis and therapy.

TH-PO218
Influence of Variations of Inflammatory Markers in the Prediction of Cardiovascular Events from CKD Non-Dialysis Patients
\[ Borja Ostrigo, Marian Groscochea, Soledad Garcia de Vinuesa, Ursula Verdiales, Claudia Yuste, Daniel Barraca, Nayara Panizo, Jose Luno.\]

Hospital General Universitario Gregorio Marañón.

Background: In CKD patients on dialysis, plasma IL-6 levels have been shown to better predict death than IL-1β, TNF-α and CRP levels. However, only one study has demonstrated the predictive role of IL-6 in non-dialysis CKD patients. Besides, the impact of intra-individual changes in inflammation markers on cardiovascular events in CKD patients is unknown.

Methods: The aim of this study was to analyze what is the best inflammatory marker of cardiovascular outcome in non-dialysis CKD patients and to examine whether the simple determination of an inflammatory marker is a better predictor than the variability of the serum levels of the marker over a 6-month period.

Ninety patients (mean age: 68.5±12.8 years) at different stages (1-4) of CKD were evaluated. Serum CRP, IL-6, IL-1β and TNF-α were measured basally and after six months. Three patterns were defined for each inflammatory marker: baseline measurement, mean of two measurements and variation: increase or decrease after six month.

Results: During the follow-up period of the study (mean time of 72±19.8 months), 14 patients died, 11 patients were included on dialysis program and 29 patients suffered a cardiovascular event. Patients with persistently elevated IL-6 values had higher risk of cardiovascular events (OR: 1.21 (1.11-1.32), p=0.000). Mean of two measurements of IL-6 was a significantly better predictor than CRP, TNF-α and IL-1β. Patients with a mean IL-6 above 6 pg/mL and patients with previous peripheral vascular disease had an increase risk of having cardiovascular events (2.34 (1.05-5.22), p=0.037 and 2.95 (1.27-6.93), p=0.011, respectively).

Conclusions: IL-6 is better inflammatory marker than PCR, TNF-α and IL-1β to predict cardiovascular events in CKD non-dialysis patients. Mean of two measurements is better than simple determinations to predict cardiovascular outcome. Moreover, IL-6 values persistently elevated predicted cardiovascular events in CKD patients.

TH-PO219
Predicting Significant Coronary Angiographic Disease in Patients with Chronic Kidney Disease Using Nuclear SPECT Myocardial Perfusion Imaging
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Background: Chronic Kidney Disease(CKD) is considered an independent risk factor for coronary artery disease (CAD). The gold standard test to diagnose CAD is coronary angiography. Patients with a Glomerular Filtration Rate (GFR) less than 60 cc/ min/1.73 m² are at an increased risk of worsening renal failure and bleeding during this
procedure. Nuclear SPECT MPI remains an attractive modality to evaluate ischemia in this population, given the high prevalence of diabetes and other comorbid conditions. Further work is needed to determine predictors of significant angiographic disease in patients with CKD using Nuclear SPECT MPI.

Methods: We retrieved from our patient population all patients with a GFR < 60 cc/min/1.73m2, a nuclear SPECT MPI stress test and coronary angiography within 2 years of the test over the last 4 years. Statistical analysis was performed using bivariate analysis and multivariate regression models.

Results: A total of 65 patients were identified among 400 patients. Abnormal myocardial perfusion was considered significant in 46 of them. Of those patients 34 had significant >50% CAD seen on angiography. Significant patient characteristics that predicted angiographic disease were history of hyperlipidemia (p=0.0268) and hypertension (p value =0.0405). However, the stress test predictor for significant disease was abnormal wall motion on gated stress imaging (HR 4.24 ; 95% CI 1.39-12.9, p value=0.0109). Usual high risk predictors, such as sum stress, rest and difference score or transient ischemic dilation failed to predict significant angiographic disease (p value=0.14-0.98).

Conclusions: In patients with CKD the presence of abnormal wall motion during stress was the only reliable predictor of significant CAD.

TH-PO220

Cornell Product Is The Best Electrocardiographic Surrogate of Left Ventricular Mass in Non-Dialysis Dependent Chronic Kidney Disease Patients

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Background: Left ventricular mass indexed to body surface area (LVM/BSA) by echocardiography (ECHO) is the most efficient method for left ventricular hypertrophy diagnosis. However, electrocardiographic (ECG) criteria may also be useful. We sought to determine associations between ECG criteria and LVM/BSA in CKD patients (pts).

Methods: In 151 non-dialysis dependent CKD stages 3-5 pts (median age: 59 years [52-67], 96 men), ECO was performed to assess LVM/BSA and ECG was used to assess Cornell and Sokolow-Lyon voltages [respectively CV and SLV] and products [respectively CP and SLPI]. Analyses were done by the same, blinded, physican.

Results: All ECG criteria were associated with LVM/BSA in sex adjusted linear regressions (r2 = 0.15 for SLV, 0.23 for SLP, 0.31 for CV and 0.35 for CP; P < 0.01 for all), and CP showed the strongest association. Pts were divided according to the CP sex specific tertiles (low [n=51], middle [n=52] and high [n=48]). Across the tertiles there were differences in hemoglobin (11.2 ± 2.3 vs. 12.2 ± 1.9 vs. 13.0 ± 5.6 g/dL, P=0.03) and BNP levels (56.2 [27.2-144.0] vs. 48.8 [22.9-129.0] vs. 142.6 [65.1-391.3] pg/mL; P < 0.01), as well as in LVM/BSA (122 [101-142] vs. 144 [123-166] vs. 175 [147-201] g/m2; P < 0.01). In a logistic regression analysis (using the lowest CP tertile as the reference category) CP was associated with a higher odds for LVM/BSA (95% CI BSA higher than the sex adjusted median) in the crude analysis (OR: 3.85 [1.59-9.31] and 11.39 [4.46-29.08]; respectively middle and high CP tertiles) and even after the adjustment for age, mean blood pressure, hemoglobin, BNP and CKD stages (2.89 [1.17-7.10] vs. 6.13 [2.35-15.99]; respectively middle and high CP tertiles).

Conclusions: Cornell product associates with LVM/BSA stronger than other electrocardiographic criteria. Our results reinforce the usefulness of ECG, especially assessing CP, as a simple and inexpensive diagnostic method for LVH in CKD.

Funding: Government Support - Non-U.S.

TH-PO221

Vitamin-K Epoxide Reductase Complex Subunit 1 Genetic Polymorphisms Predict Arterial Stiffness and Serum MGP Levels in Chronic Kidney Disease Patients

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Background: Arterial stiffness is an independent predictor of cardiovascular risk in chronic kidney disease (CKD). Calcium deposition is a major determinant of arterial stiffness and common polymorphisms in the Vitamin-K epoxide reductase complex subunit 1 (VKORC1) predict aortic calcification scores. We investigated the influence of VKORC1 polymorphisms (+1542G>C and +3730G>A) on arterial stiffness in 280 stage 3-5 CKD patients who are most Caucasian.

Methods: Blood pressure (BP), body mass index (BMI), pulse wave velocity (PWV), coronary artery calcification (CAC) and serum uncarboxylated matrix Gla protein (uc-MGP) were determined, and genotyping performed.

Patients: The +1542G>C and +3730G>A polymorphisms showed genotype-specific higher PWV and lower uc-MGP (P<0.05). The combined recessive allele model showed a significant step-wise reduction in PWV (P<0.005); subjects homozygous for both risk alleles had the highest PWV as compared to those who had one or none. In a regression model after adjustment for age, gender, mean BP, BMI and racial group, each +1542G allele was independently associated with a 0.8 m/s (95% CI 0.09 to 1.57) higher PWV and each +3730A allele was associated with 1.0 m/s (95% CI 0.14 to 1.98) higher PWV. Although in this cohort serum uc-MGP levels correlated inversely with CAC score (P<0.001), VKORC1 genotypes did not.

Conclusions: We have demonstrated for the first time that VKORC1 polymorphisms (+1542G>C and +3730G>A) influence aortic stiffness and serum uc-MGP levels in CKD patients. Although needing replication in an independent cohort, these findings suggest that Vitamin-K dependent processes may be important in arterial stiffness through previously un-characterized VKORC1 related mechanisms.

Multivariate analysis revealed systolic blood pressure, FGF-23 and proteinuria to be independent predictors of LVH in CKD. E-selectin and VCAM production is elevated in HUVECs cultured in high phosphate media with FGF-23 and Klotho both. Cell based ELISA for E-Selectin and VCAM was performed and results normalized to GAPDH.

Results: 50% of CKD and 56% of control patients had LVH. LVMI was similar between the two groups. Patients with CKD had significantly higher phosphate, parathyroid hormone and FGF-23 concentration (237.9RU/ml (106.9-393.3) vs. 12.5RU/ml (1.5-35.9), p<0.001). Those with CKD and LVH had higher FGF-23 concentrations than those without LVH (253.4RU/ml vs. 118.4RU/ml).

Conclusions: FGF-23 independently predicts LVH. Our preliminary data suggest that FGF-23 may be toxic causing activation of the vascular endothelium but do not prove causality with LVH. Whether lowering levels of FGF-23 translates to improved outcomes remains to be seen.

TH-PO223

Ferric Carboxymaltose-Mediated Effects on Platelet Count in Cardio-Renal Patients: Results from the FAIR-HF Trial

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Background: Chronic heart failure (CHF) is a hypercoagulable state with an increased incidence of thromboembolic events. Iron deficiency (ID) and treatment with ESAs, particularly high doses, are risk factors for the development of thrombocytosis. Patients (pts) with CHF often suffer from CKD. Both ID and the use of ESAs are common in these cardio-renal pts. Using data from FAIR-HF, we analyzed the effects of intravenous (IV) iron as ferric carboxymaltose (FCM) on platelet count (plt ct) in CHF pts with ID and mild to moderate CKD.

Methods: FAIR-HF was a randomized, double-blind, placebo-controlled trial of IV iron as ferric carboxymaltose (FCM) on platelet count (plt ct) in CHF pts with ID and mild to moderate CKD.

Conclusions: FGF-23 independently predicts LVH. Our preliminary data suggest that FGF-23 may be toxic causing activation of the vascular endothelium but do not prove causality with LVH. Whether lowering levels of FGF-23 translates to improved outcomes remains to be seen.
iron as FCM in 459 pts with CHF of NYHA class II or III, LVEF ≤40% (NYHA II) or ≤45% (NYHA III), ID (ferritin <100 µg/L or 100–299 µg/L, iFTAT ≤20%), and Hb 95 to 135 g/L. Baseline characteristics: age 68±11y; NYHA III 82%; LVEF 32±6%; Hb 119±13 g/L, eGFR 64±23 mL/min (42% <60 mL/min).

**Results:** Baseline plt cts were available for 434 (94.6%) pts, 89% of which were within the normal range (140-370 x10^9/L), with 5% below and 6% above this range. Mean plt ct at baseline was 246±86 in the FCM and 238±60 x10^9/L in the placebo group (p=NS). The mean changes in baseline plt ct in the FCM vs placebo group were -27±54 vs -4±48 (4 wk), 24±59 vs -4±46 (12 wk) and -25±56 vs 0±53 (24 wk).

**Conclusions:** FCM effectively treats ID and reduces the risk of thrombocytopenia by decreasing the mean plt ct by 9-10%. This reduction is seen as early as 4 wk after treatment initiation and remains stable over time. This effect may improve potentially detrimental rheological features that are present in pts with cardio-renal syndrome and hence improve their outcomes.

**Funding:** Pharmaceutical Company Support

**TH-PO224**

**Vitamin D Supplementation and the Cardiac Autonomic Response to Angiotensin II in Vitamin D Deficient Humans**

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**Background:** Cardiovascular disease (CVD) is the leading cause of death in chronic kidney disease (CKD) patients. Vitamin D (Vitamin D) deficiency is a risk factor associated with unfavourable cardiac autonomic function. In this study, we evaluated the effect of Vitamin D supplementation on the cardiac autonomic response in Vitamin D deficient humans.

**Methods:** 9 Vitamin D deficient subjects (4 men, 5 women) were included in this study. The study included a washout period of 4 weeks and a placebo period of 4 weeks. After the placebo period, Vitamin D was administered at a dose of 50,000 IU/day for 4 weeks. The cardiac autonomic response was assessed at baseline and after supplementation using a protocol that included heart rate variability, respiratory sinus arrhythmia, and baroreflex sensitivity.

**Results:** Heart rate variability increased significantly after Vitamin D supplementation (p<0.05). The respiratory sinus arrhythmia and baroreflex sensitivity also improved significantly (p<0.05). The postural blood pressure also increased significantly (p<0.05). The changes in cardiac autonomic function were correlated with changes in serum Vitamin D levels (p<0.05).

**Conclusions:** Vitamin D supplementation improves cardiac autonomic function. Further studies are needed to determine the optimal dose and duration of Vitamin D supplementation for improving cardiac autonomic function.
because of the inability to perform Valsalva maneuver. Based on previous studies, presence of cardiac dysautonomia is considered to be positive for cardiac dysautonomia.

**Results:** Fifty patients completed the tests successfully. 26 were male (52%), 32 were diabetic and the mean age was 43.9 years. Forty four (88%) were found to have cardiac dysautonomia. Abnormal heart rate response to deep breathing (E:I ratio less than 1.17) was found in 47 patients (94%), abnormal Valsalva ratio (longest to shortest R-R ratio less than 1.2) in 45 patients (90%), abnormal 30:15 ratio (30th to 15th R-R on standing less than 1.03) in 34 patients (68%). We found 3 abnormal tests in 32 patients (64%), 2 abnormal tests in 12 patients (24%), 1 abnormal test in 5 patients (10%) and no abnormal test in 1 patient (2%). Overall, 44 (88%) of the patients had cardiac dysautonomia.

**Conclusions:** This single center study finds a high incidence of cardiac dysautonomia in chronic hemodialysis patients. Future studies should investigate the association between cardiac dysautonomia and sudden cardiac death.

**TH-PO228 Optimising the Accuracy of Blood Pressure Monitoring in Chronic Kidney Disease:** The Utility of BpTRU in Kidney Disease

**Background:** Accurate blood pressure (BP) monitoring is critical in CKD. However, management is often based on a single BP reading performed in an uncontrolled setting; this will over-diagnose hypertension in up to 30% and miss around one third who are truly hypertensive. Therefore, we compared BP obtained by the routine clinical pathway with BP obtained by BpTRU, which carries out multiple BP measurements to derive a fixed reading and has been validated in a non-CKD setting as being equivalent to (daytime) 24-hour ambulatory BP monitoring (ABPM).

**Methods:** Patients (n=45) attending renal outpatient clinics had standard BP measurements with a calibrated DINAMAP PRO400 in a clinical assessment room. The patients then underwent repeat assessment with BpTRU.

**Results:** The clinic mean (± SD) systolic BP (149.7 ± 18.5 range 117–209 mmHg) was significantly higher than the BpTRU reading (122.0 ± 13.9 (96 – 150) mmHg; P < 0.001). In a subgroup (n=24), the clinic mean systolic (143.8 ± 15.5 mmHg) was significantly higher than a repeat standard BP in a quiet room (129.9 ± 19.9 mmHg; P < 0.001), but the BpTRU reading was significantly lower than both (117.3 ± 14.1 mmHg; P < 0.001). The clinic mean diastolic (82.4 ± 11.2 (49 – 100) mmHg) was significantly higher than the BpTRU reading (78.4 ± 10.0 (53 – 97) mmHg; P < 0.001; n = 45). Out of 29 patients with a clinic BP above the threshold for altering antihypertensive medication (>130/80 mmHg), only 3 were hypertensive with BpTRU. Finally, clinic BPTRU measurements in this setting (CKD) were not significantly different to the day-time mean of 24 hour ABPM.

**Conclusions:** Standard BP measurements are significantly higher than measurements using a BpTRU machine in a quiet room, which accurately reflects the day-time mean of 24-hour ambulatory BP monitoring in CKD. Adjusting clinical protocols to utilise the most accurate BP device available is a simple manoeuvre that could deliver major improvements in clinical practice.

**TH-PO229 Early Detection of Atheromatous Disease at All Stages of Chronic Kidney Disease Improves with Simultaneous Femoral and Carotid Ultrasound**

**Background:** Atheromatous disease occurs early and at high frequency in the course of chronic kidney disease (CKD). The location of the atheromatous lesion in the vascular territory determines the type of adverse cardiovascular events and mortality rates.

**Methods:** Cross-sectional study to examine whether femoral (common and superficial) ultrasound could help enhance the detection of atheromatous disease achieved with carotid (common, bulb and internal) ultrasound. Left and right carotid and femoral ultrasounds were obtained and analyzed by the same operators from 1785 patients, CKD stages 3 (38%), 4 & 5 (32%), and stage 5 undergoing dialysis (30%), average age: 57 years±13; 62.4% men; 25% diabetics, from the Spanish Multicenter Study NEFRONA.

**Results:** The % of patients with arterial plaques is shown in the table below, stratified by gender and stage of CKD.

<table>
<thead>
<tr>
<th>Stage 3</th>
<th>Stages 4&amp;5</th>
<th>Stage 5D</th>
</tr>
</thead>
<tbody>
<tr>
<td>men</td>
<td>women</td>
<td>men</td>
</tr>
<tr>
<td>carotid only</td>
<td>22%</td>
<td>19%</td>
</tr>
<tr>
<td>femoral only</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>carotid &amp; femoral</td>
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<tr>
<td>total carotid</td>
<td>30</td>
<td>31</td>
</tr>
<tr>
<td>total femoral</td>
<td>75</td>
<td>60*</td>
</tr>
</tbody>
</table>

*p<0.01 compared to men with the same stage of CKD

**Conclusions:** These results demonstrate that femoral plaques are more prevalent in men at all CKD stages prior to renal replacement. Thus, exclusive carotid ultrasound underestimates atheromatous disease in men. Also, simultaneous femoral and carotid ultrasound increased the detection of atheromatous plaques by 15-20% in both genders at all stages of CKD. Thus, the screening for an early detection of atheromatous disease should include both carotid and femoral ultrasound.

**Funding:** Pharmaceutical Company Support

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**

**TH-PO230 Relationship between Coronary Califications and Osteoporosis in Patients with CKD-5**

**Background:** Cardiac and pulmonary events account for 60% of all-cause mortality in patients with CKD, and elevated coronary calcifications (CAC) are associated with a high risk of cardiovascular events. In a previous study, we evaluated the presence and extent of coronary calcifications in patients with CKD using multi-slice computed tomography (MSCT). In the current study, we aimed to evaluate the association between CAC scores and bone mineral density (BMD) by dual-energy x-ray absorptiometry (DXA).

**Methods:** We included 45 patients with CKD-5D who underwent MSCT and DXA within the same session. CAC scores were calculated using the Agatston score, which is the sum of the area (in mm²) and density (in HU) of each calcification. CAC scores were categorized into three groups: 0, 1-400 (low risk for CVE), and 401-1600 (high risk for CVE).

**Results:** The mean age of the patients was 64 ± 13 years, and 60% were men. The mean serum creatinine was 2.1 ± 0.8 mg/dL, and 78% were on dialysis. The mean BMD of the femoral neck was 0.71 ± 0.19 g/cm², and 42% of patients were osteoporotic. The mean CAC score was 35 ± 37 Agatston units, and 34% of patients had CAC scores above 1600. The mean BMD of the lumbar spine was 0.73 ± 0.13 g/cm², and 52% of patients were osteoporotic. In a subgroup analysis, the mean BMD of the femoral neck was lower in patients with CAC scores above 400 (0.69 ± 0.16 g/cm²) compared to those with CAC scores below 400 (0.75 ± 0.20 g/cm², p=0.03). Similarly, the mean BMD of the lumbar spine was lower in patients with CAC scores above 400 (0.70 ± 0.12 g/cm²) compared to those with CAC scores below 400 (0.75 ± 0.13 g/cm², p=0.01).

**Conclusions:** Our study showed a significant association between coronary calcifications and osteoporosis in patients with CKD-5D, with a higher risk of cardiovascular events in patients with high CAC scores. These findings highlight the importance of assessing both cardiac and osteoporotic risk factors in patients with CKD.
Indeed, the use of 'off-pump' surgery was not independently associated with long term mortality. Only Euroscore (a model use to estimate risk of death following cardiac surgery) HR 1.14 (CI 1.07 to 1.22) and the development of AKI HR 2.38 (CI 1.67 to 3.38) were independently associated with long term mortality.

Conclusions: The use of 'off-pump' surgical techniques do not seem to be associated with improved prognosis within our cohort of patients with renal impairment. The time has come for an adequately powered randomised control trial to definitely determine the value of 'off-pump CABG surgery in patients with renal impairment.

TH-PO232
Heart Rate and Blood Pressure Variability in Children with Chronic Kidney Disease: Report from CKiD Study
Gina-Marie Barletta,1 Joseph T. Flynn,2 Mark Mitsnefes,3 Derek Ng,4 Bradley A. Warady,5 Joshua A. Samuels,6 Tim Poffenbarger,7 Susan L. Furtth,1 Phoenix Children’s Hospital; 1Children’s Hospital and Regional Medical Center; 2Cincinnati Children’s Hospital; 3Children’s Mercy Hospital; 4University of Texas, Houston; 5Children’s Hospital of Philadelphia.

Background: Alterations in autonomic nervous system function, including decreased heart rate (HR) variability & differences in blood pressure (BP) variability, are predictors of future cardiovascular events in adults, particularly in those with underlying chronic kidney disease (CKD).

Methods: We examined the degree of HR & BP variability in children with CKD. HR & BP variability were evaluated by 24 hour ambulatory BP monitoring in 104 participants not receiving antihypertensive medications enrolled in CKiD, an observational cohort study of children (1-16 yrs) with Schwartz estimated GFR 30-90 mL/min/1.73 m2. Variability was assessed by coefficient of variation (CV) for HR, systolic (SBP) & diastolic (DBP), and compared across categories of GFR and hypertensive (HTN) status, with adjustment for age, gender & race.

Results: Median age was 11 years (interquartile range: 7, 14), 37% female, 20% black, 58% were HTN, median iohexol GFR: 48 mL/min/1.73 m2 (36, 59); 22% had GFR<60 mL/min/1.73m2, 60 % between 30-60, and 18 % GFR<30.

HR variability was significantly lower in HTN vs. normotensive children when adjusting for age, gender & race, and was independent of activity state and GFR level. Other comparisons are outlined in Table.

Independent variables
<table>
<thead>
<tr>
<th>Variable</th>
<th>Difference (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR CV Relative %</td>
<td>16.6% (4.8, 28.5)</td>
</tr>
<tr>
<td>SBP CV Relative %</td>
<td>3.1% (12.6, 25.1)</td>
</tr>
<tr>
<td>DBP CV Relative %</td>
<td>3.1% (2.2, 4.1)</td>
</tr>
<tr>
<td>GFR&lt;30 vs GFR&gt;60</td>
<td>1.9% (0.5, 3.5)</td>
</tr>
<tr>
<td>GFR 30-60 vs GFR&gt;60</td>
<td>1.8% (0.5, 3.2)</td>
</tr>
<tr>
<td>Bold indicates p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: The degree of variability in both HR and BP is different among HTN and normotensive children with CKD. These findings are similar to that encountered in adults with CKD and may emphasize the childhood origin and progression of adverse cardiovascular outcomes.

Funding: NIDDK Support

TH-PO233
Suprarenal Aortic Clamp in Open AAA Repair Decreases Postoperative Renal Function
Satoshi Unuma,1 Masao Takeo,1 Tetsuya Imai,2 Yoshitsugu Satoh,3 Tetsuya Hayakawa,1 Tetsuya Kondo,1 Tomohiro Ohno,1 Norio Yamashita,1 Tetsuya Nishimura,1 Tomohiro Nakamura,1 Hirohide Sawa,1 Tetsuro Miyata,1 Toshiro Fujita,1 Takamoto Ohse.2

Background: Open repair of abdominal aortic aneurysm (AAA) requires infrarenal (IR) or suprarenal (SR) aortic clamping. Since SR leads to renal ischemia, open AAA repair with SR may cause the decrease in renal function. The detail statistical analysis in the change of renal function was performed in this study to clarify the risk factor of AAA repair.

Methods: Retrospective analysis was performed with 178 patients undergoing open AAA repair in the University of Tokyo Hospital between January 2004 and December 2008. After the application of the criteria, 134 patients were enrolled into the 2 weeks study (15.5 days after the operation) and 99 patients were into the 1 year study (360±180 days). We evaluated the change of estimated glomerular filtration rate (eGFR) from the preoperative points to these two points. Furthermore, we conducted multivariate regression analysis with six factors (age, gender, preoperative eGFR, site of clamping, operative duration, diameter of aneurysm) as variables.

Results: The present study showed that 1) plasma S100A12 levels in HD patients with PAD (n = 26; 21.9 [13.6–33.4] ng/ml) were significantly higher than those in HD patients without PAD (n = 107; 16.7 [7.2–16.7] ng/ml; P < 0.001), 2) in multivariate logistic regression analysis, the plasma S100A12 levels (odds ratio [OR], 7.81; 95% confidence interval [CI], 1.32–46.2; P = 0.023) as well as the presence of diabetes mellitus (OR, 3.77; 95% CI, 1.04–13.6; P = 0.043), high-sensitivity CRP levels (OR, 3.02; 95% CI, 1.01–9.04; P = 0.048), and ABI (OR, 0.53; 95% CI, 0.38–0.74; P = 0.001) were identified as independent factors associated with PAD prevalence, 3) in another multiple logistic analysis using categorized clinical factors, the higher plasma S100A12 level was associated with PAD prevalence (OR, 5.15; 95% CI, 1.43–18.6; P = 0.012).

Conclusions: These findings indicate that plasma S100A12 is strongly associated with the predicting PAD in ESRD patients.

Funding: Other U.S. Government Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Occupational Risk and Chronic Kidney Disease: A Population-Based Study in US Adult Population

**Background:** Previous studies on occupational risk for CKD have been limited in the range of occupations with a focus on nephrotoxins. There has not been a population-based study conducted on a nationally representative sample of the US adult population that investigates the association between a comprehensive list of occupations and risk of CKD.

**Methods:** This population-based complex survey study uses the National Health Interview Survey data from 2004 to 2008 that randomly sampled non-institutionalized adults in the United States. Main outcome measure was self-reported CKD defined as having weakening/failing kidneys in the past 12 months as diagnosed by a physician. Occupation categories were based on US census bureau coding of Standard Occupation Classification. Univariate analysis was used to identify high risk occupations, multivariate logistic regression applying weights necessary to make accurate population prevalence estimates was used to assess the relative risk.

**Results:** 91,340 adults completed the question of both CKD status and occupation. CKD was reported in 1,197 (1.3%) participants. After applying appropriate weights, controlling for age, gender, hypertension status and education, and with physical and social science occupation as a reference group, respondents working in Building, Grounds Cleaning and Maintenance Occupations were 4.3 times (95% CI: 1.1-17.7) more likely to develop CKD, while the likelihood of developing CKD were 4.4 times higher in Healthcare Practitioners and Technical Occupations (95% CI: 1.1-18.2), 4.7 times higher in Transportation and Material Moving Occupations (95% CI: 1.2-19.0), 4.7 times higher in Computer and Mathematical Occupations (95% CI: 1.1-20.7), 4.8 times higher in Production Occupations (95% CI: 1.2-19.7), 5.3 times higher in Food Preparation and Serving Related Occupations (95% CI: 1.3-20.8), 6.1 times higher in Healthcare Support Occupations (95% CI: 1.5-25.3), and 6.1 times higher (95% CI: 1.2-30.3) in Legal Occupations.

**Conclusions:** This study compares the prevalence of CKD among US adults in different occupations. Research on characteristics of high risk occupations is needed for guiding prevention in US job settings.

Joint Associations of Gout and Serum Uric Acid on Mortality among Subjects with Reduced Kidney Function in the General Population

**Background:** Gout is a chronic inflammatory disorder of uric acid metabolism and is associated with increased cardiovascular (CV) risk. Whether gout and hyperuricaemia contribute independently and synergistically to total and CV mortality has not been previously studied.

**Methods:** A cohort of 15,773 subjects age ≥20, and representative of the U.S. population, was identified from the Third National Health and Nutrition Examination Survey (1988-1994). Vital status was obtained through linkage with the National Death Index through to 2006. Serum uric acid and physician-diagnosed gout was modeled with total and CV mortality according to estimated glomerular filtration rate (eGFR) <60, 60-90, >90 ml/min. Cox regression modeled mortality relationships.

**Results:** In an average of 10 years of follow-up, total mortality was 9.7 % of which 4.4 % were cardiovascular. Adjusting for confounding, the multivariable relative risks (RR) for subjects with gout were 1.30 (95% Confidence Interval [CI] 1.03-1.65) for total mortality and 1.50 (95% CI 1.08-2.07) for CV mortality. The relative mortality risks per 1 mg/dl increase in serum uric acid were 1.12 (95% CI 1.07-1.17) for total mortality and were 1.10 (95% CI 1.06-1.15) for CV mortality. These relationships were significant for subjects with eGFR <60 and 60-90 ml/min but not >90 ml/min. In the conjoint analysis, the multivariable mortality risks for subjects with gout and increasing quartile of serum uric acid were significantly greater than for subjects without gout.

Short Sleep Duration as a Novel Predictor of Proteinuria

**Background:** Although multiple studies revealed that sleep duration was a predictor of cardiovascular diseases and also mortality, few studies reported an association between sleep duration and chronic kidney disease characterized by impaired renal function and proteinuria.

**Methods:** The present retrospective cohort study included 6020 employees of Osaka University aged 19-55 years, who visited Osaka University Healthcare Center for their mandatory annual health examination between May 2006 and September 2010. Questionnaires about their lifestyle, including sleep duration, and blood and urine examinations at the first examination during study period were retrospectively collected as the baseline date. The outcome of interest was time to development of proteinuria defined as ≥1+ by dipstick test after the baseline examination. An association between the baseline sleep duration and the outcome was assessed using conventional multivariable Cox proportional hazards model, propensity score-based models, and disease risk score-based models.

Hypoaalbuminemia Is Prevalent and Predictive in Appalachian Patients with Chronic Kidney Disease (CKD)

**Background:** Chronic kidney disease (CKD) has reached epidemic proportions worldwide and in the United States, minorities of race and ethnicity reach end stage renal disease (ESRD) at a disproportionate rate. A rural state in the heart of Appalachia, West Virginia leads the nation in rates of incident ESRD, despite its predominantly Caucasian population.

**Methods:** This study compares the prevalence of CKD among US adults in different occupations. Research on characteristics of high risk occupations is needed for guiding prevention in US job settings.

Hypoalbuminemia Is Prevalent and Predictive in Appalachian Patients with Chronic Kidney Disease (CKD)

**Background:** Hypoaalbuminemia, hypocalcemia, hyperparathyroidism and anemia all independently correlated with reduced survival and more rapid progression to ESRD. Compared to those from more affluent counties, patients from counties of low socioeconomic status had lower albumin levels (p<0.0001). Hypoaalbuminemia, hypocalcemia, hyperparathyroidism and anemia all independently correlated with reduced survival and more rapid progression to ESRD (p=0.0001). Compared to those from more affluent counties, patients from counties of low socioeconomic status had lower albumin levels (p<0.0001). Compared to those from more affluent counties, patients from counties of low socioeconomic status had lower albumin levels (3.36±0.014 vs 3.68±0.079 mg/dl; p=0.04) and higher rates of progression to dialysis or death (p=0.016).

**Results:** Patients were predominantly Caucasian (94.3%), with a mean age of 60.1±16.7, a 39% prevalence of diabetes; 39% presented with an albumin level ≤3.5 g/dl. Patients with higher presenting albumin levels had better survival and less progression to dialysis than those with lower albumin levels (p=0.0001). Hypoaalbuminemia, hypocalcemia, hyperparathyroidism and anemia all independently correlated with reduced survival and more rapid progression to ESRD (p<0.0001). Compared to those from more affluent counties, patients from counties of low socioeconomic status had lower albumin levels (3.36±0.014 vs 3.68±0.079 mg/dl; p=0.04) and higher rates of progression to dialysis or death (p=0.016).

**Conclusions:** In this north central West Virginia region of Appalachia, CKD patients were predominantly white, often hypoaalbuminemic and most hailed from counties of low socioeconomic status. Hypoaalbuminemia and residence in a socioeconomically disadvantaged county independently predicted overall survival and progression to dialysis, suggesting that poverty and culture, irrespective of race, warrant further study for their impact on outcomes in patients with CKD.
Results: Self-reported baseline sleep duration was 6.0 ± 0.9 hours, which reflected the mean duration during the median 2.0 (interquartile range 1.2 to 3.0) years of observational period. After the baseline examinations, proteinuria was observed in 498 employees (8.3%). A multivariate Cox proportional hazards model clarified that shorter sleep duration was associated with proteinuria in a stepwise fashion, even after adjustment for clinically relevant factors (vs. 7 hours; ≥73 hours, hazard ratio 2.76 [95% CI 0.86 to 8.87], P = 0.09; 4 hours, 1.65 [1.08 to 2.54], P = 0.02; 5 hours 1.30 [1.01 to 1.68], P = 0.05; 6 hours, 1.06 [0.84 to 1.34], P = 0.6; ≥8 hours, 0.96 [0.51 to 1.79], P = 0.9). Propensity-score based models and disease risk score-based models also ascertained ≥5 hours of sleep duration were significantly associated with proteinuria.

Conclusions: Short sleep duration, especially ≤5 hours, was a novel predictor of proteinuria.

TH-PO240
Albuminuria, Kidney Function and Risk of Stroke in the REGARDS Cohort

Orlando M. Gutierrez, Suzanne E. Judd, Dana Rizk, Paul Muntner, William M. McClellan, David G. Warnock, UAB; Emory; Vermont

Background: Chronic kidney disease associates with stroke risk but the independent contributions of higher urinary albumin/creatinine ratio (AeGFR) and lower estimated glomerular filtration rate (eGFR) are unclear.

Methods: Associations of AeGFR and eGFR with incident stroke were examined in the REGARDS for Geographical and Racial Differences in Stroke (REGARDS), a national prospective cohort of 30,239 black and white adults. After excluding those with prevalent stroke, stage end renal disease, or missing data, 24,777 participants were analyzed. Cox hazards analysis. Interaction terms were tested to assess whether the association of BMD with fractures differed in those with and without CKD.

Results: Of the 2,754 with BMD and laboratory values, mean age was 73.6 ±2.9, 51% were women, 40% were black, 60% were white, 21% had CKD (6% with stage 3). There were 465 incident non-spine fractures. Femoral neck BMD (FNBDM) and Total Hip BMD (THBMD) were associated with non-spine fracture, regardless of CKD status. After adjustment for age, race, gender, BMI, high PTTH (≥65 pg/ml) and low 25-vitamin D (>20ng/ml) the hazard ratio per standard deviation higher FNBDM was 0.40 (0.30, 0.54) and 0.52 (0.44, 0.60) for those with and without CKD, respectively (p for interaction 0.93). Likewise, the hazard ratio for THBMD for fracture was 0.42 (0.31, 0.57) and 0.49 (0.42, 0.58) for those with and without CKD (p for interaction 0.66).

Conclusions: AeGFR measurement provides information on a patient’s risk for fractures in individuals with or without moderate CKD.

TH-PO242
The Structure of Health-Related QOL Varies According to Underlying Diseases of CKD: Results from the CKD-JAC Study

Yasuo Ohashi, Satoshi Imurou, Tadato Akizawa, Enyu Imai, Seichi Matsuo, Tsyoshi Watanabe, Kosaku Nitta, Hirofumi Makino, Akira Hishida. Univ Tokyo, Japan; CKD-JAC Study Group

Background: CKD-JAC was established to prospectively study the renal and cardiovascular outcomes in 2,977 Japanese patients with CKD stages 3-5. CKD is caused by various underlying diseases, and the breakdown of Health Related HR-QOL is expected to vary depending on these diseases.

Methods: Data at registration and HR-QOL data were analyzed. In addition, canonical discriminant analysis(CDA) was performed for multidimensional analysis.

Results: Of the 2,526 patients analyzed, 36% had diabetes. The patients were divided into four major groups by disease. As the CKD stages progressed, almost all of the SF-36 domains worsened, especially in GH. PSQI and BDI also worsened. As for disease groups, SF-36, PSQI, and BDI were worse in those with diabetes.

CDA was performed about CKD stages [1] or the disease groups [2] as the criterion variables.

1:One significant canonical discriminant function was obtained. The standardized discriminant coefficients of GH, RE, and PSQI were large and the mean values of the discriminant function for each stage were 0.14(Stage 3), -0.03(Stage 4), and -0.32(Stage 5).

2:Two significant discriminant functions were obtained. The first one determined the presence of diabetes, and the second determined the presence of GN or DN(Table1).

Table1
Canonical discriminant analysis
standardized discriminant coefficient

<table>
<thead>
<tr>
<th>var</th>
<th>jux1</th>
<th>jux2</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF-36</td>
<td>Physical functioning(PF)</td>
<td>0.883</td>
</tr>
<tr>
<td></td>
<td>Role physical(RP)</td>
<td>0.011</td>
</tr>
<tr>
<td></td>
<td>Bodily pain(BP)</td>
<td>0.297</td>
</tr>
<tr>
<td></td>
<td>General health(GH)</td>
<td>0.026</td>
</tr>
<tr>
<td></td>
<td>Vitality(VT)</td>
<td>-0.465</td>
</tr>
<tr>
<td></td>
<td>Social functioning(SF)</td>
<td>-0.157</td>
</tr>
<tr>
<td></td>
<td>Role emotional(RE)</td>
<td>0.069</td>
</tr>
<tr>
<td></td>
<td>Mental health(MH)</td>
<td>-0.077</td>
</tr>
<tr>
<td></td>
<td>Pittsburgh Sleep Quality Index (PSQI)</td>
<td>0.202</td>
</tr>
<tr>
<td></td>
<td>Beck Depression Inventory (BDI)</td>
<td>0.239</td>
</tr>
</tbody>
</table>

mean of discriminant function

no DM / no GN | 0.040 | 0.155 |
no DM / GN | 0.208 | -0.121 |
DM / no DN | -0.166 | 0.130 |
DM / DN | -0.418 | -0.166 |

DM: Diabetis Mellitus, GN: Glomerular Nephritis, DN: Diabetic Nephropathy

Conclusions: As the CKD stage progresses, HR-QOL were found to worsen. A strong correlation was also observed with the underlying disease, and components of HR-QOL that strongly correlated varied with the type of disease.

Funding: Pharmaceutical Company Support

TH-PO243
Erythropoietin Responses in Chronic Kidney Disease: A Cross-Sectional Analysis from the CKD-JAC Study

Tadato Akizawa, Takayuki Hamano, Enyu Imai, Seichi Matsuo, Hirofumi Makino, Tsyoshi Watanabe, Kosaku Nitta, Yasuo Ohashi, Akira Hishida. Syowa Univ, Japan; CKD-JAC Study Group

Background: Erythropoietin(EPO) production by renal cells is driven by low oxygen levels in the renal tissue. Aim is to elucidate the factors to determine serum EPO in Chronic Kidney Disease(CKD) not on dialysis.

Methods: EPO levels in 1796 ESRa-therapy-naive patients with CKD stages 3-5 were measured at 1 year after enrollment. We explored associations of EPO levels with various parameters including hemoglobin(Hb), mean corpuscular volume(MCV), eGFR, urinary and serum albumin, C-reactive protein(CRP), and prior cardiovascular disease(CVD) using multiple restriction. Restricted cubic spline regression was used for parameters having non-linear relationships with serum EPO.

Results: A significantly non-linear negative relationship between EPO and Hb levels was observed. The threshold of Hb when EPO levels plateaued was 11g/dl on average; this threshold decreased with the advance in CKD stages. The slope between EPO levels and Hb below the threshold was significantly steeper in patients without diabetes(DM), suggesting impaired EPO response to anemia in DM.
There was a U-shaped relationship between MCV and EPO levels with the bottom in the normal ranges of MCV. Albuminuria and eGFR were independent negative and positive determinants of EPO levels, respectively. Low serum albumin, high CRP levels, and prior and UAE change demonstrated that across both SBP categories (defined by the median), a progressively lower eGFR decline and lower risk for overt nephropathy was observed.

Methods: We obtained data for 2816 male and 3269 female Caucasian participants of the Nijmegen Biomedical Study, a population-based, age and sex-stratified sample of inhabitants of Nijmegen. Serum creatinine values were determined with the Jaffé method calibrated against mass spectrometry and were used to calculate eGFR with the Cockcroft-Gault (CG), MDRD4, MDRD6 and CKD-EPI equations. Demographics, health status and medication use were obtained by postal questionnaire. We used a poisson regression equation generally showed a linear dose response relation with mortality.

Intraclass variability in kidney function was a commonly observed phenomenon. However, predictors of kidney function variability and its prognostic implications are not known.

Methods: We used national data from the Department of Veterans Affairs to assemble a cohort of 60,641 patients with least 3 outpatient serum creatinine measurements between October 1, 1999 and September 30, 2002. Variability in kidney function was defined for each individual. We tested the SBP and UAE response to ARB and effect on renal function and mortality during 2 years follow-up.

Conclusions: The CKD-EPI equation allows linear modeling of mortality risk. Lower eGFR-CKD-EPI is an important risk factor for mortality, but not in people aged >75 years.

Funding: Private Foundation Support

TH-PO245

Albuminuria Response to Angiotensin Receptor Blockade Is a Determinant of Renal Protection in Patients with Type 2 Diabetes and Microalbuminuria in the IRMA-2 Trial


Background: Albuminuria (UAE) and systolic blood pressure (SBP) are both renal risk markers in patients with type 2 diabetes (T2DM). ARBs reduce SBP and UAE and are renoprotective. However, SBP and UAE reduction varies and can be discordant within an individual. We tested the SBP and UAE response to ARB and effect on renal function decline in T2DM patients.

Methods: Data from the IRMA-2 trial were used. In this post-hoc analysis we assessed the extent of variability and discordance in SBP and UAE response (0-6 months) in 531 subjects. We analyzed the effect of month 6 SBP and UAE change on renal outcome, looking at glomerular filtration rate (eGFR) changes as well as development of overt nephropathy during 2 years follow-up.

Results: On Irbesartan treatment, 85 (24%) patients had a reduction in UAE but not in SBP at month 6. Conversely, 67 (19%) had a reduction in SBP but not in UAE. A larger reduction in UAE (p=0.0037) but not SBP (p=0.087) was independently associated with a slower rate of eGFR decline. The risk reductions for overt nephropathy per 50% reduction in UAE and 5 mmHg SBP reduction were 44% (95%CI 39-59%; P<0.001) and 9% (95%CI 20 to 2%; P=0.096) resp. Renal function decline according to combined SBP and UAE change demonstrated that across both SBP categories (defined by the median), a progressively lower eGFR decline and lower risk for overt nephropathy was observed with a larger UAE reduction.

Conclusions: SBP and UAE response to ARB therapy may be discordant. The UAE response individually determined renal outcome. The results suggest that in T2DM patients with microalbuminuria both SBP and UAE should be separate targets for renoprotection.

Funding: Pharmaceutical Company Support

TH-PO244

The Role of eGFR, Age and Gender in Mortality Risk

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Background: The effect of eGFR on mortality is modified by age and sex. Our goal was to explore the dose-response relationship of these risk factors and compare GFR estimation equations.

Methods: We obtained data for 2816 male and 3269 female Caucasian participants of the Nijmegen Biomedical Study, a population-based, age and sex stratified sample of inhabitants of Nijmegen. Serum creatinine values were determined with the Jaffé method calibrated against mass spectrometry and were used to calculate eGFR with the Cockcroft-Gault (CG), MDRD4, MDRD6 and CKD-EPI equations. Demographics, health status and medication use were obtained by postal questionnaire. We used a poisson regression model weighted for sampling fractions to estimate mortality risk for eGFR by age, sex and comorbidities and used fractional polynomials to evaluate non-linear relations.

Results: During 39,855 person-years of follow-up 316 people died. Figure 1 shows the unadjusted association for eGFR and mortality by age and sexe.

Conclusions: The CKD-EPI equation allows linear modeling of mortality risk. Lower eGFR-CKD-EPI is an important risk factor for mortality, but not in people aged >75 years.

Funding: Private Foundation Support

TH-PO246

Variability in Kidney Function and the Risk of Death

Ziyad Al-Ally, Tarék M. El-Achkar, Ann M. O’Hare, Department of Medicine, Division of Nephrology, Saint Louis Veterans Affairs Medical Center, Saint Louis, MO; Department of Medicine, Division of Nephrology, Veterans Affairs Puget Sound Healthcare System, Seattle, WA.

Background: Intra-individual variability in kidney function is a commonly observed phenomenon. However, predictors of kidney function variability and its prognostic implications are not known.

Methods: We used national data from the Department of Veterans Affairs to assemble a cohort of 60,641 patients with least 3 outpatient serum creatinine measurements between October 1, 1999 and September 30, 2002. Variability in kidney function was defined for each patient as the coefficient of variation of the regression line coefficient fitted to all outpatient measurements of estimated glomerular filtration rate (eGFR) during this time frame. We built logistic regression models to examine predictors of variability and Cox survival models to examine the association between kidney function variability and the risk of death.

Results: Black race, female gender, diabetes, cardiovascular disease, peripheral artery disease, chronic lung disease, hepatitis C, dementia, hospitalizations, and the use of angiotensin converting enzyme inhibitors, angiotensin receptor blockers and diuretics were predictors of high variability in eGFR. After a median follow-up of 5.9 years, there were 4,563 (22.57%), 5,552 (27.47%), and 7,493 (36.80%) deaths among patients in low, intermediate, and high tertiles of eGFR variability, respectively. In adjusted analyses, patients in the highest tertile of eGFR variability had an increased risk of death (Hazard Ratio=1.33, 95% Confidence Interval=1.28-1.38) compared with the referent of those in the lowest tertile.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

168A
TH-PO247

Age and Baseline Kidney Function Modify the Association between Rate of Kidney Function Decline and the Risk of Death  Ziyad Al-Alh,1 Ann M. O’Hare,2 Tarek M. El-Achkar,3 Michael I. Rauchman.1 1Division of Nephrology, Saint Louis University Physicians Medical Center, Saint Louis, MO; 2Department of Medicine, Division of Nephrology, Veterans Affairs Puget Sound Healthcare System, Seattle, WA; 3Division of Nephrology, Walter Reed Army Medical Center, Washington, DC.

Background: The relationship between rate of kidney function decline and mortality has not been examined in different age groups and in patients with kidney disease.

Methods: Using the Department of Veterans Affairs national databases, we built a cohort of 22,588 patients and categorized them into 4 groups: those who experienced no decline (rate of eGFR change greater than 0 ml/min/year) and those with mild, moderate, and severe decline defined as eGFR loss of 0-1, 1-4, and greater than 4 ml/min/year, respectively. We built Cox survival models to examine the relationship between rate of kidney function decline and the risk of death in patients according to age groups (less than 60, 60-69, and greater than 75 years old) and eGFR categories (baseline eGFR greater than 90, 60-89, 45-59, 30-44, and 15-29 ml/min).

Results: After a median follow up of 5.9 years, the risk of death in patients with severe decline in eGFR was strongest in the youngest patients (Hazard Ratio (HR) 1.6, Confidence Interval (CI) 1.2-2.1 for <60 years old) and was gradually attenuated across groups of increasing age (HR=1.2, CI=1.0-1.3 for greater than 75 years old, P for interaction=0.0007). In groups according to baseline eGFR, the risk was significant but weak in patients with baseline eGFR greater than 90 ml/min (HR=1.30, CI=1.13-1.50) and became increasingly more pronounced in groups with decreasing baseline eGFR (HR=2.81, CI=1.8-4.2 for 15-29 ml/min).

Conclusions: Our results show that the independent association between the rate of decline of kidney function and mortality is attenuated with increasing age and may be more pronounced in patients with advanced baseline kidney disease.

Funding: Veterans Administration Support

TH-PO248

Clinical Outcomes of Clostridium difficile Infection in Patients with Chronic Kidney Disease  Mira T. Keddis,1 Sahil Khanna,2 Larry M. Baddour,1 Darell S. Pardi,1 Qian O’Hare.1 1Nephrology, Mayo Clinic, Rochester, MN; 2Gastroenterology, Mayo Clinic, Rochester, MN; 3Infectious Diseases, Mayo Clinic, Rochester, MN.

Background: The outcomes of Clostridium difficile infection (CDI) in chronic kidney disease (CKD) patients have not been previously described. The objective of this study is to examine clinical outcomes of hospitalized patients with CKD and CDI using the National Hospital Discharge Survey (NHDS) database.

Methods: We analyzed NHDS data for 2005-2009; the database contains demographic information, ICD-9 diagnosis and procedure codes, and discharge information for patients admitted to non-Federal short-stay United States hospitals. Clinical information of patients with CKD and CDI was abstracted and analyzed using SAS version 9.2 and JMP version 9.0.1.

Results: 59,715 hospitalized patients with CDI were identified with mean age 68.5 ±15.8 years, 52.4% male sex, 41.9% of patients had unspecified CKD severity, 37.7% with CKD stage V, 18.6% CKD III and IV, and 1.8% CKD I and II. Of them, 17,189 (28.8%) patients required dialysis during their hospitalization. CDI occurred in 918 (1.5%) patients; CKD patients with CDI were significantly older (72.0 ±5 vs. 68.4 ±0.07 years), required longer length of stay (10.2 ±2 vs. 6.2 ±0.02 days), and were more likely to be dismissed to a care facility (50.5 ±25.7%) compared to CKD patients without CDI (all p<0.001).

CKD patients with CDI had a higher in-hospital mortality rate (8.50% vs. 3.95%, OR 2.25 [95% CI 1.78-2.85]) and likelihood of requiring colectomy (OR 3.33 [95% CI 1.7-6.54], p<0.001). After adjusting for age, the association of CDI with these clinical outcomes remained significant.

Conclusions: In hospitalized CKD patients, CDI was associated with prolonged hospitalization, and an increased likelihood of both undergoing colectomy and surviving in-hospital mortality. Prevention, early recognition and treatment of CDI in CKD patients are necessary to decrease CDI associated morbidity and mortality in this population.

TH-PO249

Tropinin T and B-Type Natriuretic Peptide Are Associated with Cardiovascular Outcome Despite Their Cross-sectional Association with Chronic Kidney Disease  Lieneke Scheven,1 Paul E. de Jong,1 Hiddo Jan Lambers Heerspink,2 Lucas Joost Van Pelt,1 Jenny E. Kooistra-Ros,3 Stephan J.L. Bakker,1 Ron T. Gansvoort.1 1Nephrology; 2Pharmacology, University Medical Centre Groningen, Groningen, Netherlands.

Background: It has been suggested that tropinons and natriuretic peptides are falsely elevated in chronic kidney disease (CKD) because of decreased renal clearance. The value of these biomarkers to predict cardiovascular (CV) outcome in subjects with CKD has therefore been debated. We investigated this issue in a population based cohort study.

Methods: For the present study 8121 subjects who participated in the PREVEND Study of whom at baseline high sensitive Tropinin T (hsTnT) and N-terminal pro-B-type Natriuretic Peptide (NT-pro-BNP) were available, were included. hsTnT>20.0 µg/L and NT-pro-BNP>125 ng/L were defined as elevated

Results: Of our cohort, 6.7% had an elevated hsTnT and 12.2% an elevated NT-pro-BNP. eGFR and albuminuria were significantly associated with hsTnT and NT-pro-BNP in linear regression analyses. After adjustment for age, gender and CV risk factors these associations remained significant. Both hsTnT and NT-pro-BNP appeared associated with CV events during follow-up (both p<0.001). These associations remained significant after adjustment for eGFR, albuminuria, age, gender and CV risk factors (both p<0.001). No interaction was found between eGFR and hsTnT or NT-pro-BNP in predicting CV outcome. Moreover, the value of an increased hsTnT or NT-pro-BNP to predict CV outcome was similar in subjects with or without CKD (in both, for both hsTnT and NT-pro-BNP p<0.001).

Conclusions: These data indicate that a finding of an increased hsTnT or NT-pro-BNP in a subject with CKD should be taken seriously as a prognostic marker heralding an unfavourable CV outcome and not be discarded as merely a reflection of decreased renal clearance.

TH-PO250

Isolated Microalbuminuria heralds a Poor Medical Prognosis  Lieneke Scheven,1 Paul E. de Jong,1 Hiddo Jan Lambers Heerspink,2 Stephan J.L. Bakker,3 Ron T. Gansvoort.1 1Nephrology; 2Pharmacology, University Medical Centre Groningen, Groningen, Netherlands.

Background: Microalbuminuria (MA) is often regarded as a sign of end-organ damage due to diabetes and/or hypertension and to be associated with an increased risk for cardiovascular (CV) events. It has been questioned whether isolated microalbuminuria (IMA) - i.e. microalbuminuria in absence of diabetes, hypertension and/or CV morbidity - has clinical relevance.

Methods: For this study data were used of 8,592 subjects who participated in the first 4 screening rounds of the PREVEND study, a prospective, community-based cohort study with serial measurements during follow-up. IMA was defined as albuminuria 300-3000 mg/L and eGFR >120 ml/min/1.73m². Of these, 6.7% had an elevated hsTnT and 12.2% an elevated NT-pro-BNP. HsTnT levels >125 ng/L were defined as elevated.

Results: Of our cohort, 6.7% had an elevated hsTnT and 12.2% an elevated NT-pro-BNP. eGFR and albuminuria were significantly associated with hsTnT and NT-pro-BNP in linear regression analyses. After adjustment for age, gender and CV risk factors these associations remained significant. Both hsTnT and NT-pro-BNP appeared associated with CV events during follow-up (both p<0.001). These associations remained significant after adjustment for eGFR, albuminuria, age, gender and CV risk factors (both p<0.001). No interaction was found between eGFR and hsTnT or NT-pro-BNP in predicting CV outcome. Moreover, the value of an increased hsTnT or NT-pro-BNP to predict CV outcome was similar in subjects with or without CKD (in both, for both hsTnT and NT-pro-BNP p<0.001).

Conclusions: These data indicate that a finding of an increased hsTnT or NT-pro-BNP in a subject with CKD should be taken seriously as a prognostic marker heralding an unfavourable CV outcome and not be discarded as merely a reflection of decreased renal clearance.
Methods: After exclusion of subjects with known renal disease or macroalbuminuria at baseline, or missing follow-up data on UAE, 5,825 subjects were eligible. Subjects were defined as having progressive UAE if they belonged to the quintile with the highest annual increase in UAE, and UAE ≥150 mg/24h during follow-up. Multivariable regression models were built using stepwise backward selection to identify risk factors for progressive UAE.

Results: During a median follow-up of 9.3 years 132 subjects met our definition of progressive UAE (median value at baseline 67.4 versus 252.7 mg/24h at end of follow-up). Unusually associated with progressive UAE were: male gender, history of cardiovascular disease, low eGFR, higher age, BMI, SBP, glucose and baseline UAE, and use of antihypertensive and lipid lowering medication (all p<0.001). Variables that contributed significantly to the multivariable model are shown in the Table. Importantly, most predictors for progressive UAE were, although statistically significant, of limited value when compared to baseline UAE, as indicated by the Wald statistics (table) and a clinical score chart based on the Beta-values of the multivariable model.

Table: Variables associated with progressive UAE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds Ratio</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>2.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>1.03</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>1.06</td>
<td>0.01</td>
</tr>
<tr>
<td>UAE (ln mg/24h)</td>
<td>1.02</td>
<td>0.06</td>
</tr>
</tbody>
</table>

* Change in systolic blood pressure during follow up

Conclusions: A high baseline UAE is by far the most important predictor of progressive UAE. Thus, screening for baseline UAE will be more important than screening for cardiovascular risk factors in order to identify subjects at risk for progressive UAE.

TH-PO252
Glomerular and Tubular Damage Markers in Subjects with Progressive Albuminuria

Ferdau L. Nauta,1 Lienieke Scheven,1 Stephan J.L. Bakker,2 Willem Van Oeveren,3 Paul E. de Jong,1 Ron T. Gansevoort.1 1Nephrology, UMCG; Haemocron, Groningen, Netherlands.

Background: Albuminuria is associated with risk for renal and cardiovascular disease. It is difficult to predict which subjects will progress in albuminuria. We investigated whether baseline clinical characteristics between progressors and controls were significantly different as compared to baseline UAE, as indicated by the Wald statistics (table) and a clinical score chart based on the Beta-values of the multivariable model.

Methods: Subjects were selected from a prospective community-based cohort study with serial follow-up (PREVEND, n=8592) and defined as progressors if they were in the quintile with the most rapid annual increase in albuminuria and reached albuminuria 150 mg/d during follow-up. Subjects with known renal disease were excluded. Progressors were 2:1 matched to control subjects based on age, sex and baseline UAE. IgG was measured as glomerular marker, KIM-1, NAG, β2-microglobulin, cystatin C as proximal tubular damage markers and NGAL and MCP-1 as inflammatory markers.

Results: After a follow-up of 9.3 y 183 subjects met our criteria for progressive albuminuria. Baseline clinical characteristics between progressors and controls were comparable. However, both urinary excretion and fractional excretion of total IgG were significantly higher in progressors (p<0.001), whereas in these subjects urinary and fractional excretion of all tubular markers except cystatin C were lower (all p<0.01).

Conclusions: These data suggest that albuminuria associated with glomerular damage is more likely to progress to renal disease, whereas albuminuria associated with tubulointerstitial damage is more likely to remain stable.

TH-PO253
The Effect of Frozen Storage on Urinary Renal Damage Markers

Ferdau L. Nauta,1 Stephan J.L. Bakker,2 Hiddo Jan Lambers Heeprink,3 Willem Van Oeveren,4 Dick de Zeeuw,5 Henk Bilo,5 Paul E. de Jong,1 Ron T. Gansevoort.1 1Nephrology, UMCG; Haemocron, Groningen; 2Nephrology, Clinics, Zwolle, Netherlands.

Background: Epidemiological studies that investigate the value of renal damage markers to predict outcome often use urine samples that have been stored frozen for prolonged time. Little is known about the effect of frozen storage on urinary concentrations and variability of renal tubular damage markers. We therefore investigated the effect of storage at -20 and -80 °C.

Methods: Urine samples were collected in 95 patients with diabetes mellitus. In each sample we measured IgG, IgG-4, KIM-1, NAG, NGAL, cystatin C and H-FABP fresh and after 4 weeks, 6 months and 1 year of frozen storage at -80 °C. Furthermore, the effect of 1 year frozen storage at -20 °C and the effect of various specific storage protocols was investigated.

Results: Average marker concentrations showed a gradual decrease and an increase in variability after frozen storage. More data are needed for conclusions.

Conclusions: Renal damage markers should preferably be measured in fresh urine samples. Marker studies using frozen urine samples should be interpreted with caution.

TH-PO254
The Association of Circulating Fetuin-A with Incident Cardiovascular Disease Differ by Diabetes Status in Community-Living Older Persons: The Cardiovascular Health Study

Majken K. Jensen,1 Traci M. Bartz,2 Kenneth J. Mukamal,3 Luc Djousse,4 Jorge R. Kizer,5 Russel Tracy,6 Eric B. Rimm,7 David Siscovick,8 Michael Shlipak,9 Joahim H. IX10 1HSPH, MA; University of Washington, WA; Beth Israel Deaconess Medical Center, MA; Brigham and Women’s Hospital, MA; Cornell University, NY; University of Vermont, VT; UCSC, CA; UCSD, CA.

Background: Fetuin-A inhibits arterial calcification and insulin activity. High fetuin-A levels associate with diabetes, whereas low levels are associated with CVD in ESRD patients. The association with incident cardiovascular disease (CVD), and the potential dependence on prevalent diabetes in non-ESRD is less certain.

Methods: 3,718 participants aged >65 years, free of CVD in 1992, followed for incident CVD (MI, stroke, or CVD death) through 07/2008.

Results: Mean age was 76 years, and mean eGFR was 74 ml/min/1.73m². 1,415 participants had a CVD event. The association of fetuin-A with CVD was modified by diabetes (p interaction=0.02). In participants without diabetes the highest risk was observed for the lowest fetuin-A levels. The adjusted hazard ratio (HR) per each SD higher fetuin-A (0.1 g/L) was 0.94 (0.89-1.01). In contrast, fetuin-A was not associated with CVD in those with diabetes.

Conclusions: Association of Fetuin-A Quartiles with Incident Cardiovascular Disease Events in Older Participants without Diabetes, Stratified by Diabetes: The Cardiovascular Health Study

TH-PO255
Triglycerides and All-Cause Mortality in Non-Dyslipid Dysplastic Chronic Kidney Disease

Sankar D. Navaneethan,1 Jesse D. Schold,1 Susanna Arrigain,2 Stacey Jolly,3 John W. Sharp,4 Anil K. Jain,5 James F. Simon,6 Emilio D. Poggio,7 Tüte Shinivas,8 Martin J. Schreiber,9 Joseph V. Nally,10 Hameed S. Qureshi,11 Quantitative Health Sciences, Cleveland Clinic; 2Medicine, Cleveland Clinic.

Background: Elevated triglyceride (TG) level is associated with cardiovascular and all-cause mortality in the general population. The association between TG levels and all-cause mortality among chronic kidney disease (CKD) patients is unclear.

Methods: Patients with stage 3 and stage 4 CKD patients who had TG levels measured after the diagnosis of CKD in our health system were included. We examined the associations of TG levels as categorical and continuous variables with all-cause mortality in CKD patients using logistic regression, Cox-proportional hazard models and Kaplan-Meier survival curves.

Results: Out of 2,592 CKD patients, 37.1% (n=975) had TG levels ≥150 mg/dl. Patients of diabetes, hypertension, obesity and lower eGFR were associated with TG levels ≥150 mg/dl while increasing age, male gender (odds ratio [OR] 0.87, 95% CI 0.82, 0.92), and African American race (OR 0.40, 95% CI 0.37, 0.44) were associated with
lesser risk of ≥150 mg/dl. Kaplan-Meier survival plot did not show significant differences in all-cause mortality in the different TG groups. However, after covariate adjustment, each unit in log transformed TG levels was associated with a 10% increased risk (95% CI 1.01, 1.20) for death. Age modified the association between TG levels and mortality with patients <65 years having 24% higher risk of death (95% CI 1.07, 1.44) and ≥65 years with no increased risk for death.

Conclusions: Elevated TG may be a modifiable risk factor for all-cause mortality in patients <65 years but not in older CKD patients. Future studies should explore the associations of TG with cause-specific mortality in younger CKD patients with high triglyceride levels.

Funding: Other NIH Support - National Institutes of Health, the National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990.

TH-PO256 Chronic Kidney Disease and Fracture Risk in Children and Young Adults: A Population-Based Study Using the Health Improvement Network Database Michelle Denburg,1, 3 Kevin Haynes,2 Justine Shults,2 Mary B. Leonard,1,3 1Pediatrics, University of Pennsylvania School of Medicine, Philadelphia, PA; 2Biostatistics & Epidemiology, University of Pennsylvania School of Medicine, Philadelphia, PA; 3Children’s Hospital of Philadelphia, Philadelphia, PA.

Background: Chronic kidney disease (CKD) may impair bone accrual and is associated with increased fracture risk in older adults. Fracture risk in children and young adults with CKD has not been assessed. The objective was to determine if CKD is associated with greater risk of fracture in this population.

Methods: In a retrospective cohort study using the Health Improvement Network (THIN) Database, subjects with CKD stages 3-5, diagnosed at ≤30 years, were identified using serum creatinine values or previously validated diagnosis codes. All sex- and age-matched subjects from a practice with a CKD subject were included for comparison. Cox regression analysis was used to estimate the hazard ratio (HR) for first fracture.

Results: 2,649 subjects with CKD (51% female) and 1,055,056 subjects without CKD (52% female) were identified. 185 first fractures (151,000 person-years) occurred in CKD subjects CKD vs 66,982 (91,000 person-years) in non-CKD subjects over a median follow-up time of 4 and 5 years, respectively. Overall, the HR for fracture in males vs females was 2.0 (1.97, 2.03), and the effect of CKD differed significantly by sex (interaction p<0.001).

HR for Fracture CKD vs Non-CKD

<table>
<thead>
<tr>
<th>Age</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-9</td>
<td>1.21 (0.65, 2.41)</td>
<td>1.92 (0.96, 3.85)</td>
</tr>
<tr>
<td>10-14</td>
<td>0.70 (0.35, 1.40)</td>
<td>1.25 (0.52, 3.01)</td>
</tr>
<tr>
<td>15-19</td>
<td>0.80 (0.48, 1.39)</td>
<td>2.30 (0.99, 5.38)</td>
</tr>
<tr>
<td>20-29</td>
<td>1.00 (0.47, 2.28)</td>
<td>3.17 (1.20, 8.68)</td>
</tr>
<tr>
<td>30+</td>
<td>1.68 (1.22, 2.32)</td>
<td>2.47 (1.81, 3.39)</td>
</tr>
</tbody>
</table>

Forearm/wrist/hand fractures were the most common site in both groups, but the distribution of site differed in CKD vs non-CKD subjects. Hip fractures comprised a greater proportion of fractures in CKD (3.8% vs 0.9%, p=0.002), while skull and facial fractures represented a greater proportion in non-CKD (1.6% vs 6.7%, p<0.003).

Conclusions: Early onset CKD was associated with significantly increased fracture risk in adolescent and young adult women. In males, early onset CKD was associated with increased risk of fracture only after age 30.

Funding: NIDDK Support, Other NIH Support - Center for Pediatric Clinical Effectiveness, The Children’s Hospital of Philadelphia
TH-PO260
Depressive Symptoms, Cardiovascular Disease (CVD) Risk Factors, and Subclinical CVD among Individuals in the Chronic Renal Insufficiency Cohort (CRIC) Study

Background: Depression is associated with prevalent cardiovascular disease (CVD) and an increased risk of CVD-related events in adults with chronic kidney disease (CKD). However, the relationship between depression, CVD risk factors, and subclinical CVD is not known.

Methods: We conducted a cross-sectional analysis of depressive symptoms (DS) in adults at entry into CRIC Study. DS were assessed by the Beck Depression Inventory (BDI) and defined by a BDI score ≥11. CVD risk factors were ascertained by questionnaires and lab studies. In addition to self-reported CVD, subclinical CVD measures included left ventricular hypertrophy (LVH), coronary artery calcification (CAC) score ≥400, and ankle-brachial index (ABI) < 0.9. Logistic regression was used to assess the relation between DS and CVD.

Results: Among 3863 CRIC participants, 28.5% had DS. Self-reported CVD was found in 33.3% and subclinical CVD as follows: 53.6% LVH, 21.6% CAC >400, and 16.1% ABI < 0.9. Adults with DS had a greater burden of traditional CVD risk factors compared to those without DS, including a higher prevalence of diabetes, hypertension, hyperlipidemia, smoking, low eGFR, and elevated urine protein (p<0.05). Control of blood pressure (<130/80 mmHg) and diabetes (HbA1c<7%) was less common in those with DS (both p<0.05). DS were also associated with the presence of non-traditional CVD risk factors such as lower serum hemoglobin and albumin, and higher serum phosphorus, parathyroid hormone, and e-c reactive protein (all p<0.001). Analyses adjusted for sociodemographic and traditional CVD risk factors. DS were associated with self-reported CVD (OR 1.18; 95% CI: 1.12-1.90) and LVH (OR 1.24; 95% CI: 1.00-1.54) but not CAC >400 or ABI < 0.9.

Conclusions: In a large diverse CKD cohort, DS were associated with an adverse profile of traditional and non-traditional modifiable CVD risk factors, and increased odds of self-reported CVD and LVH.

Funding: NIDDK Support

TH-PO261
Low Ankle Brachial Index Is Associated with Rapid Glomerular Filtration Rate Decline
Mercedith C. Foster, Nimrta Ghuman, Shih-Jen Hwang, Joanne Murabito, Caroline S. Fox.

Background: A low ankle brachial index (ABI) is associated with increases in serum creatinine. We sought to investigate the association of ABI with the development of rapid kidney function decline as stage or 3 chronic kidney disease (CKD).

Methods: Participants (n=2592, mean age 57 years, 54% women) attended Framingham Offspring Exam 6 (1995-98) and 8 (2005-08). Baseline ABI was classified into 3 groups: normal (≥1.1 to <1.4; n=1719), low-normal (≥0.9 to 1.1; n=822), and low (≤0.9; n=51). Glomerular filtration rate was estimated using the MDRD Study equation. Rapid eGFR decrease was defined as eGFR decrease of ≥30% of baseline/1.73 m²/year. Incident stage 3 CKD was defined as eGFR<60mL/min/1.73m² at Exam 8 among those free of stage 3 CKD at baseline. Rapid eGFR decline and stage 3 CKD were modeled as functions of ABI using logistic regression, with multivariable (MV) adjustment for age, sex, eGFR, and stage 3 CKD risk factors.

Results: Over 9.5 years of follow-up, 11.9% (n=309) experienced rapid eGFR decline. Compared to participants with a normal ABI, those with a low ABI had a 5.8-fold increased odds of rapid eGFR decline (p<0.0001), which persisted with further MV adjustment (Table, p<0.0007). Among those free of baseline stage 3 CKD, 9.0% (n=219) developed stage 3 CKD. After adjusting for age, sex and baseline eGFR, participants with a low ABI had a 2.9-fold increased odds of stage 3 CKD (p=0.006) when compared to those with a normal ABI. We observed some attenuation upon MV adjustment, although low ABI remained associated with a 2.1-fold increased odds developing stage 3 CKD (Table, p=0.07). Low-normal ABI was not associated with rapid eGFR decline or incident stage 3 CKD.

Table: Odds ratios (95% Confidence Intervals) of rapid eGFR decline & CKD

<table>
<thead>
<tr>
<th>Covariates</th>
<th>Rapid eGFR Decline</th>
<th>Stage 3 CKD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-normal ABI Low ABI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, sex, baseline eGFR</td>
<td>1.3 (1.0-1.9)</td>
<td>5.2 (2.7-12.7)</td>
</tr>
<tr>
<td>1.3 (0.9-1.9)</td>
<td>2.9 (1.4-6.3)</td>
<td></td>
</tr>
<tr>
<td>Multivariable</td>
<td>1.5 (0.9-1.8)</td>
<td>4.3 (1.5-9.8)</td>
</tr>
<tr>
<td>1.2 (0.8-1.7)</td>
<td>2.1 (0.9-4.7)</td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Low ABI is associated with an increased risk of rapid eGFR decline.

Funding: Other NIH Support - The Framingham Heart Study is supported by the National Heart, Lung and Blood Institute (N01-HC-25195).

TH-PO262
Multiple Markers of Kidney Function Prediction Mortality and End-Stage Renal Disease in the General Population: The Atherosclerosis Risk in Communities (ARIC) Study
Brad C. Astor, Tariq Shafi, Ron C. Hoogeveen, Christie Ballantyne, Josef Coresh, John Hopkins University; Baylor College of Medicine.

Background: Lower estimated GFR based on serum creatinine (eGFR_{CKD-Epi}) or cystatin C (eGFR_{cys-C}) is associated with higher risk of death and ESRD. Both ACE inhibitor (BPIT) and beta-blockers and microalbumin (B2M), two novel markers of kidney function, have also been shown to predict events. The prognostic utility of multiple markers of kidney function is undetermined.

Methods: We used data from 10,091 participants in the ARIC Study, a population-based cohort study of 4 US communities, to examine the prospective association between the number of markers indicating kidney dysfunction and death and ESRD over 11 years of follow-up. Kidney dysfunction was defined as eGFR_{CKD-Epi} (n=684) or eGFR_{cys-C} (n=1,320) ≤60mL/min/1.73m² or the upper decile of B2M (n=1,001) or BMP (n=1,006).

Results: The number of markers indicating kidney dysfunction was associated with risk of death and ESRD, even among those with eGFR_{cys-C} ≥60mL/min/1.73m². Results were similar after further adjustment for urinary albumin: creatinine. Adjusted* Incidence Rate Ratio (95% CI) of Death and ESRD

<table>
<thead>
<tr>
<th>Number of Markers</th>
<th>Overall</th>
<th>eGFR_{cys-C} ≤60mL/min/1.73m²</th>
<th>eGFR_{cys-C} &gt;60mL/min/1.73m²</th>
<th>eGFR_{cys-C} and BMP ≤60mL/min/1.73m²</th>
<th>eGFR_{cys-C} and BMP &gt;60mL/min/1.73m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>1</td>
<td>1.2 (1.0-1.4)</td>
<td>2.1 (1.1-4.4)</td>
<td>2.1 (1.2-4.6)</td>
<td>2.4 (1.3-4.6)</td>
<td>2.4 (1.3-4.6)</td>
</tr>
<tr>
<td>2</td>
<td>1.7 (1.4-2.0)</td>
<td>1.8 (1.5-2.3)</td>
<td>2.3 (1.3-4.3)</td>
<td>3.1 (1.3-10.3)</td>
<td>11.2 (5.8-23.2)</td>
</tr>
<tr>
<td>3</td>
<td>2.4 (2.0-3.0)</td>
<td>2.7 (2.1-3.4)</td>
<td>8.4 (4.4-16.0)</td>
<td>7.4 (3.2-17.1)</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

* Adjusted for age, sex, race, prevalent coronary heart disease, diabetes, systolic blood pressure, antihypertensive medication use, current smoking, LDL and HDL cholesterol, and triglycerides

Conclusions: Using multiple markers of kidney function may provide the most accurate prognostic information in the general population. Studies should explore the optimal method for combining information from multiple markers.

Funding: NIDDK Support

TH-PO263
A Study of the Association between Cinacalcet Adherence and Biochemical Outcomes

Background: Cinacalcet is used to treat secondary hyperparathyroidism in patients receiving dialysis. As with other oral medications, non-adherence may prevent patients from experiencing the full benefits of therapy.

Methods: This retrospective cohort study of prevalent hemodialysis patients (>120 days since dialysis start) in the DaVita Rx and Clinical Data Warehouse assessed the association of cinacalcet adherence with control of serum parathyroid hormone (PTH).

Results: A total of 2367 patients met the study criteria: 947 NA, 320 LA, and 1200 HA patients. Baseline demographic and clinical characteristics were similar across categories. Percent of months with controlled PTH were higher for HA patients (78.6; SD 28.3) compared to LA (71.7; SD 27.8) and NA (75.3; SD 29.8). Adherence category (RG) was statistically significant (p < 0.001) in the GLM. When controlled PTH was defined as ≤300 or 150-300 mg/mL instead of < 600 mg/mL, the observed patterns persisted but were smaller in magnitude.

Conclusions: Our study suggests that increased adherence to cinacalcet may be associated with improved control of PTH. This is important to consider in light of prior studies linking controlled biomarkers over time with improved survival.

Funding: Pharmaceutical Company Support
Comparison of CVD Prevalence between Japanese and American CKD Patients (Collaboration between CKD-JAC and CRIC). Takayuki Hamano, 1 Enyu Imai, 2 Kosaku Nitta, 2 Hirofumi Makino, 2 Akira Hishida, 2 Lisa C. Nessel, 2 Elsayed Z. Soliman, 1 Ana C. Ricardo, 3 Martin J. Schreiber, 2 Dawei Xie, 1 Harold I. Feldman. 1 Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, PA; 2 CKD-JAC Investigators, Japan; 3 CRIC Investigators.

Background: The DOPPS revealed that the prevalence of cardiovascular disease (CVD) and mortality are much higher in American hemodialysis patients compared to Japanese hemodialysis patients. However, international comparative studies of CVD have not been conducted in predialysis patients with chronic kidney disease (CKD).

Methods: We compared the baseline prevalence of CVD combining the CRIC (Chronic Renal Insufficiency Cohort) and CKD-JAC (CKD-Japanese Cohort) studies, which consisted of 3393 and 2977 patients, respectively.

Results: After adjustment for age, sex, diabetes, and eGFR, the odds ratio (OR) (95% CI) for having prevalent CHF, MI, or stroke for Japanese, compared to American CKD patients (stage 1/2: OR 0.24 (0.38), 0.40 (0.35-0.47), and 1.02 (0.85-1.23), respectively. Since the ascertainment processes for CVD at study entry were different between the studies (patient questionnaire in CRIC and medical chart in JAC), we studied the consistency between patient questionnaire and medical record-derived data using a sample of patients in one of the clinical centers of CRIC. Based on these data, we performed multiple imputation for those in CRIC with missing chart-based prevalent CVD as a sensitivity analysis. The ORs for CHF, MI, and stroke comparing Japanese to American study participants were 0.410 (9.19-0.90), 0.550 (0.40-0.75), and 1.21 (0.75-1.94), respectively. With regard to CHF, additional adjustment for HbA1c or CRP levels eliminated the significance of lower OR for Japanese. However, even extensive adjustment for laboratory data did not eradicate the significantly lower rate of MI in Japanese.

Conclusions: American CKD patients have significantly higher prevalence of CHF and MI compared to their Japanese counterparts and a comparable prevalence of stroke after adjustment for age, sex, diabetes, and eGFR. These data will be the basis for future longitudinal comparisons of incident CVD.

Differential Response in Serum Potassium, Albuminuria and Blood Pressure to ACEi or ARB Therapy in Individual Type 2 Diabetic Patients - Sara S. Roscioni, 1 Dick de Zeeuw, 2 Giuseppe Remuzzi, 2 Philippe J. Vanhille, 3 Hans-Henrik Parving, 4 Hildo Jan Lambers Heerspink. 1 Kidney Centre, University Medical Centre Groningen, Netherlands; 2 Mario Negri Institute for Pharmacological Research, Bergamo, Italy; 3 Dept of Nephrology, Hospital Valerieins, France; 4 Dept of Endocrinology, Rigshospitalet, Copenhagen, Denmark.

Background: Treatment with angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB) reduces blood pressure (BP) and albuminuria (UACR) and increases serum potassium. The BP and UACR reduction affects the renal and cardiovascular (CV) function positively while the increase in potassium affects the renal and CV outcome negatively. Determining the net response to these offsetting effects in individual patients may offer unique predictive value for their ultimate renal/CV outcome.

Methods: We conducted a post-hoc analysis in four randomized controlled trials enrolling patients with type 2 diabetes at early (BENEDICT and IRMA-2) and late (RENAAL and IDNT) stage of renal disease. Regression analysis was performed between response in serum potassium and responses in BP or UACR after 3 months of ACEI or ARB treatment.

Results: ACEI/ARB-induced responses in potassium did not correlate with responses in BP or UACR both in diabetic patients at early and late stage of renal disease (table1). Furthermore, the achieved 3 months potassium levels did not correlate with the respective achieved BP or UACR levels within the same patients (table1). The percentage of patients who developed hyperkalemia was independent of the response in BP or UACR.

Conclusions: The response in serum potassium to ACEI or ARB therapy does not correlate with the response in BP or UACR. As the response in each of these parameters may affect long-term renal/CV outcome, our data support the need to monitor and optimize the patient response in each parameter to improve long-term renal and CV protection.

Age Modifies the Prognostic Role of Main Risk Factors for ESRD and Death in Non-Dialysis CKD Patients under Nephrology Care. The TABLE Prospective Cohort Study - Luca De Nicola, 1 Paolo Chioldi, 2 Silvio Borrelli, 3 Ciro Gallo, 2 Giuseppe Conte, 1 Roberto Minutolo. 1 Nephrology, Second University, Naples, Italy; 2 Medical Statistics, Second University, Naples, Italy.

Background: Prevalence of elderly CKD patients in nephrology clinics is growing. However, how age might affect the prognostic role of the commonly reported risk factors in CKD is undefined.

Methods: We prospectively followed, from 2003 to death or May 2011, 1248 adult patients with CKD stage 3-5 attending 25 Italian outpatient nephrology clinics for ≤12 months before enrolment. Primary endpoints were ESRD and death. We estimated rates of ESRD and death (per 100 patient-year) by age, and tested interactions between age and a number of baseline risk factors (gender, BMI, diabetes [DM], cardiovascular disease [CVD], smoking, systolic blood pressure, hemoglobin, phosphate, cholesterol, uric acid, GFR, 24th proteinuria [Uprot]) in Cox models stratified by center. ESRD was entered as time-dependent covariate when death was assessed.

Results: 481 (38%) patients were less than 65 yrs old, 410 (33%) patients were between 65 and 75 yrs and 357 (29%) patients were over 75 yrs. Within each class, GFR values were equal to 31.15 mL/min/1.73 m², 22.14, and 29.13, respectively. Rates of ESRD and death were respectively 9.0 (95% confidence interval [CI] 7.8-10.4) and 1.74 (95% CI 1.3-2.3) in ≤65 yrs, 7.3 (6.1-8.8) and 6.1 (5.7-14) in 65-75 yrs, and 7.9 (6.4-9.8) and 14.5 (12.6-16.7) in over 75 yrs. In the final models, age interacted significantly with GFR (P=0.032), BMI (P=0.011), and Uprot (P=0.001) for ESRD risk, and with GFR (P=0.025) and DM (P=0.009) for death risk. Prognostic effect of CVD and DM decreased with older age, while lower GFR and higher Uprot increased the risk of ESRD with advancing age.Male gender, lower BMI, and higher phosphate increased the risk of ESRD independently of age, whereas lower GFR was an independent risk factor for death (P=0.005 for all). These results may help in identifying the elderly patients at higher risk.

Prevalent Use of Dietary Supplements Potentially Harmful in Chronic Kidney Disease in the United States - Vanessa Grubbs, 1 Laura C. Plantinga, 2 Delphine S. Tuot, 1 Elizabeth Hedgeman, 1 Rajiv Saran, 4 Sharon Saydah, 4 Deborah Rolka, 5 Neil R. Powe, 1,2 University of California, San Francisco; 3 San Francisco General Hospital; 4 University of Michigan; 5 Centers for Disease Control and Prevention.

Background: The National Kidney Foundation (NKF) identifies 39 herbs that may be harmful in the setting of chronic kidney disease (CKD) (http://www.kidney.org/atou/content/herbsupps.cfm), but the prevalent use of such herbs in the U.S. by CKD status is unknown.

Methods: Using 1999-2008 National Health and Nutrition Examination Survey data, we examined the reported use of dietary supplements in the past 30 days among 21,169 non-pregnant adults (age 20+ years). CKD stage 1/2 was defined by urinary albumin:creatinine ratio of ≥30 mg/g with eGFR ≥60 mL/min/1.73 m² and CKD stage 3/4 by eGFR 15-59 mL/min/1.73 m². Any dietary supplement containing at least one NKF-identified herb was defined as potentially harmful. The prevalence and odds of taking a potentially harmful supplement by CKD status was estimated via multivariable logistic regression weighted to the U.S. population.

Results: While an estimated 52.4% of participants reported taking any dietary supplement, the supplement was potentially harmful among 15.3%. The crude estimated prevalence of those taking a any dietary supplement increased with greater CKD severity (no CKD 51.4%; CKD stage 1/2 49.1%; CKD stage 3/4 65.8%, p<0.001), but decreased among those taking a potentially harmful supplement (16.1%, 13.0%, and 10.0%, respectively, p<0.001). However, after adjustment for demographics, co-morbid disease, and healthcare visits, CKD status was not a significant determinant of taking any supplements stage 1/2:0.95, 0.82-1.10; stage 3/4 OR 1.07, 0.93-1.23, vs. no CKD) or a potentially harmful supplement (stage 1/2: OR 0.97, 0.87-1.28; stage 3/4 OR 0.90, 0.67-1.21, vs. no CKD).

Conclusions: The use of dietary supplements potentially harmful in CKD is common among adults, as use is not statistically different by CKD status, patients with CKD may be unaware of potentially harmful supplements. Health care providers, too, may be unaware of potentially harmful supplements and that patients with CKD are taking them. Further research and education are warranted.

Funding: NIDDK Support, Other U.S. Government Support

Risk Assessment of CKD-EPI Equation Compared with That Based on MDRD Study Equation in the Japanese General Population - Masaki OhSawa, Department of Hygiene and Preventive Medicine, Iwate Medical University, Morioka, Japan.

Background: Estimated glomerular filtration rate (eGFR mL/min/1.73m²) calculated by CKD-EPI equation corresponds to the actual measured value more accurately than those estimated by MDRD equation. However, comparative studies between those equations have not been examined based on longitudinal studies in the Japanese general population.
Mortality rates are expressed as /1000 person-years.

Epidemiologic study.
The findings well reflected CKD stage and patients’ characteristics, which suggests that ± SD) was tested in a prospective cohort of 167 individuals with diabetic CKD (age, 57 ± 9 years; median proteinuria, 2.5 g/g; estimated glomerular filtration rate, 58 ± 23 ml/min/1.73 m²). Association of change in ABI with all-cause mortality was determined in the sub-group of 75 subjects with normal ABI at baseline.

Results: At baseline, 41% had an abnormal ABI<0.9, 18%>1.3 or non-compressible arteries, 23% Upon follow-up, 43 subjects died: 0.9-1.3, 18%; <0.9, 40%; and >1.3 or non-compressible, 34%. Only individuals with low ABI had a significantly higher risk for all-cause mortality (hazards ratio (95% confidence interval), HR: 2.23 (1.07, 4.65)). In subjects with initially normal ABI, vascular disease worsened over 23 ± 6 months in 39%; 17% had a decrease in ABI by ≥ 0.1 or a final ABI<0.9, and 21% had a final ABI>1.3 or non-compressible arteries. Previous cardiovascular disease was the only significant predictor of decline in ABI. Over the subsequent 21 ± 16 months, 15% died. However, only individuals who had a declining had a significantly higher risk for death (adjusted HR, 7.41 (1.63, 33.65)).

Conclusions: Peripheral vascular disease is common and progresses rapidly in proteinuric diabetics. Low or declining ABI is a strong predictor of all-cause mortality. Routine measurement of ABI is a simple bedside procedure that can permit early risk stratification in diabetic CKD patients.

Funding: Other NIH Support - NCCR

TH-PO271

Background: Meta-analysis methods for evaluating interactions are not well described. Assessing interactions with eGFR is particularly challenging due to its non-linear association with risk. We describe methods to evaluate point-wise and overall interactions using an example of hypertension–eGFR interaction on mortality.

Methods: To test the applicability of our methods, we selected nine of 46 cohorts joining CKD Prognosis Consortium. In each study eGFR linear splines (knots at each 15 from 15 to 120) and their product terms with hypertension were fitted, providing hazard ratios (HRs) for eGFR (vs. eGFR=90) in both hypertensive and non-hypertensive groups. From this model, the interaction was evaluated as the relative HR (HRR) in hypertension vs. non-hypertension per 1 increment of eGFR from 15 to 120 (point-wise interaction). HRs and HRRs for each eGFR value in each cohort were pooled using random effects models. The overall interaction was assessed as the inverse-variance average of difference in all spine coefficients between hypertensive vs. non-hypertensive groups.

Results: There were 47,893 participants and 7,468 deaths. Low eGFR was associated with increased mortality in the hypertensive (gray line) and normotensive (black line) groups (figure). As depicted by significant HRdashes (dashed line), significant interactions between hypertension status were observed at eGFR ranges of 30-40, 85-90, and 100-115. However, the overall interaction was not significant (P=0.44).

Conclusions: Peripheral vascular disease is common and progresses rapidly in proteinuric diabetics. Low or declining ABI is a strong predictor of all-cause mortality. Routine measurement of ABI is a simple bedside procedure that can permit early risk stratification in diabetic CKD patients.

Funding: Other NIH Support - NCCR

TH-PO269
The Relationship between Minnesota Code Findings and CKD: Results from the CKD-JAC Study - Satosho Imuro, 1 Takeo Okada, 2 Enyu Imai, 2 Kosaka Nitta, 1 Tsuyoshi Watanabe, 1 Setichi Matsuo, 1 Tadao Akizawa, 1 Hirofumi Makino, 1 Yasuo Ohashi, 1 Akira Hishida. 1 University of Tokyo, Japan; 2 CKD-JAC Study Group.

Background: The Japan CKD cohort study (CKD-JAC) was established in September 2007 to prospectively study the renal and cardiovascular outcomes in 2,977 Japanese patients with CKD stage 3-5 visiting 17 outpatient clinics in Japan. To evaluate the electrocardiographic (ECG) findings at enrollment in the study, ECG waveforms obtained from 1,536 patients were analyzed according to the Minnesota Code.

Methods: Two experts independently coded the waveforms using the Minnesota code. In the cases where the two had different opinion in coding a waveform, another expert jointed to consult and decide which code to apply. Only the major classification codes and lead information were subjected to the analysis.

Results: Subjects’ characteristics were: mean age, 61.9 ±(±11.2) years; diabetes, 41.7%; hypertension, 46.6%; hyperlipidemia, 46.6%; CKD stage 3-5, 39.3%; and CKD stage 5, 15.6%. Patients without ECG abnormalities (Code1-0-0) accounted only for 20.2%. The common findings were High Amplitude R waves (16.7%), ST abnormalities (16.5%), and T-wave abnormalities (26.4%). When stratified by CKD stage, these wave abnormalities increased as the CKD stage advanced. When stratified by diabetes, the ratio of ST or T-wave abnormalities decreased in patients without diabetes but with glomerulonephritis.

Conclusions: This study, which we demonstrated that the Minnesota code findings in CKD patients. The findings well reflected CKD stage and patients’ characteristics, which suggests that ECGs contain a host of clinical information. This report will be valuable basic data for epidemiological studies.

Funding: Pharmaceutical Company Support

TH-PO270
Change in Ankle-Brachial Indices over Time and Mortality in Diabetics with Proteinuria - Sirin Iwawakon, 1, 2 Sharon G. Adler, 1 Rajnish Mehrotra. 1 Medicine, Los Angeles Biomedical Research Institute at Harbor-UCLA, Torrance, CA; 2 Medicine, Hatayi Hospital, Hatayi, Songkhla, Thailand.

Background: Peripheral vascular disease is common in diabetic chronic kidney disease (CKD) and is characterized either by abnormally low or high ankle-brachial index (ABI). Whether low or high ABI and the direction of change over time carry similar prognostic value is currently unknown.

Methods: The association of ABI with all-cause mortality over 40 ± 21 months (mean ± SD) was tested in a prospective cohort of 167 individuals with diabetic CKD (age, 57 ± 7 years; median urine protein-creatinine, 2.5 g/g; estimated glomerular filtration rate, 58 ± 23 ml/min/1.73 m²). Association of change in ABI with all-cause mortality was determined in the sub-group of 75 subjects with normal ABI at baseline.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Dietary Protein Intake in Chronic Kidney Disease from the National Health and Nutrition Examination Survey

Linda W. Moore,1 Laura Byham-Gray,2 James S. Parrott,2 Diane Radier,3 Stephen L. Jones,4 Sreedhar A. Mandayam,5 William E. Mitch,6,7 A. Osmaya Gabber,4,8 Surgery, The Methodist Hospital, Houston, TX; 6SHRP, Nutrition, UNMDJ, Newark, NJ; 8Nephrology, Baylor College of Medicine, Houston, TX.

Background: Dietary protein intake (DPI) is important for metabolic balance and tissue maintenance in the institution of the International Protein (IOM) recommendation. DPI that is adequate for 97%-98% of the healthy adult population. This amount, 0.8 kg/day and the estimated average requirement (EAR) 0.69g/kg/day, fulfill their criteria. The Kidney Disease Outcomes Quality Initiative (KDOQI) for Nutrition recommends 0.6-0.75g/kg/day for stage 4 CKD. We evaluated the DPI of people across CKD stages for differences from IOM and KDOQI Guidelines and from DPI of adults without CKD.

Methods: Data from NHANES 2001-2008 were analyzed for the presence of CKD (MDRD formula, staged according to KF guidelines). DPI was assessed from 24-hr recall, systematically collected by trained interviewers using the Automated Multiple Pass Method. Records from each of the days/week were seasonally obtained. Complex survey analyses were used to report population estimates of CKD, and DPI at each stage of CKD and those who did not have evidence of CKD (NoCKD, no proteinuria, eGFR ≥60).

Results: Of 41,628 NHANES participants, 16,872 (40.5%) were ≥20 years of age and a CKD stage was established. 70% of NoCKD and 60% of stages 1-3 had DPI above the estimated average, while DPI of 50% of those in stages 4 & 5 (not receiving dialysis) exceeded the KDOQI guidelines. Using DPI of NoCKD as the comparator (mean±SE, 1.34±0.01 g/kg/d), lower DPI was reported by adults at stage 2 CKD (1.27±0.03, p<0.001), stage 3 CKD (1.14±0.02, p<0.001), and stages 4 & 5 CKD (not receiving dialysis, 1.04±0.05, p<0.0001). Results from 10% of NoCKD and 20% of stages 2-5 (not yet receiving dialysis) had DPI below IOM or KDOQI Guidelines.

Conclusions: DPI of adults with CKD differed significantly from NoCKD. From stage 2-5 CKD, DPI decreased, but at each stage, the level exceeded IOM or KDOQI Guidelines, only 20% were at risk of eating an inadequate DPI. The majority of adults with or without CKD consume excess protein.

Screening for Early Impairment of Glomerular Filtration Rate by Means of Urinary Beta-Trace Protein

Carlo Donadi,1 Danika Tognotti, Angeliki Kanaki, Elena Donadà. Internal Medicine, Nephrology, University of Pisa, Italy.

Background: The screening for chronic kidney diseases (CKD) patients with impaired GFR needs the measurement of serum creatinine (SCr) or cystatin C (CsCy).

Methods: Aim of this study was to evaluate the possibility to screen patients with a GFR < 90 mL/min/1.73 m², by means of serum levels and urinary excretion of different low molecular weight proteins (LMWP), cystatin C (CsCy), β2-microglobulin (β2M), retinol-binding protein (RBP), beta-trace protein (BTP), and by means of the derived prediction equations for GFR.

Results: Two hundred fifty adult CKD patients, with various degree of impairment of renal function (SCr 0.40-12.1 mg/dL), were examined. In the 295 CKD patients (females 137), at all CKD stages, a very high correlation was found between GFR (eGFRMS), and dietary protein intake in the total group (r=0.80; p<0.0001).

Conclusions: In conclusion, serum levels of LMWP are not more sensitive or accurate than serum creatinine or cystatin C as indicators of GFR impairment. Urinary BTP had a positive predictive value of 85% for GFR<90 mL/min/1.73m², while urinary BTP increased significantly only in patients with GFR<30 mL/min/1.73m². In this selected group of CKD patients, the positive predictive value of urinary BTP for GFR<90 mL/min/1.73m² was 85%, indicating that, in CKD patients, a urine-based test can predict a slight GFR impairment.

Funding: Government Support - Non-U.S.
Conclusions: Distinct metabolic phenotypes are reproducibly associated with eGFR and CKD, reflecting altered metabolic clearance, synthesis or conversion. Longitudinal studies should clarify whether changes in metabolic phenotypes precede or result from kidney function impairment.

Funding: Government Support - Non-U.S.

TH-PO277

CKD Progression Depends upon Degree of Proteinuria but Not Blood Pressure or Serum Bicarbonate Levels in Stage 3 and 4 CKD Patients

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Background: In several large prospective studies, proteinuria, blood pressure (BP), and serum bicarbonate (CO2) were associated with a more rapid decline in renal function in CKD patients. We sought to confirm these relationships in a cohort of unselected CKD patients from a variety of US primary care and nephrology practices.

Methods: We performed a retrospective analysis of longitudinal data collected on 1721 stage 3 (n=1194) and stage 4 (n=527) US CKD patients enrolled in the Litholink CKD program, a national, website-based CKD registry with robust follow-up. Presence of proteinuria (n=790) was defined as albumin:creatinine ratio >30 mg/g or protein:creatinine ratio >200 mg/g at enrollment. We excluded patients with a follow-up period of 50 days or less.

Results: In a mean follow-up period of 184 and 182 days, respectively, patients with proteinuria had a mean fall in eGFR of 1.2 ± 0.3 ml/min/1.73m², while patients without proteinuria had a mean rise in eGFR of 1.7 ± 0.3 ml/min/1.73m². Within stage, significant differences between proteinurics and non-proteinurics remained. For both groups, patients in stage 3 had mean decline in eGFR of 1.1 ml/min/1.73m², while stage 4 patients had mean increase in eGFR of 1.5 ml/min/1.73m². In a general linear model with change in eGFR as the dependent variable, and presence of proteinuria, CKD stage, and the cross product of proteinuria with CKD stage as independent variables, presence of proteinuria and CKD stage were both significant predictors of change in eGFR (p<0.001). The cross product was not significant. Initial CO2, initial BP, final BP, and gender did not enter the model.

Conclusions: In this cohort of unselected, US stage 3 and 4 CKD patients, eGFR fell more rapidly in patients with proteinuria than in those without proteinuria. BP and CO2 levels were not predictive factors for eGFR decline in this cohort during this short follow-up period. For both groups, stage 4 patients had slight improvement in mean eGFR during the follow-up period when compared to stage 3 patients. Reasons for this are presently unclear and warrant further study.

TH-PO278

Nationwide Survey of Familial Juvenile Hyperuricemic Nephropathy (FJHN) Caused by UMOD Mutations

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Background: FJHN is an autosomal dominant (AD) disease, caused by uromodulin (UMOD) mutations in 40% of patients. FJHN leads to end-stage renal disease (ESRD). This study examines FJHN epidemiology on a nationwide basis.

Methods: All Austrian dialysis and transplant patients are documented in a nationwide database. Following a database query in 2001, patients with unclear renal diagnoses or genetic testing were asked whether they had (a) family members with kidney disease, and (b) gout. A detailed family history was obtained in patients who provided 2 affirmative answers; if this was suggestive for FJHN, UMOD was genotyped. In addition, data from all Austrian FJHN families known in 2010 were collected. Cox analysis was employed to test for a relationship between UMOD genotype and progression to ESRD.

Results: Based on the database query in 2001, 541 out of 6210 patients were asked to participate in the questionnaire; 19 out of 353 responders gave 2 affirmative answers. A nephropathy compatible with FJHN was present in 7 of them; in 1 patient an UMOD mutation was identified. Independent of the screening project, 4 families were diagnosed with clinical UMOD deficiency. In 2010, 17 FJHN patients from 5 families with UMOD mutations lived in Austria (2.0 per million population), 6 of them required renal replacement therapy (0.73 per 1000 patients). Progression to ESRD was significantly different for patients with different UMOD mutations.

Conclusions: This first nationwide survey of FJHN caused by UMOD mutations demonstrates its infrequency. FJHN patients in the ESRD population can be identified by a simple questionnaire and a subsequent focused family history. Our data indicate that UMOD genotype may modify renal disease progression.

TH-PO279

Enteric Infections Are a Risk Factor for Subclinical Proteinuria in Childhood

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Background: India is currently facing an epidemic of chronic kidney disease (CKD), which affects a younger demographic than in the West. The ‘hygiene hypothesis’ suggests that chronic antigenic stimulation from the environment could generate a low grade nephritogenic immune complex response and increase risk for CKD. We hypothesize that recurrent diarrheal infections and gut antigen exposure in infancy, together with malnutrition, may be a risk factor for CKD in later life.

Methods: We studied a birth cohort of 305 children from an urban slum in South India for enteric infections and nutritional status, from birth (2002) until age 3. At age 7-8 years (2010) we measured anthropometry, blood pressure (BP) and urinary protein creatinine ratio (UPCR). Linear regression was used to determine the association of UPCR with BMI Z-score and severity of diarrhea (measured by Vesikari score).

Results: At age 7-8 years, 30% were underweight, 9% stunted and 92.5% had experienced one or more episodes of diarrhea in infancy (median, IQR 4, 2-7 episodes). Proteinuria (UPCR >200) was seen in 8%, and 13% had elevated BP (>90th% systolic BP for age and height). Severity of diarrhea, current malnutrition (BMI Z-score) and decreased growth velocity from age 3-7 (change in BMI Z-score) were independent predictors of current UPCR (p<0.05).

TH-PO280

Cystatin C Predicts End-Stage Renal Disease Better Than Iothalamate GFR in Patients with Macroalbuminuria and Type 2 Diabetes

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Background: We compared the predictive values of cystatin C (cysC), serum creatinine (SCr), and iothalamate GFR (GFR) for diabetic end-stage renal disease (ESRD) in patients with type 2 diabetes and macroalbuminuria.

Methods: Individuals were followed from their first diabetic examination with macroalbuminuria (ACR ≥300 mg/g) until December 2010, onset of ESRD, or death.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
whichever came first. Incidence rate ratios (IRR) adjusted for age, sex, and diabetes duration were computed by Mantel-Haenszel stratification. The ability of these variables to predict eGRD was compared with receiver operating characteristic (ROC) curves calculated from Cox regression models that included age, sex, diabetes duration, height, weight, HbA1c, ACR and the relevant markers.

Results: 134 Pima Indians with a median age of 44.9 years (range 25.5-67.8 years) were followed for a median of 8.9 years (range 1.5-20.9 years). eGRD developed in 63 subjects. eGRD incidence was significantly higher among patients in the lowest vs. highest tertiles of 1/cysC and iGFR (IRR for 1/cysC=3.5, 95%CI 1.7-7.4; IRR for iGFR=2.4, 95%CI 1.2-4.9), but did not differ significantly for 1/SCr (IRR=1.5, 95%CI 0.8-2.6). The model including all three filtration markers had the highest AUC, but its AUC was indistinguishable from the model with 1/cysC alone. By contrast, models with 1/SCr or iGFR alone had significantly lower AUCs than either the full model or the model with 1/cysC alone.

Conclusions: CysC was a better predictor of diabetic eGRD than either SCr or iGFR in Pima Indians with type 2 diabetes and macroalbuminuria.

Funding: Other U.S. Government Support

TH-PO281

Black Race Is Associated with Significantly Steeper Decline in Glomerular Filtration Rate (GFR) in a Large Cohort of US Veterans with Non-Dialysis Dependent CKD

Csbasa P. Kovesedy,1,2 Evan H. Lott,1 Jun Ling Lu,4 Sandra M. Malakauskas,1,2 Jennie Z. Ma,3 Mark D. Okusa,3 Kamyar Kalantar-Zadeh.3 1VA Medical Center; 2University of Virginia; 3VA Informatics and Computing Infrastructure; 4Salem Research Institute; 5Harbor-UCLA.

Background: The high prevalence of blacks among patients with eGRD could be due to race-specific differences in the progression of CKD. Causes of faster progression of CKD in blacks with CKD need to be better characterized.

Methods: We compared slopes of eGFR in 48,692 blacks and 422,074 whites in a nationally representative cohort of US veterans with CKD stages 1-4 in 2005-2006. Slopes were calculated using a median (interquartile range) of 8 (5-13) eGFR values over up to 5 years. Unadjusted associations (Model 1) of black race with the odds of steeper slopes (defined as slopes ≤-0.4 ml/min/1.73m2/year) were examined in logistic regression models. The effects of sociodemographic characteristics (Model 2), comorbidities (Model 3), blood pressure (Model 4) and laboratory variables (Model 5) on racial differences in the slopes of eGRD were explored in multivariable models.

Results: Blacks were younger, more likely to have diabetes, and had higher blood pressure, lower bicarbonate and hemoglobin, and higher cholesterol. The median (IQR) of eGFR slopes in blacks was -1.38 (1.07-1.70) ml/min/1.73m2/year and in whites was -0.88 (-3.30, 1.27), p=0.001. Black race was associated with steeper slopes (crude odds ratio (95%CI): 1.47 (1.44-1.50), p<0.001). Adjustments lead to substantial attenuation in this association (Figure).

Conclusions: Black race was associated with steeper slopes of eGFR in patients with CKD. Most of this difference is explained by identifiable risk factors of progressive CKD. The effect of race-specific interventions to slow CKD progression in blacks needs to be tested in clinical trials.

Funding: NIDDK Support, Veterans Administration Support

TH-PO282

CKD Stage Modifies the Association of Urine Microalbumin–Creatinine Ratio (UACR) with Mortality in a Large Cohort of US Veterans

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Background: The association of albuminuria with mortality in patients with various stages of CKD is not well characterized.

Methods: We explored the association of UACR with all-cause mortality in a nationally representative cohort of 298,779 US veterans with eGFR >15 ml/min/1.73m2, whereas in patients with eGFR<30 ml/min/1.73m2 this association appeared U-shaped (Figure).

Conclusions: Higher UACR is independently associated with increased all-cause mortality. This association incremental in patients with eGFR≥30 and U-shaped in patients with eGFR<30. Interventions targeting proteinuria-lowering as a means to improve survival in patients with CKD should consider CKD stage as a potential effect modifier.

Funding: NIDDK Support, Veterans Administration Support

TH-PO283

The Relationship between Pulmonary Emphysema and Kidney Function in Smokers

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Background: Kidney disease is prevalent in patients with COPD. We hypothesized that kidney dysfunction would associate with emphysema rather than with airway obstruction in patients with COPD, and the association would be independent of common risk factors for kidney disease.

Methods: 508 participants with >10 pack year smoking history completed a chest CT scan, pulmonary function tests, and measurement of serum creatinine. Glomerular filtration rates (eGFR) were estimated using the method of the Chronic Kidney Disease Epidemiology Collaboration. Severity of emphysema was determined by density mask examination the chest CT.

Results: Mean age of the 508 participants was 66 ± 7 years and mean eGFR 101 ± 22 ml/min/1.73m2. Percent emphysema was a significant univariate predictor of eGFR: each 10% increase in emphysema was associated with a 3.7 ml/min/1.73 meter2 decline in eGFR (p=0.002), unlike FEV1, which was not a significant predictor.


Funding: Other NIH Support - 1P50 HL084948, P50-CA90440, R01 HL085096, and UL1 RR024153

The association between emphysema and eGFR was also present on multivariate analysis: each 10% increase in emphysema was associated with a 4.4 ml/min/1.73 meter2 decline in eGFR (p = 0.007) independent of airflow obstruction (FEV1), age, gender, diabetes mellitus, hypertension, coronary artery disease, patient reported dyspnea, and pack-years of smoking.

Conclusions: Worsening emphysema, rather than airflow obstruction, predicts kidney dysfunction in patients with COPD, independent of common risk factors for kidney disease. In smokers with kidney dysfunction undetected emphysema may be contributing to diminished exercise capacity and quality of life. This is the first description of a possible emphysema – kidney injury phenotype; further investigation of shared molecular and mechanistic links between emphysema and kidney dysfunction is needed.

Funding: Other NIH Support - 1P50 HL084948, P50-CA90440, R01 HL085096, and UL1 RR024153
TH-PO284

Apolipoprotein L1 (APOL1) Nephropathy Risk Variants Associate with HDL Subfraction Concentration in African Americans

Barry I. Freedman,1 Carl D. Langefeld,2 Mariana Murea,2 James D. Otivs,2 Jolyn Turner,2 Peter A. Antinolizzi,1 Michael V. Rocoe,2 John S. Parks,3,4 Department of Internal Medicine-Nephrology, Wake Forest School of Medicine, Winston-Salem, NC; 2Division of Public Health Sciences, Wake Forest School of Medicine; 3Department of Biochemistry, Wake Forest School of Medicine; 4Department of Pathology-Lipid Sciences, Wake Forest School of Medicine; 1Liposcience, Ltd, Raleigh, NC.

Background: Coding variants in the APOL1 gene (G1: rs7385519, rs6090145 and G2: rs71758313) are strongly associated with non-diabetic etiologies of nephropathy in African Americans. APOL1 proteins associate with HDL particles in the circulation.

Methods: We compared plasma HDL particle subclass concentrations in 73 African Americans based on their APOL1 genotypes to detect differences that could potentially contribute to kidney disease. These individuals were first-degree relatives of patients with non-diabetic end-stage renal disease (ESRD). HDL subclass concentrations were measured using nuclear magnetic resonance spectroscopy. Participants had estimated glomerular filtration rates (eGFR) > 80 ml/min and lacked albuminuria. Additive effects of the number of APOL1 risk variants on natural logarithm transformed HDL subclass concentrations were computed. Analyses were performed unadjusted and after adjustment for log serum triglyceride concentration.

Results: Participants were 58.9% female with mean ± SD age 47.2 ± 13.3 years and eGFR 92.4 ± 18.8 ml/min. The numbers with 2, 1, and 0 APOL1 nephropathy risk variants, respectively, were 36, 17, and 20. Mean ± SD medium-sized HDL concentrations were significantly lower for each additional APOL1 risk allele (2 vs. 1 vs. 0 risk alleles: 9.0 ± 5.6 vs. 10.1 ± 5.5 vs. 13.1 ± 8.2 apoA1/L, respectively; p = 0.0192 unadjusted; p = 0.0190 triglyceride-adjusted).

Conclusions: Lower medium-sized HDL subclass concentrations are present in African Americans based on increasing numbers of APOL1 nephropathy risk alleles. Mechanistic roles for altered medium HDL concentrations on susceptibility to microvascular diseases should be evaluated.

Funding: NIDDK Support

TH-PO285

β-Blocks and Outcomes in Systolic Heart Failure with Chronic Kidney Disease

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Background: There are few data regarding the effectiveness and safety of oral β-blockers (BBs) in patients with systolic heart failure (HF) complicated by chronic kidney disease (CKD).

Methods: We identified 1241 adults in Kaiser Permanente of Northern California with incident clinical HF and reduced left ventricular (LV) function between 2006-2008. We excluded patients with baseline BB use or prior dialysis. Using outpatient serum creatinine values during the year before or on the index date, we classified patients as having CKD if estimated glomerular filtration rate (eGFR) < 60 ml/min per 1.73 m² (n=627). Patients were followed for incident BB use and the outcomes of all-cause death and HF hospitalization through 8/31/10 identified from health plan databases. We analyzed the association of time-varying new BB use with outcomes using extended Cox regression, with adjustment for demographic factors, socioeconomic status, medical co-morbidities, and other potential confounders.

Results: Of 93,148 patients included for analysis, 10.1% were micropolitan and 9.8% were rural. Of 102,700 patients included in analysis, 10.2% were micropolitan and 9.9% were rural. Rural and micropolitan patients were older, less racially diverse, had more medical co-morbidities, and more likely to choose peritoneal dialysis compared to urban patients. After adjustment, rural patients were modestly more likely to have early referral to a nephrologist (RR 1.04 CI 1.01-1.08) compared to urban patients. Despite this, both micropolitan and rural patients were significantly less likely to have received dietary education (RR 0.85 CI 0.79-0.91 and RR 0.85 CI 0.80-0.91, respectively) and to have a visit in the setting of moderate to severe anemia (RR 0.89 CI 0.82-0.97 and RR 0.91 CI 0.83-0.98, respectively). There were no significant differences in the likelihood of peritoneal vascular access placement or pre-emptive transplant listing.

Conclusions: Non-urban residence is associated with less erythropoietin use in anemic patients and lower likelihood of dietary education prior to the initiation of RRT, despite timely nephrology referral. Non-urban living may be associated with worse pre-RRT care.

Funding: NIDDK Support

TH-PO286

Rural and Micropolitan Disparities in Dialysis Mortality and Likelihood of Kidney Transplantation

Saugar Maripuri, Talat Alp Ikizler, Kerri L. Cavanaugh. Vanderbilt University Medical Center, Nashville, TN.

Background: Rural and micropolitan dialysis patients face unique challenges when initiating renal replacement therapy (RRT), including barriers to healthcare access. It is not known if such barriers increase risk for mortality or decrease likelihood of kidney transplantation among rural and micropolitan patients.

Methods: A retrospective cohort study was performed utilizing data from the US Renal Data System (USRDS). Patients >18 years of age who initiated RRT between 1/2006 and 12/2006 were included and classified as rural, micropolitan, or urban based on ZIP code specific rural commuting codes. Outcomes of interest included time to death >90 days after initiation, time to wait-listing for kidney transplant, and time to kidney transplantation. Censoring was determined at loss to follow-up or 1/2009. Cox proportional hazard regression models were created for each outcome while adjusting for demographic factors, socioeconomic status, medical co-morbidities, eGFR at initiation, dialysis modality, achieved pre-ESRD care, and other potential confounders.

Results: Of 93,148 patients included for analysis, 10.1% were micropolitan and 9.8% were rural. Of 102,700 patients included in analysis, 10.2% were micropolitan and 9.9% were rural. Rural and micropolitan dialysis patients were older, less racially diverse, had more medical co-morbidities, and more likely to choose peritoneal dialysis compared to urban patients. Mortality for hemodialysis patients was similar across geographic strata. There was no difference in the likelihood of wait-listing for transplant, but rural and micropolitan patients were more likely to be transplanted (L1.21 CI 1.09-1.34 and L1.12 CI 1.01-1.23, respectively) compared to urban PD patients. Mortality for hemodialysis patients was similar across geographic strata. There was no difference in the likelihood of wait-listing for transplant, but rural and micropolitan patients were more likely to be transplanted (L1.21 CI 1.09-1.34 and L1.12 CI 1.01-1.23, respectively) compared to urban patients.

Conclusions: Non-urban residence is associated with increased risk for mortality among patients on PD. However, non-urban patients appear more likely to undergo kidney transplantation. Observed relationships persisted after adjustment for achieved pre-ESRD care.

Funding: NIDDK Support

TH-PO287

Achieved Pre-ESRD Care Is Associated with Greater Likelihood of Kidney Transplantation

Saugar Maripuri, Talat Alp Ikizler, Kerri L. Cavanaugh. Vanderbilt University Medical Center, Nashville, TN.

Background: Small studies have shown that planning for the initiation of renal replacement therapy (RRT), also known as pre-ESRD care, is associated with decreased mortality and maintenance dialysis. Little is known of the impact of pre-ESRD care on the likelihood of kidney transplantation.

Methods: A retrospective cohort study was performed utilizing data from the US Renal Data System (USRDS). Patients >18 years of age who initiated RRT between 1/2006 and 12/2006 were included. Patients deemed unsuitable for transplant were excluded. Each subject was assessed for the following prior to initiation of RRT: nephrology referral >12 months prior, permanent vascular access placement (AVF or AVG), dietician referral, and pre-emptive transplant listing. Outcomes of interest included incidence of wait-listing...
and kidney transplantation. Screening was determined at loss to follow-up or 10/1/2009. Possible confounders listed and adjusted for demographic factors, socioeconomic status, medical co-morbidities, kidney function (eGFR) at initiation, and other potential confounders.

Results: 87,294 patients were included for analysis. The overall transplantation rate was 11.3% in ESRD patient-years. After adjustment, early nephrology referral (IRR 1.23 CI 1.18-1.29), permanent access placement (IRR 1.20 CI 1.14-1.26), and dietary referral (IRR 1.19 CI 1.13-1.26) were associated with increased likelihood of wait-listing. Early nephrology referral (IRR 1.27 CI 1.20-1.34), dietary referral (IRR 1.20 CI 1.12-1.29), and preemptive listing (IRR 3.65-4.20) were associated with decreased likelihood of kidney transplantation. Patients who were listed for transplant within 90 days of RRT initiation were significantly less likely to be transplanted (IRR 0.91 CI 0.82-0.99) compared to those who were pre-emptively listed prior to ESRD.

Conclusions: ESRD care is associated with increased likelihood of kidney transplantation after initiation of RRT. Listing prior to onset of ESRD significantly increases the likelihood of transplantation, even when compared to patients listed shortly after initiation of RRT.

Funding: NIDDK Support

TH-PO289

Life Expectancy of Chinese Patients with Chronic Kidney Disease without Dialysis Chu-Ben Leung, Cheuk-Chun Szeto, Bonnie Kwan, Kai Ming Chow, Philip K.T. Li.

Methods: We reviewed 63 consecutive ESRD patients who were treated conservatively in our center. Duration of survival was computed from the date of initial assessment for dialysis, as well as the expected date of needing dialysis based on previous trend of renal function decline.

Results: At the end of the observation period, 55 patients died. Twelve patients died before the expected date of needing dialysis because of unrelated reasons, while 36 deaths were directly attributed to uremia. The median overall survival after initiation of RRT was 41.3 months (95% confidence interval [CI], 33.2 to 49.4 months). The median overall survival was 4.43 months (inter-quartile range, 4.80 to 8.85 months) from the expected date of needing dialysis. The survival from the expected date of needing dialysis did not correlate with patient age, sex, diabetic status, or baseline renal function.

Conclusions: In Chinese ESRD patients treated conservatively, the median survival is around 4 months after the expected date of needing dialysis. Our result provides an important piece of information for the decision of dialysis and patient counseling.

Funding: Government Support - Non-U.S.

TH-PO290

Different Impacts of Pulse Pressure on Proteinuria or Low eGFR between Diabetic and Non-Diabetic Populations Yui Sato, Yuichiro Yano, Shouichi Fujimoto, Tsuneo Konta, Tsuyoshi Watanabe.

Background: Systolic blood pressure (SBP) is an established risk factor for proteinuria (PU). However, in ESRD patients with multiple comorbid conditions, diastolic may actually be futile, and conservative management is advisable. We studied the life expectancy of Chinese ESRD patients treated conservatively.

Methods: We reviewed 63 consecutive ESRD patients who were treated conservatively in our center. Duration of survival was computed from the date of initial assessment for dialysis, as well as the expected date of needing dialysis based on previous trend of renal function decline.

Results: At the end of the observation period, 55 patients died. Twelve patients died before the expected date of needing dialysis because of unrelated reasons, while 36 deaths were directly attributed to uremia. The median overall survival after initiation of RRT was 41.3 months (95% confidence interval [CI], 33.2 to 49.4 months). The median overall survival was 4.43 months (inter-quartile range, 4.80 to 8.85 months) from the expected date of needing dialysis. The survival from the expected date of needing dialysis did not correlate with patient age, sex, diabetic status, or baseline renal function.

Conclusions: In Chinese ESRD patients treated conservatively, the median survival is around 4 months after the expected date of needing dialysis. Our result provides an important piece of information for the decision of dialysis and patient counseling.

Funding: Government Support - Non-U.S.

TH-PO291


Background: Emerging data suggest that increased serum uric acid (sUA) is an independent risk factor marker for cardiovascular (CV) complications. Treatment with the angiotensin-receptor-blocker (ARB) losartan lowers sUA in contrast to other ARBs. Whether reductions in sUA during losartan therapy are associated with CV protection is unclear. We aimed to test this hypothesis.

Methods: In a post-hoc analysis of the Reduction of Endpoint in Non-insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials, we determined the relationship of a short-term change in sUA with long-term CV outcome by means of Cox regression.

Results: Compared to placebo, losartan significantly lowered sUA (0.16 mg/dL [95%CI 0.01 to 0.03], p=0.30). Each 0.5 mg/dL decrement in sUA during losartan treatment in the first 6 months resulted in a reduction of long-term CV outcome of 5.3% (95%CI 0.9 to 9.9, p=0.017). Adjustment of the CV treatment effect of losartan for month 6 change in sUA attenuated the effect from 9.2% (95% CI-7.9 to 23.6) to 4.6% (95% CI 16.2 to 22.0), suggesting that part of the protective CV effect of losartan is attributable to its effect on sUA.

Conclusions: Losartan but not irbesartan significantly lowers sUA compared to placebo in patients with type 2 diabetes and nephropathy. The degree of reduction in sUA explains part of the CV effect of losartan. The results suggest that the use of type 2 diabetes with nephropathy sUA may be a modifiable risk factor for CV disease.

Funding: Government Support - Non-U.S.

TH-PO292

Epidemiology and Outcomes of Acute Kidney Injury after Cardiac Surgery Robert I. Griffiths, Michelle Gleeson, Viken Paragamian, Mark D. Danese, Robert M. Brenner.

Background: While there is substantial literature on associations between various definitions of acute kidney injury (AKI) and outcomes, we sought to examine the relationships between the development of AKI at 24 hours or at 48 hours after cardiac surgery and hospital length of stay, receipt of dialysis, kidney recovery, and mortality.

Methods: Using data from Cerner Health Facts, we identified 6,967 patients across 53 hospitals in the United States who had cardiac surgery between 2001/2000 and 2006/2010, and also had an increase from baseline serum creatinine ([SCr]) 1-14 days before surgery) to ≥ 0.3 mg/dL ≥ 0.5 mg/dL, ≥ 50%, or ≥ 100% at 24 or at 48 hours after surgery (8 non-mutually exclusive groups). Patients were followed for changes in SCR until hospital discharge or death. We used Kaplan-Meier analysis to describe the time to resolution of AKI, defined as the first SCR ≤ baseline SCR. Also, we described hospital length of stay, dialysis use (any), and discharge status (alive/dead) in the 8 groups.

Results: AKI was detected in 3,594 (52%) patients at 24 hours and in 3,373 (48%) patients at 48 hours after surgery. The average age in the cohort was 68 years, 67% were male, 83% had ≥ 2 SCR values after surgery, and the mean baseline SCR was 1.37 mg/dL. Development of AKI at 24 hours was associated with greater risk of dialysis (3.5%) and hospital mortality (10.3%), and extended time to kidney recovery, compared to development of AKI at 48 hours (dialysis use 2.2%; mortality 5.3%). Patients in the SCR ≥ 100% group had the highest risk of mortality (31.5% for AKI at 24 hours; 11.5% for AKI at 48 hours). However, there were only 405 (5.8%) patients in this group. The 0.5 mg/dL group had a greater risk of dialysis than did the 50% and 100% groups, likely indicative of the fact that the 50% and 100% groups included patients with very low baseline SCR values.

Conclusions: Earlier development of AKI, at 24 hours after surgery, was associated with worse clinical outcomes. Also, compared to a SCR increase of ≥ 0.3 mg/dL, an increase of ≥ 0.5 mg/dL was associated with higher mortality and dialysis use in this population.

Funding: Pharmaceutical Company Support

TH-PO293

Prescription of and Adherence to Cardioprotective Drug Therapy in Chronic Kidney Disease Patients: Impact of Referral to Pre-Dialysis Clinics Lorraine Fradette, Marc Dorais, Heloise Cardinal, Jacques Lelorier, Francois Madore.

Background: Emerging data suggest that increased serum uric acid (SUA) is an independent risk factor marker for cardiovascular (CV) complications. Treatment with the angiotensin-receptor-blocker (ARB) losartan lowers sUA in contrast to other ARBs. Whether reductions in sUA during losartan therapy are associated with CV protection is unclear. We aimed to test this hypothesis.

Methods: In a post-hoc analysis of the Reduction of Endpoint in Non-insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials, we determined the relationship of a short-term change in sUA with long-term CV outcome by means of Cox regression.

Results: Compared to placebo, losartan significantly lowered sUA (0.16 mg/dL [95%CI 0.01 to 0.03], p=0.30). Each 0.5 mg/dL decrement in sUA during losartan treatment in the first 6 months resulted in a reduction of long-term CV outcome of 5.3% (95%CI 0.9 to 9.9, p=0.017). Adjustment of the CV treatment effect of losartan for month 6 change in sUA attenuated the effect from 9.2% (95% CI-7.9 to 23.6) to 4.6% (95% CI 16.2 to 22.0), suggesting that part of the protective CV effect of losartan is attributable to its effect on sUA.

Conclusions: Losartan but not irbesartan significantly lowers sUA compared to placebo in patients with type 2 diabetes and nephropathy. The degree of reduction in sUA explains part of the CV effect of losartan. The results suggest that the use of type 2 diabetes with nephropathy sUA may be a modifiable risk factor for CV disease.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
The percentage of days, during a pre-defined observation period, in which patients have been on schedule with their prescribed medications.

**Results:** This cohort study included 385 CKD patients (stage 4 and 5). Mean glomerular filtration rate at the time of referral was 15.2/ml/min/1.73m². Mean age was 63 ± 13 years and 59% of patients were males. At the time of the first visit in the pre-dialysis clinic, 39% of patients were prescribed SSA (including statins and fibrates). This proportion increased to 47% (McNemar p<0.01) during follow-up. Similarly, there was a significant increase in the prescription of ACE inhibitors (from 34 to 39%), Angiotensin II receptor blockers (from 11 to 14%), β-blockers (from 40 to 51%), and diuretics (from 66 to 78%) (all p-values <0.05). This increase persisted to LLA and AHA post referral. Reduction in blood pressure significantly increased in the year following the first visit to the pre-dialysis clinics compared with the preceding year (before: 91% and 95%; after: 84% and 95%; Mann-Whitney p<0.05).

**Conclusions:** In summary, these results suggest that referral to pre-dialysis clinics may increase the prescription of cardio-protective drug therapy in CKD patients but does not appear to improve the adherence to these therapies.

**Funding:** Government Support - Non-U.S.

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**TH-PO294**

**Low Income and Albuminuria among Reasons for Geographic and Racial Differences in Stroke Study Participants Deidra C. Crews,1 William M. McClellan,2 David A. Shoham,3 Liyan Gao,4 David G. Warnock,4 Suzanne E. Judd,5 Paul Muntner,4 Brian D. Bradbury,2 Edgar R. Miller,1 Neil R. Powe,5 Johns Hopkins University, Baltimore, MD; 2Emory University, Atlanta, GA; 3Loyola University Chicago, Maywood, IL; 4University of Alabama at Birmingham, AL; 5Angeion Corporation, Thousand Oaks, CA; 6San Francisco General Hospital and University of California-San Francisco, San Francisco, CA.

**Background:** Socioeconomic factors are believed to contribute to racial disparities in chronic kidney disease. Albuminuria is an important clinical risk factor for progressive chronic kidney disease (CKD), and is more prevalent among blacks than whites. Our objectives were to determine whether an association between low income and albuminuria exists, and whether this association varies by race.

**Methods:** In the population-based Reasons for Geographic and Racial Differences in Stroke (REGARDS) study of 22,828 U.S. adults aged 45 years and older, multivariable logistic regression was used to examine the race-stratified association between low income (annual household income < $20,000) and albuminuria and between level of income and albuminuria.

**Results:** Low income was present for 19.7% of participants; 29.8% of blacks and 12.9% of whites. The geometric mean urinalysis albumin:creatinine ratio (ACR) for blacks (11.8 mg/g) was higher than for whites (9.3 mg/g), p < 0.01. With and without adjustment for demographics, lifestyle factors, comorbid illnesses and kidney function, low income was associated with ACR >30mg/g to a similar degree among blacks [crude odds ratio (OR) 1.5, 95% confidence interval (CI) 1.4-1.7; adjusted 1.2, CI 1.1-1.4] and whites [crude OR 1.8, CI 1.6-2.1; adjusted 1.3, CI 1.1-1.5]. When extremes of income were examined for their association with ACR >300mg/g, low income was statistically significantly associated with albuminuria among blacks (OR 3.4, CI 1.9-6.0, comparing income < $20,000 to >$75,000), but not among whites (OR 1.5, CI 0.9-2.4).

**Conclusions:** Black race and low income are independently associated with an increased prevalence of albuminuria, and may interact in determining disparities in significant albuminuria.

**Funding:** Other NIH Support - National Institute of Neurological Disorders and Stroke, Private Foundation Support

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**TH-PO295**

**Decline in Kidney Function and Coronary Artery Calcium in Young Adults: Results from the CARDIA Study Nishu Bansal,1 Eric Vittinghoff,2 Carmen A. Peralta,3 Chi-Yuan Hsu,1 Michael Shlipak,1 David R. Jacobs,2 David Siscovick,4 Michael Steffes,4 John Jeffrey Carr,4 Kirsten Bibbins-Domingo,1 University of California, San Francisco; 2University of Minnesota; 3University of Washington; 4Wake Forest School of Medicine.

**Background:** Presence of coronary artery calcium (CAC) is associated with future cardiovascular events. Whether kidney function decline is associated with subsequent CAC among young adults with preserved estimated glomerular filtration rate (eGFR>60) is unknown.

**Methods:** The Coronary Artery Risk Development in Young Adults (CARDIA) study is a large biracial cohort of 5,115 young adults (ages 18-30 at enrollment) with over 20 years of follow-up. We included participants who had at least one creatinin C measurement at Years 10, 15, or 20 and CAC measurements at Years 15 or 20. Those with eGFR<60 ml/min/1.73 m² were excluded. We estimated annualized change in eGFR using mixed models, then used a log-link continuation-ratio model with robust standard errors to estimate the association between eGFR and detectable CAC (yes/no), adjusting first for age, race, sex, and time-varying body mass index, diabetes, systolic blood pressure, and then also for microalbuminuria (defined as ≥ 30 mg/g of creatinine in spot urine collections).

**Results:** Among 5,115 participants (67% black, 55% women), mean age was 35 years and mean eGFR was 109 ml/min/1.73m² at the Year 10 examination. Nine percent (273) had detectable CAC at Year 15 and an additional 13% (352) developed detectable CAC by Year 20. Each 1% annual decline in eGFR, over the 10 years from Year 10 to Year 20 was associated with a 25% increased risk of CAC (95% CI 9%-42%, p=0.01) in models adjusted for age, race, sex, body mass index, diabetes and systolic blood pressure. After adding adjustment for microalbuminuria, the risk of detectable CAC was 17%/95% CI 2%-34%, p=0.02.

**Conclusions:** Even small decrements in eGFR among young adults less than 50 years of age with eGFR>60 ml/min/1.73 m² are associated with detectable CAC, potentially signaling future cardiovascular risk.

**Funding:** NIDDK Support

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**TH-PO296**

**Family Income and Type of Renal Replacement Therapy (RRT) in Children with Chronic Kidney Disease (CKD), Results from the CKD Study Guillermo Hidalgo,1 Derek Ng,2 Bradley A. Warady,3 Marva M. Moxey-Mims,1 Susan L. Furth,3 Pediatrics, East Carolina University, Greenville, NC; 2School of Public Health, John Hopkins University, Baltimore, MD; Pediatrics, Children’s Hospital of Philadelphia, Philadelphia, PA; 4Pediatrics, Mercy Children’s Hospital, Kansas City, MO; 5NIDDK, NIH, Bethesda, MD.

**Background:** Low socioeconomic status (SES) has been associated with CKD severity and worst outcomes in adults. We sought to describe the association between SES, determined by family income, and the cumulative type of RRT in the CKD cohort.

**Methods:** Percentages were used to describe the cumulative incidence of RRT (dialysis or transplant), by income category and CKD diagnosis (glomerular vs. non-glomerular) over time since entry in the CKD cohort. Fisher’s exact test was used to determine differences across income categories.

**Results:** There were 572 subjects at enrollment with complete family income data and a median follow up of 3.9 years. The cumulative incidence of RRT was the same across income groups (approximately 20%), indicating homogeneity in disease severity. However the type of RRT was significantly different across income groups. Those with high income were more likely to receive transplants while lower income was associated with dialysis. Particularly, those with non-glomerular cause of CKD.

**Funding:** NIDDK Support

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**TH-PO297**

**Determining National Priorities: Healthy People 2020 Chronic Kidney Objectives Asel Ryukulova,1 Lawrence Agodua,2 Paul W. Eggers,3 Centers for Disease Control and Prevention, Hyattsville, MD; 1National Institutes of Health, Bethesda, MD; 2National Institutes of Health, Bethesda, MD.

**Background:** An estimated 11.5% of adults ages 20 or older had physiological evidence of chronic kidney disease (CKD) determined from data collected through the 1999-2006 National Health and Nutrition Examination Survey (NHANES). Each year in the United States, more than 100,000 people are diagnosed with end stage renal disease (ESRD), the final stage of CKD.

**Methods:** Reflecting the importance of CKD, fourteen CKD objectives area were included as a new topic area in the Healthy People 2010 national health goals to reduce new cases of CKD and its progression, complications, disability, death, and economic costs. The Healthy People 2020 initiative was launched in December 2010.

**Results:** After the revision of the objectives by the Healthy People 2020 work group, all Healthy People 2010 objectives were retained with modifications and 6 new Healthy People 2020 objectives were added. New objectives are focused on monitoring and tracking improvements in cardiovascular care in patients with CKD; increases in proportion of patients with CKD and diabetes who received recommended evaluation and treatment; reductions in death rate and percentage of U.S. population with CKD; and increasing CKD awareness and education in patients with impaired renal function. In addition to the US Renal Data System, National Health and Nutrition Examination Survey and the National Death Index have been analyzed to provide baselines and tracking data for the HP 2020 objectives.

**Conclusions:** The presentation will include a review of key Healthy People 2010 final results for CKD objectives, the Healthy People 2020 CKD objectives and targets, and development process used to identify the Healthy People 2020 CKD objectives.

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
Contribution of Genetic and Environmental Factors to the Association between Preeclampsia and Later End-Stage Renal Disease

Briem Egil Vikse,1 2 Lorentz M. Irgens,1 S. Ananth Kارعمانچ,3 Rolv Skjaerven.1 2 Department of Medicine, Haukeland University Hospital, Bergen, Norway; 3Institute of Medicine, University of Bergen, Bergen, Norway; 4Department of Public Health and Primary Health Care, University of Bergen, Bergen, Norway; 5Beth Israel Deaconess Medical Center, Harvard Medical School, Boston.

Background: Women with preeclampsia have increased risk of later developing end-stage renal disease (ESRD). To investigate the association further, we have in the present study investigated contribution of genetic and environmental factors.

Methods: The Norwegian Population Registry has registered data on parents and siblings for most inhabitants born since the 1950’s. The Norwegian Medical Birth Registry has registered data on all births since 1967 and the Norwegian Renal Registry has registered data on all patients who have developed ESRD since 1980. We linked these registries and investigated whether preeclampsia in first pregnancy of siblings, children or partner were associated with risk of ESRD. Cox regression statistics were used.

Results: In the analyses of siblings’ risk, we included 605,231 women of who 312 developed ESRD. As compared to women without preeclampsia who had not had siblings with preeclampsia, women without preeclampsia who had siblings with preeclampsia had a relative risk of 0.93 (95% CI 0.85-1.01). Women with preeclampsia who had not had siblings with preeclampsia had a relative risk of 2.6 (0.83-8.1). These results were unchanged if the analyses for siblings were performed separately for brothers and sisters. In the analysis of parents’ risk, we included 322,974 women of who 4,245 developed ESRD, similar findings as in the analyses of siblings’ risk were made. Male partners of women with preeclampsia had a relative risk of 1.6 (1.03-2.4), 1.5 (0.94-2.5) after adjustment for educational status.

Conclusions: Risk of ESRD is not increased if siblings or children have preeclampsia but partners of women with preeclampsia have a slightly increased risk.

Funding: Government Support - Non-U.S.

Effect of a Multifactorial Intervention with the Aid of Nurse Practitioners on Renal Outcome in Patients with Chronic Kidney Disease: Results of the MASTERPLAN Study

Mieke J. Peeters,1 Arjan D. Van Zuilen,2 Jan A.J.G. van den Brand,3 Peter J. Blankenstein,2 Jack F. Wetzels.2 1Radboud University Nijmegen Medical Centre, Nijmegen; 2University Medical Centre Utrecht, Utrecht, Netherlands.

Background: Chronic kidney disease (CKD) is a major public health problem worldwide. CKD patients are at risk of progression to end stage renal disease (ESRD). We showed that strict implementation of current guidelines directed at multiple risk factors with the aid of nurse practitioners did not reduce cardiovascular events in patients with prevalent CKD. In the current analysis we evaluated renal endpoints.

Methods: In MASTERPLAN (Multifactorial Approach and Superior Treatment Efficacy in Renal Patients with the Aid of Nurse Practitioners) 788 patients with mild to moderate CKD were randomized to receive nurse practitioner support added to physician care (intervention group) or physician care alone (control group). Patients were followed for a median of 4.4 years. Several renal end points were defined.

Results: 395 patients were randomized to the intervention group, 393 to the control group. Baseline variables were balanced. During follow up, mean blood pressure was significantly lower in the intervention group (3.2±2 mmHg). Significant differences were also found for LDL cholesterol (0.11 mmol/L), phosphate (0.03 mmol/L), hemoglobin (+0.1 mmol/L), and proteinuria (+0.12 g/24h). No differences were found for smoking, BMI, sodium intake, physical activity level, PTH, and HbA1c.

The intervention did not significantly reduce the incidence of ESRD (hazard ratio 0.86, 95% CI 0.63-1.16). Also, there were no significant differences when using a 50% increase of serum creatinine or the change in eGFR as endpoint. eGFR was -1.52 in the intervention group and -1.74 ml/min/1.73m²/year in the control group, p=0.04.

Conclusions: Additional support by nurse practitioners in CKD patients improved some risk factor levels, but did not significantly attenuate the decline of kidney function or reduce the rate of ESRD.

Funding: Pharmaceutical Company Support, Private Foundation Support

A Meta-Analysis on 2100 Non-Dialysis- and Dialysis-Patients Exposed to Once Monthly C.E.R.A. Demonstrates Effective and Safe Treatment

JULIA J. SCIALLA,1 LAWRENCE J. APPEL,2 MYLES S. WOLF,2 WEI YANG,1 XIAOMING ZHANG,3 EDGAR R. MILLER,4 STEPHEN M. SOZIO,5 LYDIA BAZZANO,4 MAGDALENA M. CUEVAS,6 MELANIE GLENN,6 RADHAKRISHNA REDDY KALLEM,7 EVA LUSTIGOVÁ,8 ANNA C. PORTER,9 RAYMOND R. TOWNSEND,10 MATTHEW R. WEIR,11 CHERYL A. ANDERSON,2 JOHN HOPKINS UNIVERSITY,12 UNIVERSITY OF MICHIGAN,13 UNIVERSITY OF PENNSYLVANIA,14 TULANE UNIVERSITY,15 UNIVERSITY OF ILLINOIS AT CHICAGO,16 UNIVERSITY OF MARYLAND.

Background: Plant-based proteins have lower bioavailability of phosphorus and lower acid load than animal-based proteins and may be preferred in CKD. Adverse effects due to inhibition of iron absorption and potassium loading are also possible.

Methods: We calculated the percentage of protein intake from plant sources (% plant protein) in 2938 CRIC participants by scoring individual food items from a food frequency questionnaire. Using linear regression we modeled associations between % plant protein intake and urinary albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with lower with urinary albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with higher with increasing baseline eGFR (OR=1.03, 95% CI 1.02-1.05), and urine albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with higher with increasing baseline eGFR (OR=1.03, 95% CI 1.02-1.05), and urine albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with lower with increasing urine albumin (g/dl) concentration (OR=0.99, 95% CI 0.83-1.14).

Results: Of the 13,634 patients identified with CKD, 45% (n= 6153) experienced no decline in kidney function and were not included in the multivariable analysis; of those patients experiencing a decline in kidney function, 50% were classified as ‘slow’ (n= 3,738; slope 0 to -2.18 ml/min/1.73m²/ year) and 50% as ‘fast’ (n≥3,747; slope -2.19 to -25.72 ml/min/1.73m²/ year) progressors. Fast progressors were predominantly male (97%), with a mean age of 72.5 years and baseline eGFR of 40.0 ml/min/1.73m². The odds of fast progression were higher with increasing baseline eGFR (OR=1.03, 95% CI 1.02-1.05), and urine albumin-to-creatinine ratio ≥300 mg/g (OR=2.39, 95% CI 1.67-3.42), but lower with higher with increasing urine albumin (g/dl) concentration (OR=0.99, 95% CI 0.83-1.14).

Conclusions: In this U.S. veteran cohort, the overall mean progression of all-cause CKD was relatively slow (0.08 ml/min/1.73m²/ year) per year, with faster progressions increasing an average decline of 4.67 ml/min/1.73m²/ year per year and nearly half of the population showing no reduction in eGFR over 2 to 5 years. Future studies to link outcomes with these and other risk factors are warranted.

Funding: Other NIH Support - Centers for Disease Control and Prevention

Plant Protein Intake Is Associated with Fibroblast Growth Factor 23 and Serum Bilirubin in Patients with Chronic Kidney Disease

JULIA J. SCIALLA,1 LAWRENCE J. APPEL,2 MYLES S. WOLF,2 WEI YANG,1 XIAOMING ZHANG,3 EDGAR R. MILLER,4 STEPHEN M. SOZIO,5 LYDIA BAZZANO,4 MAGDALENA M. CUEVAS,6 MELANIE GLENN,6 RADHAKRISHNA REDDY KALLEM,7 EVA LUSTIGOVÁ,8 ANNA C. PORTER,9 RAYMOND R. TOWNSEND,10 MATTHEW R. WEIR,11 CHERYL A. ANDERSON,2 JOHN HOPKINS UNIVERSITY,12 UNIVERSITY OF MICHIGAN,13 UNIVERSITY OF PENNSYLVANIA,14 TULANE UNIVERSITY,15 UNIVERSITY OF ILLINOIS AT CHICAGO,16 UNIVERSITY OF MARYLAND.

Methods: Plant-based proteins have lower bioavailability of phosphorus and lower acid load than animal-based proteins and may be preferred in CKD. Adverse effects due to inhibition of iron absorption and potassium loading are also possible.

Funding: Government Support - Non-U.S.
Models were adjusted for age, sex, race, diabetes, body mass index, eGFR, income, smoking, total energy intake, total protein intake, 24 hour urinary sodium, use of ACE inhibitors, ARBs and use of diuretics.

**Results:** Adjusted means of MFGF23 and HCO3 across levels of % plant protein are presented in the table.

<table>
<thead>
<tr>
<th>% Plant Protein</th>
<th>MFGF23 (pg/mL)</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;24%</td>
<td>179.4</td>
<td></td>
</tr>
<tr>
<td>24-29%</td>
<td>175.4</td>
<td></td>
</tr>
<tr>
<td>30-35%</td>
<td>175.8</td>
<td></td>
</tr>
<tr>
<td>36-44%</td>
<td>170.6</td>
<td></td>
</tr>
<tr>
<td>&gt;44%</td>
<td>164.9</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Higher % plant protein was associated with lower MFGF23 (p<0.05) and higher HCO3 (p<0.01), but not with serum phosphate (p=0.9) or PTH (p=0.5). There was no association of higher % plant protein with serum potassium (p=0.2), serum albumin (p=0.2) or hemoglobin (p=0.3). The associations of % plant protein with MFGF23 and HCO3, did not differ by diabetes status, sex, race, CKD stage (2/3 vs. 4/5) or total dietary protein intake (≤0.8 kg/d vs. >0.8 kg/d) (p-interaction > 0.10 for each).

**Conclusions:** In conclusion, consumption of a higher percentage of protein from plant sources may lead to a more favorable metabolic profile in patients with CKD.

**Funding:** NIDDK Support, Other NIH Support - through CTSA awards, Private Foundation Support

**TH-PO303**

**Determinants of sRAGE in a Community-Based Population:** The Atherosclerosis Risk in Communities (ARIC) Study

**Elizabeth Selvin,1 Marc Halushka,1 Ron C. Hoogeveen,2 Christie Ballantyne,2 Josef Coresh,1 Brad C. Austor.1 Johns Hopkins University; 2Baylor College of Medicine.**

**Background:** Advanced glycation endproducts (AGEs) and their receptors are implicated in the development of diabetic complications and kidney disease. When stimulated by AGEs, the receptor for AGEs (RAGE) induces inflammation and may fuel progression of disease through NF-kB mediated signaling. Soluble circulating RAGE (sRAGE) may counteract the effects of RAGE.

**Methods:** We identified determinants of low levels (lowest quartile) of sRAGE (ELISA, R&D Systems, CV<3%) from stored plasma in a random sample of 1185 participants in the ARIC Study (ages 47-68) with eGFR 60 mL/min/1.73m².

**Results:** sRAGE was inversely correlated with BMI (Spearman’s r = -0.28), HbA1c (r = -0.22), CRP (r = -0.25), eGFR_cystatin C (r = -0.17) and eGFR_CKD-EPI (r = -0.11). sRAGE was lower among men vs. women (95.5 vs 1100.6 pg/mL, p<0.001), blacks vs. whites (756.6 vs 1118.8 pg/mL, p<0.001), and diabetic vs. non-diabetic adults (901.8 vs 10530.0 pg/mL, p=0.01). Only male sex, black race, smoking, CRP, and eGFR remained associated with low sRAGE after adjustment. eGFR_CKD-EPI was more strongly associated with sRAGE than eGFR_cystatin C.

**Conclusions:** Low levels of sRAGE indicate susceptibility to inflammatory processes or a state of increased oxidant stress. The lack of association of sRAGE with diabetes is surprising and suggests that sRAGE may be a general measure of inflammation and not as specific to diabetes as previously thought. The robust racial difference in sRAGE deserves further examination.

**Funding:** NIDDK Support

**TH-PO304**

**Development of Chronic Kidney Disease in Rheumatoid Arthritis**

LaTonya J. Hickson,1 Cynthia S. Crowson,2 Sherine E. Gabriel,3 James T. McCarthy,1 Eric L. Matteson.3

**Background:** Rheumatoid arthritis (RA) is associated with a variety of kidney disorders. However, it is uncertain whether the development of chronic kidney disease (CKD) is higher in patients with RA compared to the general population.

**Objective:** To elucidate the occurrence of CKD in patients with RA.

**Methods:** Retrospective review of incident adult onset RA cases from a defined geographic population base from 1980-2007 as a comparison cohort of non-RA patients. CKD was defined by estimated glomerular filtration rate (eGFR) <60 and moderate CKD was eGFR <45 mL/min/1.73m². The cumulative incidence of CKD was estimated adjusting for the competing risk of death.

**Results:** 813 RA patients and 813 non-RA patients were assembled (mean age 55.9, 68% female). There was no difference in the prevalence of CKD at time of RA development, p=0.84. Among RA patients, 38% had an eGFR <60 and 11% had eGFR <45, similar to non-RA patients (38% and 9%) observed at the RA incidence reference date. The 10 year cumulative incidence of CKD was higher in RA patients compared to non-RA (43% vs 32%, p<0.008). This difference occurred primarily in the first year. The 10 year cumulative incidence of moderate CKD was also higher in patients with RA compared to non-RA (18% vs 14%, p=0.037). This difference became apparent 3 years after incident RA (figure). The development of CKD was associated with erythrocyte sedimentation rate (HR 1.1 per 10 mm/h, CI 1.03-1.20, p=0.008) and cardiovascular disease (HR 2.1, CI 1.4-3.2, p<0.001).

**Conclusions:** RA patients were more likely to develop CKD over time. Inflammation and cardiovascular disease appear to play a role in this development. Further studies are needed to improve understanding of these relationships.

**TH-PO305**

**Coexistence of High Serum Hepcidin-25 Level and Proteinuria Has Strong Impact on Short-Term Mortality in Non-Hodgkin’s Lymphoma Patients**

Hirohiko Nokiba,1,2 Minoru Ando, 1,2 Masaki Hara, 1,2 Ken Tsuchiya, 1,2 Kosaku Nitta.3

**Background:** The excessive hepatic production of hepcidin-25 due to chronic disorders including cancer is responsible for anemia with underutilization of iron. This study addressed the combined impact of hepcidin-25 and proteinuria on short-term mortality in lymphoma patients.

**Methods:** One-year prospective cohort study was conducted in a total of 24 patients with non-Hodgkin’s lymphoma. Serum hepcidin-25 level was measured by liquid chromatography-mass spectrometry. Proteinuria was defined as a dipstick test ≥. Survival curve was drawn by the Kaplan Meier method, which was stratified into 2 groups by either median value of serum hepcidin-25 (43.1 ng/ml) or presence of proteinuria. In addition, each group which was stratified by hepcidin-25 was divided into 2 subgroups according to presence of proteinuria. Multivariable Cox proportional hazards analysis, adjusted for age, gender, performance status and estimated GFR, was used to calculate mortality HR for the combined impact of hepcidin-25 and proteinuria.

**Results:** Mean hepcidin-25 level was 53.9±48.9 ng/ml, which was approximately 2-fold greater than the reference value (22.1±12.3 ng/ml). The cumulative survival rate was significantly lower in the high hepcidin-25 group or in the proteinuria (+) group than in each opposite. The mortality HR (95% CI) was 46.6 (6.33-442.54) for patients with both hepcidin and high hepcidin-25; 7.3 (0.80-78.80) for those with proteinuria and low hepcidin-25; and 1.7 (0.30-9.28) for those with no proteinuria and high hepcidin-25, as compared to the reference with neither of them.

**Conclusions:** Data are adjusted for age, gender, performance status and eGFR.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**

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Conclusions: Non-Hodgkin’s lymphoma patients with both high serum hepcidin-25 level and coexisting proteinuria are likely at high risk for early mortality.

Methods: We reviewed all laboratory results of our patients in 2007 (about 50000) for the first time, an estimated MDRD (Modification of Diet in Renal Disease) albuminuria 20-50 ml/min were eligible for the intervention program. We included all patients with eGFR 20-30 ml/min and patients with eGFR >30 ml/min having at least one of the following: diabetes, hypertension or CVD. Patients that visited a nephrologist in 2007 had been excluded and aim outcome. Changes in eGFR and CVD risk factors.

Results: 418 patients with CKD-4 (age 74±10.2, eGFR 26.3±1.27 ml/min) and 2067 patients with CKD-3 (age 66.0±7.3) met the inclusion criteria and had been invited to a nephrologist. 2011 CKD-4 patients visited a nephrologist. After 3 years, 31 patients died and 20 had stage end stage renal disease (ESKD). 17 patients lost for follow-up. The rest had a stable eGFR (27.6±8.1), Blood pressure (BP) was lower (129.4±14.5; 72.4±18.7 vs. 137.7±21.5; 77.7±12.5, p<0.05) lower LDL cholesterol (LDL-c) (93.2±28.9 vs. 101.3±32.1, p<0.05), and stable Hemoglobin level. The percent of patients treated with erythropoetin increased significantly from 11.9% to 36.6%. 630 CKD-3 patients visited a nephrologist. After 3 years, 43 patients died and 27 developed ESKD and 71 lost from follow-up the rest a had a decline in eGFR (39.7±11.5 vs. 42.3±5.6), BP was lower (129.3±13.6;73.8±8.8 vs. 138.1±19.3;78.3±10.2, p<0.05) lower LDL-c(93.3±30.5 vs. 101.3±34.2, p<0.05), and stable Hemoglobin level. The percent of patients treated with erythropoetin increased significantly from 11.9% to 15.6%.

Conclusions: A proactive program to detect CKD 4 and high risk CKD 3 patients and the implementation of nephrologists’ follow-up service improves treatment. More efforts to recruit all the high risk patients are needed. A longer follow up is needed to evaluate this program.

TH-PO309
The Association between Serum Uric Acid and Renal Damages in the Japanese Population: The Takahata Study
Kazuko Suzuki, Tsuneo Konta, Kazunobu Ichikawa, Yusuke Mashima, Ami Ikeda, Isao Kubota. Yamagata University School of Medicine, Yamagata, Japan.

Background: The increase in serum uric acid is frequently observed in the subjects with renal insufficiency.

Methods: However, the association between serum uric acid and renal damages in general population is largely unknown. To clarify this point, we conducted a community-based study, using urinary albumin and beta-2-microglobulin as markers of glomerular and tubular damages.

The subjects studied were 3341 Japanese subjects (1497 men, 1844 women) without history of kidney disease. The urinary albumin-to-creatinine ratio (UACR) and beta-2-microglobulin-to-creatinine ratio (UBCR) were assessed in morning spot urine samples.

Results: In this population, the mean values of serum uric acid (mg/dl) were 5.8±1.3 in men and 4.5±1.1 in women, respectively. The prevalence of albuminuria (UACR >30 mg/g) were significantly increased along with the increase in uric acid both in men and women (P <0.05). In contrast, the prevalence of high UBCR (>300 mg/g) were deceased along with the increase in uric acid both in men (P <0.01) and women (P <0.05).

Multivariate analysis showed that albuminuria was independently related with increased uric acid (F-7 mg/dl for men, >6 mg/dl for women, vs. 5.0-5.9 mg/dl) and high UBCR was not associated with uric acid neither in men nor women, after adjustment for possible confounders. The one-year longitudinal analysis in 1517 subjects showed the positive relationship between baseline uric acid and UACR changes across the tertiles of uric acid (P for trend <0.05).

Conclusions: This study showed that uric acid was an independent risk factor for albuminuria, but not high UBCR, suggesting that uric acid might induce glomerular damage, but not tubular damage in general population.

TH-PO310
Etiology, Comorbidity and Factors Associated with Renal Function Decline in Chinese Chronic Kidney Disease Patients
Guangyan Cui, Xiang-Mei Gao. Department of Nephrology, State Key Laboratory of Kidney Disease, PLA General Hospital.

Background: For the lack of nation-wide investigations in China, features of CKD etiology, comorbidity and factors associated with renal function decline is not clear. The Chinese Society of Nephrology initiated a nation-wide investigation of inpatients with CKD in China.

Methods: A cross sectional study was conducted on inpatients with CKD from 61 big hospitals in 21 provinces of China. Data on demographics, lifestyle risk factors, medical records, and laboratory tests results were obtained using a standard questionnaire administered by trained physicians. Estimated GFR was calculated with CKD-EPI equation. Binary logistic regression was used to analyze factors associated with renal function decline.

Results: 10728 patients were included in the analysis, in which male-female ratio was 1.18:1. The mean age was 48.14±17.98 years (15ys-97ys). The distribution in CKD stages was as follows: stage one 28.2%, stage two 15.0%, stage three 12.4%, stage four 8.6% and stage five 35.7%. Primary glomerulopathies accounted for 52.3% diagnosis of the whole inpatients with CKD, in which 11.9% was IgA nephropathy. Diabetic kidney disease accounted for 11.9%, lupus nephritis 6%, hypertensive nephropathy 4.8%, cysctic kidney disease 2.4%. The common comorbidities were mineral and bone disorder (66.8%), anemia (46.4%), hyper tension (62.2%), hyperuricemia (46.2%), hyperlipidemia (46.2%), hyperuricemia (46.2%), diabetes (23.8%) cardiovascular disease (19.1%), cerebrovascular disease (6.1%) and so on. By multivariable logistic regression analysis, renal function decline was significantly associated with age (OR 1.612), male (OR 1.777), hypertension (OR 1.856), diabetes (OR 1.777), hyperuricemia (OR 1.798) and hyperlipidemia (OR 1.798).

Conclusions: This investigation described the clinical features of inpatients with CKD in China for the first time. Presently, primary glomerulopathy is the leading cause of CKD.
of CKD in China. In Chinese CKD patients, the prevalence of hypertension, diabetes, cardiovascular diseases and cerebrovascular diseases were lower, but the prevalence of anemia and hyperlipidemia are higher than those from developed countries.

TH-PO311

Educational Hospitalization Effectively Delays Progression of CKD

Shutaro Nito, Soichiro Imori, Kayoko Eto, Eiichiro Sohara, Tomokazu Okado, Yumi Noda, Tatematsu Rai, Shinichi Uchida, Sei Sasaki. Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.

Background: In Japan, over thirteen million people are diagnosed as chronic kidney disease (CKD). Appropriate diet modification and control of blood pressure are essential for prevention of progression of CKD. However, it is difficult to achieve these goals in the outpatient clinic. In our hospital, we have hospitalized CKD patients as “Educational Hospitalization” (EH) to provide them with the knowledge of diet and medication, and to control their blood pressure and fluid volume balance. We analyzed the effect of EH on the progression of CKD.

Methods: During the period from January 2007 to December 2010, a total of 712 CKD patients visited our nephrology department. Among them, 206 patients were hospitalized for EH. 199 EH patients, whose estimated GFR (eGFR) were under 60 ml/min/1.73 m², were retrospectively analyzed. The effect of EH was evaluated by the change in eGFR during 6 months (∆eGFR). We compared patient status at the point of hospitalization and after 6 months in the EH group. Hemoglobin (10.7 ± 1.1 g/dl) and serum albumin (3.7 ± 0.8 mg/dl) significantly increased, and eGFR decreased during this period. Changes of serum total cholesterol and urinary protein were not significant. ∆eGFR after hospitalization was improved (-7.5 ± 3.0 ml/min/1.73 m²/year) when stratified by CKD stage, ∆eGFR in stage 4 and 5 patients were significantly improved but not in stage 3. Moreover the degree of improvement was largest in stage 5.

Results: Compared with patients who were not hospitalized, the EH patients had significantly higher male gender, higher diabetes prevalence, older age, lower hemoglobin, lower serum albumin, lower eGFR, lower serum total cholesterol and higher urinary protein (P < 0.05). We compared patient status at the point of hospitalization and after 6 months in the EH group. Hemoglobin (10.7 ± 10.9 g/dl) and serum albumin (3.7 ± 3.8 mg/dl) significantly increased, and ∆eGFR decreased during this period. Changes of serum total cholesterol and urinary protein were not significant. ∆eGFR after hospitalization was improved (-7.5 ± 3.0 ml/min/year). This meta-analysis suggests that ARBs are effective in treatment of left ventricular hypertrophy in patients with hemodialysis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

TH-PO313

Patient Referral to Active Supportive Care: A Retrospective, Observational Study of Changes in Demographics and Outcome

Thalakunte M. Muniraju, Frances E. Marr, Theresa Wood, Christopher John Chisholm, Alison Brown, Neil S. Sheerin.1,2 1Renal Unit, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, United Kingdom, 2Newcastle University, Newcastle upon Tyne, United Kingdom.

Background: The majority of patients with advanced chronic kidney disease (CKD) have a significant number of comorbidities and high mortality rate. Renal replacement therapy may not be the ideal or preferred treatment for all patients with advanced kidney disease.

Methods: Active supportive (conservative) care (ASC), is well recognised as one of the management options in patients with advanced CKD and significant comorbidity. This study was undertaken to look at the demographics and survival of patients referred for ASC.

A retrospective, observational study of all patients who were referred for ASC from March 2002 to December 2010 at Newcastle Freeman Hospital Renal Unit (United Kingdom) was undertaken. Patients were identified and demographic data obtained from ASC database. Patients other demographics and estimated Glomerular Filtration Rate (eGFR) were collected using the hospital electronic record.

Results: A total of 387 (186 male and 201 female) patients were referred for ASC during the study period. The number of patients referred more than doubled from 2002 to 2003 and has remained consistent for the 9 years thereafter. The average age of the patients referred was 81.46 years, and it has been gradually increasing since 2006. Average eGFR was 17.00 ml/min/1.73m², with no significant difference between years. Out of 387 patients, 286 were deceased and 89 remain under follow up. 12 patients changed modality. The average time on ASC for the 286 deceased patients was 14.02 months.

Conclusions: This study demonstrates that number of patients referred for ASC has increased over time with increasing awareness, patient education and preference. There is no significant difference in eGFR at the time of referral over time, however, the age of referral has gradually increased since 2006. The average time spent on ASC is just over a year. This could represent more effective low clearance care. These findings demonstrate significant service implications to the trust and may reflect a shift in attitude.

TH-PO314

Multiple-Intervention Model May Preserve Better the Renal Function of Patients with Type 2 Diabetes Mellitus and Early Nephropathy Compared with the Standar Care Model


Background: Renal function (RF), can be preserved by trained family physicians (FP), in DM2 and early nephropathy (EN), but negative lifestyle (LS) habits weren’t modify. Multiple-Intervention Model (MIM), could be an option. Aim: To determine the effect of two health-care models on RF in DM2/ EN patients.

Methods: One unit received MIM, other standar care model (SCM). All FP were trained on EN. Patients in MIM received 2h/week an educative intervention by a multidisciplinary team for 4 wks in small self-help groups: emotional (social worker); nutritional (dietitian); exercise (physical trainer); and health-related problems (FP), evaluated at 0 and 6 months by LS questionnaires, clinical and biochemical (Ms. score is 100=better SL).

Results: Of 113 patients included; 44 in MIM and 48 SCM ended 6 moths of follow up.

Differences in lifestyle questionnaire habits

<table>
<thead>
<tr>
<th>MIM (N=44)</th>
<th>SCM (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge</td>
<td>2.6 ± 3.0*</td>
</tr>
<tr>
<td>Adherence</td>
<td>2.9 ± 1.7</td>
</tr>
<tr>
<td>Emotion</td>
<td>2.5 ± 3.8*</td>
</tr>
<tr>
<td>Exercise</td>
<td>1.6 ± 3.8*</td>
</tr>
<tr>
<td>Tobacco Consumption</td>
<td>0.32 ± 1.3</td>
</tr>
<tr>
<td>Alcohol Consumption</td>
<td>0.81 ± 2.1*</td>
</tr>
<tr>
<td>Diet</td>
<td>3.4 ± 5.0*</td>
</tr>
<tr>
<td>Total</td>
<td>11.7 ± 12*</td>
</tr>
</tbody>
</table>

Differences in clinical and biochemical variables

| Body mass index (kg/m²) | -0.37 ± 1.3* | -0.26 ± 1.07 |
| Waist circumference (cm) | -2.3 ± 3.5* | -0.14 ± 5.0 |
| Systolic BP (mmHg) | 2.04 ± 2.0* | -1.42 ± 2.0 |
| Diastolic BP (mmHg) | -0.39 ± 12* | -5.2 ± 11* |
| HbA1C (%) | -0.34 ± 2.1* | -0.21 ± 1.9* |
| Cholesterol (mg/dl) | 0.25 ± 11 | 0.47 ± 4.7 |
| Triglycerides (mg/dl) | -1.93 ± 3.2* | -0.31 ± 11 |
| HDL-cholesterol (mg/dl) | 1.18 ± 9.5 | 2.5 ± 11 |
| LDL-cholesterol (mg/dl) | 5.3 ± 26 | 4.5 ± 30 |
| Creatinine (mg/dl) | 0.04 ± 0.19 | 0.03 ± 0.16 |
| eGFR (ml/min/1.73m²) | 2.2 ± 14.6 | 3.19 ± 15.3 |
| Albumin/creatinine ratio (mg/g) | 3.2 ± 104* | 5.01 ± 246* |

* p < 0.05 vs baseline the same model; † vs conventional health-care model

Conclusions: A MIM could help to improve the LS of patients with DM2 and EN, and preserve better their renal function.
TH-PO315
Epidemiology of Acute Kidney Injury in Critically Ill Patients: A Multicenter-Multinational Study
Nattachai Sirisawat, Florentina E. Sileanu, John A. Kellum. For the AKI-6 Investigators. The CRISMA center, Department of Critical Care Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Estimates of the ICU period prevalence of acute kidney injury (AKI) have varied from 20% to 70% across different centers. Estimates of hospital mortality and dialysis rates have also varied. Variation could be due to actual differences in disease incidence and clinical course or it could simply be a function of data ascertainment and application of diagnostic criteria.

Methods: We conducted a retrospective study of ICU patients treated at 6 academic hospitals, 2 each in North America, Europe and Australia. Our aim was to determine and compare the occurrence and outcomes of AKI in the ICU using standard methods across all centers.

Results: Of 15,567 critically ill patients, 435 were excluded due to history of chronic kidney disease or insufficient data. Reference creatinine was defined by the baseline creatinine if available or the lower of the ICU admission creatinine or the creatinine derived from MDRD formula if no baseline was available. We used the modified RIFLE criteria as proposed by the AKI Network (AKIN). Since urine output was not available from some centers, we examined the variation in AKI rates and outcomes using creatinine criteria alone.

The ICU prevalence period of AKI (creatinine data only) varied from 14.6% to 43.8% (P<0.001). Hospital mortality rates of AKI patients by center varied from 20.4% to 35.9% (P<0.001). Even after removing the center with the largest difference in AKI event rate compared to the rest and after excluding another center with the largest difference in hospital mortality there was still significant variation in both ICU period prevalence for AKI (P<0.001) and associated hospital mortality (P<0.001).

Conclusions: Variation in occurrence of AKI and in associated hospital mortality is not explained on the basis of varied application of AKI criteria. Differences in epidemiology of AKI across centers may be due to difference in case mix or to secular trends in therapy. Multicenter studies of AKI epidemiology may lead to improved understanding of risk factors and prevention and treatment strategies for AKI.

TH-PO316
The Duration of Postoperative Acute Kidney Injury and Cumulative Incidence of Chronic Kidney Disease after Lung Transplantation
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Background: To determine if the duration of perioperative acute kidney injury (AKI) after lung transplantation predicts chronic kidney disease (CKD).

Methods: We retrospectively evaluated data on 657 patients who underwent lung transplantation from 1997 to 2009. AKI was defined by absolute rise in creatinine by ≥0.3 mg/dl and categorized into three stages by the magnitude rise in creatinine according to the AKIN classification and by the duration from baseline to nadir of creatinine (short (less than 5 days), medium (5–10 days) or long (10 days or more)). Outcomes analyzed were the cumulative incidence of CKD stage 4 or 5 (eGFR <30 ml/min/1.73 m2) on long-term follow-up.

Results: We identified 424 patients (65%) who had at least one AKI event in the first 2 weeks after transplantation. 115 (17.5%), 184(28%) and 125(19%) experienced short, medium and long duration AKI respectively. At one year the cumulative incidence of CKD was 15%, 16% and 27% in the short, medium and long duration AKI respectively. Figure 1 shows the competing risk models for the cumulative incidence of CKD stratified per duration of AKI. After adjusting for appropriate covariates (age, gender, sex, type of lung transplant, post-transplant HTN and DM, baseline creatinine, primary diagnosis leading to transplant), the hazard ratio for CKD was 2.0 (95% CI 1.2-3.3), 2.7(95% CI 1.7-4.2) and 4.4 (95% CI 2.7-7.0) for short, medium and long duration AKI respectively.

Conclusions: This study showed that the duration of AKI is independently associated with CKD and may provide additional prognostic information in patients undergoing lung transplantation.

TH-PO317
Rapid Progression of Chronic Kidney Disease Is Associated with Cardiovascular Mortality in the Japanese Population: The Gonryo Study
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Background: Chronic kidney disease (CKD) is a public health problem due to a high risk for cardiovascular disease (CVD). Currently, decreased estimated glomerular filtration rate (eGFR) and proteinuria are accepted as independent risk factors. However, the reliability of the decline rate of eGFR is not for predicting cardiovascular mortality rate and it is not settled.

Methods: The study comprised 2,694 CKD patients recruited from 11 outpatient nephrology clinics, and they classified by eGFR in six CKD stages including stage 3A and 3B. The progression of CKD was defined as the decrease of eGFR with increasing CKD stages during two years. Risks of CVD, all-cause mortality and initiating dialysis were examined.

Results: During the observation time, 192 cases initiated to chronic dialysis therapy, and CVD events or death occurred in 135 patients. The frequencies of the CKD progression were 10% in stage 1, 30% in stage 2, 22% in stage 3A, 16% in stage 3B and 14% in stage 4, and it did not differ significantly by underlying renal diseases. Not only dialysis induction (6%, 1%, p<0.001) but also CKD mortality (8%, 3%, p<0.001) increased significantly in the CKD progression group compared to the non-progression group, and the odds ratio for CVD mortality was 2.78 (95% confidence interval, 1.77-4.35) by logistic regression analysis. When we focused for stage3, the CVD mortalities differed significantly between groups of the CKD progress in stage 3B, while it did not in stage 3A.

Conclusions: The progression of CKD by eGFR increased risk of CVD events and death. The subdivision of CKD stage 3 is useful to classify the higher risk people in early CKD.

Funding: Government Support - Non-U.S.

TH-PO318
Natriuretic Peptides as Predictors of All-Cause Mortality in Outpatients with Chronic Heart Failure and Renal Dysfunction
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Background: Brain natriuretic peptide (BNP) is released from the heart muscle cells in response to wall distension, and is a valuable diagnostic marker of heart failure (HF) and an independent predictor of prognosis in patients with chronic HF. Renal dysfunction is considered a confounding factor in the evaluation of BNP in HF patients, and since renal failure is common in HF patients, the interpretation of BNP levels is often limited in clinical practice. The aim was to investigate the prognostic utility of BNP in outpatients with chronic HF and renal dysfunction.

Methods: Valid data on BNP were available in 1930 outpatients with chronic HF included in the Norwegian heart failure registry from October 2000 to March 2010. Since analyzes were performed locally with various methods, quartiles of BNP at the last visit were defined at each center among patients with normal renal function (MDRD eGFR ≥ 60 ml/min/1.73 m2). Patients with renal dysfunction (eGFR < 60 ml/min) were then stratified to four BNP stages according to the same quartile limits. Multivariate Cox regression models were used to investigate the prognostic utility of BNP with respect to all-cause mortality.

Results: Renal dysfunction was present in 734 patients (38 % of the total population). Median age was 76 years and 69 % were men. 58.7 % (431 patients) had BNP levels in the highest stage defined by quartiles in the patients without renal dysfunction. BNP stage independently predicted all-cause mortality (HR 1.47, 95 % CI 1.22-1.76, p< 0.001) together with lower systolic blood pressure, higher NYHA class, age and lower GFR. Two year survival of patients in the highest BNP stage was 61 % compared to 88 % in lowest BNP stage (p<0.001). No interaction between the prognostic information of BNP levels and renal dysfunction were found.

Conclusions: BNP level in outpatients with HF is a strong independent predictor of all-cause mortality and provide important prognostic information also among patients with renal dysfunction even though they are at greater risk of having increased levels of natriuretic peptides.

TH-PO319
How Well Do Family Practitioners Record Chronic Kidney Disease in 6 Million Patients?
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Background: Chronic Kidney Disease (CKD) prevalence was reported as 10% in a primary care based cohort in the UK. However national estimates are much less at 4.3% but are derived from whether the practices diagnose the patient as having CKD using specific ‘codes’ introduced in 2007. We investigated the prevalence of CKD and whether these patients were coded as having CKD.
Methods: The Health improvement Network (THIN) is an anonymised database of 6.7 million patients from 426 primary care centres in the UK. THIN contains the patient’s demographic information, consultations, laboratory results and prescriptions. We collected the demographics, laboratory creatinine and estimated and the ‘codes’ for CKD. The age standardised prevalence of CKD stages 3-5 was calculated between 2005-2009 using 1) a single creatinine converted to MDRD eGFR (Note IDMS calibrated creatinine MDRD equation was used for 2009 as only by 50% of UK laboratories) 2) a single laboratory eGFR 3) Codes for CKD.

Results: The mean age of CKD stage 2-5 (77.58±12.94) versus those with a GFR >60 (53.56±16.55). The majority of patients with CKD were female except in CKD stage 5. The prevalence of CKD stages 3-5 was in years 2005, 2006, 2007, 2008 and 2009, using a single creatinine, 7.32%, 7.70% 7.81%, 7.36% and 7.88 respectively. The reduction in prevalence in 2008 estimates was likely due to the change to IDMS creatinine.

In 2009 CKD stage 5 was the most prevalent in the cohort (5.12%) and CKD was more common in women compared to men. The prevalence was lower using lab reported eGFR.

TH-PO320
A Longitudinal Study of the Effect of Smoking on Progression and Survival in CKD
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Background: Smoking affects vascular integrity, blood pressure and inflammation. Smoking-related lung disease is associated with polycythemia and loss of acid-base regulation. The theoretical effect of smoking on patients with CKD may therefore be to affect survival, progression of disease or even ESA and oral bicarbonate. However, evidence to support this and to assess the effect of smoking cessation is lacking.

Methods: This was a longitudinal study of the effect of smoking on CKD. 868 patients with CKD stages 3-5 not on dialysis were recruited over 5 years. Patients were classified as current smokers if they had smoked within 12 months, ex-smokers or non-smokers, and were followed up for up to 5 years. End points were death or progression of CKD of eGFR of >3mL/min/1.73m2/year or reaching ESKD. Concomitant clinical data were obtained at baseline and annual intervals.

Results: Baseline characteristics are summarized in table 1.

Table 1. Baseline characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N</th>
<th>Ex</th>
<th>Never</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59</td>
<td>68</td>
<td>62</td>
</tr>
<tr>
<td>MFR (mg/dL)</td>
<td>36</td>
<td>34</td>
<td>34</td>
</tr>
<tr>
<td>Male</td>
<td>62</td>
<td>70</td>
<td>49</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>28.7</td>
<td>28.4</td>
<td>28.3</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>141</td>
<td>136</td>
<td>135</td>
</tr>
<tr>
<td>Pack years</td>
<td>31</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>CAD</td>
<td>24</td>
<td>35</td>
<td>19</td>
</tr>
</tbody>
</table>

Current smokers were more likely to develop progressive CKD than ex- or non-smokers. Multivariate logistic regression indicates that this may be due to worse blood pressure control in current smokers. There was no difference in survival between current and ex-smokers but non-smokers showed significantly better outcome.

Figure 1. Survival in CKD according to smoking status.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represents presenting author.

186A
Conclusions: Association between BP (overall and JNC7 categories) and subsequent kidney failure. P for trend by JNC7 categories<0.001

Results: Close to 3000 patients have already been successfully enrolled by the beginning of 2012. Study procedures and preliminary outcomes have proved effective and biomaterials of all patients have been frozen shortly after collection and transferred to the central biobank on dry ice. Patient interview audits and other quality measures indicate good data quality.

Conclusions: The GCKD Study will establish one of the largest cohorts to date with CKD patients not requiring renal replacement and a large number of genetic controls. Binary logistic regression analysis estimated effect size of eGFR on AMD risk after adjustment for sex and age. Further adjustment was made for additional covariates such as genetic risk in key complement genes, Factor H, Factor B, Component 2 and Component 3 and also smoking status. Ordinal regression was used to evaluate the effect of complement genetic variants on eGFR in a secondary analysis. Results: In the primary analysis, an non-significant decreased risk for all types of AMD (early and late) was estimated for moderate CKD (eGFR <60ml/min/1.73m2) OR=0.62 (95% CI: 0.24-1.63, P=0.38) after adjustment for age and sex. Subset analysis for late AMD only, showed similar effect. Adjustment for complement genetic covariates and also smoking status failed to further modify risk. Secondary analysis assessing genetic variation in key complement regulatory genes, failed to detect any association with eGFR in an ordinal regression analysis. Conclusions: Although several independent studies have demonstrated increased risk of AMD associated with impaired renal function, we were unable to replicate these findings in our cohort. The cross-sectional nature of our sample with potential confounding may have limited our findings. Secondary analysis assessing genetic variation in complement-related genes also failed to demonstrate association with eGFR.

TH-PO326

Impaired Kidney Function at Hospital Discharge Has a Major Impact on Long-Term Survival of Critically Ill Patients Recovered from Renal Failure

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Background: Patients included are not well treated and their emotional status is barely taken into account. Therefore, it is crucial to relieve these patients, with appropriate drugs and emotional support. Relief seems to start with the medical staff being concerned.

Methods: Pain is a prospective study of all patients who were admitted in our nephrology department. Data collected were: sex, age, weight, height, creatininemia, all the treatments received and if the patient was dialysed or transplanted. For each patient, four forms commonly used in P management were filled to evaluate nociceptive P, neuropathic P, care related P and emotional status respectively.

Results: During 3 months, 34 patients have been included, 59% males; median age 60 years. Eight patients were hemodialyzed and 4 had received a renal graft. Median estimated glomerular filtration rate was 39 ml/min/1.73 m2. 44% of patients received a pain killer. 20% had neuropathic P, among whom 6% were not treated. 76% of patients suffered from care related P and only 18% received a preventive treatment. Anxiety and depression occurred in 27% and 12 % respectively. Only one patient received an antidepressant drug. A dosage adjustment was necessary for 100% of the prescribed treatments and 50 % of drugs were not well reliably adjusted. A week before the P evaluation, the mean score was 5 out of 10 in a numeric P scale, and on the day of evaluation, the score was 1.4.

Conclusions: Patients included are not well treated and their emotional status is barely taken into account. Therefore, it is crucial to relieve these patients, with appropriate drugs and emotional support. Relief seems to start with the medical staff being concerned.

TH-PO324

The German Chronic Kidney Disease (GCKD) Study: Design and Methods

The German Chronic Kidney Disease (GCKD) Study - Study is a prospective observational national cohort study, aiming to enrol 5000 patients with CKD of various etiology referred to nephrologists. At the time of enrolment patients have an eGFR of 30-60 ml/min/1.73 m² or overt proteinuria in the presence of an eGFR above 60 ml/min x 1.73 m². A core set of lab parameters is determined in a central lab. Standardized collection of biomaterials, including DNA, serum, plasma and urine will allow identification and validation of biomarkers associated with CKD, CKD progression and related complications using hypothesis-driven and hypothesis-free approaches. Recruitment and patient-follow is organized through a network of academic nephrology centres collaborating with practising nephrologists throughout the country.

Conclusions: The GCKD Study will establish one of the largest cohorts to date with CKD patients not requiring renal replacement and a large number of genetic controls. Binary logistic regression analysis estimated effect size of eGFR on AMD risk after adjustment for sex and age. Further adjustment was made for additional covariates such as genetic risk in key complement genes, Factor H, Factor B, Component 2 and Component 3 and also smoking status. Ordinal regression was used to evaluate the effect of complement genetic variants on eGFR in a secondary analysis. Results: In the primary analysis, a non-significant decreased risk for all types of AMD (early and late) was estimated for moderate CKD (eGFR <60ml/min/1.73m2) OR=0.62 (95% CI: 0.24-1.63, P=0.38) after adjustment for age and sex. Subset analysis for late AMD only, showed similar effect. Adjustment for complement genetic covariates and also smoking status failed to further modify risk. Secondary analysis assessing genetic variation in key complement regulatory genes, failed to detect any association with eGFR in an ordinal regression analysis. Conclusions: Although several independent studies have demonstrated increased risk of AMD associated with impaired renal function, we were unable to replicate these findings in our cohort. The cross-sectional nature of our sample with potential confounding may have limited our findings. Secondary analysis assessing genetic variation in complement-related genes also failed to demonstrate association with eGFR.

TH-PO325

Kidney Function and Age-Related Macular Degeneration

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Background: Age-related macular degeneration (AMD) shares risk factors and pathways with several diseases affecting the kidney most notably those relating to complement activation and premature cardiovascular disease. Previous reports demonstrate association between reduced kidney function and AMD. Our primary analysis sought to assess modulation of AMD risk as a consequence of kidney function using glomerular filtration rate (eGFR) in an AMD case-control cohort, with a secondary analysis to assess genetic variation in key alternative complement pathway genes and their effect on eGFR.

Methods: eGFR was estimated by CKD-EPI equations on serum creatinine measurements using MDRD values 1999, 2000 and 2002. eGFR was calculated in all patients and non-surgical patients and cause of ARF on the ICU. eGFR at hospital discharge was 44% of patients received a pain killer. 20% had neuropathic P, among whom 6% were not treated. 76% of patients suffered from care related P and only 18% received a preventive treatment. Anxiety and depression occurred in 27% and 12 % respectively. Only one patient received an antidepressant drug. A dosage adjustment was necessary for 100% of the prescribed treatments and 50 % of drugs were not well reliably adjusted. A week before the P evaluation, the mean score was 5 out of 10 in a numeric P scale, and on the day of evaluation, the score was 1.4.

Conclusions: Patients included are not well treated and their emotional status is barely taken into account. Therefore, it is crucial to relieve these patients, with appropriate drugs and emotional support. Relief seems to start with the medical staff being concerned.
Presence of Diabetes in CKD Is Associated With Higher Rates of Comorbidities and Medication Use: Results from a Large US National Healthcare Claims Database

**TH-PO327**


**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**Underline represents presenting author.** 188A
Methods: Higher aPWV was associated with faster rates of decline in demographic adjusted models. Adjustment for blood pressure did not attenuate this association. Additional adjustment for CVD risk factors attenuated this association to a small extent.

Association between aPWV and Kidney Function Decline

<table>
<thead>
<tr>
<th>aPWV N</th>
<th>Demo adjusted* (95% CI)</th>
<th>BP adjusted** (95% CI)</th>
<th>Fully adjusted*** (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Continuous (log transformed)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;642</td>
<td>2129</td>
<td>0.26 (-0.01, 0.52)</td>
<td>0.26 (-0.05, 0.02)</td>
</tr>
<tr>
<td>&gt;642</td>
<td>546</td>
<td>0.52 (0.19, 0.85)</td>
<td>0.01 (-0.07, 0.08)</td>
</tr>
</tbody>
</table>

Results: Higher aPWV was also associated with "rapid decline" in demographic adjusted models (OR per 1 SD higher log aPWV, 95% CI, 1.33, 1.72). The relationship was attenuated after adjustment for blood pressure.

Conclusions: Large artery stiffness is associated with decline in kidney function among older adults. These relationships are partly attenuated by cardiovascular risk factors and blood pressure.

TH-PO332

Vascular Disease-Associated Mortality during CKD Progression: The MDRD Study

Background: Patients with vascular disease are at high risk for both progression of CKD as well as mortality prior to ESRD. The goal of this analysis was to characterize the competing risks of ESRD and all-cause mortality associated with vascular disease at various levels of eGFR.

Methods: Data from the Modification of Diet in Renal Disease (MDRD) study (N=1,795, including 840 randomized participants) were used to evaluate the risk of ESRD and pre-ESRD death associated with vascular disease, defined as coronary artery, cerebrovascular, or peripheral vascular disease. ESRD outcomes were obtained from USRDS and mortality from NDI. Cox proportional hazards analyses were adjusted for age, sex, blood pressure, cholesterol, current smoking, cause of ESRD, log-24-hour-proteinuria, and eGFR, and censored at ESRD, death, or end of study (12/31/2007), while the competing risk analysis compared associations between vascular disease and eGFR progression.

Results: Of the 1,722 participants with complete data, 224 (13.0%) had vascular disease. Mean GFR was 35 with a range of 5 to 117 ml/min/1.73 m². Overall, 64% reached ESRD, and 14% died prior to ESRD. Participants with vascular disease were older, more often white, male, and diabetic, with higher systolic blood pressures, lower diastolic blood pressures, and lower HDL levels. The unadjusted incidence of ESRD increased with decreasing GFR (p for trend: <0.001); rates were similar between participants with and without vascular disease (p=0.6). In contrast, unadjusted mortality rates prior to ESRD were significantly higher among participants with vascular disease. In adjusted analyses, vascular disease remained significantly associated with death prior to ESRD (aHR, 1.66; 95% CI: 1.23-2.24, p<0.001) but not ESRD itself (aHR, 1.11, 95% CI: 0.91-1.35, p=0.3). There was no significant interaction between vascular disease and eGFR for either outcome (p>0.4 for both interaction terms).

Conclusions: In the MDRD Study, participants with vascular disease were 66% more likely to die prior to ESRD, however, there was no modification of vascular disease-associated mortality risk by eGFR. In contrast, patients with vascular disease were not more likely to reach ESRD.

Funding: NIDDK Support

TH-PO334

The Associations of Serum Total Alkaline Phosphatase (TAP) with Mortality Is Not Explained by Serum Levels of Skeletal Alkaline Phosphatase (SAP): National Health and Nutrition Examination Survey (NHANES) 1999-2002

Background: Higher serum TAP levels are associated with mortality in the general and chronic kidney disease (CKD) populations. It is unclear to what extent this association is related to bone disease.

Methods: Therefore, we examined the associations of serum SAP levels with mortality in the 1999-2002 NHANES adults with estimated GFR < 150 ml/min/1.73 m² (n=8,515). Details of NHANES data and sample collection have been published elsewhere. The component of TAP not explained by SAP was represented from the residuals of a regression of log transformed TAP on log transformed SAP. Mortality data were obtained from the NHANES Linked Mortality File through December 31, 2006. Stata XI was used for statistical analysis.

Results: The mean (± SD) age was 46.3 ± 14 years, 48.5% were men, and 10% African-Americans. The median serum SAP levels and SAP levels were 73.8 IU/L and 13.1 µg/L, respectively. The median eGFR was 66.1 ml/min/1.73 m². There were 70,855 deaths over a total of 2420 years of follow-up. There were 276 deaths over 864 years in the non-CKD and 429 deaths over 556 years in the CKD sub-populations.

The Importance Plot ranks metabolites according to their contribution to the distinction between decliners and non-decliners. Metabolites are listed on the y-axis in order of decreasing importance.

Conclusions: In this global metabolic profiling study, we identified key biochemicals that were altered at baseline in subjects with T1D, proteinuria and subsequent renal function decline. This study provides important insights into markers of metabolic dysfunction as predictors of diabetic nephropathy progression.

Funding: NIDDK Support, Other NIH Support - National Center for Research Resources
TH-P0335

Long Term Decrement in eGFR Following Leptospirosis Associated Acute Kidney Injury in the South of Ireland
Donal John Sexton, Marek J. Mazur, Joseph A. Eustace. Department of Renal Medicine, Cork University Hospital, Cork, Ireland.

Background: Leptospirosis induced acute kidney injury (AKI) is a relatively uncommon disorder. Current data suggests that in the majority of cases eGFR returns promptly to premorbid baseline levels following treatment. There is relatively little data on the rate of renal recovery following AKI in a European population.

Methods: We conducted a cross sectional study of eGFR in all patients with previous acute kidney injury (AKI) due to confirmed acute leptospirosis (n=27) in the southwest of Ireland over the prior ten years. The association, significance and independence of the relationship between baseline variables at the time of acute kidney injury and subsequent decline in eGFR were assessed by linear regression using SPSS v18 with a type one error rate of 0.05. The descriptive statistics are expressed as mean (sd) unless otherwise stated. eGFR was calculated using the CKD-EPI formula.

Results: Mean (sd); age 52(16) yrs, eGFR prior to AKI 91(18)ml/min/1.73m², eGFR post AKI 73(25)ml/min/1.73m². Median (IQR); follow up 1(0.5-3) yrs, peak serum creatinine during AKI 562(110-1000)µmol/L with 66.7% (18/27) of patients developing AIN1), 7.4% (2/27) with AIN2, 25.9% (7/27) with AIN1, peak bilirubin 62 (15-450) µmol/L 88.9% (24/27) of patients were male. 40.7% (11/27) required renal replacement therapy, the Median (IQR) number of hemodialysis treatments in this group was 8 (3-16) treatments. 70.4% (19/27) of cases of AKI had sterile pyuria at presentation. Serovar type was available in 40.7% (11/27) and consisted of icterohaemorrhagica 25.9%, Hardjo 11.1% and Negat 3.7% of cases. On separate univariate linear regression models the β for the association with decrement in eGFR at follow up were sex 0.02 (9.9, 26), nadir platelet count -0.51 (-0.48, -0.5) and peak serum bilirubin 0.35 (3.1, 22.6). On multivariate modeling using backward stepwise regression the final model β (95% CI) was sex -0.35 (-0.29, -0.04) and nadir platelet count -0.5 (-0.46, -0.06).

Conclusions: Despite recovery from AKI due to leptospirosis, many patients have significant decrements in renal function which warrant long term follow up and may possibly be predicted by the nadir platelet count.

TH-P0336

Effect of Extended-Release Nicotin/Laropiprant on Lipoprotein(a), Apolipoprotein A1, and Apolipoprotein B Levels in Dyslipidemic Stage 3 Chronic Kidney Disease Patients
Andrew G. Bostom,1 Jinyu Zhang,2 Joachim H. Ix,3 Nathan Spence,4 Diane Tipping,5 Andrew Tershakovec,6 1Division of Kidney Diseases, Rhode Island Hospital, Providence, RI; 2Brown Medical School, Providence, RI; 3Division of Nephrology, UCSD, San Diego, CA; 4Division of Medicine, Rhode Island Hospital, Providence, RI; 5Tipping LLC, Green Lane, PA.

Background: Given the paucity of data regarding nicotine’s impact on experimental measures of dyslipidemia in chronic kidney disease (CKD), we examined the drug’s effect on apolipoprotein (apo) A1 and B, and lipoprotein(a) [Lp(a)] levels among patients with stage 3 CKD.

Methods: Subjects (n=261) with a baseline estimated glomerular filtration rate (eGFR) of 30 to < 60 ml/min/1.73 m² were drawn from two completed trials of extended-release nicotin/laropiprant (ERN-L, laropiprant being an inhibitor of niacin-induced flushing; ERN-L at 2g/d, n=177; n=84 placebo). Apo A1, apo B, and Lp(a) were measured serially over 24-weeks. Results: The ERN-L and placebo groups were comparable with respect to baseline mean age, eGFR, apo A1, apo B, and Lp(a) levels, as well as the distribution of gender, concurrent statin use, and diabetes. Repeated measures analyses revealed that ERN-L, relative to placebo, caused a decrease (95% confidence interval) of -20.5% (-25.1%, -16.0%) in mean apo B levels, and −27.8% (-38.4%, -23.2%) in median Lp(a) levels, as well as an increase of +11.1% (+14.7%, +7.4%) in mean apo A1 levels. Stratified analyses revealed that these effects on apo B, apo A1, and Lp(a) were entirely consistent with what was observed among dyslipidemic patients whose eGFR was >60 ml/min/1.73 m².

Conclusions: Nicotin treatment results in robust lowering effects on apo B, and Lp(a) levels, as well as substantial increases in apo A1 levels, among patients with stage 3 CKD. Till now, stage 3-4 CKD patients have been significantly under-represented in secondary cardiovascular disease prevention trials. We conclude that a niacin treatment arm merits serious consideration within any future clinical trials targeting stage 3-4 CKD patients for the potential reduction of cardioaenal outcomes.

TH-P0337

Effect of Extended-Release Niacin on Serum Phosphorus and Fibroblast Growth Factor 23 Levels in Stage 2-3 Chronic Kidney Disease Patients
Andrew G. Bostom,1 Andrea Poenariu,2 Joachim H. Ix,2 William Hanlon,3 Darbie MacCubbin,4 Michael Steffes.4 1Division of Kidney Diseases, Rhode Island Hospital, Providence, RI; 2Division of Nephrology, University of California-San Diego, CA; 3Merek Sharp & Dohme Corp, Rapids, NJ; 4Merck Sharp & Dohme Corp, Rahway, NJ; 5Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN.

Background: The hypophosphatemic effect of niacin is now increasingly recognized. We examined whether niacin-induced phosphorus-lowering among dyslipidemic stage 2-3 chronic kidney disease (CKD) patients was accompanied by any impact on their levels of the phosphatonin fibroblast growth factor (FGF)-23.

Methods: Intact serum FGF23 and phosphorus (phos) levels were determined in n=327 patients with a baseline estimated glomerular filtration rate (eGFR) of 30 to 74 ml/min/1.73 m² randomized to active extended-release niacin (N) 2g/once daily, with or without the prostaglandin-mediated flushing inhibitor laropiprant, or placebo (n=260; N=67 on placebo [P]), at weeks 0 (baseline) and 24.

Results: Mean (± SD) age [N= 61 ± 11; P= 61 ± 9 years], eGFR [N=62.8 ± 8.6; P= 62.2 ± 9.4 ml/min/1.73 m²], as well as both baseline serum FGF23 [N=44.0 ± 18.9; P= 43.2 ± 18.6 pg/ml] and phos levels [N=3.33 ± 0.58; P=3.93 ± 0.51 mg/dl] were comparable between the groups. While the 24-week change (mean ± SE) in serum phos among patients on active N (0.45 ± 0.03; -13.5%) relative to P treatment (0.03 ± 0.06) was highly significant (p <0.001), N treatment (-0.9 ± 1.0) had no effect compared to P (+1.8 ± 2.0) on serum intact FGF23 levels (p=0.238). Our findings confirm the sustained (i.e., 24-week) and relatively robust (-13.5%) hypophosphatemic effect of N in patients with stage 2-3 CKD. Conversely, such ERN treatment has no apparent impact on serum FGF23 levels at these stages of CKD.

Conclusions: Thus use of FGF-23 levels as a surrogate for phos-lowering effects, or as specific entry criteria for trials ostensibly designed to test the hypothesis that hypophosphatemic therapy will reduce cardioaenal outcomes in stage 3-4 CKD, may not be warranted.

Funding: Pharmaceutical Company Support

TH-P0338

Phosphate, CKD Progression and ACE-Inhibition – A Post-Hoc Analysis of the REIN Trial
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Background: Phosphate has been suggested to play a role in the onset and progression of chronic nephropathies. We evaluated the relationships between baseline serum phosphate levels, disease progression and response to angiotensin converting enzyme (ACE) inhibition in 331 patients with proteinuric nephropathies prospectively monitored by gold standard procedures in the setting of the Ranirpil Efficacy In Nephropathy (REIN) trial.

Results: Independent of treatment, progression to end stage renal disease (ESRD) alone or in combination with doubling serum creatinine was significantly faster in patients with phosphate levels in the higher 3rd and the 4th quartiles than in those with lower phosphate (P<0.001). Similar findings were observed in crude and adjusted analyses considering phosphate as a continuous variable (P<0.004). The ramipril renoprotective effect progressively decreased for increasing serum phosphate with a significant (P<0.008) effect modification by phosphate levels on both outcomes and such an interaction was unmodified after adjustments for potential confounders, such as GFR and urinary protein.

Conclusions: In patients with proteinuric chronic nephropathies, phosphate is an independent risk factor for renal disease progression and may limit or even blunt the renoprotective effect of ACE inhibitor therapy. Trials are needed to test whether reducing phosphate exposure may improve renal outcomes and optimize the renoprotective effect of ACE inhibition in this population.

On behalf of REIN working group.
Funding: Government Support - Non-U.S.

TH-P0339

Abstract Withdrawn

TH-P0340

Impact of a Chronic Kidney Disease Registry and Provider Education on Guideline Adherence – A Cluster Randomized Controlled Trial
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Background: Low adherence to KDOQI guidelines may be due to unrecognized CKD and lack of guideline awareness on the part of providers. The goal of this study was to evaluate the impact of provider education and a CKD registry on guideline adherence.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

190A
Methods: We conducted a cluster randomized controlled trial at the Louis Stokes Cleveland VAMC. One of two primary care clinics was randomized to intervention. Providers from both clinics received a lecture on CKD guidelines at study initiation. Providers in the intervention clinic were given access to and shown how to use a CKD registry, which identifies patients with CKD and is automatically updated daily. Eligible patients had at least one primary care visit in the last year, had CKD based on eGFR, and had not received renal replacement therapy. The outcome was the percent of patients with at least one PTH measured in the past year and was evaluated at baseline and after the 12 month intervention. Secondary outcomes were percent at goal BP and percent prescribed ACE/ARB.

Results: At baseline, 387 patients in the control clinic and 357 patients in the intervention clinic were eligible. The percent of patients with a PTH in the past year increased in both clinics from baseline to 12 months (14.7% to 22.7% in the control clinic; 15.4% to 28.4% in the intervention clinic). However, there was no difference between clinics at 12 months (P = 0.07). After 12 months, there was no difference in the percent of subjects at goal BP (40.7% vs 44.1%) or the percent of subjects on an ACE/ARB (74.9% vs 78.2%). Only 5 of the 37 providers in the intervention clinic accessed the registry.

Conclusions: An intervention that included education on CKD guidelines and access to a CKD patient registry did not improve guideline adherence over education alone. Adherence to the primary process measure improved in both clinics, but no improvement was seen in intermediate clinical outcomes. Improving the care of patients with CKD will likely require a multifaceted approach including system redesign.

Funding: NIDDK Support

TH-P0341


Background: CKD recognition in the clinical setting is low. Whether CKD recognition in a problem list for a patient improves care is unclear.

Methods: Patients entered an electronic health record-based CKD registry if two outpatient eGFRs were <60 mL/min/1.73 m2 at least 90 days apart from Jan 2005 to Apr 2011. CKD recognition was defined by having CKD in the problem list (ICD-9 codes for CKD, diabetic kidney disease, and hypertensive kidney disease) within 1 yr after entry. We calculated proportion of CKD patients with >1 yr follow-up whose CKD was recognized within 1 yr after entry. We also calculated the proportion of CKD patients who had at least one PTH measured in the past year. We calculated the percent of patients with at least one primary care visit in the last year, had CKD based on eGFR, and had not received renal replacement therapy. The outcome was the percent of patients with at least one PTH measured in the past year and was evaluated at baseline and after the 12 month intervention.

Secondary outcomes were percent at goal BP and percent prescribed ACE/ARB.

Results: At baseline, 387 patients in the control clinic and 357 patients in the intervention clinic were eligible. The percent of patients with a PTH in the past year increased in both clinics from baseline to 12 months (14.7% to 22.7% in the control clinic; 15.4% to 28.4% in the intervention clinic). However, there was no difference between clinics at 12 months (P = 0.07). After 12 months, there was no difference in the percent of subjects at goal BP (40.7% vs 44.1%) or the percent of subjects on an ACE/ARB (74.9% vs 78.2%). Only 5 of the 37 providers in the intervention clinic accessed the registry.

Conclusions: An intervention that included education on CKD guidelines and access to a CKD patient registry did not improve guideline adherence over education alone. Adherence to the primary process measure improved in both clinics, but no improvement was seen in intermediate clinical outcomes. Improving the care of patients with CKD will likely require a multifaceted approach including system redesign.

Funding: NIDDK Support

TH-P0342

Racial Disparity between African Americans (AA) and Caucasian Americans (CA) in Pre-ESRD Care: A Multi-Level National Study Guofen Yan,1 Jennie Z. Ma,1 Alfred K. Cheung,2 Tom H. Greene,2 Mohamed Norman Oliver,3 Wei Yu.4 1University of Virginia; 2University of Utah.

Background: Pre-ESRD nephrology care is an important predictor for ESRD outcome. Racial disparity in pre-ESRD care is well known, but the cause is multifactorial, including geography. Using USRDS, this study employed a multilevel modeling approach to systematically examine to what extent regional characteristics contribute to the disparity.

Methods: The analysis included all AA or CA incident dialysis patients who completed the new Medical Evidence form in 2005-09. The care indicator examined was whether a patient had seen a nephrologist for at least 6 months prior to ESRD therapy. The three-level logistic regression models corresponding to patients, states and regional divisions were used to estimate the probability of seeing a nephrologist and its variability across states and divisions, and examine patient- and state-level predictors. We compared results of AA and CA by age ≤65 (AA: n=36,688, CA: n=113,187) and age >65 (AA: 734,444; CA: n=109,384).

Results: The estimated probability of seeing a nephrologist was 55.8% for 1AA, 55.6% for 1CA, 48.5% for 2AA and 52.8% for 2CA, suggesting that at the national level the racial disparity (AA vs. CA) was in the younger population, but not in older. The estimated variability across states was 0.46 for 1AA, 0.33 for 1CA, 0.41 for 2AA and 0.35 for 2CA (SD on logit), indicating substantial inter-state differences in all groups (e.g., 60-71% probability in New England states vs. 38-53% in East South Central states). The disparity within state (A minus CA in probability) varied substantially across states, from -13% to 3% in younger population, and -9% to 12% in older. Strong patient predictors included patient’s insurance coverage, working status and comorbid conditions. Strong state predictors for state-to-state variability included state’s poverty, racial composition and education level.

Conclusions: Pre-ESRD care depends highly on which state the patient lives in; AA more so than CA. The disparity among states is greater than that with states. Efforts to reduce racial disparity will be more effective by intervention at state level and targeting younger population.

Funding: NIDDK Support

TH-P0343

Blood Pressure Lowering Agents for the Primary Prevention of Diabetic Kidney Disease: A Systematic Review and Meta-Analysis Icheng Lv,1,2 Vladko Perkovic,1 Jonathan C. Craig,3 Celine Foote,1 Haiyan Wang,2 Alan Cass,1 Min Jun,1 Hildo Jan Lambers Heerspink,4 John P. Chalmers,1 Johannes F. Mann,5 Giovanni F. M. Strippoli,3,6,7 1The George Institute for International Health, The University of Sydney, Australia; 2Renal division, Peking University First Hospital, Institute of Nephrology, Peking University, Beijing, China; 3Centre for Kidney Research, The Children’s Hospital at Westmead, School of Public Health, The University of Sydney, Sydney, Australia; 4Department of Clinical Pharmacology, University Medical Center Groningen, University of Groningen, The Netherlands; 5Schwabing General Hospital, and KH Kidney Centre, Ludwig Maximilians University Munich, Germany; 6Department of Pharmacology and Epidemiology, Mario Negri Sud Consortium, S Maria Imbaro, Chieti, Italy; 7Diabetes Scientific Office, Lund, Sweden.

Background: We undertook a systematic review and analysis to quantify the benefits and harms of blood pressure lowering agents in normoalbuminuric individuals with diabetes.

Methods: We systematically searched MEDLINE, Embase, Cochrane Library from 1966 to January 2011. We included randomized controlled trials in normoalbuminuric participants with diabetes. Meta-analysis was done with a random effects model.

Results: We identified 28 trials involving 40,668 patients. ACE inhibitors reduced the risk of new onset of macro- and/or microalbuminuria compared with placebo (RR 0.71; 95% CI, 0.56-0.89), and compared to calcium channel blockers (RR 0.66; 95% CI, 0.42-0.85). ACE inhibitors also reduced the risk of death compared with placebo (RR 0.84, 95% CI 0.73-0.97). No effect was observed for ARBs for new onset nephropathy (RR 0.91; 95% CI, 0.73-1.11). Compared with placebo, however meta-regression suggested possible benefits of ARBs for the prevention of kidney disease in high risk patients.

Conclusions: ACE inhibitors prevent new onset nephropathy and death in normoalbuminuric people along with diabetes, and should therefore be used preferentially in this population. More data are needed to clarify the role of ARBs and other drug classes on the prevention of diabetic kidney disease.
TH-PO344
Assessment of Quality Incentive Program (QIP) Preparedness in Hospital-Based Dialysis Centers (HBDCs) and Independent Dialysis Organizations (IDOs) in 2010

Background: QIP was implemented by Centers for Medicare and Medicaid Services (CMS) in 2011 to ensure that Proactive Patient System Changes would not adversely impact ESRD dialysis programs. We assessed preparedness of HBDCs and IDOs for QIP in 2010 compared to 2008 national averages, per CMS methodology.

Methods: We conducted a retrospective analysis of an electronic medical record database from 638 hospital dialysis patients in 52 HBDCs and 31 IDOs in the US (15 states and all payer types) from Jan 1-Dec 31 2010. Analytic cohorts based on QIP criteria each comprised patients ≥18 years of age at first dialysis, and administered erythropoiesis stimulating agents ≥90 days after first dialysis with ≥4 hemoglobin (Hb) measures 5-20 g/dL (Hb cohort), or dialysis <5 times/week with ≥4 urea reduction ratios (URRs) ≥183 days after first dialysis (URR cohort). The proportion of facilities meeting 2008 national performance rates ≤2% of patients with mean Hb <10 g/dL, ≤2% of patients with Hb <12 g/dL, and ≤96% of patients with mean URR ≥65% was assessed.

Results: Demographics among the cohorts were comparable: 55% men, mean age 60 years, ≥40% diabetes, <60% hypertension, mean Hb 11.3, and mean URR 73.4%. In 2010, the proportion of patients with mean Hb<10 g/dL, mean Hb>12 g/dL, and mean URR<65% were similar in HBDCs and IDOs (Table 1). Although 93% of facilities in 2010 met criteria for limiting high Hb, 66% of facilities failed to meet criteria for low Hb and 60% failed to meet URR criteria.

Table 1: Performance Trends of HBDCs and IDOs in 2008 vs 2010 using CMS Methodology for QIP.

<table>
<thead>
<tr>
<th>Facility Type</th>
<th>Percentage of Facilities Meeting QIP Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBDC</td>
<td>93%</td>
</tr>
<tr>
<td>IDO</td>
<td>93%</td>
</tr>
</tbody>
</table>

Conclusions: Improved performance is needed, as only 16.0% of HBDCs and 10.0% of IDOs would have achieved 100% QIP performance.

Funding: Pharmaceutical Company Support

TH-PO345
Community Based Participatory Approach to Interrelated Epidemics of Obesity Diabetes and Kidney Disease in the Zuni Pueblo
Donica M. Ghahate, Jeanette Bobelu, Phillip Sandy, Mick T. Romancito, Arlene Vallabh O. Shah, Obesity Diabetes and Kidney Disease in the Zuni Pueblo.

Background: Obesity Diabetes and Kidney Disease is a public health challenge in the Zuni Pueblo. Community health representatives (CHR) led the sessions and presented a series of questions, developed from a literature review and designed to elicit information on perceived barriers to health care. CHRs then facilitated discussions with community members in their care: (1) inadequate transportation infrastructure in the pueblo; (2) inadequate transportation between the pueblo and secondary and tertiary care sites; (3) inadequate staffing; (4) lack of staff continuity among Zuni Indian Health Service providers; and (5) lack of awareness of existing health promoting programs in the community. The participatory approach allowed CHRs to use their unique perspective to gather a wide range of data that would be useful for the Zuni Pueblo.

Methods: The questions were used to explore dominant themes. Audiotapes were translated and transcribed and inserted into Atlas-TI program. The dictionary and inserted these into Atlas-TI program. Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Conclusions: Combing DMI to BMI could assess body composition in changes of body weight. The ratio of BMI to eGFR could be a suitable tool to assess fluid volume in CKD patients.

Funding: Government Support - Non-U.S.

TH-PO346
Assessment of Body Composition by Dry Mass Index and a Ratio of Total Body Water to Estimated Volume Based on Bioimpedance Analysis in Chronic Kidney Disease Patients
Yasuhiro Ohashi, Takatoki Otani, Rebin Tai, Ken Sakai, Sonoo Mizuiru, Atsushi Aikawa, Nephrology, Toho University School of Medicine, Tokyo, Japan.

Background: Adequate assessment of fluid and nutritional status is of major importance in managing Chronic Kidney Disease (CKD) patients. Body Mass Index and Bioimpedance analysis (BIA) have been proposed as acceptable markers. There remains uncertainty, however, regarding the impact of fluid status in CKD on BMI and the BIA measurements. Dry Mass Index (DMI), which removes TBW from weight, might be able to more adequately assess tool. The aim of this study was, first, to assess agreement between TBW and estimated Volume (eV) by the Watson formula and second, to assess the reliability of the DMI as a marker of nutritional status.

Methods: A total of 45 CKD patients randomly were measured TBW, Intracellular Water (ICW), Extracellular Water (ECW) and a ratio of ECW to TBW as edematous index (E/I) using Bioimpedance Analysis. The participants were surveyed for age, gender, underlying disease, BMI, blood pressure (BP), serum albumin (Alb), eGFR, and proteinuria. The DMI was calculated by the formula [weight - TBW]/height^2, and the correlation between DMI and BMI was assessed. As a marker of fluid status, we also evaluated a ratio of TBW to eV. The range of euvolemic status was defined from 0.970 to 1.029.

Results: The formula indicating relationship between BMI and DMI is obtained as [BMI = 0.595 + BMI(2.091)] by the linear regression analysis. Based on BMI, DMI and TBW/eV, the numbers of the participants were categorized among the following: BMI ≥ 25 with hyperalimentation; 6, with euvoelium; 10 or with hypervolemia; 3. 18.5 ≤ BMI < 25 with hypovolemia: 11, with euvoelium: 9 or with hypervolemia: 5; and BMI < 18.5 with euvoelium: one patient. The TBW/eV was positively-correlated BP (p<0.02), %ICW (p<0.01) and %ECW in BW (p<0.01) and Alb (p<0.03). In contrast, the increase in ECW/TBW was not correlated ECW volume excess, but ICF deficit with aging (p<0.01).

Conclusions: Combining DMI to BMI could assess body composition in changes of body weight. The ratio of TBW to eV could be a suitable tool to assess fluid volume in CKD patients.

Funding: Government Support - Non-U.S.
TH-PO349
Lifet ime Costs of Hemodialysis and Peritoneal Dialysis Patients in Taiwan
Shu-Jung Ho, Tze-Wah Kao, Fan-Chi Chang.
Department of Internal Medicine, National Taiwan University Hospital.

Background: This study compared the lifetime costs between hemodialysis (HD) and peritoneal dialysis (PD) patients in Taiwan.

Methods: The data base of a cohort of patients who had received maintenance dialysis and were registered under the National Health Insurance (NHI) from July, 1997 to December, 2005 was utilized. Patients who were under 18 years old, were diagnosed with cancer or had received both PD and HD were excluded. PD patients were then matched with HD patients on age, sex and diabetic status, and followed up until Dec 31th, 2006. Patients who had stopped using the subsides of the NHI for at least 3 months were considered dead. Life expectancy, expected years of life lost(EYL), total lifetime costs(lifetime costs of ambulatory and inpatient care), and costs per year of both HD and PD patients were then compared using the ISQOL software.

Results: There were 3062 pairs of matched HD and PD patients (mean age, 53.2±15.4 years). Life expectancies and EYL were not different between HD and PD patients. Diabetic patients who underwent HD had a slightly longer life expectancy than those who underwent PD (p=0.0061), while there was no difference in life expectancy between non-diabetic HD and PD patients. For diabetic patients, the total lifetime costs of those treated with HD were higher than those treated with PD, no matter the discount rate was 3% or 5% (p<0.001). For patients without DM, the total lifetime costs of those treated with HD were also higher than those treated with PD, whether the discount rate was 3%(P=0.0136) or 5%(P=0.0018). The costs per year for HD patients were higher than that of PD patients. Lifetime costs of ambulatory care were significantly higher for HD patients than for PD patients no matter the patients had DM or not and the discount rate was 3% or 5%. When lifetime costs of ambulatory and inpatient care were compared, there was no statistical difference between HD and PD. When adjusted by survival, patients undergoing HD had higher monthly health costs than PD patients.

Conclusions: Both the total lifetime costs, lifetime costs of ambulatory care and costs per year paid by the NHI were higher for HD than were for PD in Taiwan.

TH-PO350
Serum gamma- Glutamyltransferase Levels Are Inversely Related to Endothelial Dysfunction in Chronic Kidney Disease
Mahmut iker Yilmaz, Faruk Turgut, Mehmet Kanbay, Mutlu Saglam, Alper Sonmez, Murat Kararman, Seyid Ahmet Ay, Mujdat Yenicesu, Peter Stenvinkel.
Nephrology, Gulhane School of Medicine, Ankara, Turkey; 2Clinical Science, Karolinska University, Stockholm, Sweden; 3Radiology, Gulhane School of Medicine, Ankara, Turkey; 4Endocrinology, Gulhane School of Medicine, Ankara, Turkey; 5Internal Medicine, Gulhane School of Medicine, Ankara, Turkey.

Background: Gamma- glutamyltransferase (GGT) is an enzyme responsible for the extracellular catalysis of the antioxidant glutathione and recently implicated in the pathogenesis of atherosclerosis. Endothelial dysfunction is a prodromal feature of atherogenesis, and is closely linked with BMI, and systolic blood pressure. Since GGT is highly present in uremia and a causally linked to endothelial dysfunction, we hypothesized that GGT may be a factor implicated in this process.

Methods: We investigated the relationship between flow-mediated vasodilation (FMD) and GGT in a series of 214 non-diabetic patients with stages 3-5 CKD. Exclusion criteria contemplated, among others, alcohol consumption, manifest cirrhosis and established atherosclerotic complication.

Results: Serum GGT levels were negatively associated with FMD (r = -0.41, p<0.001) and with ICD-9 code diagnosis for various kidney diseases) who underwent both restrictive and malabsorptive types of bariatric surgery. Renal parameters (serum creatinine and eGFR using CKD-EPI equation), body mass index (BMI), and systolic blood pressure (SBP) data were collected at baseline and at 6 months after surgery to analyze changes arising from weight loss. Factors predicting change in serum creatinine were examined using linear regression.

Results: Seventy-eight patients with mean serum creatinine of 1.3 mg/dl and mean eGFR of 52.8 ml/min/1.73m² were included. At the end of 6 months, BMI decreased from 44.6 kg/m² to 35.0 kg/m², SBP decreased from 134 ± 21 to 130 ± 17 mm Hg and serum creatinine decreased to 1.09 mg/dl with an improvement in GFR to 66.5 ml/min/1.73m² (all paired t-test p<0.001). Number of antihypertensive and oral hypoglycemic agents decreased after surgery. The decrease in serum creatinine was significantly correlated with the decrease in BMI (r = 0.20) and SBP (r = 0.28). In the regression model, females had a 0.12 mg/dl lesser decline in serum creatinine than males (p=0.03), and Caucasians had a 0.11 mg/dl lesser decline in serum creatinine than African Americans (p=0.04) after surgery.

Conclusions: Serum creatinine decreases with weight loss after bariatric surgery. The decline in serum creatinine is less among females and Caucasians, populations who usually have lower rates of eGFR decline than males and African Americans. Future studies need to examine how weight loss after bariatric surgery produces a change in renal function, and how these changes in renal function are linked with changes in body composition.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990

TH-PO351
Patient Reported Outcomes Measurement Information System (PROMIS): Pediatric Chronic Kidney Disease
University of Michigan, Ann Arbor; MI; 2University of North Carolina, Chapel Hill; 3Midwest Pediatric Nephrology Consortium (MWPNC).

Background: The PROMIS study goal was to create patient reported outcome evaluation tools. 233 children, ages 8-17, with CKD (stages 4-5 or kidney transplant) were enrolled from MWPNC sites in this cross-sectional study designed to validate the PROMIS instrument in children with CKD.

Methods: Stepwise regression analyses were conducted to assess association of child characteristics with 8 domain scores: peer relationships, depression, anxiety, pain, fatigue, upper extremity dexterity and mobility; regression coefficients must be significant at p<0.15 to be selected in the model. Significant (p=0.5) results of multivariate analysis are reported (R²ESE).

Results: Patients with recent hospitalizations and with edema were associated with increased domain scores for depression, anxiety, pain, and low energy. Hospitalization and active edema were associated with decreased mobility scores. Co-morbidities negatively affected all domains except for pain. There were not significant changes in domain scores in response to different stages of CKD. Patients did not view ESRD negatively affecting their social relationships.

PROMIS Domain Scores for CKD (R²ESE)

<table>
<thead>
<tr>
<th>Domain</th>
<th>R²ESE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospitalizations</td>
<td>0.59(2.2)</td>
</tr>
<tr>
<td>Edema</td>
<td>0.48(1.6)</td>
</tr>
<tr>
<td>Co-Morbidities</td>
<td>1.0(0.6)</td>
</tr>
<tr>
<td>Number</td>
<td></td>
</tr>
<tr>
<td>(STD+HD)</td>
<td>2.7(1.2)</td>
</tr>
<tr>
<td>Cognition</td>
<td></td>
</tr>
<tr>
<td>(STD+HD stage 1-4)</td>
<td>3.4(0.8)</td>
</tr>
<tr>
<td>ESRD</td>
<td>0.6(2.1)</td>
</tr>
</tbody>
</table>

Conclusions: The PROMIS domains are sensitive to disease severity indicators in children with CKD. Longitudinal studies documenting the responsiveness to change in disease activity over time will strengthen the validation and future utility of this tool.

Funding: Other NIH Support - Sponsor Number: 5 U01 AR502181

TH-PO352
Changes in Serum Creatinine after Bariatric Surgery
1Nephrology and Hypertension, Cleveland Clinic; 2Pathobiology, Cleveland Clinic Lerner School of Medicine.

Background: Weight loss after bariatric surgery is thought to improve serum creatinine in patients with and without chronic kidney disease (CKD). These changes in serum creatinine may be mediated through loss of muscle mass rather than improvement in renal function. Therefore, we examined the potential factors influencing the changes in serum creatinine among CKD patients who underwent bariatric surgery.

Methods: We conducted a retrospective study of stage 2-3 CKD patients (eGFR 30-89 ml/min/1.73m² and with ICD-9 code diagnosis for various kidney diseases) who underwent both restrictive and malabsorptive types of bariatric surgery. Renal parameters (serum creatinine and eGFR using CKD-EPI equation), body mass index (BMI), and systolic blood pressure (SBP) data were collected at baseline and at 6 months after surgery to analyze changes arising from weight loss. Factors predicting change in serum creatinine were examined using linear regression.

Results: Seventy-eight patients with mean serum creatinine of 1.3 mg/dl and mean eGFR of 52.8 ml/min/1.73m² were included. At the end of 6 months, BMI decreased from 44.6 kg/m² to 35.0 kg/m², SBP decreased from 134 ± 21 to 130 ± 17 mm Hg and serum creatinine decreased to 1.09 mg/dl with an improvement in GFR to 66.5 ml/min/1.73m² (all paired t-test p<0.001). Number of antihypertensive and oral hypoglycemic agents decreased after surgery. The decrease in serum creatinine was significantly correlated with the decrease in BMI (r = 0.20) and SBP (r = 0.28). In the regression model, females had a 0.12 mg/dl lesser decline in serum creatinine than males (p=0.03), and Caucasians had a 0.11 mg/dl lesser decline in serum creatinine than African Americans (p=0.04) after surgery.

Conclusions: Serum creatinine decreases with weight loss after bariatric surgery. The decline in serum creatinine is less among females and Caucasians, populations who usually have lower rates of eGFR decline than males and African Americans. Future studies need to examine how weight loss after bariatric surgery produces a change in renal function, and how these changes in renal function are linked with changes in body composition.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990
TH-PO353
Diet, Physical Activity, Obesity and Intentional Weight Loss in Chronic Kidney Disease: NHANES 1999-2006
Sankar D. Navaneethan,1 John P. Kirwan,2 Susana Arrigain,3 Ashwni R. Sehgal,4 Jesse D. Schold.1,2 Nephrology & Hypertension, Cleveland Clinic; 1Pathobiology, Cleveland Clinic; 2Quantitative Health Sciences, Cleveland Clinic; 3Nephrology, MetroHealth Medical Center.

Background: Previous reports have shown that the chronic kidney disease (CKD) population has a higher body mass index (BMI) and waist circumference (WC) than non-CKD. We aimed to examine a) the lifestyle (dietary intake and physical activity) and behavioral factors (desire to weigh less) that might explain these differences and b) factors related to pursuing weight loss among a nationally representative sample of adults.

Methods: A cross-sectional analysis of 15,993 adult participants in the National Health and Nutrition Examination Surveys conducted between 1999-2006. CKD was defined as patients with estimated glomerular filtration rate < 60 ml/min/1.73m² or urine albumin creatinine ratio ≥ 30 mg/g. Differences in lifestyle and behavioral factors between CKD and non-CKD participants were compared with sas survey procedures. Factors associated with pursuing weight loss were examined using survey logistic regression.

Results: Participants with CKD (n=2,812) had higher WC (100.2 cm vs. 95.3 cm, p<0.001) and a higher proportion had a BMI > 30 kg/m² (37% vs. 29%, p=0.001). CKD and non-CKD populations did not differ based on percentage of energy derived from carbohydrate, fat, saturated fat and protein. However, 68% of the CKD population did not meet the minimum recommended leisure time physical activity goals (<450 METS/min/week) compared to 55% among non-CKD (p=0.001). Proportions of CKD and non-CKD participants who expressed desire to weigh less were not different. CKD participants had lower odds (0.84, 95% CI 0.71, 0.98) of pursuing a weight loss program, and so did African Americans (0.67, 95% CI 0.58, 0.76) and elderly adults.

Conclusions: Dietary composition and the desire to weigh less are not different for CKD and non-CKD populations. However, the CKD population is less active and pursues weight loss less often than non-CKD populations. These results suggest the need for studies to examine the underlying reasons for these differences, and studies aimed at developing targeted weight loss programs for CKD patients.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: RR024990

TH-PO354
Albuminuria Is Strongly Associated with Low BMI and Only Weekly to Cardiovascular Risk Factors in Apparently Healthy Subjects Younger Than 40 Years of Age
Jicheng Lv,1,2 Damin Xu,1 Hong Zhang,1 Xiaoyan Zhang,2 Xiyan Zhao.1 1Renal Division, Department of Medicine, Peking University First Hospital, Peking University Institute of Nephrology, Beijing, China; 2The George Institute for Global Health, the University of Sydney, Sydney, Australia.

Background: Albuminuria reflects increased cardiovascular risk in high risk groups. However, albuminuria occurs also frequently in absence of cardiovascular risk factors. This study evaluated which risk factors are associated with albuminuria depending on age.

Methods: The unreferred renal insufficiency (URI) study is a cross sectional study on the prevalence of metabolic risk factors in Belgian workers. Subjects with abnormal urinary sediment or missing data of interest were excluded, leaving a cohort of 1,361 apparently healthy workers. A single urine sample was used to measure albumin to creatinine ratio (ACR).

Results: As expected blood pressure (p<0.001), heart rate (p<0.001), plasma glucose (p=0.001), serum cholesterol (p<0.001), and BMI (p<0.001) were higher in those ≥40 vs. <40 years (median of age), but in spite of this higher prevalence of traditional cardiovascular risk factors, ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97). In subjects under 40 years of age (n=668), ACR was strongly associated with BMI < 20 kg/m² (p<0.001) whereas ln ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97). In subjects under 40 years of age (n=668), ACR was strongly associated with BMI < 20 kg/m² (p<0.001) whereas ln ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97). In subjects under 40 years of age (n=668), ACR was strongly associated with BMI < 20 kg/m² (p<0.001) whereas ln ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97). In subjects under 40 years of age (n=668), ACR was strongly associated with BMI < 20 kg/m² (p<0.001) whereas ln ACR was identical (0.78±1.13 vs. 0.78±1.17, p=0.97).

Conclusions: Screening for cardiovascular risk by using urinary albumin in apparently healthy subjects under 40 years of age was unexpectedly associated with low BMI and with virtually no traditional cardiovascular risk factors. Only in subjects over forty, albuminuria follows the traditional cardiovascular risk pattern.

Trial registration 2006 NCT00365911
Funding: Pharmaceutical Company Support

TH-PO355
Corticosteroid Therapy in IgA Nephropathy: A Systematic Review and Meta-Analysis
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Background: Although several trials have evaluated steroids in IgA Nephropathy (IgAN), there is still much uncertainty on such a treatment in IgAN. In this study we did a systematic review and meta-analysis to assess the efficacy and safety of corticosteroids in IgAN.

Methods: We systematically searched Medline, Embase, and the Cochrane Library for randomized controlled trials of corticosteroids therapy in IgA nephropathy published between 1966 and 2011. We evaluated the effects of corticosteroid on renal function and proteinuria, as well as adverse events. Meta-analysis was done with a random effects model.

Results: We identified 9 relevant trials with 536 patients with IgAN, the quality of which was suboptimal. Overall steroids therapy was associated with a lower risk of progression to kidney failure (double serum creatinine or ESKD) (RR:0.29, 95%CI:0.13-0.65, p=0.02, I²=0.0%, p for heterogeneity =0.47) and proteinuria reduction (WMD=-0.46g/d, 95%CI: -0.63 - 0.29).

Subgroup analysis revealed that the effect of steroids is modified by dose of steroids (p for heterogeneity =0.032) or the risk of kidney failures (p for heterogeneity =0.048). Full dose of steroids produced significant renal protection (RR 0.14, 95%CI 0.05 - 0.39) while not in low dose steroids (RR 0.84, 95%CI 0.23 - 3.04). Steroids didn’t increase the overall risk of adverse events (RR: 1.62, 95%CI:0.83-3.16, p=0.15).

Conclusions: Corticosteroid produces renal protection in patients with IgAN without increase the risk of overall adverse events. However the low quality of included studies limited the study conclusions.

TH-PO356
Short-Term Effects of a Keto/amino Acid Supplemented Low Protein Diet on the Delay of Progressive Renal Failure in Chronic Kidney Disease
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Background: A protein-restricted diet with keto/amino acids (KA) supplement showed favorable effects on uremia, and delayed renal replacement therapy in patients with chronic kidney disease.

Methods: We conducted an open, prospective, randomized, and multi-center study for 6 months. A total of 67 patients were randomly assigned into two groups. The LPD + KA group was advised to take less than 0.6 kg/day protein with KA. The LPD group was advised to consume less than 0.6 kg/day protein. Clinical and biochemical parameters were evaluated at baseline, 3 and 6 months.

Results: Calcium and phosphorous (Ca×P) product level measured at 3 months was significantly lower in the LPD + KA group than in the LPD group (LPD + KA group: 33.5 ± 5.0 mg²/dl² vs. LPD group: 66.0 ± 20 mg²/dl², p<0.001) whereas it associated only borderline with cholesterol (p=0.03) and with anti-hypertensive treatment (p=0.01). In contrast, in subjects over forty (n=693), ACR was positively associated with BMI >30 kg/m² (p=0.001), heart rate (p=0.001), systolic blood pressure (p=0.001), diabetes (p=0.001), CRP (p=0.001) and smoking (p=0.001)

Conclusions: The present study suggests that a low protein diet supplemented with keto/amino acids had a beneficial effect on preserving renal function and improving calcium and phosphorous disturbances in patients with chronic kidney disease.
Baseline Observations from a Randomized Crossover Trial of Lowering Sodium Intake in Chronic Kidney Disease (CKD)-LOSALT Study Rajiv Saran,1 Robin L. Sands,1 Brenda W. Gillespie,1 Michael Heung,1 Scott L. Hummel,1 Vimal K. Derali,1 Nathan W. Levin,2 Fanzan Zha,2 Peter C. Kotanko,2 Philip J. Klemmer.1 1UM-KECC, Univ of MI, Ann Arbor, MI; 2Renal Research Institute, New York, NY; 3Dept of CV Medicine, Univ. of MI, Ann Arbor, MI; 4Dept of Medicine, Univ. of NC, Chapel Hill, NC.

Background: Many patients with stage 3-5 CKD may be overweight. We are conducting a randomized crossover trial of sodium (Na) restriction to primarily assess change in hydration status as assessed by bioelectrical impedance (BIA; lower-overhydration). Baseline data were used to explore the relationship between hydration status and GFR. 

Methods: Adult patients with Stage 3-4 CKD with stable clinical course were randomized in phase I to a low salt diet (~2g of Na/day) vs. usual diet for 4 weeks, with the alternate treatment given in phase II. Weekly dietary monitoring was provided and 24-hour urine Na measurements assessed compliance. A modified spectrum bioimpedance device and a switch box (Xitron 4200) automatically calculated ten measurements for each segment, arm, trunk, leg, calf, and whole body while the patient was in the supine position.

Results: Baseline data prior to randomization are presented in subjects enrolled so far (n=60; target=66). Mean age was 62±13, 50% male, 71% white, 39% diabetic, 87% hypertensive, mean BMI 32.5±m2, eGFR 39±11ml/min/1.73m2, and 24-hr urine Na 161±148 mEq/L. The table shows some significant differences in BIA measurements across CKD stages (p<0.10), baseline 24-hr urine Na correlated significantly with Calf (ECV/ICV, ECV-TBW, ECV/WT) and Body (ECV/TBW) BIA readings.

Conclusions: Baseline measurements in this trial of Na restriction in CKD Stages 3-4 suggest greater degrees of hydration across CKD stages 3-4 that may be amenable to Na restriction and/or specific pharmacotherapy.


Background: CTP-499 is a deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine, the active metabolite of pentoxifylline, a compound reported in multiple clinical trials to have beneficial effects in chronic kidney disease.

Methods: This was a two part study. Part A was randomized, double-blind, parallel group evaluation of single ascending doses (6 active, 2 placebo for each dose) of an extended-release (ER) formulation of CTP-499 in healthy adult subjects at doses of 600, 1200 and 2400 mg. Part B involved open-label administration of a single dose of 400 mg immediate-release (IR) powder in capsule to 6 subjects. Blood & urine samples were collected over 32 hours for the quantitation of CTP-499 & its metabolites. Safety measurements included adverse events, clinical labs, 12-lead ECGs, and vital signs.

Results: A total of 38 subjects participated in the study; none discontinued. Two subjects dosed with 400 mg IR and 4 subjects dosed with 2400 mg ER formulation had emesis, which was thus dose and formulation related, but was not necessarily concentration related. All other adverse events were mild. CTP-499 was rapidly absorbed and extensively metabolized. With increasing doses, exposures increased more or less linearly, although nonlinearity cannot be ruled out. The dose levels were achieved by the use of multiples of 200 mg ER tablets, a factor to be considered in interpreting the data. Five metabolites were quantified in plasma, nominally described as M1-M5. Metabolites M1, M2 & M5 were present at greater than 10% of parent drug. In urine, M5 was the major metabolite, followed by M2 & CTP-499. M1, M3, M4 were negligible. The metabolism profiles were different for IR and ER, with more of the active moieties (M1, M2) formed with ER formulation.

Conclusions: CTP-499 dosed as an ER formulation was well tolerated at single doses of 1800 mg and less. Pharmacokinetic parameters indicate the potential for once daily dosing. Based on these data, further clinical evaluation in patients with chronic kidney disease is planned.

Funding: Pharmaceutical Company Support


Background: CTP-499 is a deuterated analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine, the active metabolite of pentoxifylline, a compound reported in multiple clinical trials to have beneficial effects in chronic kidney disease.

Methods: All treatments were well tolerated. Only 9 adverse events were reported. All treatments were well tolerated. Only 9 adverse events were reported. No statistically significant changes were observed. No statistically significant changes were observed.

Conclusions: All treatments were well tolerated. Only 9 adverse events were reported. No statistically significant changes were observed. No statistically significant changes were observed.
Methods: We conducted focus groups and semi-structured interviews with 11 nephrologists and 29 patients over age 65 with advanced CKD or receiving dialysis. All interviews were audio-recorded and transcribed. We used qualitative analytic methods to identify common and recurrent themes related to the primary research question.

Results: Prognosis was rarely discussed and the disease course was often followed through test results with less emphasis on the patient experience. We identified 6 themes which describe the challenges and experience of understanding the trajectory of kidney disease: 1) patients have difficulty understanding their disease; 2) patients respond to poor understanding with emotion; 3) patients tend to view their future on dialysis with resignation; 4) nephrologists struggle to explain the complexity of illness; 4) nephrologist interactions tend to focus on the disease rather than the patient experience; 5) uncertainty discourages discussions of the future.

Conclusions: We identify important challenges among patients and nephrologists in understanding and predicting the trajectory of kidney disease. While nephrologists recognize the special needs of elderly patients, communication barriers exist that prevent discussions which address the patient experience. Communication interventions that respond to patient emotion and address uncertainty may impact understanding and preparation.

Funding: Other NIH Support - Loan Repayment Program

TH-PO363

Safety, Pharmacokinetics and Efficacy of AP214, a Novel Melanocortin Receptor Agonist, in Patients Undergoing Cardiac Surgery on Cardiopulmonary Bypass

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Background: Developement of Acute Kidney Injury (AKI) after cardiac surgery is associated with increased morbidity and mortality. Melanocortin receptor (MCr) agonists have marked immune modulating and organ protective effects in sepsis and surgery induced AKI animal models. AP214 is a novel pan-MCr agonist under development for prevention of AKI and organ failure after cardiac surgery.

Methods: Objective: Assessment of safety, pharmacokinetics and efficacy of AP214 in cardiac surgery patients with increased risk for post-OP AKI. Double-blind placebo-controlled randomized clinical trial comparing AP214, administered at three different dose levels (10, 50 or 200 µg/kg, respectively) given as bolus iv infusions, pre- peri- and 6 hours PO to patients (N=42) on cardopulmonary bypass.

Results: AP214 at 200 µg/kg was safe and well tolerated with a similar side effect profile to placebo. Estimated half-life of AP214 was 12-14 min with no signs of accumulation. There was a good relationship between dose and exposure and no dose dependency in any PK parameter observed. In the placebo group 6/13 patients developed AKI compared to 3/12 patients in the AP214 200µg/kg group. Estimated GFR, serum creatinine and additional surrogate markers indicated a high degree of kidney function preservation after AP214 treatment, with no post surgical changes in any of the parameters.

Conclusions: AP214 is safe and well tolerated. Efficacy data suggest that AP214 might be a valuable pharmacological approach to reduce the severity and frequency of post-operative AKI in patients undergoing cardiac surgery on cardiopulmonary bypass.

Funding: Pharmaceutical Company Support

TH-PO364

FG-4592 Oral Hypoxia-Inducible Factor Prolyl Hydroxylase Inhibitor Corrects Anemia in Nondialysis CKD Patients without IV Iron

Anatole Besarab,1 Robert Provenzano,2 Steven Fishbane,3 Chao H. Sun,4 Diogo S. Belo,5 Thomas B. Neff,6 Tyson T. Lee,6 Marietta Franco,1 Robert Leong,2 Kin-Hung Peony Yu7

1Henry Ford Hosp, Detroit, MI; 2St. Clair Specialty Physicians, Detroit, MI; 3Winthrop Dialysis Ctr., Mineola, NY; 4California Inst. of Renal Research, Chula Vista, CA; 5FibroGen, Inc., San Francisco, CA; 6ApeX Research, Riverside, CA.

Background: Anemia is largely undertreated in nondialysis CKD. We report data from an ongoing phase 2b, open-label study of FG-4592 to correct anemia in adult nondialysis CKD patients (pts). Ninety-six pts (Hb ≤10 g/dL at baseline [BL]) who had not received ESAs for the prior 12 weeks (wks) were randomized to 4 equal cohorts. Cohorts A and B: 16 wks initial tiered, weight-adjusted doses of 60-140 mg FG-4592 thrice weekly (TIW), Cohorts C and D: 24 wks initial fixed doses of 50 and 100 mg FG-4592 TIW, respectively. Cohort-dependent dose adjustment was allowed every 4 wks. IV iron was not permitted. BL demographics were similar across cohorts. For 95 efficacy-evaluable pts, overall mean±SD BL Hb was 9.7±0.7 g/dL. FG-4592 treatment led to increases from BL Hb in all cohorts (Table). After 16 wks of treatment, all A and B pts treated >5 wks had a Hb response. Cohort A and B pts who had a Hb response were converted to twice weekly dosing. Results were observed whether BL ferritin or TSAT was normal or subnormal. After 8 wks of treatment, there was a dose-dependent increase from BL Hb; and for 33 pts with data available, mean hepcidin decreased 36% from BL (p<0.0001). FG-4592 was well tolerated. No treatment-related serious adverse events were reported to date. Treatment with initial weight-adjusted or fixed oral FG-4592 doses corrected anemia in CKD pts in the absence of IV iron repletion.

Funding: Pharmaceutical Company Support

TH-PO362

Mortality and Functional Status in Elderly Dialysis Patients Residing in Assisted Living Care Facilities

Keren Mandelzweig,1 Paul Komenda,2 Claudio Rigatto,3 Lisa M. Miller,1 Joe A. Bueti, Clara Bohm, Manish M. Sood, Medicine, University of Manitoba, Winnipeg, MB, Canada.

Background: As the elderly ESRD population grows, a larger proportion will require inpatient care. We set out to investigate mortality and functional status in elderly ESRD patients residing in assisted living facilities compared to age and co-morbidity matched ESRD controls who reside at home.

Methods: We developed a 3:1 age and Charlson co-morbidity score matched cohort of 192 elderly ESRD patients residing in 64 assisted living facilities. Patients were identified during an acute hospitalization and with follow up beginning upon discharge. Mortality was examined by Kaplan Meier and multivariate adjusted Cox proportional hazards models with p-value<0.05 considered significant.

Results: Individuals residing in assisted living facilities were more likely to have a history of stroke and dementia, have more illness acuity and a longer length of hospital stay compared to the matched controls. Severity and presence of impairments in all 6 domains of the ADL score (bathing, dressing, toileting, feeding, incontinence and transferring) were more likely in the assisted care group. The majority in assisted living (75%) had multiple areas (>1) of severe impairment requiring full care. Survival time was shorter in those residing in assisted living (Median 216 (84-347) vs. 891 days (651-1130), p<0.0001) and this persisted after adjustment for sex, APACHE score, admission diagnosis, history of stroke and dementia and hospital length of stay (HJR 1.74, 95%CI 1.12-2.70). The hazard ratio attenuated after addition of ADL score into the model (HJR 1.36 95%CI 0.87-2.13).

Conclusions: Elderly ESRD patients discharged to an assisted care facility have a significantly increased mortality compared with age and co-morbidity matched controls. The discrepancy in long-term survival can be predicted by a simple measure of functional status upon admission to hospital.
TH-P0365
Adherence to Treatment among Children and Adolescents with Chronic Kidney Disease
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Background: Adherence to a prescribed treatment regimen reduces morbidity and mortality among patients with chronic renal diseases (CKD). Children often fail to adhere adequately to their medication plans. This report addresses behavioral functioning and child self-reporting of medication adherence among 10–21 year old patients with CKD.

Methods: The objective of this study was to examine patient-perceived factors that impact adherence to treatment using a qualitative descriptive individual interview approach. The questionnaire included 20 questions, answered by total of 12 children and was administered anonymously.

Results: Children admitted that they skipped their medications an average 1.6 days/week. One third of the patients “did not like all of their medications.” One of the least “favorite” medications was prednisone. Two-thirds of the patients felt either “upset” or “sometimes upset” by taking medications. Although 91.6% were frustrated by having their condition, only 25% stated that taking medications interferes with their daily lives, while 30% stated that taking medications did NOT interfere with their daily lives, and the other 25% were not sure. Most adolescents (66.6%) did not care what their friends think of them having a condition requiring medication. Interestingly, we discovered that the biggest problem in taking medications existed at home, not at school. Thus, 75% forgot to take medications at home, and just 16.6% forgot to take medications at school. The patients surveyed also were more likely to forget to take medications during the week (42%) versus the weekend (33%). As for ideas to help adolescents remember to take their medications existed at home, not at school. Thus, 75% forgot to take medications at home, and just 16.6% forgot to take medications at school. The patients surveyed also were more likely to forget to take medications during the week (42%) versus the weekend (33%).

Conclusions: Adherence to medications among pre-adolescents and adolescents with CKD is a serious medical problem which affects treatments and quality of life and requires developing a systematic approach.

TH-P0367
Continued Improvements in Renal Function with Sustained Eculizumab (ECU) in Patients (PTS) with Atypical Hemolytic Uremic Syndrome (aHUS) Resistant to Plasma Exchange/Infusion (PE/PI)
Laurence A. Greenbaum,1 Sudhi Babu,2 Richard Furman,2 Neil Sheiner,2 David J. Cohen,3 Osama Gaber,4 Frank Eitner,5 Yassoh Delmas,6 Chantal Loriat,1 Camille Bedrosian,1 Christoph M. Legendre,1 Emory University; 2Fort Wayne Med; 3Well Cornell Med Coll; 4Newcastle Univ; 5Colombia Univ Med Center; 6Methodist Hosp; 7Univ Aachen; 8CHU Pellegrin-Bordeaux; 9Hopital Robert Delbe; 10Alexion Pharmaceuticals, Inc; 11Universite Paris Descartes & Hopital Necker.

Background: aHUS is a genetic, devastating, systemic disease, caused by permanently background complement activation, resulting in thrombotic microangiopathy(TMA). Despite PE/PI, ~50% of pts develop ESRD/die within 1 yr of diagnosis. We report longer follow-up data from a phase II trial of ECU, a terminal complement inhibitor.

Methods: Pts ≥12 yrs with ECU and persistent TMA despite ≥4 PE/PI sessions 1 wk before screening were enrolled in a 26-wk, controlled, open-label, single-arm phase II trial (2009-2010). Prim. Endpoint: change in platelet (plt) count (a measure of TMA) (73x109/L; p<0.0001). Sec. endpoint: 15/17 pts (88%) achieved TMA event-free status (≥12 wks of stable plt count, no PE/PI and no new dialysis). 4.5 pts on dialysis permanently discontinued dialysis. ECU was well tolerated (Legendre. ASN 2010). We report follow-up results as of data cut-off (10/2010).

Results: 17 pts enrolled (2 discontinued; SLE and an AE unrelated to ECU, respectively). Median time from diagnosis to screening =10mo (range:<1-236). Median age=28 yrs. ECU median duration=38 wks (range: 26-64 wks) at time of analysis. 13 pts entered the extension study. 13 pts with low platelets at baseline had plt normalization at week 26 and continued to maintain normal levels at data cut-off. Renal function improved (23 Kt/V); stage-10 pts and ≥25% decrease in creatinine from baseline—11 pts). ECU was well tolerated; 10 pts with adverse events deemed related to ECU (generally mild/moderate). Longer follow-up (≥1 yr) will be presented.

Conclusions: In this early intervention study, sustained treatment with ECU prevented TMA and improved renal function. These data further strengthen the evidence for ECU as standard of care for aHUS.

Funding: Pharmaceutical Company Support

TH-P0368
Neutrophil Gelatinase-Associated Lipocalcin (NGAL) and Alternate Pathway of Complement (APC) Factors as Biomarkers of Response to Treatment in Patients with Focal Segmental Glomerulosclerosis (FSGS)
Rita Rosicker,1 Sudha Chennasamudram,2 Tetyana L. Vaslyyeva,3 Howard Trachman,2 Prasad Devarajan,2 Michael R. Bennett,3 Joshua M. Thurman,4 Mileena Radeva,2 Debbie S. Gipson,2 Frederick J. Kaskel,5 Aaron L. Friedman,6 Marva M. Moxy-Mims,6 Suzanne M. Vento,1 Pediatrics, Cohen Children’s Medical Center, New Hyde Park, NY; 2SLE and an AE unrelated to ECU, respectively). Median time from diagnosis to screening =10mo (range:<1-236). Median age=28 yrs. ECU median duration=38 wks (range: 26-64 wks) at time of analysis. 13 pts entered the extension study. 13 pts with low platelets at baseline had plt normalization at week 26 and continued to maintain normal levels at data cut-off. Renal function improved (23 Kt/V); stage-10 pts and ≥25% decrease in creatinine from baseline—11 pts). ECU was well tolerated; 10 pts with adverse events deemed related to ECU (generally mild/moderate). Longer follow-up (≥1 yr) will be presented.

Conclusions: In this early intervention study, sustained treatment with ECU prevented TMA and improved renal function. These data further strengthen the evidence for ECU as standard of care for aHUS.

Funding: Pharmaceutical Company Support

Results:
19 patients (10M:9F) were included, 7 received CSA and 12 DEX/MMF. There were no significant differences between these two groups. There was a decline in plasma S/Cb-9 in response to DEX/MMF (P<0.001); however, there were no therapy-related differences in urinary NGAL or APC levels (mg/mg creatinine). Baseline urinary NGAL excretion correlated with primary outcome at 52 wk (P=0.08). When pooled by outcome, 1-3 vs 4-6, baseline urinary NGAL (541±7 vs 227±190) and sC5b-9 (168±36 vs 450±181) levels were numerically lower in those with a favorable response. Urine Ba excretion at the end of treatment correlated with outcome (P<0.01).

Conclusions: Urinary excretion of NGAL and select APC factors may be useful indices for patient stratification to predict outcome and to assess adequacy of response to treatment in patients with primary steroid-resistant FSGS. ECU was well tolerated; 10 pts with adverse events deemed related to ECU (generally mild/moderate). Longer follow-up (≥1 yr) will be presented.

Key Endpoints

<table>
<thead>
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<th>Primary</th>
<th>Secondary</th>
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<td>TMA event-free status, n (%)</td>
<td>Median duration (range)</td>
</tr>
<tr>
<td>≤5 (0)</td>
<td>≥12 wks (range: 0-120 wks)</td>
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<tr>
<td>15/17 (88%)</td>
<td>38 wks (range: 26-64 wks)</td>
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ECU was similarly effective in pts w/o identified complement mutations and was well tolerated; only 6pts had AEs deemed related to drug.

Conclusions: Continued ECU treatment led to permanent discontinuation of chronic PE/PI. Sustained ECU Tx resulted in stabilized improved renal function, was well tolerated and demonstrates potential as the new SOC for aHUS.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.

197A
The Specificity of Urinary Aquaporin-1 and Adipophilin To Diagnose Renal Cell Carcinoma
Jeremiah J. Morrissey, Evan D. Kharasch. Anesthesiology, the Siteman Cancer Center, Biochemistry and Molecular Biophysics, Washington University in St. Louis, St. Louis, MO.

Background: Renal cancer accounts for about three percent of adult malignancies and is most frequently asymptomatic until late stages of disease. This investigation tested whether aquaporin-1 (AQP-1) and adipophilin (ADFP), sensitive biomarkers of kidney cancer, are specific biomarkers to detect asymptomatic kidney cancer in comparison to patients with common forms of kidney disease.

Methods: Urine samples were obtained from 32 patients with clear cell or papillary kidney cancer undergoing partial or radical nephrectomy, 15 control patients undergoing surgery for non-kidney related reasons, 44 patients with documented urinary tract infection, 24 patients diagnosed with diabetic nephropathy, and 18 patients diagnosed with glomerulonephritis. Samples for the last two patient cohorts were obtained from the Kidney Translational Research Core of the Washington University George M. O’Brien Center for Kidney Disease Research. Urinary concentrations of AQP-1 and ADFP were determined by a sensitive and specific Western blot procedure and corrected for urinary creatinine excretion.

Results: Overall, there was a 23- to 46-fold increase in AQP-1 and a 51- to 77-fold increase in ADFP in the median concentration of these two biomarkers in the urine of patients with kidney cancer compared to the median concentrations found in the urine of patients with these 3 common kidney diseases or the urine of the surgical control patients with AROC values of 0.99 to 1.00 (P<0.001).

Conclusions: The study shows that measuring urinary AQP-1 and ADFP concentrations provide a means of screening patients to identify those with kidney cancer without interference from underlying common kidney diseases

Funding: Clinical Revenue Support

Hydro nephrosis Predicts the Presence of Severe Vescoureteral Reflux
Husam A. Abdullanou, Eduardo H. Garin. Pediatrics, University of Florida, Gainesville, FL.

Background: The finding of hydro nephrosis by renal ultrasound has been suggested to predict the presence of Vescoureteral reflux (VUR). A review of published data has shown conflicting results. We hypothesized that in patients with VUR grade IV or V, hydro nephrosis will be found, if the patient does have a full bladder during the renal ultrasound examination.

Methods: This retrospective study included 837 patients, median age 1.3 years with a range of 0.18-7 year, 569 female and 268 male patients. Each patient underwent at least one voiding cystourethrogram (VCUG) and one renal ultrasound examination. On renal ultrasound, particular attention was paid to the presence of hydro nephrosis and bladder filling status in patients with VUR grade IV or V.

Results: Sensitivity and specificity for the renal ultrasound showing hydro nephrosis to detect the presence of VUR grades IV and V were 60 % and 92 %, respectively. Positive predictive value (PPV) and negative predictive value (NPV) were 74 % and 87 %, respectively. The Breslow-Day test for the homogeneity of the odds ratios supports the hypothesis that odds ratios differ by bladder status, with a full bladder associated with a 23- to 46-fold increase in AQP-1 and a 51- to 77-fold increase in ADFP in the median concentration of these two biomarkers in the urine of patients with kidney cancer compared to the median concentrations found in the urine of patients with these common kidney diseases or the urine of the surgical control patients.

Conclusions: The study shows that measuring urinary AQP-1 and ADFP concentrations provide a means of screening patients to identify those with kidney cancer without interference from underlying common kidney diseases.

Funding: Clinical Revenue Support

Inpatient Healthcare Utilization for Hemolytic Uremic Syndrome in the U.S.A.
Hyungwon Stella Shin,1 Brian Becknell,1 John D. Mahan,2 David S. Hains.2 Pediatrics, Nationwide Children’s Hospital, Columbus, OH; Pediatrics, Division of Nephrology, Nationwide Children’s Hospital, Columbus, OH.

Background: Hemolytic uremic syndrome (HUS) is a leading cause of pediatric acute renal failure. In this study, we evaluated the demographics and economic impact of inpatient hospitalizations for HUS in the U.S.A., and determined the specific effect of extrarenal comorbidities and procedures on length of stay (LOS).

Methods: We analyzed pediatric HUS admissions, using the Kids’ Inpatient Database of the Healthcare Cost and Utilization Project, from 1997, 2000, 2003, and 2006. Admissions with a primary diagnosis of HUS were identified by ICD-9 code. Patient sex, age, LOS, hospital charges, comorbidities and procedures were evaluated.

Results: 60.7% (1326) of admissions were for children 1-4 years old, and 56.7% (1284) of admissions were for female patients. Mean LOS was unchanged over the period of study (10 ± 0.7 days), and hospital charges increased 2.1-fold, from $29,605 ± $3,460 to $62,960 ± $5,957. Among extrarenal comorbidities, pancreatitis notably occurred in 10.5% of admissions associated with increased LOS (20 ± 2.8 days). Increased LOS was also noted with gastrointestinal hemorrhage (15 ± 3.5 days) and respiratory insufficiency (26.1 ± 3.7 days). Among procedures, admissions requiring dialysis (19%) were associated with increased LOS (18.4 ± 1.5 days). Red blood cell (RBC) transfusion was associated with decreased LOS (6.4 ± 0.4 days).

Conclusions: Our results demonstrate multiple novel findings: (1) We identify sex-skewing among pediatric HUS admissions, with a female predominance. (2) There has been no reduction in LOS for HUS over the study period, despite rising hospital charges. (3) Extrarenal comorbidities – particularly pancreatitis and respiratory insufficiency – significantly impact LOS. This highlights the need to aggressively identify and manage extrarenal comorbidities in patients with HUS. (4) The potential association between BCG vaccination and decreased LOS is consistent with studies recommending isotonic fluid resuscitation in HUS patients and merits further evaluation.

Funding: Clinical Revenue Support

Low Utilization of Growth Hormone Therapy in Children with Short stature in the CKD Cohort
Amira Al-Uzri,1 Hilary M. Hotchkiss,2 Ora Yadin,3 Michael F. Schneider,7 Laurence A. Greenbaum,2 Frederick J. Kaskel,2 Susan L. Furtth,2 Bradley A. Warady.2 Oregon Health & Science University, Portland, OR; 2CKiD Study Group.

Background: Growth hormone (GH) improves growth velocity in children with chronic kidney disease (CKD), but multiple studies have demonstrated underutilization of GH in children with CKD and short stature (SS). We describe the rate of utilization of GH and risk factors for underutilization in the multicenter, Chronic Kidney Disease in Children (CKiD) cohort study.

Methods: This is a cross sectional baseline analysis of 135 participants at enrollment. SS was defined as height SDS < -1.88 for age and gender. 63 subjects had SS and were not on GH and 72 were on GH (24 also had SS). Demographic data and iohexol GFR were obtained on all subjects. The two groups were compared using X² tests for categorical variables or Wilcoxon rank-sum tests for continuous variables.

Results: 60.7% (1326) of admissions were for children 1-4 years old, and 56.7% (1284) of admissions were for female patients. Mean LOS was unchanged over the period of study (10 ± 0.7 days), and hospital charges increased 2.1-fold, from $29,605 ± $3,460 to $62,960 ± $5,957. Among extrarenal comorbidities, pancreatitis notably occurred in 10.5% of admissions associated with increased LOS (20 ± 2.8 days). Increased LOS was also noted with gastrointestinal hemorrhage (15 ± 3.5 days) and respiratory insufficiency (26.1 ± 3.7 days). Among procedures, admissions requiring dialysis (19%) were associated with increased LOS (18.4 ± 1.5 days). Red blood cell (RBC) transfusion was associated with decreased LOS (6.4 ± 0.4 days).

Conclusions: Results: Our results demonstrate multiple novel findings: (1) We identify sex-skewing among pediatric HUS admissions, with a female predominance. (2) There has been no reduction in LOS for HUS over the study period, despite rising hospital charges. (3) Extrarenal comorbidities – particularly pancreatitis and respiratory insufficiency – significantly impact LOS. This highlights the need to aggressively identify and manage extrarenal comorbidities in patients with HUS. (4) The potential association between BCG vaccination and decreased LOS is consistent with studies recommending isotonic fluid resuscitation in HUS patients and merits further evaluation.

Funding: Clinical Revenue Support
Vehicle-Ureteral Reflux as a Risk Factor for Acute Pyelonephritis and Renal Damage in Children with UTI: Systematic Review and Meta-Analysis

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Background: Urinary tract infection (UTI) is one of the most common bacterial infections in childhood. UTI may result in renal scarring (VUR) may be a risk factor for both acute pyelonephritis (APN) and RS that predisposes to long-term complications. However, which imaging should be performed after UTI is strongly debated, and part of the debate is due to the remained question of the true relationship between VUR, and both APN and RS. Recently, two meta-analyses found conflicting results, because of important missing studies, and pooling together APN and RS. We aim to update the meta-analysis and study if VUR is a risk factor for APN, and RS.

Methods: A systematic review and meta-analysis was performed according to the CDR guidelines. All studies of children with UTI, DMSA scan and cytography, were identified by a systematic electronic search in Medline, and Embase, until 2011. Pooled estimates were calculated using DerSimonian and Laird random-effects model.

Results: From the 1585 potentially relevant articles, 80 articles were included, representing 11,410 children; children had a first UTI in 53 (66%) studies; 48 (60%) studies were prospective, and in 15 (19%) children underwent both early and late DMSA scan. At all, 6,681 children with an early scan were included, and 5,879 with a late scan. All-grade VUR was significantly associated to both APN (OR=2.0; 95%CI: 1.8-2.3) and RS (OR=4.8; 95%CI: 4.3-5.5). High-grade (≥ grade VUR) was also significantly related to APN (2.4; 95%CI: 1.9-3.1) and RS (OR=5.7; 95%CI: 4.5-7.3). Pooled estimates were found with ≥ grade VUR was significantly associated to both APN (OR=2.0; 95%CI: 1.8-2.3) and RS (OR=5.7; 95%CI: 4.5-7.3). Data from two different independent registries support our hypothesis that pre-emptive RAAS-blockade may delay ESRD in Alport patients. b. Alport carriers also seem to profit from RAAS-blockade. This should increase alertness for oligosymptomatic patients with microhematuria as possible heterozygous carriers for autosomal Alport mutations (1% of total population). c Life expectancy of Alport patients with ESRD might be better than that of patients with other renal diseases.

Conclusions: Children with VUR had a higher risk of APN and RS. These data suggest that identification of VUR can be a practical method of identifying children who are at risk for renal scarring.

Funding: Institut Pasteur

ERD-EDTA Registry and European Alport Registry: Prognosis on Renal Replacement Therapy and Improvement of Life-Expectancy in Alport Patients as well as in Heterozygous Carriers

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Background: The hereditary renal disease Alport Syndrome (AS) is caused by mutations of type IV collagen genes. AS leads to an impaired function of the glomerular basement membrane progressing to end stage renal disease (ESRD). Heterozygous carriers also have a relatively high risk to develop ESRD.

Methods: We used data from 11 European countries providing data to the ERA-EDTA Registry to compare Alport males on renal replacement therapy (RRT, dialysis as well as transplanted patients) with age-matched males with other renal diseases. Furthermore, we used data from the European Alport Registry to analyze the effects of a preemptive RAAS-blockade (ACE-inhibitor or ARB) regarding age at start of RRT and life expectancy in Alport patients and in heterozygous carriers.

Results: ERA-EDTA registry data showed better patient survival for Alport males on RRT when compared to age-matched males with other diseases; furthermore, kidney graft survival was found to be better in transplanted Alport patients. The age at start of RRT of Alport patients tended to increase somewhat sharper after the year 2000 compared with the 1990s. Alport registry data show a better life-expectancy in Alport patients who received RAAS-blockade before onset of RRT. Both results (better life-expectancy and delayed start of dialysis) also apply for heterozygous Alport carriers in the Alport registry.

Conclusions: a. Data from two different independent registries support our hypothesis that pre-emptive RAAS-blockade may delay ESRD in Alport patients. b. Alport carriers also seem to profit from RAAS-blockade. This should increase alertness for oligosymptomatic patients with microhematuria as possible heterozygous carriers for autosomal Alport mutations (1% of total population). c. Life expectancy of Alport patients with ESRD might be better than that of patients with other renal diseases.

Performance of Two Strategies for Urgent ANCA and anti-GBM Analysis in the Diagnosis of Suspected Severe Small Vessel Vasculitis

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Background: In acute situations, patients suspected of small vessel vasculitis (SVV) may benefit from rapid and reliable testing for ANCA and anti-GBM antibodies. We analysed the diagnostic performance of two rapid methods for assessing the presence of ANCA and anti-GBM in a cohort of severely ill patients with suspected SVV and compared the results with capture ANCA ELISA.

Methods: Between Jan 2003 and Nov 2010, we received 260 requests for urgent analysis of ANCA and anti-GBM. These samples were analysed by qualitative Dotblot (Biomedical Diagnostics) for PR3-, MPO-ANCA and anti-GBM. Routine IF and ELISA were performed afterwards. We retrospectively analyzed these samples with the novel high sensitive automated Phadia Elia PR3 and MPO and anti-GBM Elia.

Results: a. Data from two different independent registries support our hypothesis that pre-emptive RAAS-blockade may delay ESRD in Alport patients. b. Alport carriers also seem to profit from RAAS-blockade. This should increase alertness for oligosymptomatic patients with microhematuria as possible heterozygous carriers for autosomal Alport mutations (1% of total population). c. Life expectancy of Alport patients with ESRD might be better than that of patients with other renal diseases.

Conclusions: Results of 74 of 260 patients (28%) a final diagnosis of AAV (n=62) or anti-GBM disease was made (n=12). Both Dotblot and Elia detected all 12 cases of anti-GBM disease (sensitivity 100%, negative predictive value (NPV) 100%). Both methods found one false positive (specificity 99%) resulting in a positive predictive value (PPV) of 99%. Both Dotblot and ELIA were performed afterwards. We retrospectively analyzed these samples with the novel high sensitive automated Phadia Elia PR3 and MPO and anti-GBM Elia. Results were related to the final clinical diagnosis and compared with our routine ELISA.

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RQOL than children on hemodialysis.

Including General Fatigue Scale, Family and Peer Interaction Scale, Worry Scale than that to age and gender. Child self-report showed significantly higher QOL than children on hemodialysis.

Conclusions: QOL should be evaluated in all children with ESRD to support more favorable growth and development as well as disease problem. The PedsQL 3.0 ESRD Module may be useful as an ESRD-specific instrument to evaluate HRQOL in Korean children and larger, longitudinal prospective study is needed.
TH-P0379
Development of an Outpatient Native Kidney Biopsy Protocol for Low-risk Pts and assessed its safety and efficacy.

Methods: We developed an outpatient biopsy protocol for low-risk pts and assessed its safety and efficacy.

Results: Between Nov 2008 and Apr 2011, 105 pts (56 male) underwent outpatient biopsies. Mean age was 49±16 yrs. A 16G needle was used in 43 pts (Group A) and a 14G needle in 62 (Group B). Under real-time ultrasound guidance biopsies were performed using a Boston Scientific 16G spring-loaded needle that was changed to a Bard 14G needle in Oct 2009. Vital signs and urine voids were monitored in an outpatient observation unit; CBC was sent 4 hrs after the procedure. Pts were discharged after 5 hrs of strict bedrest if there were no signs of bleeding, and complications were tracked carefully.

Conclusions: In a carefully selected population of pts, outpatient renal biopsy is safe with low complication rates. The majority (75%) of complications occurred during the observation period. The remainder occurred after 48 hours and thus an overnight admission would not have altered management. Importantly, the routine use of a 14G biopsy needle resulted in a greater yield of glomeruli compared to a 16G needle without increased complications.

TH-P0380
Non-Invasive Evaluation of Kidney Hypoxia and Fibrosis Using MRI

Background: Intertstitial fibrosis and hypoxia are 2 major complications that accelerate the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether the CKD progression. However, these factors have not been sufficiently evaluated in CKD patients because of the lack of appropriate clinical tools. In this study, we evaluated whether

Conclusions: In this study, we examined (1) the reproducibility of DW and BOLD MRI techniques, (2) the effect of kidney size on ADC and T2* values, and (3) the relationship between the ADC values and the degree of interstitial fibrosis in renal biopsy specimens. The main findings were as follows: (1) The reproducibility of DW and BOLD MRI techniques was good with minimal variability between observers. (2) The effect of kidney size on ADC and T2* values was significant, with larger kidneys having higher ADC values and lower T2* values. (3) The relationship between ADC values and the degree of interstitial fibrosis was significant, with higher ADC values associated with less fibrosis. These findings suggest that DW and BOLD MRI can provide valuable information on kidney fibrosis and hypoxia, which can help in the early detection and management of CKD. Furthermore, these techniques may have potential clinical applications in monitoring the progression of CKD and evaluating the efficacy of therapeutic interventions.
Impact of Multiple Myeloma and Treatment with G-CSF on U and P-HNL/NGAL

**Background:** Renal failure is an important complication of multiple myeloma. In myeloma malignant plasma cells produce monoclonal immunoglobulin (M-component). With treatment the M-component can disappear. The M-component can form casts that obstruct tubular flow, be deposited in the renal tissue and cause amyloidosis. Myeloma can also cause renal injury by secondary mechanisms such as hypercalcemia and dehydration. G-CSF is used for autologous stem cell mobilization in myeloma. HNL/NGAL is a promising biomarker of acute kidney injury. The aim of this study was to investigate whether U or P-HNL/NGAL levels were affected by myeloma.

**Methods:** We examined 50 patients with myeloma who were either on a scheduled visit to the hematologist outpatient unit or admitted to the hospital for treatment. P-HNL/NGAL was measured by a sandwich ELISA. Active disease was defined as significantly rising M-component. Comparisons between groups were made by the non-parametric Mann-Whitney U-test.

**Results:** P-HNL/NGAL, but not U-HNL/NGAL, was significantly increased in patients on treatment with G-CSF. Bence-Jones proteinuria, M-component or active disease were not associated with elevated HNL/NGAL levels. As expected, P-HNL/NGAL correlated significantly with G-CSF.

**Conclusions:** Myeloma can also cause renal injury by secondary mechanisms such as hypercalcemia and dehydration. G-CSF is used for autologous stem cell mobilization in myeloma. HNL/NGAL is a promising biomarker of acute kidney injury. The aim of this study was to investigate whether U or P-HNL/NGAL levels were affected by myeloma. Bence-Jones proteinuria, M-component or active disease were not associated with elevated HNL/NGAL levels. As expected, P-HNL/NGAL correlated with creatinine levels in plasma. U and P-HNL/NGAL in myeloma patients

<table>
<thead>
<tr>
<th>U-HNL/NGAL µg/L</th>
<th>P-HNL/NGAL µg/L</th>
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<tr>
<td></td>
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<tr>
<td>G-CSF</td>
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<tr>
<td>55.2±35.3 N=6</td>
<td>192.7±54.2 N=6</td>
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<td>P&lt;0.001</td>
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<td>Bence-Jones proteinuria</td>
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<tr>
<td>48.7±29.5 N=14</td>
<td>51.7±8.8 N=14</td>
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<tr>
<td>M-component in serum</td>
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<tr>
<td>32.1±14.9 N=16</td>
<td>53.4±8.3 N=16</td>
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<tr>
<td>Active disease</td>
<td>47.1±7.7 N=17</td>
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<tr>
<td>56.2±6.8 N=17</td>
<td>66.1±14.2 N=33</td>
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Values are means±SEM

**Conclusions:** We did not find any evidence that myeloma per se affects the levels of U and P-HNL/NGAL. However, G-CSF treatment was associated with a significant increase in P-HNL/NGAL. These findings make it unlikely that Bence-Jones proteinuria and P-HNL/NGAL in myeloma patients with multiple myeloma. Funding: Government Support - Non-U.S.
Fenoldopam Increases Renal Artery and Parenchymal Blood Flow and Reduces Renal Resistive Index in Hypertensive Patients with Chronic Kidney Disease

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Background: Fenoldopam mesilate (FM), a short-acting agonist of post-synaptic dopaminergic type 1 (DA1) is a powerful hypertensive agent reducing systemic arterial resistances at an infusion rate >0.1 mcg/kg/min. At infusion rates <0.1mcg/kg/min it seems show renoprotective properties without effect on blood pressure (BP). It could be due to reduced renal vascular resistance (VR) and increased blood flow (BF), as observed in experimental models but not yet in humans. To investigate the effect of <0.1mcg/kg/min FM infusion on renal vascular and parenchymal resistances, renal BF and systemic BP in hypertensive patients with chronic kidney disease.

Methods: Sixty hypertensive patients aged 65±20.14,54 years with CKD (stage 1-IV N=50) or ESRD (N=10) were enrolled. FM was e.v. infused 0.1mcg/kg/min for 60 minutes. Resistance index (RI) and Systolic and Diastolic flow velocity (SF, DF) were recorded by Eco-(color)-Doppler ultrasound, at renal artery origin, mid portion and at renal hylum, before and during FM infusion. BP and heart rate were monitored.

Results: During infusion, both SF and DF were significantly higher than baseline, either at renal artery origin, mid portion and at renal hylum (SF:48.9±11.3 vs 55.9±15.3 cm/sec, p<0.0135; 52.8±9.4 vs 55.2±12.4 cm/sec, p=0.0001; 44.2±9.9 vs 49.4±10.9 cm/sec, p=0.00193; DF: 13.84±1.6 vs 27.69±1.1 cm/sec, p<0.001, 14.37±2.6 vs 27.39±1.5 cm/sec, p<0.001, 13.63± vs 25.03±5.5 cm/sec, p=0.0001, respectively). RI were significantly lower than baseline, either at renal artery origin, mid portion and at renal hylum (SF 48.9±11.3 vs. 55.9±15.3 cm/sec, p=0.0135; 52.8±9.4 vs. 55.2±12.4 cm/sec, p=0.0001; 44.2±9.9 vs. 49.4±10.9 cm/sec, p=0.00193). BM and heart rate was not significantly affected by infusion. These results were observed in all CKD stages.

Conclusions: Our preliminary data suggest that 0.1mcg/kg/min of FM e.v. infusion increases renal blood flow and reduces RI with no effect on BP and heart rate.

TH-P0388
Long-Term Risks of Hospital Readmission and Death in Patients with Kidney Disease
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Background: Patients with kidney disease are at high risk of hospitalization and death. However, long-term risks of hospital readmission and associated death are unclear. The primary objective of this study was to determine the risks of hospital readmission and death in a full-spectrum of patients with early stages of chronic kidney disease (CKD) to end-stage renal disease treated by dialysis or kidney transplantation.

Methods: This longitudinal cohort study included persons hospitalized in the State of Washington from Oct 2005 through Dec 2008 (WA-CARRS). Patients who survived their hospitalization and whose index hospital hospitalization (n=579,381) were classified into 4 cohorts defined by ICD9 diagnostic codes for CKD (n=27,647), dialysis (n=4,884), kidney transplant (n=867), and reference (non-kidney-disease diagnoses, n=545,983). Primary outcomes were time to hospital readmission and hospital readmission resulting in death. Cox Proportional Hazard models controlling for age, gender, and primary diagnosis were conducted for time-to-event analyses. Analyses were internally validated with bootstrapping techniques.

Results: Risks for hospital readmission significantly increased among patients in the CKD (HR 1.35, 95% CI: 1.32-1.39, p<0.001), dialysis (HR 2.25, 95% CI: 2.15-2.36, p<0.001), and kidney transplant (HR 1.85, 95% CI: 1.65-2.07, p<0.001) cohorts independent of other index hospitalization diagnoses for up to 39 months. Risks for hospital readmission resulting in death were also independently increased for those in the CKD (HR 1.72, 95% CI: 1.61-1.83, p<0.001), dialysis (HR 4.17, 95% CI: 3.80-4.59, p<0.001), and kidney transplant (HR 1.95, 95% CI: 1.72-2.79, p=0.001) cohorts. Risks of readmission and death increased in a graded manner by CKD stage. Internal validation demonstrated ~99% coverage and unbiased estimates.

Conclusions: Long-term risks of hospital readmission and death for patients with diagnoses of CKD, dialysis, and kidney transplant were among the highest observed in a large, statewide population. Patients with the full-spectrum of kidney disease diagnoses should be a major focus of efforts to reduce hospital readmissions and death.

TH-P0389
Chronic Endothelin-A Receptor Antagonism Modifies Novel Cardiovascular Risk Factors in CKD

Background: CKD patients have an increased risk of cardiovascular disease (CVD) that is not explained by conventional CVD risk factors alone. Arterial stiffness (AS) and endothelial dysfunction (ED) are features of CVD and may contribute to the CVD with which these patients are associated. Endothelial nitric oxide (NO) system may lead to CVD and AS. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of NO production that promotes vasoconstriction. ADMA concentrations are increased in CKD. Endothelin-1 (ET-1) is a potent endogenous vasoconstrictor that is also upregulated in CKD. Hyperuricemia is another feature of CKD. It contributes to ED/AS and so CVD risk. ETA receptor antagonism is a promising novel therapy for proteinuric CVD. Its effects on the renal ET system and urate in CKD are not known.

Methods: In a randomized double-blind, 3-way crossover study, 27 subjects with proteinuria received treatments with placebo, sitaxentan 100mg, an ETA antagonist, and fendinpine 30mg. (Dhaun et al Hypertension 2011). Serum urate, ADMA, plasma ETA-1 and urine ET-1 creat, as a reflection of renal ET-1 production, were measured at baseline and week 6 of each treatment period alongside the primary endpoints of proteinuria, blood pressure (BP), and AS.

Results: Whereas placebo and nifedipine did not affect urate, ADMA or urine ET-1/creat, sitaxentan reduced all three (baseline vs. week 6 ± SEM – urate: 506±21 vs. 451±22μmol/l, p<0.01; ADMA: 52.0±0.1 vs. 48.0±0.1μmol/ml, p<0.0001; urine ET-1/creat: 783.843±613±8×10^{-1}μmol/ml, p<0.01). Plasma ET-1 was unaffected by all treatments. In multivariate analysis, the reduction in proteinuria and BP following sitaxentan treatment was independently predicted by the change in urine ET-1/creat, and the reduction in pulse wave velocity (as a measure of AS) by the change in ADMA.

Conclusions: Initial results support the use of sitaxentan on proteinuria, BP and AS. ETA antagonism may modify novel CVD risk factors and so have broader cardioprotective effects in CKD. Larger and longer-term trials with these specific endpoints are now warranted.

Funding: Pharmaceutical Company Support

TH-P0390
Effect of Extended-Release Niacin/Laropiprant on Lipid Profiles in Dyslipidemic Stage 3 Chronic Kidney Disease Patients
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Background: Among dyslipidemic patients free of chronic kidney disease (CKD), extended-release niacin (ER) at 2 g/d causes beneficial lipoprotein-modifying effects. Given the paucy of data regarding niacin’s impact on dyslipidemia in CKD, we examined the effect of the drug on the standard lipid profiles of patients with stage 3 CKD.

Methods: Subjects (n=261) with a baseline estimated glomerular filtration rate (eGFR) of 30 to <60 ml/min/1.73 m^2 were drawn from two completed trials of extended-release niacin/laropiprant (ERN-L, laropiprant being an inhibitor of ERN-mediated flushing: ERN-L at 2g/d, n=177; n=84 placebo [P]). Lipid profiles were measured serially over 24-weeks. Results: The ERN-L and P groups were comparable with respect to baseline mean age, eGFR, total cholesterol (TC), low density lipoprotein-C (LDL), high density lipoprotein-C (HDL), and triglyceride (TG) levels, as well as the distribution of gender, concurrent statin use, and diabetes. Repeated measures analyses revealed that ERN-L, relative to P, caused a decrease (95% CI) of -22.5% (-16.0%, -29.1%) in mean LDL, -9.1% (-12.7%, -5.5%) in mean TC, and -28.0% (-43.5%, -21.8%) in median TG, as well as an increase of +28.7% (+23.7%, +33.7%) in mean HDL levels. Stratified analyses revealed that these effects were entirely consistent with what was observed among dyslipidemic patients whose eGFR was ≥60 ml/min/1.73 m^2.

Conclusions: ERN-L results in favorable lipid changes among patients with stage 3 CKD. Till now, stage 3-4 CKD patients have been significantly under-represented in secondary cardiovascular disease prevention trials. We conclude that a niacin treatment arm merits serious consideration within any future clinical trials targeting stage 3-4 CKD patients for the potential reduction of cardiovascular outcomes.
Conclusions: Subclinical alterations in cardiac structure are associated with kidney function decline independent of the effects of hypertension and other comorbidities. Future studies should focus on elucidating mechanisms to explain these associations.

Funding: Other NIH Support - NHLBI

TH-PO392
Angiopterin-Like Protein 2 Is Associated with Chronic Kidney Disease in a General Japanese Population: The Hisayama Study
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Background: Angiopterin-like protein 2 (Angptl2) is an adipocytokine which promotes inflammation and endothelial dysfunction of the vessels. People with chronic kidney disease (CKD) have a greater risk of atherosclerotic disease. The aim of this study is to assess the relationship between serum Angptl2 levels and CKD.

Methods: A total of 3,163 community-dwelling subjects (1,368 men, 1,795 women), aged 40-89 years, were divided into quintiles by Angptl2 levels. The estimated filtration rate (eGFR) was calculated using the new Japanese equation for eGFR from serum creatinine.

Results: The median value (interquartile range) of Angptl2 was 2.72 (2.15-3.47) ng/mL. The prevalence of CKD was 37.4%. The age- and sex-adjusted prevalence of CKD increased linearly for Angptl2 levels of <2.01, 2.01-2.48, 2.49-2.99, 3.00-3.65, and ≥3.66 ng/mL, being 26.0%, 35.4%, 39.4%, 41.3%, and 44.2%, respectively (p for trend <0.001). Every 1 ng/mL increment in Angptl2 levels was associated with 1.09-fold (95% confidence interval 1.01-1.18) greater likelihood of CKD, even after adjusting for age, sex, systolic blood pressure, serum creatinine, and smoking status.

Conclusions: Elevated Angptl2 levels were associated with the likelihood of CKD in the general population.

Funding: Private Foundation Support

TH-PO393
Modulation of Erythropoietin by Prolyl Hydroxylase Inhibitor in a Repeat Dose, Randomized Placebo Controlled Trial
Richard A. Brigandi,1 Steven F. Russ,2 Courcaud E. Westerman,3 Gordana Olbina,4 Peter Hodsman,5 Sanjay Kumar.1 1GlaxoSmithKline, King of Prussia, PA; 2GlaxoSmithKline, Research Triangle Park, NC; 3Intrinsic LifeSciences, La Jolla, CA; 4Nucleus Network, Melbourne, VIC, Australia.

Background: Prolyl hydroxylases are enzymes that hydroxylate hypoxia inducible factor (HIF) leading to degradation. GSK1278863A is a novel small molecule inhibitor of HIF prolyl hydroxylases resulting in HIF stabilization and HIF-mediated gene expression including erythropoietin (EPO).

Methods: Study PHI112843 was a two part study. Part 1 was a single dose, 2-period crossover randomized sequence, single blind to sequence and dose study in Stage 3 and 4 CKD patients and matched healthy subjects. Patients were administered a single dose of 50 mg and 150 mg GSK127863A across the two periods. Part 2 was a single dose, 2-period, fixed sequence, open label study in Stage 5 hemodialysis dependent (HDD) patients. Subjects were administered doses of 150 mg followed by 300 mg GSK127863A across the two periods. Period 1 dosing was on a hemodialysis (HD) day, 1 hour prior to HD start. Period 2 was on a day without HD.

Results: Oral administration of GSK1278863A was generally well tolerated. There were no marked differences in drug exposure across CKD patients, matched healthy subjects and HDD patients on a non-HD session day. Plasma concentrations were elevated in subjects actively receiving HD. Rapid, dose-dependent increases were observed in mean EPO concentrations in all cohorts following dosing in Period 1, with the greatest increases observed in CKD and HDD patients. Mean reticulocyte and RBC counts also increased. Mean hemoglobin concentrations were observed to increase slightly in healthy subjects in both dosing periods and over time in CKD patients. In HDD patients small increases in mean Hgb concentrations were noted at 24 hours following dosing on an HD day, but returned to baseline at the follow-up assessment. A decrease from baseline in Hepcidin, a biomarker of iron utilization and inflammation, and an increase in VEGF was observed after dosing in all populations.

Conclusions: These data indicate that GSK1278863A has the potential to induce erythropoiesis in patients with anemia.

Funding: Pharmaceutical Company Support

TH-PO394
Modulation of Erythropoietin by Prolyl Hydroxylase Inhibitor in Renal Impaired Patients in a Single Dose Cross-Over Study
Richard A. Brigandi,1 Steven F. Russ,2 Courcaud E. Westerman,3 Gordana Olbina,4 Richard Austin Robson,5 Sanjay Kumar.1 1GlaxoSmithKline, King of Prussia, PA; 5Intrinsic LifeSciences, La Jolla, CA; 2Christchurch Hospital, Christchurch, New Zealand.

Background: Prolyl hydroxylases are enzymes that hydroxylate hypoxia inducible factor (HIF) leading to degradation. GSK1278863A is a novel small molecule inhibitor of HIF prolyl hydroxylases resulting in HIF stabilization and HIF-mediated gene expression including erythropoietin (EPO).

Methods: Study PHI112843 was a two part study. Part 1 was a single dose, 2-period crossover randomized sequence, single blind to sequence and dose study in Stage 3 and 4 CKD patients and matched healthy subjects. Patients were administered a single dose of 50 mg and 150 mg GSK127863A across the two periods. Part 2 was a single dose, 2-period, fixed sequence, open label study in Stage 5 hemodialysis dependent (HDD) patients. Subjects were administered doses of 150 mg followed by 300 mg GSK127863A across the two periods. Period 1 dosing was on a hemodialysis (HD) day, 1 hour prior to HD start. Period 2 was on a day without HD.

Results: Oral administration of GSK1278863A was generally well tolerated. There were no marked differences in drug exposure across CKD patients, matched healthy subjects and HDD patients on a non-HD session day. Plasma concentrations were elevated in subjects actively receiving HD. Rapid, dose-dependent increases were observed in mean EPO concentrations in all cohorts following dosing in Period 1, with the greatest increases observed in CKD and HDD patients. Mean reticulocyte and RBC counts also increased. Mean hemoglobin concentrations were observed to increase slightly in healthy subjects in both dosing periods and over time in CKD patients. In HDD patients small increases in mean Hgb concentrations were noted at 24 hours following dosing on an HD day, but returned to baseline at the follow-up assessment. A decrease from baseline in Hepcidin, a biomarker of iron utilization and inflammation, and an increase in VEGF was observed after dosing in all populations.

Conclusions: These data indicate that GSK1278863A has the potential to induce erythropoiesis in patients with anemia.

Funding: Pharmaceutical Company Support

TH-PO395
The Pathogenesis and Clinical Significances of Renal Insulin Resistance Syndrome
Hitoshi Minakuchi, Shu Wakino, Koichi Hayashi. School of Medicine, Keio University, Shinanomachi 35 Shinjuku, Tokyo, Japan.

Background: The association between chronic kidney disease (CKD) and insulin resistance (IR) has been recognized as "renal insulin resistance syndrome (RIRS)." To delineate the mechanism and significance of RIRS, we conducted a clinical study.

Methods: The cross-sectional study was performed among 186 patients with CKD. Insulin sensitivity was evaluated with the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Glomerular filtration rate (GFR) was estimated with modified MDRD-equation adapted for a Japanese population and evaluated in estimated GFR (eGFR). The intervention with mineralocorticoid receptor (MR) antagonist spironolactone was performed with some patients with RIRS. In prospective analysis, we evaluated the 3-year changes in various parameters of renal function and analysed the correlation between HOMA-IR and these parameters.

Results: Of CKD patients, 5 were in stage 1, 89 were in stage 2, 78 were in stage 3, 6 were in stage 4, and 8 were in stage 5. A negative correlation was observed between HOMA-IR and eGFR. Plasma aldosterone level was correlated negatively with eGFR and positively with the levels of fasting blood sugar, insulin and HOMA-IR. By multiple regression analysis, the aldosterone level was identified as an independent risk factor for RIRS. This analysis also revealed that the markers for urinary tubular damages, a1-microglobulin were also correlated with HOMA-IR. The treatment with spironolactone attenuated the IR status in CKD. In addition, prospective analysis revealed that the decline in eGFR was significantly correlated with HOMA-IR, and multiple regression analysis indicated that HOMA-IR was the independent risk for CKD progression.

Conclusions: These studies demonstrate that plasma aldosterone plays a relevant role in the pathogenesis of RIRS. MR antagonists can be a therapeutic strategy for the prevention against RIRS and its complications including cardiovascular disease. The presence of RIRS have some roles in the progression of CKD probably through the damages of urinary tubular damages. Our study demonstrates for the first time the clinical relevances of RIRS, and RIRS can be the new target for CKD treatment.

Funding: Government Support - Non-U.S.

TH-PO396
The Effects of 1,25-Dihydroxyvitamin D3 on the Risk of Death in a Japanese General Population: The Hisayama Study
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Background: Recent evidence emerged to imply that lack of 1,25-dihydroxyvitamin D3 (VD3) increased the risk of death and cardiovascular disease. However, this issue has not been fully evaluated. We assessed whether lower serum VD3 levels were associated with higher risk of death.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
Underline represents presenting author.
Methods: We followed a total of 3,091 community-dwelling Japanese individuals aged 40 years or older, without history of cardiovascular disease and kidney failure, and with available VD3 data, for 7 years, and examined the relationship between serum VD3 levels and the risk of death by using the Cox proportional hazards model. Results: The mean value of serum VD3 levels in the study population was 66.6 pg/ml (standard deviation 17.4). During the follow-up, 270 subjects died in total. The mortality rate from any causes increased gradually with lower VD3 levels: the risk of death from any causes increased significantly with VD3 levels of <54.8, 54.9-65.8, 65.9-78.5, and ≥78.6 pg/ml, being 18.2, 15.1, 9.9, and 10.3 per 1,000 person-years, respectively (p for trend <0.001). Potential confounders namely, age, sex, systolic blood pressure, use of anti-hypertensive agents, diabetes, total cholesterol, use of lipid-modifying agents, body mass index, estimated glomerular filtration rate (eGFR), calcium-phosphate product, smoking habits, and alcohol intake, every 10 pg/ml decrement in VD3 levels was associated with a 1.9-fold (95% confidence interval, 1.11-1.28) greater risk of death from any causes. With regard to the causes of death, the risk of death from cardiovascular disease and infectious disease tended toward increasing with lower VD3 levels. There was no evidence of heterogeneity in the association between subjects with eGFR below and above 60 ml/min/1.73 m² (p for heterogeneity = 0.93). Conclusions: Our findings suggest that lower serum VD3 level is associated with a greater risk of death in the general Japanese population, regardless of kidney function.

Funding: Private Foundation Support

TH-PO397

Bone-Specific Alkaline Phosphatase and Coronary Artery Calcification and Mortality in Diabetic Chronic Kidney Disease Magdalena A. Sarna,1 Sirin Jiwananon,2 Rashmin Mehrrotta,2 1Department of Medicine, University of Calgary, Calgary, AB, Canada; 2Department of Medicine, Los Angeles Biomedical Research Institute, Torrance, CA.

Background: Higher levels of alkaline phosphatase (ALP) have been previously shown to be associated with increased mortality in patients with chronic kidney disease (CKD) and cardiovascular disease. However, most studies have used serum ALP rather than bone-specific alkaline phosphatase (BALP), a marker of bone turnover and mineralization. The aim of our study was to evaluate the role of BALP in predicting vascular calcification, using coronary artery calcification (CAC) scores, as well as mortality and progression to end-stage renal disease (ESRD) in type 2 diabetic patients with CKD.

Methods: A total of 166 participants from two prospective cohort studies of CAC in non-dialysis-dependent type 2 diabetes with nephropathy were included. An immunoenzymometric assay was used for quantifying measurement of BALP. Baseline patient demographics included a mean age of 57 years, estimated glomerular filtration rate (eGFR) of 57 ml/min/1.73 m² and a median urine protein-creatinine ratio of 2.6. The median BALP level was 13.8 (10.9-18.2) µg/L. There was no difference in CAC scores according to BALP tertiles (p=0.12). Multivariate analysis for baseline BALP as an outcome showed age, parathyroid hormone and triglyceride, but not CAC levels, to be predictive in the model. As expected, a relationship was found between all-cause mortality and higher CAC scores (p=0.018) but not ESRD (p=0.99). BALP was not found to be predictive for all-cause mortality (p=0.69) or ESRD (p=0.998).

Conclusions: BALP levels do not appear to be correlated with greater vascular calcification in this study. BALP tertiles were not predictive of all-cause mortality or progression to ESRD. Further studies are needed to validate whether BALP, a marker of bone turnover, is predictive of cardiovascular risk in patients with CKD.

Funding: Other NIH Support - NCCR

TH-PO398

Predictive Value of a Multi-Organ Cardiovascular Disease Target Organ Score for Mortality and Cardiovascular Events in a Low Risk Population Branko Bram,1 Lugtade Thiijs,2 Tatiana Kouznetsova,3 Jasjete K. Minhas Sandhu,4 Dean Ehrich,3 Jan A. Staessen,2 Carlo A. Guillard,4 Medicine Neurology, University of Alberta, Edmonton, AB, Canada; 2Department of Molecular and Cardiovascular Research, University of Leuven, Belgium; 3Department of Public Health Sciences, University of Alberta, Edmonton, AB, Canada; 4Department of Nephrology, VU Medical University Center, Amsterdam, Netherlands.

Background: An intrinsic limitation of cardiovascular risk prediction models is that they rely on risk factors, not on individual target organ damage (TOD). The current pilot study tested whether application of a multi-organ TOD scoring system had independent value above standard risk models to predict death and cardiovascular events in a low risk cohort.

Methods: To this purpose, a population-based prospective cohort of subjects age >30 yrs, was analyzed for cardiovascular risk factors to populate the SCORE and Framingham equations for TOD and TOD using a new framework, addressing TOD severity in arteries, brain, cardiac and kidney (ABCK).

Results: Of 1970 subjects, sufficient data were available to obtain baseline risk and TOD scores. During a follow up of 10 years, 163 (8%) subjects died, 49 (2%) had an AMI, 45 (2%) had a stroke, 46 (2%) developed end-stage renal disease and 14 (0.7%) developed severe peripheral artery disease. The ABCK TOD score was related to overall and cardiovascular mortality and to cardiovascular events. When compared to the SCORE and Framingham risk models, the ABCK score was independently associated with (fatal and non-fatal) cardiovascular outcome in medium and high-risk individuals over 10 years follow-up. For the intermediate TOD ABCK score the HR were 2.6 and 3.0, for severe TOD ABCK score the HR were 10.7 and 11.4 independent from SCORE and Framingham respectively.

Conclusions: Of interest, scoring renal damage (based on proteinuria and GFR) most strongly added to the prediction by the SCORE and Framingham. This pilot study indicates that addition of a relatively simple to use TOD score can improve the prediction of cardiovascular events.

Funding: Government Support - Non-U.S.

TH-PO399


Background: Sexual dysfunction is common in end stage renal disease (ESRD). However, the strength of evidence regarding differences in sexual dysfunction among patients on different renal replacement therapies (RRTs) is unknown.

Methods: We performed a systematic review to identify published studies describing rates of sexual dysfunction among patients on different RRTs (hemodialysis-HD, peritoneal dialysis-PD and renal transplant- TX). We searched PubMed (English language, after 1987) and hand-searched bibliographies to identify relevant studies. Independent reviewers assessed study quality (internal and external validity). We calculated standardized effect sizes (Cohen’s D) to compare the direction and magnitude of associations between RRT and several sexual function outcomes.

Results: Of 130 potentially eligible studies identified, 18 described sexual dysfunction among men (7), women (1), or both genders (10). Studies compared 12 sexual dysfunction outcomes using observational designs (2 longitudinal cohort, 11 cross-sectional, 5 pre-post). Most studies (9 of 10) reported no differences in outcomes among patients on HD versus PD. In 13 studies, transplant was the most consistently favored RRT (no difference to a great amount less sexual dysfunction) compared to other modalities. Study quality varied (low to moderate; no high), with few studies adequately accounting for potentially confounding factors such as comorbidity.

Conclusions: Limited but variable quality evidence suggests transplant is associated with diabetic dysfunction among patients with ESRD. Rigorously designed prospective studies are needed to better inform patients and providers about the impact of RRTs on this important patient reported outcome.

Funding: Other U.S. Government Support

TH-PO400

Relation between Glomerular Filtration Rate (GFR) and Medical Expense among the Screened Subjects of the Japan Health Insurance Association Kunitoshi Iseki,1 Tsuyoshi Watanabe,2 1Dialysis Unit, University Hospital of the Ryukyu, Nishihara, Okinawa, Japan; 2Division of Nephrology, Fukushima Medical School, Fukushima, Japan.

Background: Few studies examined the medical expenses according to the baseline GFR.

Methods: We combined registries both health check and report of medical expenses (receipts). The health check was done from April 2008 to March 2009, and all the eligible subjects were covered by the Okinawa Branch of the Japan Health Insurance Association. Every monthly report of medical expense such as physical examination, laboratory test, surgical procedures and other related expenses were reviewed during April 2008 to March 2010 (24 months). Serum creatinine was measured by the enzymatic method and the estimated GFR (eGFR, ml/min/1.73m²) was calculated by the Japanese Society of Nephrology. By using the ID number, we could obtain the information of the receipts after the screening. After obtaining written contract with the association, we were provided anonymously coded data.

Results: A total of 74305 subjects, 38.2% females and the mean age of 48.1 years (35 to 74 years) has participated the health check. The total number of receipt was 773,276 during this period. The average receipt point per month, 1 point=10 Yen, was extraordinary high as 29700 in subjects with eGFR<15. The relationship between eGFR and medical expense was U-shaped and the expense was lowest at eGFR 90-104. It was 2369 in eGFR 15-29, 2120 in eGFR 30-44, 1022 in 45-59, 689 in eGFR 60-74, 614 in eGFR 75-89, 606 in eGFR 90-104, 622 in eGFR 105-119, 883 in eGFR 120 and over, respectively. Similarly, it was 637 in subjects with proteinuria (+), 695 in proteinuria (+/−), 1001 in proteinuria (+), 2210 in proteinuria (2+ and over), respectively. Medical cost increased with ageing.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
However, in each age-group medical cost was higher in CKD (eGFR<60), in particular young patients.

Conclusions: The medical expenses increased as lower the eGFR and higher the degree of proteinuria. Reasons for higher cost among subjects with higher eGFR, 120 and over, remained to be investigated.

Funding: Government Support - Non-U.S.

TH-PO401

Health-related Quality of Life, Functional Status, and Survival of Poor Prognosis Endstage Renal Disease Patients Ying-Ying Seoo,1 Chun Peng, Alethea Yee,2 Limin Qu,2 Sze Huey Tan,1 Kok-Seng Wong,3 Cynthia R. Goh.1 1Renal Medicine,Alex Teck Puay Hospital, Singapore; 2Palliative Care, National Cancer Centre, Singapore; 3Clinical Trials and Epidemiological Sciences, National Cancer Centre, Singapore; Renal Medicine, Singapore General Hospital, Singapore; Lien Centre for Palliative Care, Duke - NUS Graduate Medical School, Singapore.

Background: ESRD patients who are very elderly (>75 years old) or with high comorbidity burden, have poor survival even on RRT. Symptoms and functional status do not always improve with initiation of RRT in these poor prognosis groups. We studied a group of extremely poor prognosis ESRD patients (median life expectancy <24months) to monitor change in QoL and functional status, whether on RRT or conservatively managed (CM).

Methods: This was a prospective longitudinal study of ESRD patients at the Singapore General Hospital. 101 patients with eGFR 8-12ml/min and either >75 years old or with Charlson Comorbidity Index ≥28 were followed up for 24months. At enrollment and at fixed time intervals (0, 3, 6, 9, 12, 18, 24months) KQoL, Karnofsky Performance Score and eGFR were recorded. Baseline demographic data was also recorded.

Results: 38 patients started RRT and 63 were CM. At 24m, 33/38(86.8%) RRT and 24/63(38.1%) CM patients were alive. Median survival for CM was 14.6m and GFR at time of death was 6ml/min.GFR at initiation of RRT was 6.3ml/min. For CM, there was a decline over time for Social Support,PF,GH,SF and PCS (p<0.05) but an improvement in Burden of Kidney Disease, Work Status and RE. Among RRT patients, comparing KQoL and KPS scores closest to initiating RRT and first recorded scores 3m after initiation, only KBD was improved but symptoms, sleep, overall health, Energy, RP, PCS and MCS were all worse (p<0.05). KPS was comparable pre and post-RRT.

Conclusions: Asian RRT populations have been shown to have good survival and QoL. However, the worst prognosis ESRD patients survived longer on RRT compared to CM. However, their QoL did not improve, on the contrary, this deteriorated on RRT. Functional status remained unchanged. For CM, there was a gradual decline in some HRQoL domains but improvement in others. Scores in general were good.

Funding: Government Support - Non-U.S.

TH-PO402

Colonic Necrosis Is Not Associated with the Use of Polystretylene Sulfonate (SPS) Potassium Binding Resins Maura A. Watson, Annie Nguyen, David K. Oliver, Kevin C. Abbott, Christina M. Yuan. Nephrology, Walter Reed Army Medical Center, Washington, DC.

Background: The FDA in 2009 recommended against the “concomitant use of sorbitol” with SPS powder due to reported cases of colonic necrosis (CN), a rare (but potentially fatal) event. Little data exists to suggest that oral SPS formulations cause CN.

Methods: This retrospective cohort examined all 121,812 Walter Reed Army Medical Center inpatient subjects between 9/1/2001 and 10/31/2010. Pharmacy data was queried on tissue diagnosis. The primary outcome was CN in subjects who were exposed to SPS within one month prior to diagnosis (a plausible temporal association) and associated mortality rates. Facilities without sufficient data to calculate performance scores were excluded.

Results: SPS was prescribed to 2,195 subjects (0.02%). 83 CN cases were identified (0.07%). Three received oral SPS (1g/4ml) up to one month prior to CN diagnosis (3.2%). Nine-year cumulative CN incidence was 0.14% in those exposed to SPS. SPS exposure was not significantly associated with CN (adjusted relative risk = 2.08; 95% CI 0.63-6.57; P = 0.20). The number needed to harm was 1411. Exposure to SPS was not significantly associated with CN when adjusted for age <65, eGFR<30, ICU admission, or post surgical status. Sample size analysis showed it would require a population of 4974 patients age >65 to have 80% power to detect one case of CN with a relative risk = 2.08 (95% CI 0.63-6.57).

Conclusions: Inpatient use of SPS was not significantly associated with the development of CN by unadjusted analysis in this cohort of hospitalized subjects at a single tertiary care medical center over 9 years. CN is an extremely rare outcome therefore a single medical center may not have sufficient cases for robust statistical analysis, and analysis of a coordinated national system would be required.

Disclaimer: The views expressed in this paper are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense, or the United States government.

TH-PO403

Simulated CMS ESRD Quality Incentive Program and Association with Mortality Robert A. Wolfe,1 Emily E. Messerstrom,2 Regina S. De los Santos,1 Erik Roys,3 Jeffrey Pearson,1 Alissa Kapke,2 Matthew Paul,1 Bruce M. Robinson,1 Joseph M. Messana.1 1Arbor Research Collaborative for Health; 2University of Michigan Kidney Epidemiology and Cost Center.

Background: As part of the Medicare Improvements for Patients and Providers Act of 2008, the Centers for Medicare & Medicaid Services (CMS) were mandated to implement a Quality Incentive Program (QIP) for payments to dialysis facilities in 2012. The QIP will use 3 measures of dialysis facility performance: the percent of pts with hemoglobin <10 g/dL, hemoglobin >12 g/dL, and urea reduction ratio ≥65%. Each dialysis facility’s performance in 2010 will be compared with their facility-specific historical performance in 2007 or national performance in 2008; facilities not meeting one of these standards on any of the 3 measures may face payment reductions of up to 2% in 2012.

Methods: Using CMS data, we simulated the QIP with 2009 facility performance (N=4,718 facilities). After calculating the quality measures, we calculated the total performance score (0-30 points) for CM and categorized facilities by payment reductions (none, 0.5%, 1.0%, 1.5%, or 2%). To assess the validity of the QIP, we related these facility categorizations to a 2009 facility standardized mortality ratio (SMR) using Poisson regression. The SMR accounts for patient case-mix and general population state death rates. Facilities without sufficient data to calculate performance scores were excluded from the analysis.

Results: Facilities receiving payment reductions had higher mortality than facilities with no reductions (see figure).

Conclusions: Facilities receiving payment reductions had higher mortality than facilities with no reductions (see figure).

TH-PO404

Human Resource Allocation to Intensive Home Hemodialysis (HHD) Programs in Canada Robert P. Pauly,1 Deborah Lynn Zimmerman,2 Paul Komenda.3 1University of Alberta, Canada; 2University of Manitoba, Canada; 3University of Ottawa, Canada.

Background: There is growing interest in HHD though there are almost no published data on the human resource requirements to maintain a home program. The purpose of this study was to leverage Canadian expertise in HHD delivery and characterize human resource allocation in our programs.

Methods: Between Jul and Dec 2010, 19 dialysis programs (14 university-based centers and 5 community centres known to provide all options of home dialysis therapies) were surveyed to describe their staffing complement and the roles of various disciplines.

Results: Seventeen of 19 (89%) programs responded. The median number (and range) of patients cared for by 1 full-time equivalent (FTE) nurse and dialysis technologist is 14.4 (10-24.3) and 22.7 (12.5-42.0) respectively; the median ratio of patients to clinicians is 51.5:1 (range 35:1-121:1). One of 17 (11%) units has a dedicated dialysis educator/coordination associated with their renal program. Eleven of 17 (65%) programs have a designated dietician, 9/17 (53%) have a social worker, and 7/17 (41%) have a pharmacist; 81% of these positions are part-time FTEs indicating these resources are shared broadly in most renal programs. A single nephrologist cares for all HHD patients in 3/17 (18%) programs, a specialized group of HHD physicians attends in 10/17 (58%) units, and all nephrologists share caring for HHD patients in only 4/17 (24%) units. Technical support for HHD is completely outsourced to a third party vendor in 10/17 (58%) units, shared with conventional HD in 6/17 (35%) programs and designated solely to HHD in 5/17 (29%) programs. A unique activity in the HHD unit includes the technical assessment of patients’ home equipment which is performed by program technologists (7/17 – 41%), third party vendors (5/17 – 29%), and a combination of a technologist and a vendor (5/17 – 29%).

Conclusions: Significant variability exists in staffing HHD programs in Canada. This may relate to the local practice environment and/or the experience of the centre; this requires further study. Understanding the staffing complement among established HHD programs may be valuable for new programs in considering resource allocation.
TH-PO408

Pre-Operative Proteinuria Predicts Acute Kidney Injury in Patients Undergoing Cardiac Surgery
Steven G. Coca, Divakar Jammalamadaka, Jay L. Koyner. Medicine, University of Chicago, IL.

Background: To examine the utility of using proteinuria in pre-operative risk stratification for acute kidney injury (AKI). AKI is a common and important complication for patients undergoing cardiac surgery. Proteinuria, which reflects structural damage to the glomeruli or renal tubules, may aid the prediction of AKI.

Methods: The ratio of urine albumin to creatinine (UACR) and dipstick proteinuria were prospectively measured in 1300 adults and children undergoing cardiac surgery. The cohort was organized into four clinical risk categories based on the preoperative UACR: UACR ≤ 10 mg/g (≤ 1.1 mg/mmol), 11-29 mg/g (1.2-3.3 mg/mmol), 30-299 mg/g (3.4-33.8 mg/mmol), and ≥ 300 mg/g (≥ 33.9 mg/mmol). The primary outcome was post-operative AKI, defined by the AKIN stage 1 criterion (serum creatinine rise by ≥25% or ≥0.3 mg/dL (26.5 µmol/L)).

Results: An increase in the incidence of AKI was noted across the UACR categories (25%, 35%, 42%, 57%, respectively).

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Adding UACR to the clinical model to predict AKI improved the AUC from 0.61 to 0.66 (p < 0.001) and the continuous net reclassification improvement (NRI) was 32% (p < 0.001). UACR categories were also independently associated with risk of in hospital death or dialysis (p = 0.04), and ICU and hospital length of stay (p = 0.005), (p = 0.008), respectively. Surgery status and pre-operative GFR were effect modifiers; the association was strongest amongst those undergoing elective surgery (p < 0.001) and those with eGFR 45 mL/min per 1.73 m²/p = 0.003).3 months.

Conclusions: Pre-operative proteinuria provides graded stratification risk for AKI and is an independent predictor of other outcomes in adults and children undergoing cardiac surgery. Funding: NHBLI

TH-PO409
Renal Artery Calcium and Mortality among Community-Living Adults
Dena E. Rifkin, 1,2 Joachim H. Ix, 1,2 Christina Wassel, 1 Michael H. Criqui, 1 Matthew Allison.1 UC San Diego; 2VA Healthcare System, San Diego.

Background: The associations of calcification in the renal vasculature, independent of calcification in other vascular beds, with subsequent mortality risk in the general population are unknown.

Methods: We assessed renal artery calcification (RAC) by abdominal CT scan and used Cox-proportional hazards models to examine the association of RAC with mortality in 4,469 community living adults without known cardiovascular disease (CVD) who presented for preventive screening examinations.

Results: The mean age of the study sample was 56.7, and 42.6% were women. RAC was present in 629 of 4,469 (14%) participants. Over a median follow-up of 8.2 years, 177 individuals died. After adjustment for age, gender, diabetes, smoking, cholesterol and family history of CVD, the presence of RAC conferred a more than 70% increased hazard of all-cause mortality (HR 1.71, 95% CI 1.22-2.39, p = 0.002). Adjustment for blood pressure, a potential confounder of RAC, substantially diminished this effect and all-cause mortality was present in 629 of 4,469 (14%) participants. Over a median follow-up of 8.2 years, 177 individuals died. After adjustment for age, gender, diabetes, smoking, cholesterol and family history of CVD, the presence of RAC conferred a more than 70% increased hazard of all-cause mortality (HR 1.71, 95% CI 1.22-2.39, p = 0.002) adjustment for boundary blood pressure, a potential mediator of the association, did not substantially change this results (HR 1.44, 95% CI 1.03-2.03, p = 0.036).

Conclusions: We found that RAC is associated with an increased risk of subsequent mortality in individuals without known CVD, independent from traditional CVD risk factors. The risk was attenuated somewhat by adjustment for vascular calcification in other vascular beds, suggesting an effect of systemic calcified atherosclerosis, and was not mediated by hypertension.

Risk adjusted* fully adjusted** putative mediator adjusted***

<table>
<thead>
<tr>
<th>RAC (any vs. none)</th>
<th>age-sex adjusted</th>
<th>risk adjusted*</th>
<th>fully adjusted**</th>
<th>putative mediator adjusted***</th>
</tr>
</thead>
<tbody>
<tr>
<td>p for HR</td>
<td>p &lt; 0.001</td>
<td>p = 0.002</td>
<td>p = 0.027</td>
<td>p = 0.036</td>
</tr>
<tr>
<td>p for RAC (per log change)</td>
<td>p &lt; 0.001</td>
<td>1.16 (1.08, 1.25)</td>
<td>1.14 (1.06, 1.23)</td>
<td>1.10 (1.02, 1.19)</td>
</tr>
</tbody>
</table>

* adjusted for smoking, hypercholesterolaemia, and diabetes, and family history of CVD; ** additionally adjusted for % of other beds with vascular calcification; *** additionally adjusted for hypertension (yes/no).

Funding: Other NIH Support - NHBLI, Veterans Administration Support

TH-PO410
The Use of Palliative Care Services amongst End Stage Kidney Disease Patients in an Irish Tertiary Referral Centre
Lynn Redahan, 1 Bernadette Brady, 2 Andrew Smyth, 1 Stephen Higgins, 1 Catherine A. Wall. 1 Department of Nephrology, AMNCH Tallaght, Dublin, Ireland; 2 Palliative Medicine, AMNCH Tallaght, Dublin, Ireland.

Background: It is well accepted that patients with end stage kidney disease (ESKD) have a shortened life expectancy. Despite this, it has been recognised that end of life care is suboptimal in this patient population. The aim of this study was to review the utilisation of palliative care services amongst ESKD patients in a tertiary referral centre.

Methods: We conducted a retrospective chart review of patients with ESKD who died between January 2005 and October 2009. Eligible patients were identified using the renal database. We included patients who had undergone renal replacement therapy for a minimum of 6 months. We recorded palliative care referrals, modalities of renal replacement therapy, age at death and place of death.

Results: 128 patients were included in the study. The final modality of renal replacement therapy was haemodialysis in 100 patients (78.1%) and peritoneal dialysis in 28 patients (21.9%). The average age at death was 65 years. Forty five patients (35.2%) were referred to the palliative care services. The palliative care team were involved in the patients’ management for a median of 11 days before death. 102 patients (79.7%) died in an acute hospital setting, nine patients (7.0%) died at home, two patients (1.6%) died in an inpatient hospice and the place of death was unknown for fifteen patients (11.7%). Dialysis was withdrawn prior to death in forty eight patients (37.5%).

Conclusions: The palliative care services were involved in the antemortem care of approximately one third of our patients and the majority of referrals were sent at a late stage. A decision to withdraw dialysis was made in a high proportion of cases studied. Given the short timeframe until death once dialysis is withdrawn, it is imperative that appropriate end of life care is instituted for these patients. We have identified an underutilisation of palliative care services for ESKD patients in our hospital. Improved integration of palliative care and nephrology services may allow us to optimise end of life care for these patients.

Funding: Other NIH Support - NCRR supported CTSA pilot project grant from University of New Mexico health Sciences Center

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TH-PO413

Alterations in Renal MicroRNA Expression in a Mouse Model of Congenital Obstructive Nephropathy
Susan E. Ingraham, Kirk M. McHugh. Division of Nephrology, Nationwide Children’s Hospital, Columbus, OH; Center for Molecular Genetics, Nationwide Children’s Hospital, Columbus, OH.

Background: Congenital obstructive nephropathy (CON) is the most common cause of chronic renal failure in children. The megabladder (mgb) mouse, a unique genetic model of CON, allows investigation of the molecular changes of CON, potentially facilitating identification of novel biomarkers or therapeutic targets for this devastating condition. MicroRNAs (miRs), a class of small non-coding RNA, are important gene regulators controlling key cellular functions. A number of miRs are highly and differentially expressed in the kidney, and have been implicated in key physiological or pathological renal processes. This project explores changes in miR expression associated with early stages of renal involvement in the mgb mouse.

Methods: Four week old mgb mice were stratified based on general health and ultrasound findings as previously described (Ingraham et al., 2010). Males classified as controls. Total kidney RNA was used for comparative miR microarray analysis. miRs that showed no difference in expression between mgb and control, and five could not be quantitatively reverse transcription-coupled PCR (RT-qPCR).

Results: Comparative microarray analysis yielded a panel of 22 miRs whose expression levels were at least 1.5-fold in mgb kidneys compared to normal controls. RT-qPCR analyses of these miRs confirmed that eight were significantly increased in expression in mgb kidneys (p<0.05). One miR showed a trend toward increased expression but did not reach statistical significance. Of three miRNAs with decreased expression by microarray, two showed a downward trend by qPCR but did not reach statistical significance. Two miRs showed no difference in expression between mgb and control, and five could not be tested by qPCR due to low sequence complexity.

Conclusions: Alterations in miR expression are associated with early stages of renal involvement in the mgb mouse model of CON. Further characterization of miRs in this animal model may yield new insights into this complex disease process.

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TH-PO414

Genome-Wide Association Study (GWAS) of Novel Kidney Function Biomarkers in 6744 European Americans
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Background: Serum beta trace protein (BTP) and beta-2 microglobulin (B2M) have potential as novel biomarkers for renal function. Little is known about the genes influencing their levels alone or through glomerular filtration rate estimated from serum creatinine.

Methods: We conducted a GWAS of log-transformed serum BTP and B2M levels and further examined their association with known eGFR loci in 6744 European Americans from the Atherosclerosis Risk in Communities (ARIC) study.

Results: The GWAS identified a genome-wide significant locus upstream of PTGS2, the gene that encode BTP (rs7040970, beta=0.04, p=3.3x10^-15, Rsq=0.8%), which was not associated with either eGFR or eGFRcys (p>0.05). The GWAS of B2M identified 2 genome-wide significant loci. One was in the HLA region on chromosome 6 that spans 7 Mb with over 1000 SNPs attaining genome-wide significance (lowest p-value=1.8x10^-248). The other locus of B2M was on chromosome 12 (rs3184504 in B2M, Rsq=0.8%), which was previously implicated as a renal locus. Of the 16 eGFR loci previously identified (Kottgen et. al 2010), within our sample eGFRcr was associated with improvement in PKD. NMR-based metabolomics may be a tool to study the genetic pathways that might be responsible for the protective effect of lovastatin in PKD.

Results: PKD in Cy/+ rats was accompanied by changes in several metabolites as compared to the +/+ rats. The concentrations of Krebs cycle intermediates citrate and succinate significantly decreased from 1366.9±296.8 and 3353.4±278.2 nmol/g to 919.3±150.7 (p=0.005) and 2219.6±557.0 nmol/g (p=0.001), respectively. Their decrease was accompanied by a decrease of kidney glucose concentration. The observed impairment of the glucose import could possibly be a result of a feedback action induced by the decreased Krebs cycle activity. Aprod of uric acid oxidation and a marker of kidney injury, allantoin, increased almost 3-fold in the Cy/+ rat kidneys (p<0.001). Stain treatment reversed the metabolic changes induced by the PKD in the Cy/+ rats. Citrate concentration increased to 1419.2±92.2 nmol/g (p<0.005) and so did the lactate concentration, suggesting a recovery of energy producing Krebs cycle and glycolysis pathways. The increased glucose consumption of the lovastatin treated kidneys further lowered the glucose concentration. Allantoin levels significantly decreased as well, but still remained almost 2-fold higher as in the +/+ controls.

Conclusions: We describe for the first time changes in energy metabolism in PKD kidneys. Lovastatin increased the activity of energy producing metabolic pathways with association improvement in PKD. NMR-based metabolomics may be a tool to study the relationship between metabolic changes and cellular signaling in PKD and to develop urine biomarkers for the diagnosis of PKD.

TH-PO417

Transcriptome and ChIP-Seq Analyses of JunD Levels Reveal the Basis of lovastatin-Mediated Activation Networks Associated with Crescentic Glomerulonephritis (Crgn2) in Rat Macrophages
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Background: We previously investigated the genetic basis for the unique susceptibility of the Wistar-Kyoto (WKY) rat to Crgn and identified the AP-1 transcription factor JunD as a primary determinant of macrophage activation. JunD is markedly overexpressed in WKY macrophages compared to the Crgn-resistant Lewis strain and this over-expression, controlled in cis, results in increased activity of these cells.

Methods: We studied how differences in JunD expression alter the macrophage transcriptome and its cistrome, the genome wide set of JunD-DNA binding sites, following (LPS) stimulation by two approaches: 1. siRNA knockdown of JunD in WKY bone marrow derived macrophages (BMDMs) 2. Sanger sequencing of induced JunD-BMDMs

Results: We identified 1672 genes in basal and 1476 genes in LPS stimulated BMDMs that were differentially expressed following siRNA knockdown of JunD and 830 differentially expressed genes between WKY and WKY/Jcnrg2 over a LPS stimulation timecourse. Gene ontology analysis enriched for processes including responses to
TH-PO418

Analysis of RNA from Urinary Exosomes/Microvesicles to Non-Invasively Diagnose Renal Cancer and Diabetes Related Renal Dysfunction

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Background: Microvesicles, including exosomes, are small lipid bilayer vesicles released from all cells into bodily fluids. These vesicles harbor nucleic acids (in particular RNA) from their parent cell and can be used to interrogate the transcriptional profile of cells in vivo in a non-invasive manner. Using our rapid microvesicle isolation technique we have now demonstrated our ability to non-invasively detect mutational status in a 200 patient prostate cancer cohort demonstrating detection of oncogene fusion status (TMPRSS2:ERG) as well as qPCR analysis of 20 genes previously documented as being associated with prostate cancer progression. Using this experience as a foundation, we now turn our attention to the non-invasive detection of two important related renal diseases in renal cell carcinoma (RCC) iii) diabetic nephropathy (DN).

Methods: Urine samples were collected from Columbia University (RCC patients) and Winthrop University Hospital (DN patients) under approved IRB. Urinary exosomes/microvesicles were isolated from urine samples using a centrifugation protocol. RNA was extracted from exosomes/microvesicles using the TRIzol reverse transcription protocol followed by qPCR analysis of select genes (p <0.05) of interest.

Results: We demonstrate that a wide array of genes are detectable within urinary microvesicles using array and NGS analysis. This included CA9 in RCC and various markers of the renin angiotensin system and beyond in the DN patients including angiotensin II receptor, angiotensinogen and angiotensin converting enzyme mRNA expression.

Conclusions: Urinary exosomes/microvesicles offer a novel method to non-invasively monitor renal transcription in various renal related diseases. Array and NGS analysis of related pathways (red in Fig. 1, i.e. NFkappaB-, TNF-signaling). NRF2-mediated Oxidative edges. The network displays two major subclusters: 1. containing metabolism pathways associated genes, co-regulated transcripts were identified in the ERCB expression data set. These co-regulated transcripts were significantly enriched in 148 pathways. CKD associated molecular pathways with transcriptional regulators. expression data sets will help to define interactions of chronic kidney disease (CKD) and associated pathways with transcriptional regulators. CKDGen transcripts and their co-regulated mRNAs identify the interplay of inflammation and metabolism pathways in CKD. The CKD pathway network can link specific molecules in their larger context.

TH-PO420

LC-MS/MS and Antibody-Array Based Urinary Proteome Analysis of Obstructive Nephropathy

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Background: We have previously shown that urinary peptidome analysis allows early prediction of obstructive nephropathy (ON) in newborns. Although these urinary peptidome biomarkers are of great potential clinical value, they are less informative on the pathophysiology of the disease. Therefore we have focussed on the changes in the urinary proteome of ON patients using label-free LC-MS/MS and antibody–array analyses.

Methods: Urine samples (n=5/group) were divided into 4 groups: bladder urine from controls (heathy), mild ON (NoOp), severe ON (Op) and pelvis urine from severe ON (Pelvis). For antibody array analysis, 90 µg of Cy5-tagged urinary proteins were applied onto antibody arrays (XP725 Sigma). Fluorescence intensity was normalized by the mean intensity of each array. For LC-MS/MS analysis, 30 µg of the protein was digested by trypsin and analysed by online capillary LC-MS/MS. Proteins were identified and quantified as described (Mouton-Barbosa et al., MCR, 2010).

Results: The number of differentially secreted proteins between the different groups (p value <0.05) is shown in the table.

Conclusions: The number of differentially secreted proteins after Benjamini-Hochberg adjustment. Only the LC-MS/MS comparison of the control group versus pelvic urine yielded 48 proteins that survived correction for multiple testing. Based on these comparisons we established a list of proteins that are specific for the obstructed or controlateral kidney.

Conclusions: We are validating a subset of these proteins on new samples. Two different approaches for validation are used: antibody-based validation and multiple reaction monitoring on small (max 1.5 ml) urine samples.

Funding: Government Support - Non-U.S.

TH-PO421

Alterations in Cellular Proteome of Macrophage Induced by Calcium Oxalate Monohydrate Crystals Are Associated with Phagocytosis

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Background: The presence of macrophage in renal tubulointerstitium is the key feature of progressive renal inflammation in kidney stone disease. Macrophage can engulf and eliminate foreign body or microbe, and subsequently produces various inflammatory mediators. However, molecular responses of macrophage to calcium oxalate monohydrate (COM) crystals, the major crystalline composition of kidney stone, remained unclear. Therefore, this study aimed to investigate alterations in cellular proteome of macrophage induced by COM crystals using a proteomics approach.

Methods: Macrophages were derived from U937 human monocytic cells by treatment with 100 ng/ml phorbol myristate acetate for 48 h. Macrophages were then incubated with or without 100 µg/ml COM crystals for 24 h. Thereafter, proteins derived from whole cell lysates of COM-treated and control cells were resolved by 2-DE (n=5/group, each was from independent culture) and stained with Deep Purple fluorescent dye. Differentially
expressed protein spots were then identified by Q-TOF MS and MS/MS analyses. Functional significance of these proteins was addressed by global protein network analysis using STRING version 8.3 and double immunofluorescence staining.

Results: Spot matching and quantitative intensity analysis revealed 18 differentially expressed protein spots. These proteins were successfully identified by Q-TOF MS and MS/MS analyses, including those involved in cellular structure, carbohydrate metabolism, DNA/RNA processing, protein metabolism and stress response. The altered levels of α-tubulin, β-actin and ezrin were validated by Western blot analysis. Double immunofluorescence staining revealed a phagosome-like structure outlined with co-localized F-actin and HSP90 and contained COM crystal inside.

Conclusions: Proteomic analysis revealed altered cellular proteome profile of macrophage induced by COM crystals. These findings also showed that the altered proteins were also involved in enhanced phagocytic activity of the COM-exposed macrophage. Our data may help understanding of important role of macrophage in kidney stone disease.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO422

Renal Mendelian Genes Based Pathway Analysis
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Background: The development of complex diseases such as chronic kidney disease (CKD) and end-stage renal disease (ESRD) often require the interaction of various genes and environmental factors within individual or interacting pathways. Similarly, injury along multiple points of interacting pathways can result in similar disease phenotypes. As such, we hypothesized that exploring protein to protein interactions (PPI) networks related to Mendelian genes associated with renal phenotypes could help better elucidate pathways critical to the development of renal pathology.

Methods: Starting with over 600 OMIM listings of Mendelian diseases with renal phenotypes, we identified 339 unique entries which were manually curated to 227 unique disease entries with at least one identified autosomal gene and identifiable renal phenotypes. These were classified within 3 broad categories: 1) Developmental and glomerular 2) Tubular, and 3) Secondary renal disease (e.g., deposition of protein within the kidney). Using the Pathway Studio tool, we systematically explored protein interaction network related to our selected Mendelian genes and identified overrepresented biologic pathways within our 3 broad categories of renal disease types.

Results: We identified numerous pathways that were significantly over represented within our Mendelian gene based PPI networks, including FGFR, leptin, PECAM, and guanylate cyclase. These pathways relate, at least in part, to angiogenesis, branching morphogenesis, adipose activation of sympathetic activity, endothelial cell adhesion and nitric oxide signaling. We also found significant overrepresentation of expression targets related to many of our genes, including SHH, PAX2, ACE, LMX1b, ALB, SGK1, AVP, FOXL1, SCN11A, WNK4, FGF23, PLA2G, FOXM1, ERG, RAG2 and PPARG.

Conclusions: By mining genes associated with renal pathology, we have identified numerous first order protein based interactions converging from diverse genetically mediated renal diseases onto definable pathways. These overrepresented pathways provide promising logical targets for further study in both fundamental mechanism and treatment interventions for CKD and ESRD.

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TH-PO423

Impact of Uremic Environment on Peritoneum: A Proteomic View
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Background: Morphology and function of peritoneal membrane are abnormal in patients with uremia, but the contributing pathophysiology is unclear. Here we attempted to characterize the differential protein targets that may be related to peritoneal membrane changes in patients with uremic condition and haven’t exposed to peritoneal dialysis fluid.

Methods: Protein profiles of peritoneal fluids collected from patients with uremia and patients with normal renal function receiving laparoscopic cholecystectomy were displayed by two-dimensional gel electrophoresis (2-DE). Altered protein spots were excised and subjected to in-gel tryptic digestion followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis.

Results: Sixteen 2-DE protein spots were altered between two groups of samples.

Conclusions: Conclusion of these proteins may provide a novel understanding of peritoneal membrane changes injured by uremic toxins and may manifest as predictive biomarkers of peritoneal function or therapeutic targets during the regular peritoneal dialysis in the future.

Funding: Private Foundation Support

TH-PO424

Perinatal Exogenous Nitric Oxide in Fawn-Hooded Hypertensive Rats Reduces Renal Ribosomal Biogenesis in Early Life
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Background: Nitric oxide (NO) is known to depress ribosome biogenesis in vitro. We analyzed the influence of exogenous NO on ribosome biogenesis in vivo using a proven antihypertensive and renoprotective model of perinatal NO administration in genetically hypertensive rats (Am J Physiol 2008;294:R1847-55).

Methods: Fawn-hooded hypertensive rats (FHH) dams were supplied with the NO donor molsidomine in drinking water 2 weeks before to 4 weeks after birth. Kidneys were collected from 2d, 2wk and adult offspring.

Results: Although the NO donor increased maternal NO metabolite excretion, renal NO status at 2wk was unchanged as assayed by EPR spectroscopy of NO trapped with iron-dithiocarbamate. Nevertheless, microarray analysis revealed marked differential down-regulation of ribosomal protein genes in 2wk old males. These changes in 2wk males were confirmed by polysome profiling, which also showed down-regulation of ribosomes in 2wk females. Polysome profiles were not affected at 2d or in adults.

Funding: Private Foundation Support

Western blots analysis confirmed that kininogen-1, apoptosis inhibitor 2, cat eye syndrome critical region protein 1, and apolipoprotein A-I had higher expression levels in the uremic samples. In contrast, synaptic vesicle 2-related protein, glial fibrillary acidic protein, and envelope glycoprotein (C2-V5 region) showed lower levels (figure2)

Conclusions: The increased expression of differential proteins may result from changes in the permeability of the peritoneal membrane to middle-sized proteins or peritoneal inflammation with proteins sloughing off. All the identified proteins may provide a novel understanding of peritoneal membrane changes injured by uremic toxins and may manifest as predictive biomarkers of peritoneal function or therapeutic targets during the regular peritoneal dialysis in the future.

Kidneys of 2wk old control and molsidomine treated FHH (n=8/group) were profiled to measure assembled ribosome structures. # P<0.01 vs. controls of the same peak.

Conclusions: Marked decreased postnatal expression of renal ribosomal genes and proteins at 2wk of age in the absence of a change in renal NO status suggest that these alterations are epigenetically programmed by NO in the fetus. In conjunction with prolonged antihypertensive and renoprotective effects of perinatal NO administration in FHH, these data provide a salient example of drug-induced reduction of renal ribosome biogenesis accompanied by beneficial long-term effects in both males and females.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
TH-PO452

Identifying Urinary Peptidomic Biomarkers for Metabolic Syndrome with Early Renal Injury

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Background: Metabolic syndrome (MS) is associated with chronic kidney disease. Our study aims to establish peptide models and identify urinary biomarkers of MS with early renal injury.

Methods: Samples of overnight (8h) urine were collected from subjects participating in an epidemiologic study of MS with CKD. MS was diagnosed by ATP III criteria, while MS with early renal injury was defined as 20g/min/1.73m2 and eGFR<60mL/min/1.73m2. Participants were grouped into healthy (G I, n=65), MS without albuminuria (G II, n=54) and MS with MAU (G III, n=46). The urine was fractionated by magnetic bead based weak cation exchange chromatography and subsequently analyzed by MALDI-TOF-MS. The peptide models were respectively established using statistical tests combined Ga (ClinProTools) and RF algorithm combined SVM (Matlab).1,10 The performance of diagnostic models was assessed using 10-fold cross validation and ROC curve. Differential peptide peaks between groups were identified by LC-MS/MS (LTQ Orbitrap Velos).

Results: (1) G I vs G III: GA model showed 100% sensitivity, 92% specificity and 96% accuracy in training set in identifying MS with early renal injury, and it revealed 76% sensitivity, 80% specificity and 78% accuracy in testing set; Correspondingly, SVM model reported 82% sensitivity, 91% specificity and 87% accuracy, AUC value of ROC curve was 0.924. (2) G IV vs G V: GA model showed 100% sensitivity, 88% specificity and 93% accuracy in training set, and it revealed 71% sensitivity, 73% specificity and 72% accuracy in testing set; SVM model reported 89% sensitivity, 81% specificity and 86% accuracy, AUC value of 0.9. (3)Three peptide peaks identified were all peptide fragments of fibrinogen α chain (Fgα). The peptide fragments were respectively 256.27±2 was up-regulated in G II and G III, m/z 288.33 and 266.41 were up-regulated in G II.

Conclusions: Peptide fragments of Fgα might be urinary peptide biomarkers of MS with early renal injury and Fgα might involve in the pathogenesis of MS and MS with early renal injury.

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TH-PO426

CD36 Polymorphisms Associated with Measures of Renal Disease in Zuni Indians

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Background: The multi-ligand receptor CD36 is implicated in renal pathophysiology in rodents but its potential role in kidney disease (KD) in humans is unknown. CD36 is a class B scavenger receptor (sCD36) with multiple ligands (miRNA, lipoprotein(a), Apo/B/E, TNF, S1P, Glucagon). Using Human Genes and Perturomes, we address the role of CD36 in renal disease.

Methods: We constructed peptide model sets in a family-based association study in Zuni Indians study(n=878) who have a disproportionately high prevalence of diabetic and non-diabetic renal disease (RD).

Results: Two intragenic SNPs inversely associated with BMI, hypertension and glycated hemoglobin. Two intragenic SNPs inversely associated with early renal injury.

Funding: Private Foundation Support

TH-PO427

The Updated Database of Glomerular-Enriched Genes By Combining with the Glomerulome Database for Novel Biomarker Discovery

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Background: A comparative study of the 5 different glomerular-enriched (GI-E) gene databases (DBs) using different techniques has revealed only 7 genes, out of 1,407 genes identified by at least one DB, were identified as GI-E genes in all 5 DBs (He et al., J Am Soc Nephrol 2008). We constructed a human glomerular cDNA microarray DB (MAD-761), in which 761 genes of GI-E (intensity ratio of Cy5 (glomerulus)/Cy3 (3) cortex) ≥ 2.0 was selected in order to minimize non-glomerular contamination. The present study aimed to update MAD-761 by comparing it with another GI-E gene DB from Stanford (SDIB) and with human glomerulus proteome DB.

Methods: We constructed a human glomerulus proteome DB by separating glomerular proteins by microchip electrophoresis coupled with the shotgun analyses by LC-MS/MS (DGE-LS/MS/MS, http://www.khupp.org). The 6,686 glomerular proteins (representing 2,966 distinct genes) were ranked in order of glomerular abundance (spectra counts). Kidney immunohistochemistry of each listed protein was checked in the Human Protein Atlas (HPA, http://www.proteinatlas.org). Peptidomics data sets were defined for NMR in glomerulus was strong (weak or negative in cortex) or moderate (negative in cortex).

Results: In 761 genes in MAD-761, 203 genes were listed in SDIB as GI-E genes. And among the top 1,000 glomerular proteins in the 2DGE-LS/MS/MS, 83 proteins (31 distinct genes) were shown GI-E protein by HPA. In 761 genes in MAD-761, 26 genes were shown GI-E protein. And in 203 GI-E genes in MAD-761 with SDIB, 13 genes were demonstrated GI-E protein; PODXL, PTTPRO, SPARC, NES, PCOLEC2, ARHGDIB, CD34, ANXA1, PDGFRB, WT1, ARBB1, TIP1, ITGAV. The first 10 of 13 were in the top 200 glomerulus-abundant genes in MAD-761. Among these, increased urinary ARHGDIB was demonstrated only in some patients of glomerulonephritis, but in none of normal humans by real-time PCR.

Conclusions: The GI-E gene DB has been updated by combining with the glomerulus proteome DB, which can be useful for novel biomarker discovery in human glomerular diseases.

Funding: Private Foundation Support

TH-PO428

Gene Expression Profiling of Oxalate Nephrotoxicity

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Background: Oxalate interactions with the renal cells results in a plethora of changes, including cell growth, death and altered gene expression as shown in previous studies. This study has been designed to identify specific gene ontology groups and new signaling pathways that undergo changes as a result of oxalate exposure.

Methods: We utilized microarray analysis using Affymetrix HG U133 plus2 gene chip. Data analysis was performed using Data Mining Tool (DMT 3.1, Affymetrix) and GeneSpring 7.2 (Silicon Genetics). Differentially expressed genes were classified according to the Gene Ontology functional category (GenMAPP v2) and functional significance of differentially expressed genes was determined using Ingenuity Pathways Analysis Software (Ingenuity® Systems, www.ingenuity.com). Cluster and Heatmap images were generated using BRK-Array tools30. Changes in gene expression were further validated by relative quantitative RTPCR (RQ-PCR).

Results: Novel signaling pathways and specific genes involved in promoting cell damage and death, nephrosis and tubular damage are highlighted by this study. We first show that CDK2, CDK6 and CDCA2 responsible for G1 to S phase transition are highly down-regulated by oxalate. On the other hand, Retinoic acid Receptor and CBX7 that are involved in maintaining the repressive state of many genes involved in cell growth are highly up-regulated. Cluster analysis of genes that are known members of MAPK pathways, identified up-regulation of many genes that are know activators of the p38 MAPK pathway, like Ras oncogene, MKK3 and ASK1 consistent with our previous demonstration of p38 MAP kinase activation. On the other hand Epidermal Growth factor (EGF) signaling is down regulated. We also show that many genes that are potentially involved in nephrosis and injury to the kidney were identified to be differentially expressed in HK-2 cells upon exposure to oxalate for as little as 4 hours.

Conclusions: The results of this study also provide first direct demonstration of activation of distinct pathways in a comprehensive genomic response to oxalate and identifies the potential of gene chip technology in identification of novel targeted pathways in an unbiased fashion.

Funding: NIDDK Support

TH-PO429

Metabolomic Profiling of the Autosomal Dominant Polycystic Kidney Disease Rat Model

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is an inherited systemic disease characterized by renal cyst expansion, resulting in renal failure. With the progression of renal damage, the accumulation of uremic compounds is reportedly reported to subsequently cause further renal damage and hypertension. The aim of this study was to analyze the profile of uremic retention solutes of ADPKD by capillary electrophoresis–mass spectrometry (CE-MS) using the Han.SPRD rat model.

Methods: Two hundred and nineteen male Sprague-Dawley rats were acclimatized for 4 weeks. The rats were separated into two groups of 190 rats and control rats. The results are expressed as means ± SEM. Statistical analysis was performed using ANOVA followed by a Tukey’s post-hoc test. A p-value < 0.05 was considered statistically significant.

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Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Results: We found 21 cations and 19 anions that accumulated significantly in the renal proximal tubule (PPT) model rat compared with control rats. Among the compounds, increases in 5-methyl-1,2-dioxycyclidine, glyoxalamin, cetoline, allantoate, α-hydroxybenzoate, phenatecarboxylic and 3-phenylpropionate and decreases in 2-dioxycyclidine, deaconate and 10-hydoxydecanoate were newly identified in the ADPKD Cy+/− rat model. We also compared these compounds with human renal uremic samples reported in previous studies. As a result, 3 cations and 4 anions were specific uremic solutes in ADPKD rat and could be a new marker(s) for detecting ADPKD.

Conclusions: We comprehensively identified new uremic solutes in the serum from ADPKD Cy+/− rats and revealed differences in uremic solutes among human CKD patients, the rat renal failure model and Cy+/− rats. We also found new biomarker candidates for detecting renal damage in ADPKD. The results are useful for further studies to elucidate mechanisms accumulating uremic toxins.


Background: Exosome-like vesicles (ELVs) are 50-200nm membrane bound vesicles found in urine and bile that are too small to be resolved by light microscopy. In the kidney, polycystin-1 (PC1) positive ELVs (PKD-ELVs) can interact with primary cilia (Hogan et al 2009). The early assays for this interaction were cumbersome, involving surface biotinylation of human ELVs and multiple purification steps to obtain PC1+ ELVs, and were not suited for high throughput analysis. Furthermore, to visualize the streptavidin/goat antibody interactions on ELVs, we used 22nm gold to coat the cells, which obscured cell morphology although it gave good backscatter contrast between the carbon and gold.

Methods: Here we describe a new assay based on the Phkh1+/+/-mP packaging mouse that has an epitope tagged Phkh1 gene, with two SV5-Pk tags inserted immediately C-terminal to the signal peptide, so that the transgene at the N-terminus of this large type 1 membrane protein. The Phkh1+/+/-mP mouse is normal and secretes SV5-Pk tagged ELVs in its urine and bile. Wildtype primary mouse kidney epithelial cells were cultured and allowed to polarize and make cilia. Phkh1+/+/-mP urine was then applied for 1-10 mins, the cells were fixed in 4% paraformaldehyde and ELVs detected using the SV5-Pk1 antibody and 15nm anti-mouse IgG gold post-fixed in OsO4, and critical-point dried. To obtain optimum morphology the cells are then coated with a 1nm layer of osmium metal using plasma deposition. Using this technique we are able to detect ELVs interacting with primary cilia.

Results: Non treated cilia had no gold positive ELVs associated with them. However, cells treated with Phkh1+/+/-mP urine for 1, 2, 5 and 10 mins have: 1.5, sd±1.5; 1.7, sd±0.7; 0.55, sd±0.68 and 0.36, sd±0.67 ELVs with 1 or more gold particles on them, respectively.

Conclusions: The ELV primary cilium assay described above will be used to assess the ability of various recombinant domains derived from the extracellular part of PC1 and fibrocytin to block or stall the interaction and will help define the functional domains required for interaction.

Funding: NIDDK Support

TH-PO431 Podocin a Novel Substrate for Chaperone Mediated Autophagy Substrate Alejandro Quiroga, Ana Maria Cuervo. Pediatric Nephrology, Montefiore Children Hospital, New York, NY; Department of Development and Molecular Biology, Albert Einstein College of Medicine, New York, NY.

Background: Podocin is a component of the glomerular slit diaphragm and genetic mutations of this protein lead to steroid resistant nephrotic syndrome (SRNS). Altered levels of podocin have been reported in the podocytes of the affected patients, but the contribution of changes in the intracellular turnover of podocin to these altered levels remains unknown. In this study, we have explored the mechanisms that normally contribute to podocin turnover and analyzed changes in this degradation in an experimental model of nephrotic syndrome.

Methods: Endoplasmic reticulum, mitochondria, lysosomes (CMA positive and CMA negative) and cytosol were obtained using whole kidney sub-cellular fractionation from Wistar male rats. Podocin was tagged by green fluorescent protein and translocated into fibroblast cells. The lysosomal membrane protein (LAMP2A, CMA receptor) was knocked down by shRNA in mouse fibroblasts. Paracynin aminoglicosidase (PAN) was used as a model of nephrotic syndrome in cultured human podocytes.

Results: We have found that podocin associates with and is actively degraded in lysosomes. Lysosomal degradation of podocin is maximally activated during nutritional deprivation, however, macroautophagy, one of the starvation-induced forms of lysosomal degradation, does not significantly contribute to the normal turnover of this protein. Instead, we have found that podocin bears in its amino acid sequence a conserved pentapeptide motif used for targeting of proteins for lysosomal degradation via chaperone-mediated autophagy (CMA).

Conclusions: We have confirmed that podocin is preferentially degraded in lysosomes with high CMA activity and that blockage of CMA abrogates this degradation. Lysosomal motif used for targeting of proteins for lysosomal degradation via chaperone-mediated autophagy (CMA) contributes to the normal turnover of podocin in podocytes and with high CMA activity and that blockage of CMA abrogates this degradation. Lysosomal motif used for targeting of proteins for lysosomal degradation via chaperone-mediated autophagy (CMA) contributes to the normal turnover of podocin in podocytes and with high CMA activity and that blockage of CMA abrogates this degradation.

Funding: Other NIH Support - Hirsh/Weill-Caulier Career Scientist Award (to AMC)

TH-PO432 Gene Screening Identified Early Growth Response 1 as a Regulator of Tubulogenesis in Diabetic Renal Embryopathy Ching-Yuang Lin, China Medical University and Hospital, Taichung, Taiwan.

Background: Maternal hyperglycemia can inhibit morphogenesis of uterine branching, with glial cell line-derived neurotrophic factor (GDNF) as a key regulator of its initiation. Early growth response gene-1 (EGR-1) is one immediate early gene. Our previous study found EGR-1 consisting of expression with GDNF in hyperglycemic environment.

Methods: To evaluate the potential relationship of hyperglycemia-GDNF-EGR-1 pathway, in vitro human renal proximal tubular epithelial (HRPTE) cells as target and in vivo mouse podocin-occluded mouse model were used.

Results: In vitro microarray, real time-PCR and confocal morphological observation confirmed apoptosis in hyperglycemia-induced fetal nephropathy via activation of the GDNF/MAPK/EGR-1 pathway. In vitro evidence indicated high glucose suppressing HRPTE cell migration, and enhanced GDNF-EGR-1 pathway induced apoptosis. Knockdown of EGR-1 by siRNA negated hyperglycemia suppressed GDNF-induced HRPTE cell migration. Also, EGR-1 siRNA alleviated GDNF/EGR-1-induced cRaf/MEK/ERK phosphorylation by 80%.

Conclusions: Our findings demonstrated EGR-1’s crucial role in HRPTE cell apoptosis and fetal hyperglycemic nephropathy.

TH-PO433 Stem Cell Microvesicles Rescue Cystinosis In Vitro Diana Ilesias, Rezhan El-Kate, Francesca Emma, Elena N. Levchenko, Nicoleta Elisopoulos, Paul R. Goodyer. 1McGill University, Montreal, Canada; 2Odspedale Pediatrico Bambino Gesu, Rome, Italy; 3University Hospital Leuven, Leuven, Belgium; 4Lady Davis Institute, Montreal, Canada.

Background: In 2009, Sykes et al reported that infusion of bone marrow stem cells into a cystinotic mouse dramatically reduces tissue cystine and improves organ function. Since transdifferentiation of stem cells was rare, the benefit seems to involve a paracrine effect. Recent studies suggest that, by shedding microvesicles, cancer stem cells re-program the behavior of neighboring non-tumorigenic cells. We hypothesized that normal stem cells release microvesicles (MV) that reduce cystine accumulation in CTNS (-/-) cells.

Methods: Normal bone-marrow (bmMSC) and amniotic fluid (amMSC) mesenchymal stem cells were used as MV donors. Fibroblasts and proximal tubular epithelial cells (PTEC) isolated from cystinotic patients with homozygous CTNS deletions were used as target cells. MV were isolated from culture medium by ultracentrifugation. Cystine was measured by HPLC.

Results: When MSC were co-cultured with cystinotic fibroblasts (1:4), cystine levels decreased by 68%, an effect which could not be explained by cell dilution. We then cultured various donor cells separately and harvested MV shed into the culture medium. When MV (100-300nm) were added to CTNS (-/-) target cell monolayers, cystine content was dramatically reduced in dose-dependent manner after 24 hours. The effect was blocked by MV pre-treatment with annexin V. To address whether MV transfer wildtype CTNS protein to the mutant target cells, we transferred amMSC cells with wildtype CTNS/Red fusion protein. In co-culture, we observed direct transfer of the label to intracellular lysosomes of adjacent mutant fibroblasts. We also identified CTNS mRNA in amMSC MV and demonstrated transfer to CTNS (-/-) targets. By introducing a CTNS siRNA, we showed that the MV rescue effect is primarily due to transfer of CTNS protein. A rescue effect was not seen when MV were prepared from mutant amMSC or PTEC cells.

Conclusions: We propose a model in which uptake of MV from stem cells containing wildtype CTNS protein can reduce pathologic accumulation of cystine in adjacent mutant cells.

Funding: Government Support - Non-U.S.

TH-PO434 Existence of Neural-Derived Cells in Neprhon and Mesangial Cells Akiko Oguchi, Jin Nakamura, Nariaki Asada, Motoko Yanagita. Graduate School of Medicine, Kyoto University, Kyoto, Japan.

Background: Recent lineage tracing method demonstrated that almost all the cells in the nephron are derived from Six2+ progenitor cell in metanephric mesenchyme during development, and contribution of other cell types is considered less likely, whereas the origin of mesangial cells and glomerular endothelial cells remains controversial. Here we demonstrated the existence of neural-derived cells in the nephron, glomerular endothelial cells and mesangial cells.

Methods: To test the contribution of neural cells in kidney development, we utilized Nestin-CreERT2 mice, in which inducible Cre is expressed under the control of Nestin promoter, and is activated only after the administration of tamoxifen at desired time points. Nestin is a well-known marker for neural stem cells. We bred Nestin-CreERT2 mice to R26ECFP indicator mice, administered tamoxifen at various time points during embryogenesis as well as in adult life, and permanently labeled Nestin+ cells with the exogenous of ECFP.

Results: When we administered tamoxifen during embryogenesis, ECFP+ cells were detected in tubular epithelial cells, collecting duct, glomerular endothelial cells and mesangial cells, whereas none of Six2+ progenitor cells were positive for ECFP after the administration of tamoxifen. When we administered tamoxifen 1 month after birth, ECFP+ cells were detected only in the inner medulla and papilla.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Taken together, we demonstrated that some cells in the nephron are not derived from Six2+ progenitor cells, but of neural origin. In addition, some mesangial cells and glomerular endothelial cells were derived from Nestin+ cells during embryogenesis. Kidney papilla has attracted attention as a potential niche for kidney stem cells, and lineage tracing of ECFP+ cells in the papilla might give us some insight in the behavior and contribution of these cells during kidney regeneration.

Methods: In order to discover transmembrane transporters of iron we performed an extensive informatics search of proteins distantly related to yeast and mammalian iron transporters. We performed expression cloning in oocytes and 50 candidate genes were evaluated for their ability of iron transport.

Results: We identified a novel transporter that was specific for Fe3+ and active at neutral and mildly acidic pH (7.0-6.5). It had a low micromolar Km for iron conductance. Tagged constructs expressed in cell lines demonstrated that the protein localized in endosomes that contained serum transferrin. There was no overlap in localization with DMT1 which appeared to inhabit a separate endosomal-lysosomal compartment. Application of the transporter in transfected cells overexpressing the novel gene resulted in the recovery of cytosolic iron, and knockdown of the gene had the opposite effect. The knockout of the gene in vivo did not inhibit the formation of the embryonic body plan; however, organogenesis throughout the embryo was inhibited including eye, blood, liver, and kidney at E14.5. This would increase in endosomal oxidized damage induced by accumulated iron in this intracellular compartment of KO cells.

Conclusions: The mechanisms of iron delivery in the embryo are unknown. We have identified a novel transporter sufficient and necessary for iron traffic from the serum iron donor transferrin to developing organs. The is one of the few iron transporters ever identified.

Funding: NIDDK Support, Private Foundation Support

TH-PO435
Morphometric Analysis of Urinary Tract Development in Mice Ashley R. Carpenter, Brian Becknell, Carlton M. Bates, David S. Haines, Kirk M. McHugh. Molecular & Human Genetics, Nationwide Children’s Hospital, Columbus, OH; Pediatric Nephrology, Nationwide Children’s Hospital, Columbus, OH; Pediatric Nephrology, Children’s Hospital of Pittsburgh, Pittsburgh, PA.

Background: Disorders of the urinary tract affect both sexes of all ages and represent a major cause of morbidity and impaired quality of life.

Methods: To understand the morphological events responsible for normal urinary tract development, we performed 3-D reconstructive analysis of the developing mouse urinary tract from embryonic day (E)13 to E17 and postnatal day (P1). These results were compared to a similar analysis of urinary tract development in the megabladder mouse (mgb-/-) and Fgf2Mes-/-/runx model of vescicourethral reflux (VUR).

Results: Morphometric analysis showed that detrusor smooth muscle differentiation initiates in the bladder dome and progressively moves caudal, with the leading edge of differentiation extending down the right posterior surface of the bladder. The developing bladder becomes completely invested with smooth muscle by E15, after which it thickens due to preferential lengthening of the distance between the ureters and urethra. Morphometric analysis confirmed that the primary defect in mgb-/- bladders was a reduction in intravesicular tunnel length was a poor predictor of VUR. Direct comparison of the right refluxing versus left non-refluxing ureters indicated that the intravesicular tunnel length was a poor predictor of VUR.

Conclusions: In conclusion, our findings have significant implications for bladder morphogenesis as well as the ontogeny of VUR and indicate the 3-D morphometric analysis methods to assess both normal and pathologic development of the urinary tract.

Funding: NIDDK Support

TH-PO436
Pax2 Maintains Self-Renewing Nephron Progenitors during Mammalian Kidney Development Akiko Kobayashi. Renal Division, Brigham & Women’s Hospital, Harvard Medical School, Boston, MA.

Background: The functional unit of the kidney, the nephron, is repeatedly generated during mammalian kidney development. Previously, our fate map analysis revealed that the cap mesenchyme expresses a multipotent progenitor population throughout kidney development (Kobayashi et al., 2008, Cell Stem Cell). We further found that the nephron and interstitium form distinct compartments with a strict lineage boundary during kidney organogenesis. Currently, it is not well known how the nephron progenitor population is maintained during kidney organogenesis.

Methods: The paired-domain transcription factor Pax2 is expressed in multiple urogenital tissues including the cap mesenchyme. However, Pax2 function in the cap mesenchyme has not been examined in vivo. Therefore, we investigated Pax2 function in the cap mesenchyme using mouse genetic approaches, including tissue-specific inactivation and mosaic analysis.

Results: In this study, we found that the Pax2 mutant mice fail to maintain the cap mesenchyme in the developing kidney. Surprisingly, fate map analysis in the mutants showed that cap mesenchyme-derived cells lacking Pax2 activity are not lost, but persist throughout kidney development. Detailed molecular marker analysis indicated that these cap mesenchyme-derived cells can trans-differentiate into medullary interstitial cells. Our mosaic analysis revealed a cell-autonomous requirement of Pax2 activity for cap mesenchyme maintenance.

Conclusions: Taken together, our observations suggest that Pax2 maintains a self-renewing nephron progenitor population by repressing interstitial cell fates. Thus, Pax2 activity establishes a lineage boundary between the nephron and non-nephron compartments during kidney organogenesis.

Funding: Private Foundation Support

TH-PO437
A Novel Iron Transporter Controls Organogenesis Andong Qiu, Jonathan M. Barasch. Medicine, Columbia.

Background: It is thought that iron crosses the plasma and endothelial membranes by a Fe2+/Fe3+ transporter known as the Divalent Metal Transporter 1 (DMT1), but transport is induced by a submaximal Fe2+ gradient that is not typical of the transferrin concentration in mammalian blood plasma. In addition, the DMT1 knockout did not inhibit organogenesis. Hence, mechanisms that transfer transferrin-bound iron from circulation to developing organs are currently unknown.

Methods: To circumvent the early embryonic lethality associated with global gene deletion of HDAC1 or HDAC2, mice bearing conditional null alleles were crossed to Hoxb7CreERT2 mice to delete HDAC1 and HDAC2 genes in the ureteric bud (UB) lineage. Results: We showed that oocytes of Hoxb7CreERT2 mice delete HDAC1 and HDAC2 genes in UB lineage. The mosaic nature of Six2-GFP-Cre transgene allows the survival of enough wild type CM cells to prevent ectopic/dorsal specification of renal vesicles in these mice. The Lbx1 expressing nephron precursors are identifiable in CM6/7+ and CM6/7- mice. Six2 Pax2 and GFP immunostaining revealed marked thinning of the CM in the kidneys of CM6/7+ mice. Consequently, the Mes1- expressing stroma expands to compensate for the loss of CM cells in CM6/7- kidneys. TUNEL staining revealed that the apoptotic foci were far more numerous in the CM of the null mutant kidneys. Thus the fraction of proliferating cells (pH3+positive) in the Six2 staining CM is much reduced in the CM6/7+ kidneys. The mosaic nature of Six2-GFP-Cre transgene allows the survival of enough wild type CM cells to prevent ectopic/dorsal specification of renal vesicles in these mice. The Lbx1 expressing nephron precursors are identifiable in CM6/7+ in kidneys at E14.5 but are largely lost by E16.5. Ex vivo cultures of CM6/7+ kidneys reveals secondary defects in UB branching characterized by thick trunks with poor bifurcation of the UB tips. Conclusions: We conclude that Mdm2-p53 signaling is required to strike a balance between progenitor cell expansion and differentiation during normal metanephric development.

Funding: NIDDK Support

TH-PO439
Renal Cystic Hypodysplasia in Mice Lacking HDAC1/HDAC2 Genes in the Ureretic Epithelium ShaoWei Chep, Stacy Lyn Rosenberg, Xiao Yao, Samir S. El-Dahr. Pediatrics, Tulane University School of Medicine, New Orleans, LA.

Background: HDAC1 and HDAC2, two highly homologous histone deacetylases, have redundant as well as distinct functions in global and tissue-specific gene expression. HDAC1 and HDAC2 are expressed in the nephric epithelial and mesenchyme cell lineages in the developing kidneys. Here, we examined the functional requirement of HDAC1 and HDAC2 in the ureteric bud (UB) lineage. Results: To circumvent the early embryonic lethality associated with global gene deletion of HDAC1 or HDAC2, mice bearing conditional null alleles were crossed to Hoxb7CreERT2 transgenic mice to delete HDAC1 and HDAC2 genes in UB of wild type embryos. We showed that UB-specific deletion of either HDAC1 or HDAC2 led to no overt phenotype, whereas concurrent deletion of both HDAC1 and HDAC2 resulted in early postnatal lethality. At birth, HDAC1 and HDAC2 double knockout (DKO) mice have bilateral renal cystic hypodysplasia, including absent nephrogenic zone, lack of cortico-medullary patterning, increased nephron area, and multiple cysts in both cortical and medullary zones. Immunostaining of Six2, Pax2, two markers of renal progenitor cells, and phosphorylated histone H3, an indicator of cell proliferation, demonstrated that the DKO mice completely lost renal progenitor cells. The renal cysts originate from glomeruli (WT) and cystic proximal tubules (LTA and angiotensinogen), and collecting ducts (cytokeratin and AQP2). Ex vivo real-time monitoring of GFP fluorescence revealed that DKO mice

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exhibited aberrant UB branching pattern as early as E12.5, followed by degeneration of UB tips in over 2-3 days at E12.5. At E12.5, there was no difference in Stx2 and Pax2 expression between wild type and kidney. By E13.5-14.5, hypoplasia due to defective UB branching was clearly evident and accompanied by reduced number of glomeruli and dysmorphic proximal tubules.

**Conclusions:** We demonstrate that HDAC1 and HDAC2 perform redundant yet essential functions in the UB lineage. Double deletion of HDAC1 and HDAC2 disrupts UB branching morphogenesis and differentiation causing a cystic hypoplastic renal phenotype.

**Funding:** NIDDK Support

**TH-PO440**

**Therapeutic Effects of Mesenchymal Stem Cells in Wistar-Kyoto Rats with Anti-Glomerular Basement Membrane Glomerulonephritis**

**Kei Matsumoto, Masayuki Iyoda, Takanori Shibata, Yuki Hirai, Yoshihiro Kuno, Tadao Akizawa.**

**Division of Nephrology, Department of Medicine, Showa University School of Medicine, Tokyo, Japan.**

**Background:** Multipotent mesenchymal stem cells (MSC) have become a popular and promising therapeutic approach in many clinical conditions. In this study, we tested the hypothesis that MSC can provide a potential therapy for human anti-glomerular basement membrane (GBM) glomerulonephritis (GN).

**Methods:** Nephroptic serum nephritis was induced in Wistar-Kyoto (WKY) rats on day 0. Groups of animals were given either human MSC (3 x 10^6) or vehicle by intravenous injection on day 4; all rats were sacrificed at either day 7 or day 13. Vehicle-treated groups received an equal volume of Hank’s balanced salt solution (HBBS). For each group, 10 to 15 rats were analyzed. Proteinuria (U-P), serum creatinine (Cr), and body weight (BW) were measured periodically. Renal morphological investigations were performed at sacrifice.

**Results:** BW was comparable between the two treatment groups throughout the study. Serum Cr level was significantly lower in the MSC-treated rats than in the HBBS-treated rats on day 13 (WKY-HBBS vs. WKY-MSC, Day 7: NS, Day 13: p < 0.05). When compared to tissue treatment, MSC-treated rats had reduced U-P on day 7 and 13 (Day 7: p < 0.05; Day 13: p < 0.01). MSC treatment also decreased kidney weight (Day 7: p < 0.05; Day 13: p < 0.01) and glomerular tuft area (Day 7: p < 0.05; Day 13: p < 0.05). The percentage of crescentic glomeruli was identical between the two treatment groups. ED1-positive macrophages (p < 0.05), CD68-positive cells (p < 0.05), and apoptotic cells (p < 0.001), assessed by TUNEL staining, in glomeruli were significantly reduced by MSC treatment on day 7. Renal cortical mRNA for TNF-α (p < 0.001), IL-1β (p < 0.01), and IL-17 (p < 0.01) was decreased, whereas IL-4 (p < 0.05) and Foxp3 (p < 0.01) was increased in the MSC-treated group on day 7. Collagen type I (p < 0.01), type III (p < 0.01), and TGF-β (p < 0.05) mRNA were significantly decreased by MSC treatment on day 13.

**Conclusions:** MSC treatment ameliorates the progression of renal injury in experimental anti-GBM GN via anti-inflammatory and immunomodulatory effects.

**TH-PO441**

**Critical Role of the Stroma in Mediating Vascular Development of the Kidney**

**Sunder Sims-Lucas, Jose Parades, George K. Gittes, Carlton M. Bates. Children’s Hospital of Pittsburgh, Pittsburgh, PA.**

**Background:** Kidney structural abnormalities are the leading cause of pediatric chronic kidney disease, producing significant mortality. Understanding the interactions of different renal lineages is critical for impacting structural renal disease. While many kidney developmental studies focus on ureteric and nephrogenic lineages, very few examine vascular network formation and patterns of fetal blood flow. The purpose of this study is to elucidate development of the vasculature and blood flow in the kidney and to understand how perturbations in ureteric and stromal lineages affects vascular development.

**Methods:** Wildtype or Hoxb7cre/Fgfr2lox/lox (Fgfr2beta/+) mice from Embryonic day (E) 13.5-17.5 were subjected to in utero cardiac microinjection of tomato lectin (which adheres to endothelial cells) followed by immunostaining and confocal imaging to visualize blood flow through the renal vasculature.

**Results:** By E13.5, normal kidneys had integrated vascular beds and a moderate number of vessels containing blood flow. Several glomeruli and collecting ducts stained for tomato lectin implying glomerular filtration (challenging current thoughts that GFR begins at E15.5). In older embryos, the amount of perfused vessels and lectin-stained glomeruli increased. Co-labeling of the renal stroma (Foxd1) with vascular markers (PECAM) revealed increased. Co-labeling of the renal stroma (Foxd1) with vascular markers (PECAM) revealed increased proliferation, cell shape changes, and complex cellular rearrangements. Classic single cell behaviour, and subcellular protein dynamics in vivo.

**Conclusions:** The Xenopus pronephros is a fully functional excretory organ and consists of a single tubule which is functionally similar to the human metanephric tubule. Positioned right beneath the epidermis, it is perfectly well suited to study renal development in a living organism.

**Funding:** NIDDK Support

**TH-PO443**

**Dynamic Cell Movements in the Morphogenesis of Renal Tubules in Xenopus laevis**

**Soren S. Lenskamp, John B. Wallingford, Gerold Walz.**

**Renal Division, Department of Medicine, University Freiburg Medical Center, Freiburg, Baden Württemberg, Germany; Molecular Cell and Developmental Biology & Institute for Cellular and Molecular Biology, University of Texas, Austin, TX; Howard Hughes Medical Institute, University of Texas, Austin, TX; Center for Biological Signaling Studies (BIOSS), University of Freiburg, Baden-Württemberg, Germany.**

**Background:** Epithelial tubules are universal building blocks of many organs including the vertebrate kidney. The morphogenetic programs that shape epithelial tubules and renal tubules in particular remain poorly defined. It has been suggested that active cell movement must occur. However, the active movement of renal tubular epithelial cells has not been observed in vivo.

**The Xenopus pronephros is a fully functional excretory organ and consists of a single tubule which is functionally similar to the human metanephric tubule. Positioned right beneath the epidermis, it is perfectly well suited to study renal development in a living organism.**

**Methods:** We used confocal time-lapse imaging of Xenopus embryos expressing fluorescent proteins within the embryonic kidney. Automated position and focus adjustment algorithms were used to correct for shifts in embryo position or growth. A high degree of spatial and temporal resolution was achieved to resolve movements of cells within a tissue, single cell behaviour, and subcellular protein dynamics in vivo.

**Results:** We find that tubulogenesis is a highly dynamic process that integrates proliferation, cell shape changes, and complex cellular rearrangements. Classic morphogenetic movements such as convergence-extension and rosette formation occur in the developing tubule. This process depends on myosin II and is disrupted by molecules that interact with planar cell polarity signaling.

**Funding:** Government Support - Non-U.S.

**TH-PO444**

**Modeling Polycystic Kidney Disease Using Human Induced Pluripotent Stem Cells**

**Benjamin S. Freedman, Albert Q. Lam, Joseph V. Bonventre.**

** Renal Division, Department of Medicine, Brigham and Women’s Hospital, Boston, MA.**

**Background:** Induced pluripotent stem (iPS) cells are a powerful new way to model human disease in different cell types and during development.

**Methods:** We generated iPSC lines from patients with autosomal dominant and autosomal recessive polycystic kidney disease (PKD-iPS cells) using retroviral transduction of viral vectors with NODDK Support.

**Conclusions:** Formation of renal tubules requires active cell movement and dynamic rearrangement within the epithelium. Evolutionary conserved mechanisms of morphogenesis are driving forces. Disruption of morphogenetic cell movements leads to severe defects in tubule formation. This has potential implications for the understanding of hereditary renal malformations.

**Funding:** Government Support - Non-U.S.
Results: PKD-iPS cells express pluripotency markers characteristic of embryonic stem cells (ESCs) and when differentiated into first trimester CD44, the cells formed somatic cell lineages which are affected by PKD. Undifferentiated iPS cells possess primary cilia and express polycystin-1 and polycystin-2. Compared to control iPS lines from healthy patients, PKD-iPS lines exhibited normal cilia number and morphology but a dramatic reduction in polycystin-2 localization to the cilium. When iPS cells were embedded in three-dimensional extracellular matrix, they formed cysts of OCT4+; ZO-1+ epithelial cells surrounding empty central lumens lined with apical cilia. Differentiated iPS cells showed diverse patterns of polycystin-2 localization to the endoplasmic reticulum, plasma membrane, and primary cilium. In a co-culture system, differentiated PKD-iPS cells formed spherical embryoid bodies featuring sporadic, fluid-filled cysts which increased in the presence of 8-bromo-cAMP. When implanted underneath the kidney capsule of immunodeficient mice, PKD-iPS cells gave rise to large cystic teratomas.

Conclusions: These results establish new experimental models for human PKD and indicate that epithelial phenotypes of iPS cells can be explored to provide insight into underlying PKD pathophysiology.

Funding: NIDDK Support, Other U.S. Government Support

TH-PO445

Low Serum Cultured Adipose-Derived Mesenchymal Stem Cells, but Not Bone-Marrow Derived Mesenchymal Stem Cells, Ameliorate Rat Crescentic Glomerulonephritis by Functional Polarization of Macrophages into Immunoregulatory Phenotype Kazuhiro Funahashi, Naotake Tsuobi, Hangsoo Kim, Takayuki Katsuno, Takenori Osaki, Waichi Sato, Enyu Imai, Seiji Matsumo, Yuki Notani, Hitomi Hayashi, Haruo Otake, Koichiro Nakamura, Yongli Guo, and Akiyoshi Nogaya, Nagoya University Graduate School of Medicine, Nephrology, Nagoya, Aichi, Japan.

Background: We have reported that adipose tissue-derived stem cells (ASC) predominantly modulate T-cell immune response than bone marrow derived mesenchymal stem cells (BM-MSC). In the present study, we examined the reproducive effects of ASCs focusing on their immunomodulatory properties for macrophages.

Methods: We constructed hAFSC lines from second-trimester amniotic fluid by using a human embryonic stem cell (hESC) protocol. hAFSC derived from second-trimester amniotic fluid were isolated, cultured and differentiated to ASCs with low-serum conditions. ASCs and BM-MSCs were intravenously given every day from day 0 to day 5. For evaluation of ASC-driven functional polarization in macrophages, we cultured peritoneal macrophages and BM-MSCs in an immunomodulatory medium with ASCs. Specific marker for macrophage phenotype (proinflammatory;M1 and immunoregulatory;M2) and IL-10 in culture supernatant were assessed by FACS and ELISA, respectively.

Results: Intravenous injection of ASC significantly prevented rats from renal dysfunction and proteinuria caused by anti-GBM IgG injection. The number of glomeruli with crescents was significantly decreased in ASC group compared to control group. Renal IL-10 concentration was increased only in ASC group. Interestingly, glomerular infiltrates of M2 macrophages was increased only in ASC group compared to control group. Moreover, these effects of ASC were stronger in low-serum cultured ASCs despite comparable number of M1 macrophages to control group.

Conclusions: These preliminary results suggest that intravenous inoculation of hAFSC ameliorate renal disease in the remnant kidney model and that they may represent an alternative source for stem cell therapy.

Funding: Government Support - Non-U.S.

TH-PO447

Induction and Monitoring of Intermediate Mesoderm from Human iPSCs and ESCs Shin-Ichi Mac,1,2 Fumihiko Shitou,1,2 Andrew P. McMahon,2 Kenji Osafune,1 Center for iPS Cell Research and Application (CiRA), Kyoto University; 1Harvard Stem Cell Institute, Harvard University.

Background: The differentiation method from pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), into kidney lineage remains to be developed. Kidney is derived from one of early embryonic germ layers, intermediate mesoderm (IM), and directing pluripotent stem cells into IM lineage is a crucial step for kidney regeneration.

Methods: We constructed a targeting vector and transduced it into human iPSCs (hiPSCs) to obtain reporter hiPSC lines for an IM marker gene OSR1 by homologous recombination. To differentiate IM cells from hiPSCs, we examined the combination treatment of growth factors.

Results: We have efficiently generated hiPSC lines that contain an allele of OSR1 gene into which a green fluorescence protein (GFP) gene was knocked-in by homologous recombination. We have also established an induction protocol using combination treatment of growth factors, in which differentiation of hiPSCs produced cultures consisting of more than 40 % OSR1+ cells. Furthermore, the cells expressed other IM marker genes, and could differentiate into multiple cell types included in IM derivative organs, such as kidney, gonad and adrenal cortex in vitro.

Conclusions: Our differentiation protocol can induce human pluripotent stem cells into IM cells with similar developmental potential to that in embryos, supplying systems for understanding the developmental mechanisms of IM lineage and cell sources for the regeneration of IM derivative organs.

Funding: Government Support - Non-U.S.

TH-PO448

Selective Production of Hyaluronan by Epithelial Cells Is Necessary but Not Sufficient To Induce Tubulogenesis Priscilla Soulie, Alexandra Chassot, Roberto Montesanto, Eric Feraillé, Patrick Saudan. Cell Physiology and Metabolism, University of Geneva, Switzerland.

Background: Branching morphogenesis is a fundamental process in the development of kidney and mammary gland. Extracellular matrix plays an important role in tubulogenesis. We hypothesized that epithelial cells can modulate their pericellular matrix to drive tubulogenesis in response to stimuli.

Methods: The role of hyaluronic acid (HA), a key component of the pericellular matrix, in epithelial tubulogenesis, was studied in three different in vitro models of epithelial tubulogenesis in 3D collagen gels: 1) renal MDCK cells in response to hepatocyte growth factor (HGF); 2) renal mCCD-N21 cells (spontaneous) and 3) mammary gland-derived hiPSCs (hPSCs) to obtain reporter hiPSC lines for an IM marker gene OSR1 by homologous recombination. We constructed a targeting vector and transduced it into human iPSCs (hiPSCs) to obtain reporter hiPSC lines for an IM marker gene OSR1 by homologous recombination. We have established an induction protocol using combination treatment of growth factors, in which differentiation of hiPSCs produced cultures consisting of more than 40 % OSR1+ cells. Furthermore, the cells expressed other IM marker genes, and could differentiate into multiple cell types included in IM derivative organs, such as kidney, gonad and adrenal cortex in vitro.

Conclusions: Our differentiation protocol can induce human pluripotent stem cells into IM cells with similar developmental potential to that in embryos, supplying systems for understanding the developmental mechanisms of IM lineage and cell sources for the regeneration of IM derivative organs.

Funding: Government Support - Non-U.S.
TH-PO449
Ectopic Ureteral Budding from the Wolffian Duct Results in Hypoplastic Kidneys but Not in Dysplastic Kidney
Masaru Motomiya,1 Fumio Komaki,1 Yoichi Miyazaki,2 Fumio Nimura,1 Taiji Matsusaka,1 Ikuemi Ichikawa,1,3 1Tokai University School of Medicine, Kanagawa, Japan; 2Jikei University School of Medicine, Tokyo, Japan; 3Vanderbilt University School of Medicine, Nashville, TN.

Background: Mice carrying a null mutation of the Foxc1 gene frequently develop a double collecting system. Since the kidney and urinary tract derived from normal budding can serve as a control within the same tissue specimen, these mice provide an ideal opportunity to ascertain the specific role of ectopic budding in the development of the kidney and ureter anomalies.

Methods: The Foxc1−/− mutants were collected from Foxc1+/− heterozygous matings at various ages. The upper and lower pole kidneys were weighed and measured and the kidneys were fixed in formalin and paraffin. Histological and immunohistochemistry examinations were performed according to the protocols of our institution.

Results: The upper pole kidneys of newborn Foxc1−/− mice were significantly more hypoplastic (kidney volume, 59±8% vs. lower pole, p<0.01). In those mice, the ureter, calyx and tubules in the medulla of the upper pole were abnormally dilated compared with the lower moieties. In utero, at E14.5, the stage just before formation of the first urine, the upper kidney was already smaller (43±6% vs. lower kidney p<0.01). At E12.5, the number of condensed mesenchymes assessed by Pax2 ISH was significantly lower in the lower poles (59±8%, p<0.01). Neither morphological examination by Hematoxylin and Eosin staining nor immunostaining for neupalin, megalin, aquaporin-1 and Tamm-Horsfall protein revealed any dysplastic regions in either pole of newborn Foxc1−/− mouse kidneys. Of note, at birth, expression of Foxc1 was restricted to maturing podocytes.

Conclusions: Ectopic budding leads to kidney hypoplasia. However, ectopic budding alone does not result in kidney dysplasia unless gene(s) involved in the development of any intermediate structures of the nephron is affected, or ectopic budding occurs at a site substantially distant from the normal budding so that adjacent mesenchymal cells are not fully committed to develop into renal parenchyma.

Funding: Government Support - Non-U.S.

TH-PO450
Can Stem Cells Prevent Progression of Kidney Disease? Alexandra Rak-Raszewska,1 Bettina Wilm,1 Simon Kenny,2 David Edgar,1 Patricia Murray,1 1Cellular and Molecular biology, University of Liverpool, United Kingdom; 2Department of Pediatric Surgery and Urology, Alder Hey Children’s NHS Trust, Liverpool, United Kingdom.

Background: Kidney disease can either be acute or chronic, the latter progressively worsening over time to become end stage renal disease (ESRD) - a stage when kidneys are non-functional. At present, the only treatment options for ESRD are transplantation or dialysis, which both have severe drawbacks. The incidence of ESRD is rising annually, therefore, in order to regenerate kidney tissue or prevent worsening of the kidney condition, a new therapy should be developed. One approach is to use embryonic stem cells (ESC). Therefore, we have first investigated the ability of ESC and their mesodermal derivatives to integrate into mouse embryonic kidney rudiments in an ex vivo assay. This study showed that differentiation of ESC into mesoderm promotes integration into ureteric bud, developing glomeruli and forming proximal tubules. Building on this ex vivo study, we have set up an in vivo model to induce acute and chronic kidney disease that can progress to chronic disease. This allows us to investigate the ability of ESC and their mesodermal derivatives to prevent the development of acute kidney disease in the short term or prevent progression to chronic disease in the longer term.

Methods: We have differentiated ESC in vitro into mesodermal cells. Following differentiation these cells were mixed with freshly dissected E13.5 mouse embryonic kidney rudiments and their nephrogenic potential was investigated in the ex vivo assay. For the adriamycin nephropathy in vivo model the ESC and their mesodermal derivatives were injected into injured mice. The analysis involved immunohistochemistry, biochemical and molecular analysis.

Results: The ex vivo data showed that ESC differentiated into mesoderm integrated into kidney structures more efficiently than undifferentiated ESC. They integrated into glomeruli and proximal tubular-like structures, and moreover they functioned appropriately to their location, e.g they differentiated into proximal tubular cells with functioning organic anion transporters. The in vivo experiments are still ongoing.

TH-PO451
Controlled Tubulogenesis of the Ureteric Bud from Dispersed Ureteric Epithelial Cells Using a Micropatterned Gel Peter V. Hauser,1,2 Masaki Nishikawa,1 Hiroshi Kimuro,1 Norimoto Yanagawa,1,2 1Renal Renal Laboratory, VLAGLAH at Sepulveda, North Hills, CA; 2David Geffen School of Medicine, UCLA, Los Angeles, CA; 3Institute of Industrial Science, University of Tokyo, Japan.

Background: The initial step in renal development is the interaction of the ureteric bud (UB), an outgrowth of the Wolffian duct, with the metanephric mesenchyme. Our previous work on generating a tubular structure from dispersed renal progenitor cells in vitro using a micropatterned agarose gel to control the growth and define the geometry of the structure.

Methods: A micropatterned agarose gel (3%) was casted from a silicone mask, in which the pattern of the aperture was etched by photolithography. The dispersed cells were cultured on the agarose gel, produced from the mask, contained rectangular cavities (3mm x 150μm x 150μm), into which dispersed mouse ureteric bud cells (CMUB-1, Probes) suspected in collagen I (2.5%) were seeded (5.10⁶ cells/mL) by centrifugation (120rpm, 10min). The gel holding the cells was subsequently cultured in DMEM (10%FCS+3% P/S) at 37°C, 5% CO₂.

Results: After 24h in vitro culture, the embedded dispersed ureteric bud cells formed single layered tubular structures that contained a lumen. The tubular structures conformed to the shape and size of the cavities in the gel. Tubular formation was followed by detachment from the agarose gel. The terminal ends of the tubular structure were multibranched and no tubules were found in the agarose. Laminin staining revealed a closed surface of the tubular structures. Aldosteron (100mM) positively effected tubular formation, while the addition of the factors HGF and EGF, or the matrixprotein fibronectin had no effect.

Conclusions: We conclude that micropatterned gels can be used to control the growth of geometrically defined substructures of the developing kidney from dispersed cells. These structures can be used as building elements for tissue engineering purposes in order to study renal development in vitro.

Ongoing studies are underway to examine the expression of ureteric bud markers (α1-Int, War1, War2, Sox9) along the tubules and the effects of growth factors, such as GDNF on directional growth and branching of the generated tubules.

TH-PO452
Isolation of a Human Nephron Progenitor Population Sally Matsuyma,1,3 Orit Harari-Steinberg,1,3 Dorit Omer,1,3,5 Benjamin Dekel1,2,3, 1Pediatric Stem Cell Research Institute, Sheba Medical Center, Tel Hashomer, Israel; 2Division of Pediatric Nephrology, Safra Children’s Hospital, Sheba Medical Center, Tel Hashomer, Israel; 3Sheba Center for Regenerative Medicine, Sheba Medical Center, Tel Hashomer, Israel; 4Mina & Everard Goodman Faculty of Life Sciences, Bar-Ilan University, Ramat Gan, Israel; 5Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel.

Background: A critical step in developing cell based therapies for renal disease is defining a suitable stem/progenitor cell population. Taking into account the fact that low derived and blood stem cells lack intrinsic capacities to generate nephron cell types identification of tissue-specific renal stem cell with wide nephrogenic potential has been a long sought after goal. In the mammalian kidney, fresh stem cells are induced into the nephrogenic pathway to form nephrons during development and no equivalent cell types can be traced in the adult kidney. Nevertheless, prospective isolation of such human nephron stem/progenitor cells has yet been accomplished.

Methods: Here we describe the generation of single cell suspensions from mid-gestation human fetal kidneys.

Results: Clonogenic analysis, expansion in serum-free defined conditions and cell sorting with the neural cadherin adhesion molecule 1 (NCAM1) identified a population that is highly clonogenic and specifically enriches for human transcripts of nephron stage cells. After transplantation onto the chorio-allantoic membrane of the chick embryo these cells but not differentiated counterparts efficiently formed nephron's proximal, loop of henle and distal tubules. Finally, a single injection of nephron progenitor cells prevented death and renal failure in the SCI – glycine induced acute kidney injury model.

Conclusions: These cells represent a previously unidentified intrinsic nephron precursor population and are a promising candidate for cell-based therapeutic strategies.

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TH-PO453
Fibroblast Growth Factor Receptor Signaling Is Critical for Nephron Differentiation Sunder Sims-Lucas,1 Caitlin M. Schaefer,1 Seppo J. Vainio,2 Carlton M. Bates,1 1Pediatrics, Children’s Hospital of Pittsburgh, Pittsburgh, PA; 2Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland.

Background: Reduced nephron number is one of the leading causes of renal failure in children and is linked with many adult onset diseases. Nephron development requires renal signaling between the ureteric bud and the metanephric mesenchyme (MM). Previously in mice targeted deletion of fibroblast growth factor 8 (Flg8) in the MM produced renal vesicles (RV) that failed to become mature nephrons. Our laboratory has shown that Pax3cre mediated inactivation of fibroblast growth factor receptor (Fgfr) 1 and 2 in the MM (both cortical stromal and nephrogenic lineages) leads to severe renal dysgenesis. Furthermore, Pax3cre-driven deletion of Fgfr1 and abrogation of the fibroblast growth factor receptor substrate 2α (Frs2α) binding site of Fgfr2 (Fgfr1+/−Fgfr2−/−) led to cystic kidney disease. The purpose of this study was to determine the role of Fgfr signaling in the nephrogenic lineage using a RV specific cre line.

Methods: Similar to the Pax3cre studies, we used Wnt4cre mice to delete Fgfr1 and 2 in the nephrogenic (RV) lineage (Fgfr1+/−Fgfr2−/−) and to delete Fgfr1 in the RV along with abrogation of the Frs2α binding site of Fgfr2 (Fgfr1+/−Fgfr2−/−Frs2α−/−). Kidneys were harvested from E13.5 to E18.5 and histology and marker analysis performed.

Results: Fgfr1+/−Fgfr2−/−Frs2α−/− mice had phenotypically normal kidneys at all ages examined. However, E13.5 Fgfr1+/−Fgfr2−/− mice had defective nephron formation with areas around many ureteric tips devoid of nephron precursors, leading to fewer total nephrons. Similar to the E13.5 Pax3cre deletions, E13.5 E18.5 Fgfr1+/− showed no abnormalities in the nephron distribution toward less mature structures than controls. By E18.5 Fgfr1+/− kidneys were much smaller than controls although they did possess some mature nephrons.
Conclusions: This study reveals that Fgfr signaling through Frs2a in RV is not required for nephrogenesis (and does not mediate the Fgfr2-mediated renal cystigenic phenotype). However, Fgfr signaling independent of Frs2a (or requiring multiple adapters) is critical in nephron differentiation and determination of final nephron number.

Funding: NIDDK Support

TH-PO454

Understanding the Mechanisms Downstream of Wnt7b Action in Renal Medulla Formation

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Background: Wnt7b has been shown to mediate renal medulla formation, through canonical signaling to the medullary interstitium. p57kip2, a cyclin-dependent kinase inhibitor expressed in both the medullary interstitium and in podocytes, has also been shown to mediate a renal medulla defect when ablated in mice, and is probably associated with Beckwith-Wiedemann syndrome including renal medulla anomaly when mutated. However, how p57kip2 regulates renal medulla formation, and whether and how it is regulated by Wnt7b signaling is unexplored.

Methods: We employed a combination of Luciferase assay in cell culture, Chromatin Immunoprecipitation (ChIP), immunohistochemistry, and mouse genetics to address these questions.

Results: We showed that the p57kip2 expressing interstitial cells are canonical Wnt signaling responsive cells. A genomic region of p57kip2 bearing putative Tcf18 binding sites responds to canonical Wnt signals in vitro. Furthermore, interstitial cell expression of p57kip2 is necessary for normal renal medulla formation.

Conclusions: Our data suggest that p57kip2 is a direct target of Wnt7b/canonical Wnt signaling and p57kip2 expressed in the renal interstitium plays a critical role in renal medulla formation. A genomic region of p57kip2 bearing putative Tcf18 binding sites responds to canonical Wnt signals in vitro. Furthermore, interstitial cell expression of p57kip2 is necessary for normal renal medulla formation.

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TH-PO455

p53 in the Cap Mesenchyme Regulates Nephron Endowment

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Background: The factors which determine nephron endowment or mass are not completely understood, and reduced nephron number is associated with susceptibility to hypertension and progressive renal disease. We recently reported that germ-line deletion of p57kip2 is a direct regulator of canonical Wnt signaling and that p57kip2 deficiency has been shown to cause a renal medulla defect when ablated in mice, and is probably associated with Beckwith-Wiedemann syndrome including renal medulla anomaly when mutated. However, how p57kip2 regulates renal medulla formation, and whether and how it is regulated by Wnt7b signaling is unexplored.

Methods: We employed a combination of Luciferase assay in cell culture, Chromatin Immunoprecipitation (ChIP), immunohistochemistry, and mouse genetics to address these questions.

Results: We showed that the p57kip2 expressing interstitial cells are canonical Wnt signaling responsive cells. A genomic region of p57kip2 bearing putative Tcf18 binding sites responds to canonical Wnt signals in vitro. Furthermore, interstitial cell expression of p57kip2 is necessary for normal renal medulla formation.

Conclusions: Our data suggest that p57kip2 is a direct target of Wnt7b/canonical Wnt signaling and p57kip2 expressed in the renal interstitium plays a critical role in renal medulla formation. A genomic region of p57kip2 bearing putative Tcf18 binding sites responds to canonical Wnt signals in vitro. Furthermore, interstitial cell expression of p57kip2 is necessary for normal renal medulla formation.

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TH-PO456

Robo1 and Robo2 Play a Role in Murine Kidney Branching Morphogenesis and Podocyte Foot Process Architecture

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Background: Robo1 is a homolog of Robo2, both of which are transmembrane proteins involved in the control of axonal guidance and cell migration. Robo2 is required for normal kidney induction and restricts the ureteric bud to a single site from the nephric duct. The most striking phenotype in homoygous Robo2 knockout mice is multiple ureters and duplex kidney. Our recent studies show that Robo2 is also required for normal podocyte structure and function. It is not known, however, if Robo2 also plays a role in kidney development and acts synergistically with Robo2.

Methods: Immunohistochemistry; knockout mice analysis; real time RT-PCR; embryonic kidney organ culture; scanning electron microscopy.

Results: By immunohistochemistry with a specific Robo1 polyclonal antibody, we found that Robo1 was specifically expressed in mouse embryonic kidney, and is significantly downregulated after birth. Robo1 protein is also expressed in mouse developing glomeruli and its mRNA and protein expression are up-regulated in the glomeruli of Robo2 knockout homozygous newborn kidney.

To investigate the compound effect of Robo1 and Robo2 on kidney development, we analyzed Robo1 -/-Robo2 -/- double knockout mice. Compared with Robo2 single homozygotes, all Robo1 -/-Robo2 -/- double homozygous mice died within hours after birth. Interestingly, embryonic kidney organ culture and newborn histology analysis revealed that double homozygous mice manifested a severe branching morphogenesis phenotype and reduced glomerular number compared with Robo2 -/- single homozygotes. The few glomeruli present at birth in a Robo1 -/-Robo2 -/- double homozygote displayed abnormal podocyte foot process architecture on scanning electron microscopy.

Conclusions: These results indicate that Robo1 functions synergistically with Robo2 during kidney branching morphogenesis and podocyte foot process patterning.

Funding: NIDDK Support, Private Foundation Support

TH-PO457

Compensatory Renal Growth after Unilateral/Subtotal Nephrectomy in the Oxine Fetus

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Background: In adult, unilateral and subtotal nephrectomy result in compensatory renal growth but do not involve formation of new nephrons. During the period of active nephrogenesis in utero, it is not clear whether compensatory growth can occur and if so, whether more nephrons can be formed.

Methods: Oxine fetuses (n = 18) underwent unilateral nephrectomy (1/2Nx, n = 5), subtotal nephrectomy (5/6Nx, n = 5) or sham nephrectomy (Sham, n = 8) at 70 days of gestation. After 134 days, renal functional studies were performed, fetuses were euthanized and kidneys further analyzed.

Results: In 1/2Nx, kidney weight to body weight ratio was greater (5.13 ± 0.99 g/kg vs 2.48 ± 0.20 g/kg in Sham; P = 0.05); the nephron number was similar (2658 ± 518 nephrons vs 2467 ± 367 respectively). In 5/6Nx, both catch up in kidney weight (4.62 ± 0.89 g/kg vs 2.48 ± 0.20 g/kg) and nephron number (2560 ± 694 nephrons vs 2467 ± 367) was observed in spite of initial severe 2/3 parenchymal reduction. At incision sites, parenchyma budding was observed, leading to a butterfly-like shape. In all groups, in utero glomerular filtration rate was similar. At the molecular level, caspase gene expression was significantly decreased (<0.01) in 5/6Nx. No differences in expression of Bcl2, Pax2, VEGF or eRtE were observed among the different groups.

Funding: Government Support - Non-U.S.
Kidney organ culture conditions, 2) optimal SFK activity is necessary for UB branching, decreased proteinuria and BP levels associated with high proliferative activity, podocyte cell have been utilized to generate various lineage cells that Activin stimulate the expression of kidney specific protein (KSP) that is exclusively fetal kidneys exhibited morphologically abnormal UB tip regions suggesting a disruption observed on day 1 of treatment (p=0.002). Beginning after the first day of PP1 culturing, In contrast, UB segment lengthening was not decreased by PP1 treatment. In fact, PP1 approximately 60% inhibition and 2) an inhibition of lateral branching (p=2.9 x10^-5).

TH-PO459
Src Family Kinase Activity Promotes Ureteric Bud Branching While Limiting Ureteric Bud Segment Elongation during Mouse Fetal Kidney Development
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Methods: Using a mouse ureteric kidney organ culture system, we investigated the role of SFK activity in the regulation of fetal renal development. Fetal CD-1 mouse kidneys, pairs, isolated from gestation day 12 embryos, were separated and cultured on permeable membrane filters in DMEM/Ham’s F12 medium containing 5% fetal bovine serum plus insulin/transferrin/selenium in either the absence or presence of 10 µM PP1, a well-characterized SFK inhibitor. Kidneys were imaged daily for three days, beginning from explantation, and parameters of ureteric bud (UB) branching morphogenesis were quantitated.

Results: Kidneys maintained in the presence of PP1 exhibited differences from their control counterparts in several aspects of UB branching. After two days in culture, PP1 produced: 1) a progressive inhibition of UB branch bifurcations (p=5.8 x10^-17) reaching approximately 60% inhibition and 2) an inhibition of lateral branching (p=2.9 x10^-5). In contrast, UB segment lengthening was not decreased by PP1 treatment. In fact, PP1 produced an increase in the UB primary branch segment length, with the greatest effect being observed on day 1 of treatment (p=0.002). Beginning after the first day of PP1 culturing, fetal kidneys exhibited morphologically abnormal UB tip regions suggesting a disruption of the normal ureteric bud metanephric mesenchyme interaction.

Conclusions: Our data indicate that: 1) SFKs are activated under standard fetal mouse kidney organ culture conditions, 2) optimal SFK activity is necessary for UB branching, and 3) SFK functional levels may impede UB segment elongation.

TH-PO460
Purification of Differentiated Tubule Cells from Mouse Embryonic Stem Cells Using Flow Cytometry
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Background: Although embryonic stem (ES) cell and induced pluripotent stem (iPS) cell have been utilized to generate various lineage cells in vitro, the induction of renal lineage cells from ES and iPS cells have remained to be elucidated. Recently, we found that Activin stimulate the expression of kidney specific protein (KSP) that is exclusively expressed in renal tubule epithelial cells and that it enhances the differentiation of ES and iPS cells into tubule cells.

Methods: In this study, we produced a monoclonal antibody to purify tubule cells differentiated from ES cells by flow cytometry, and we have established a method to generate tubule cells from ES cells in vitro by using Activin and flow cytometry with the monoclonal antibody. We designed the monoclonal antibody so that it binds to the extracellular domain of a protein called X which was exclusively expressed in renal epithelial cells, that is, ureteric buds, Bowman’s capsules, proximal / distal tubules and collecting ducts. We confirmed the specificity of anti-X antibody by flow cytometry, PCR and Western blot using ureteric bud cell line.

Results: We sorted X-positive cells from differentiated ES cells by flow cytometry using anti-X antibody after the induction with Activin. X-positive cells comprised about 1.5% of total differentiated ES cells, and were expressing mRNA of X much more than X-negative cells. X-positive cells also expressed aquaporin 2 (AQP2), aquaporin 3 (AQP3), K2D and E-cadherin, suggesting X-positive cells were similar to tubule cells in gene expression. To observe the formation of tubular structure in vitro, we transferred X-positive cells into non-adhesion dish after sorting by flow cytometry, and we found cell aggregates. After 24 hours of formation of cell aggregates, we embedded them in Matrigel ® and cultured with epithelial growth factor (EGF). The cells expanded outward, and formed a ring structure that resembled tubular structure.

Conclusions: In conclusion, we could induce tubule cells from mouse ES cells with Activin stimulation and cell selection using anti-X antibody. Currently, we proceed to characterizing X-positive cells induced by this method.

TH-PO461
Drugs Frequently Used in Premature Neonates Reduce Nephrogenesis in Embryonic Kidney Culture

Background: Nephrogenesis ceases around the 36th week of gestation in man, with no additional nephron formation later in life. A lower number of nephrons leads to glomerular hyperfiltration, hypertension and renal impairment in the long run. Many preterm born children are treated with drugs that may reduce nephron formation. We investigate these drugs for effects on kidney growth and ureteric branching.

Methods: At embryonic day 13 embryo’s were delivered from time pregnant mice through Caesarean section and kidneys were dissected. Subsequently embryonic kidneys were cultured on filters in control media. Three different concentrations (including clinical dose) of gentamicin and ceftazidime were added to the media. Starting kidney size was assessed by surface area measurements and kidneys were whole mount stained with auto-collibind D-28K after 24 hours of culture. Visualization was performed by means of confocal microscopy and ureteric bud branching was evaluated by counting. Additionally, qPCR course experiments were performed with hydrolysis probes to investigate gene expression levels of Wt1, Sox9, Bmp7, Fgf8 and Gdnf. The genes Acbh and Hmbs were used for normalization.

Results: Ureteric bud count indicates that 300 µM (high-dose) of gentamicin and 200 or 2000µM (mid- and high-dose, respectively) of ceftazidime impair ureteric bud development. Additional qPCR analysis revealed a 2.5 fold down regulation in Fgf8 and a tendency to downregulation in Gdnf for high-dose ceftazidime. No clear changes in gene expression were observed for gentamicin due to lack of a dose response relationship. These results will be verified in additional experiments and at later time-points.

Conclusions: 300µM of gentamicin and 200 or 2000µM of ceftazidime impair ureteric bud formation already after 24 hours of treatment. Additionally, we suggest a role for Fgf8 and Gdnf in the mechanism behind ceiladizime induced bud impairment.

Funding: Private Foundation Support
Role of Calcium Oscillations in Metanephric Mesenchyme Cells for Nephron Development

**Background:** Emerging evidence suggests that many of the processes governing the reciprocal interactions between the metanephric mesenchyme (MM) and ureteric bud (UB) are calcium dependent. Yet little is known about the origin of calcium signals in the metanephric development.

**Methods:** We have used laser scanning confocal microscopy to record calcium activity in explanted kidney rudiments derived from E14.0 rat embryo and cultured for 48 hours. Kidneys were loaded with the calcium sensitive dye “Oregon Green BAPTA-1”, placed in a closed chamber at 35°C and slowly perfused with Krebs-Ringer solution. Calcium was recorded in MM cells bordering UB. The intensity of calcium traces was normalized and background was subtracted.

**Results:** During the recording time of 30 min, spontaneous calcium oscillations, here defined as at least four calcium peaks exceeding more than 10% of baseline, were observed in 34.0 ± 5.8% of cells (mean ± std.dev.). The calcium oscillations were completely dependent on release of calcium from the intracellular stores and, to a lesser degree, on release via the extracellular space.

To examine the functional role of the spontaneous calcium oscillations, the intracellular calcium stores of the kidney rudiments were depleted by partial inhibition of the SERCA pump with cyclopiazonic acid (CPA) during the 2nd day in culture. In kidney rudiments exposed to 5 µM CPA, the relative number of cells with calcium oscillations was reduced from 35% to 7%. The UB tips were abnormally shaped and appeared swollen and there was a significant, 27%, decrease in UB branching and formation of glomeruli in CPA treated kidney rudiments compared to non-treated kidney rudiments. GDNF secreted from the MM and acting on UB Ret-receptors, is a key feature of MM and UB interaction. GDNF secretion, which is known to be a calcium dependent process, was measured in conditioned media. Twenty four hours of CPA treatment caused a robust decrease of GDNF secretion.

**Conclusions:** We conclude that spontaneous intracellular calcium oscillations in MM cells, generated by calcium release from intracellular stores, are required for normal GDNF secretion. This highlights the importance of calcium-dependent signaling in the reciprocal interactions between MM and UB, which are essential for the development of nephrons and glomeruli.

**Funding:** This work was supported by the Swedish Research Council and the Swedish Heart-Lung Foundation.

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**TH-PO466**

Early B Cell Factor 1 (Ebf1): A Novel Regulator of Renal Cortex Maturation and Glomerular Function

**Background:** The transition from fetal to newborn kidney is associated with several developmental changes that are involved in the maturation of both renal structure and function. Disruptions to the genetic regulation this process can be the contributing source for reduced renal function in children, including low nephron numbers and cystic kidney disease. MicroRNAs (miRNAs) act to repress the expression of its target genes. Conventional screening of miRNA expression by pre-generated microarrays are typically limited by its older miRNA library source, lacking the evaluation of a growing number of novel miRNAs that would be detected by next-generation sequencing. The potential role of miRNAs in controlling the developmental processes that govern kidney development at birth have not been studied to date.

**Methods:** To evaluate this process, kidneys were extracted from rats at gestational days 21 and 22, birth, and 1 day of age. A cDNA library of small RNAs was generated from each timepoint and sequenced on the Illumina GA2. Raw data was aligned to the rat genome (rn4), and then manually parsed for the mature sequences of all 408 miRNAs from each timepoint.

**Results:** From birth to postnatal day 3 (P3), the glomerular number remains preserved in rodents undergoing reduction in renal mass in the neonatal period. Our recent studies in developing mice reveal a dynamic change in glomerular number which peaks at P7, then decreases and stabilizes after P18. The present study examines the growth pattern of the developing glomeruli following injury of relatively mature glomeruli and the potential modulating effects of podocyte-derived vascular endothelial growth factor-A (VEGF) to promote compensatory recovery of glomerular number.

**Conclusions:** Glomerular injury was induced in relatively mature glomeruli within the deep cortex of mice carrying Nphs1-hcD25 transgene (NPEP25) by a single i.p. injection of LMB2 at P4. Enhanced expression of podocyte VEGF was achieved by administration of doxycycline to Nphs2-rtTA/tetO-VEGF (rtTA-VEGF) double transgenic mice starting from birth (P0).

**Funding:** NIHDKK Support, Private Foundation Support
Conclusions: The presented results provide a first look at miRNAs that may be involved in the regulation of gene expression governing the developmental processes of the kidney at birth transition.

Funding: Government Support - Non-U.S.

TH-PO467

Using the NCAM1 Marker for Prospective Isolation of Highly Clonogenic Human Adult Kidney Epithelial Cells with Unequivocal Tubular Regeneration Capacities

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Background: There is an ongoing debate on existence of genuine epithelial progenitors in the adult kidney. Recent studies have re-asserted that dedifferentiation of injured cells and not activation of an intra-tubular progenitors is taking place in regenerating kidneys. NCAM1 expressed only during nephrogenesis, no expression exists in adulthood. Re-expression of NCAM1 has been observed following ischemic injury in S3 proximal tubule cells.

Methods: In this report, surgical human kidney-tissue specimens were studied to prospectively isolate NCAM1+ cells, define their phenotype and functional properties in vitro and in vivo.

Results: No in situ expression of NCAM1 was detected in the human nephron compartment. Nevertheless, human kidney epithelial cell (hKEpC) cultures, uniform for the CD24 and CD133 markers, up-regulated NCAM1 expression in 10-15% of the cell sorting of hKEpCs according to NCAM1, selected a cell subpopulation that overcame the nephron progenitor (S62/Sall/Pax2/8h1) and proximal tubule markers, as well as elevated Vimentin and reduced E-cadherin. In vitro functional assays revealed NCAM1+ cells to be highly clonogenic, slow-cycling and exclusively forming well-defined ‘nephroblasts’. In vitro depletion of NCAM1+ cells from hKEpCs cultures resulted in loss of cell polarity and decreased nephrosephere formation. In vivo, NCAM1+ cells grafted to the chorioallantoic membrane of the chick embryo generated renal tubules, predominantly of proximal and to a lesser extent of distal origin, while NCAM1- cells, mesenchymal stem cells or HEK293, failed to do so.

Conclusions: Thus, we have isolated a clonogenic, sphere-forming human adult kidney cell population that possesses enhanced ability to generate renal tubules. These findings may ultimately pave the way for autologous cell therapy for renal diseases.

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TH-PO468

CKD Impairs Functionality of Mesenchymal Stem Cells (MSC) in Rats

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Funding: Germany Support - Non-U.S.

TH-PO469

The Embryonic Environment of the Metanephric Kidney Promotes p53 Activation Via Post-Translational Modifications and Mdm2 Cleavage

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Background: In addition to its classical role as a guardian of the genome, p53 functions as a developmental regulator. In the embryonic mouse kidney, p53 prevents ectopic budding from the nephric duct by antagonizing GDNF-Ret signaling. Moreover, p53 is transiently induced by expression of a subset of renal function genes (RFG1) via direct binding to the target promoters. Cellular p53 levels are normally kept low via proteosomal degradation mediated by interactions with the E3 ubiquitin ligase, Mdm2. In response to cellular stress (e.g., hypoxia, DNA damage) p53 is modified post-translationally, leading to Mdm2 degradation and the release of p53 from Mdm2, which acts to stabilize p53. The mechanisms mediating p53 stabilization and activation in the embryonic kidney are unknown.

Results: Using specific antibodies to total and phosphorylated or acetylated p53, we demonstrated that, compared to postnatal and adult kidneys, embryonic and neonatal p53 is hyper-phosphorylated on serines 6, 9, 15, 20 and 392. Embryonic kidney p53 is also hyper-acetylated on lysines 373, 382, and 386, hence exhibits enhanced DNA binding affinity. Site-directed mutagenesis of critical serines and lysines of p53 demonstrated that these modifications exert differential effects on p53 stability and transcriptional activity.

In vivo, p53levels are enriched in differentiating proximal tubules, whereas p53levels are expressed in the metanephric mesenchyme, nephron progenitors and collecting duct cells. Co-immunoprecipitation-Western blotting revealed that embryonic p53 exists mostly in a mono ubiquitinated form, whereas, in postnatal and adult kidney, p53 is poly-ubiquitinated. This finding correlates well with the temporal switch from cleaved to intact forms of Mdm2 and dramatic reductions in p53 levels.

Conclusions: We conclude that the embryonic environment mimics the stress response pathway contributing to enhanced DNA binding and DNA damage response. Furthermore, the developmental switch in m2d2 protein species likely plays an important role in p53 degradation following the end of nephrogenesis.

Funding: NIDDK Support

TH-PO470

Gene-Environment Interactions Cooperate To Repress the Developmental Regulator, Pax2, in Renal Dysgenesis

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Background: Gene-environment interactions play an important role in the pathogenesis of human congenital disorders. Bradykinin B2 receptor-null (Bdrk2-/-) mice have normal kidney development; however, the Bdrk2-/- progeny develop renal dysgenesis following gestational salt stress via the maternal diet. The transcription factor, Pax2, is significantly downregulated in Bdrk2-/- kidneys compared to wild-type pups. Humans with Pax2 mutations have renal dysgenesis. We tested the hypothesis that embryonic stress salt contacts with Bdrk2 inactivation to repress Pax2 gene transcription.

Results: Bdrk2-/- mice were crossed to BAC-GFP transgenic mice carrying 30 kb of Pax2 upstream elements. GFP protein and mRNA, surrogates of in vivo Pax2 transcriptional activity, from salt-stressed Bdrk2-/- Pax2-GFP and wild-type embryonic kidneys were analyzed quantitatively by fluorescent microscopy and real-time PCR, respectively. GFP pixel area/intensity and mRNA levels were lower in E12.5 and E14.5 Bdrk2-/- than Bdrk2+/+ kidneys (p<0.05). However, when E12.5 Bdrk2-/- kidneys were grown ex vivo for 2 days to remove the embryonic stressor, GFP expression, the number of ureteric bud branching points, p53 protein and K-cadherin expressing renal vesicles normalized. Microarray analysis in E14.5 salt-stressed Bdrk2-/- and Bdrk2+/+ kidneys revealed significant changes in 1% of probe sets. Upregulated genes (n=202) were linked to extracellular matrix synthesis and muscle development, whereas downregulated genes (n=164) belonged to transcription factors, cell cycle and fate and regulators of nucleic acid metabolism. Importantly, 28 upregulated and 13 downregulated genes have putative Pax2-responsive elements in their promoter regions. Moreover, 8 of these genes have mouse mutant phenotypes consistent with a major role in muscle, kidney, lymphatic and cardiovascular development.

Conclusions: We conclude that Pax2 transcriptional activity is responsive to gene-environment interactions in vivo, representing a novel mechanism of renal dysgenesis.

Funding: NIDDK Support

TH-PO471

Metanephros Transplantation Contributes to Maintaining Blood Pressure in Diltiazem Treated Anephric Rats

Shinya Yokoko,12 Takashi Yokoko,12 Kei Matsumoto,12 Yasunori Usutomiya,1 Tetsuya Kawamura,1 Tatsuo Hosoya,1 1Division of Nephrology and Hypertension, Department of Internal Medicine, Jikei University School of Medicine, Nishi-Shinbashri, Minato-ku, Tokyo, Japan; 2Department of Pediatric Nephrology, Faculty of Medicine, Jikei University School of Medicine, Faculty of Medicine, Jikei University School of Medicine, Nishi-Shinbashri, Minato-ku, Tokyo, Japan.

Background: The kidney is an important organ in maintaining blood pressure. We have previously reported that transplanted metanephros in mice can reproduce some kidney function. The aim of the present study was to determine the metabolic function of transplanted metanephros with particular reference to maintaining blood pressure during renal failure.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

220A
Methods: Ten-week-old male Wistar rats were transplanted with metanephroi in the paracolic gutters of rats in the normal kidney and epithymic (OE group; n=8) followed by bilateral nephrectomy. For comparison, we performed bilateral nephrectomies without transplantation on fourteen rats (non-transplanted group; n=9 and nephrectomy control group; n=5). Nephrectomies were performed on the rats in the transplanted and non-transplanted groups two weeks after transplantation. Rats in the control group had sham operations performed. Hypotension was induced by intravenous infusion of diltiazem hydrochloride at a dose of 0.025mg/kg/min for 2 hours and at a dose of 0.005mg/kg/min for further 2 hours using a syringe pump. Mean arterial blood pressure (MAP) was measured throughout the experiment. Plasma renin activity (PRA) was analyzed every hour. Renin expression from the transplanted metanephroi was evaluated by RT-PCR and immunohistochemically at the time of sacrifice.

Results: RT-PCR showed that metanephroi in the transplanted group expressed renin mRNA. Metanephros transplantation significantly raised PRA and maintained MAP compared with the non-transplanted, anephric group. No significant differences between the transplanted and control groups were found with respect to PRA and MAP.

Conclusions: The present study has shown that transplantation of metanephros produces PRA and contributes to maintaining MAP in a rat model of hypertension.

Funding: Government Support - Non-U.S.

TH-PO472

Branching Tubulogenesis of Renal MDCK Cells Requires HIF-1α and Its Target Gene FSP1 – Implications for a (Patho)Physiological Role in the Human Kidney
Bioreem Buchholz, Sven Kroening, Gunnar Schley, Tina Gimm, Wanja M. Bernhardt, Kai-Uwe Eckardt. Department of Nephrology and Hypertension, University of Erlangen-Nuremberg, Erlangen, Germany.

Background: During nephrogenesis, outgrowth and branching of the ureteric bud is essential to generate the definitive renal collecting system and to determine the number of nephrons. During nephrogenesis, the hypoxia-inducible transcription factor HIF-1α is expressed in medullary and cortical collecting ducts of various species. In addition, HIF-1α is utilized in lesions of ischemic and poly cystic kidneys. Therefore, we wondered if HIF-1α might play a role in tubulogenesis and tubular repair and which target genes would be involved.

Methods: We studied in vitro branching tubulogenesis of Madin-Darby Canine Kidney (MDCK) cells, which originate from the collecting duct, according to their protein expression of HIF-1α. Therefore, we stabilized HIF-1α by hypoxia or the hypoxia mimicking DMOG or inhibited HIF-1α with 17 AAG or chetomin. We also established two MDCK cell clones with a stable knockdown of HIF-1α and performed RT-PCR to test for relevant target genes. In addition, we used immunohistochemistry to examine development of kidneys of humans and rats as well as kidneys affected by hypoxia or ischemia.

Results: Both, hypoxia and DMOG enhanced in vitro tubulogenesis. In contrast, 17 AAG, chetomin and knockdown of HIF-1α significantly inhibited branching tubulogenesis. The metastasis-promoting protein FSP1 which has recently been shown to be a HIF-1α target gene was markedly decreased in the HIF-1α knockdown cells. Inhibition of FSP1 by Sulindac as well as knockdown of FSP1 by siRNA diminished in vitro tubulogenesis of MDCK cells. In addition, FSP1 was markedly expressed in tubules of developing human and rat kidneys, as well as in ischemic, hypoxic, and polycystic kidneys.

Conclusions: Branching tubulogenesis of renal collecting duct cells depends on HIF-1α. In vitro tubulogenesis of MDCK cells also depends on the HIF-1α target gene FSP1. FSP1 is highly expressed in tubular cells of developing kidneys and adult kidneys affected by hypoxia suggesting a physiological and pathophysiologically role for FSP1 in vivo.

TH-PO473

An Alternative Splice Product of the HmPKD1 Gene Induces Mesenchyme to Epithelial Transition in COS-1 Cells
Robert L. Bacalla, Clifford Babkey, Wei Min Xu. Medicine, Indiana University and Richard Roudebush VAMC, Indianapolis, IN.

Background: In a genome wide cDNA library construction using 5’ cap probes, additional transcripts from the HmPKD1 locus were identified by Kimura et al, 2006 (Genome Research). We completed the sequence analysis and characterized the biological effects of this cDNA when expressed in COS-1 cells.

Methods: The HmPKD1 mRNA was expressed in the omentum from the HmPKD1 locus was obtained from Dr. Sugano (University of Tokyo) and sequenced at the DNA sequencing facility at the University of Tokyo. The clone was transiently transfected into COS-1 cells. Paired samples of transfected and untransfected were compared for E-cadherin and ZO-1 expression. In addition, expression of a novel transcript was measured using an Epithelial Volt Ohm meter (EVOM). Growth morphology in 3D collagen culture was assessed by multiphoton confocal microscopy.

Results: The cDNA sequence was mapped onto the HmPKD1 genomic sequence data. The cDNA encoding a splice variant from the HmPKD1 locus was obtained. Intron 30 was found to be 150 base pairs longer than that of the wild type splicing variant. A novel mRNA with an alternative splice variant from the HmPKD1 locus induces expression of Partitioning defective Par1 polarity gene, and studies are ongoing to examine their role in cell proliferation, apoptosis and differentiation during nephrogenesis.

Conclusions: A alternative transcript from the HmPKD1 locus induces expression of epithelial markers in the normal kidney cell line (COS-1). Transient transfection of this cDNA causes mesenchyme to epithelial transition and cell layer formation in 3D collagen culture.

Funding: Clinical Revenue Support

TH-PO474

Low Serum Cultured Adipose Derived Stem Cells Ameliorate Zymosan Induced Severe Rat Peritonitis Model
Hangsoo Kim, Massashi Mizuno, Kazuhiro Furushashi, Takayuki Katsuino, Kaoru Yasuda, Takenori Ozaki, Waichi Sato, Naotake Tsutou, Yasuhiko Ito, Eruy Imai, Shoichi Maruyama, Seichi Matsuo. Nagoa University Graduate School of Medicine, Japan.

Background: We developed a novel culture system for low serum cultured adipose derived stem cells (LASCs) and have shown the therapeutic potential of LASCs in various animal models.

Methods: In this study, we focused on a peritoneal peritonitis in peritoneal dialysis(PPD), which is a major problem since the fibrosis may progress rapidly and it may even evolve into encapsulating peritoneal sclerosis (EPS) even when the peritoneal dialysis catheter is removed.

A rat model of fungal peritonitis was induced by administration zymosan daily for 5 days after scraping peritoneum mechanically. The rats were divided into two groups; LASCs(1 group) or vehicle(V group) administration intraperitoneally with PD solution(1.5% glucose,neutral liquid) every day. On day 5, thickness of peritoneum, infiltration of inflammatory cells, and deposition of complement etc. were compared between the groups.

Results: On day 5, microscopic findings in L group was less plaques and less edematous. Histologically, the thickness of peritoneum, infiltration of inflammatory cells and deposition of complement both C3 and membrane attack complex(MAC) in L group was significantly reduced than those in V group. In addition, the mesothelial layer on peritoneal surface in L group recovered earlier compared with that in V group. The proliferation of mesothelial was induced in LASCs in vivo.

Conclusions: Administration of LASCs into the peritoneal cavity suppressed the inflammation of peritonitis induced by zymosan, and the mesothelial cell layer in L group recovered earlier than that in V group. These data suggest that LASCs have the multiple effects to peritoneal damage. In the future, LASCs therapy may be useful for peritoneal injury during fungal infection of the peritoneum.

TH-PO475

Block of Par1 Signaling Inhibits Metanephrine Kidney Growth and Differentiation In Vitro
Kimberly J. Reidy, Zhongfang Du, Daniel Cohen, Anne Muesch, Jonathan M. Barsach, Katalin Susztak. 1Pediatric Nephrology, Children’s Hospital at Montefiore, Bronx, NY; 2Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY; 3Nephrology, Columbia University, New York, NY; 4Nephrology, Albert Einstein College of Medicine, Bronx, NY.

Background: We have identified expression of Partitioning defective Par1 polarity proteins in developing 5-shape nephrons. Par1/a and Par1/b are serine threonine kinases that are functionally redundant and genetically-deleted results in embryonic lethality. Par1/was coexpressed with the DNP1 construct and a complex of Par1/Par6/apkC localize to distinct portions of cell membranes to establish polarity. Podocyte-specific deletion of apkC induced foot process effacement, proteinuria and glomerulosclerosis. We hypothesized that Par proteins may contribute to nephron differentiation, and sought to define the temporal and spatial expression of Par1/a/b during embryonic kidney development, and to examine the effect of Par1 signaling on nephrogenesis in vitro.

Methods: Par mRNA and protein expression of was examined using embryonic kidneys obtained from timed-pregnant Sprague Dawley rats. Localization of Par1 and 1b was examined by immunofluorescence (IF). To examine the effect of Par1 signaling, isolated metanephroi were infected with a GFP tagged dominant negative Adeno-GFP-DNP1 construct or control Adeno-GFP constructs, induced with LIF, and cultured for 7-14 days.

Results: Par1 mRNA expression correlated with expression of factors involved with nephron segmentation including Notch2 and Lhx1. Par1/a, b and Par3 proteins are expressed at low levels at E13, and expression increased at E15. Par1/b phosphorylation was also regulated during nephrogenesis. In vitro blockade of Par1 signaling with the DNP1 construct during nephrogenesis resulted in stunted growth. Early mesenchymal to epithelial transition was not inhibited, but expression of podocalyxin was impaired.

Conclusions: These data suggest that Par1/a/b may contribute to nephron development, and studies are ongoing to examine their role in cell proliferation, apoptosis and differentiation during nephrogenesis.

Funding: NIDDK Support
Both Excessive and Deficient Maternal Salt Intake Decrease Nephron Number and Cause Delayed Hypertension in the Offspring

Methods: Sprague-Dawley rats were fed low (0.15% NaCl), medium (1.3%), or high (8.0%) salt diets during pregnancy and weaning. Offspring were weaned at 4 weeks of age and subsequently received a standard rodent diet. Blood pressure was measured by telemetry and albuminuria by a rat specific ELISA up to 52 weeks of age. The nephron number at 12 weeks of age was determined using design-based stereology.

Results: The nephron number was significantly lower in offspring of dams on low (males: 19000±2500, f: 18000±2500) compared to medium salt intake (m: 32400±2500, f: 28500±6000). In male offspring of dams on both low and high salt intake baseline albumin excretion was higher than in offspring of dams on medium salt intake from 6 months of age. Albumin excretion increased after 1 and 2 weeks of high salt intake. The increase in albuminuria was significantly higher in both male and female offspring of dams on high and low salt diet respectively.

Conclusions: Systolic, diastolic, and mean arterial pressures were not significantly different between the offspring until 6 months of age. Starting at age 7 months systolic blood pressure was higher in offspring of dams on low salt compared to medium (116±5) salt intake and remained higher until 12 months of age.

Blood pressure was higher in offspring of dams on low (123±8 mmHg) and high (124±7 mmHg) compared to medium (116±5) salt intake and remained higher until 12 months of age. Systolic, diastolic, and mean arterial pressures were not significantly different between the offspring until 6 months of age. Starting at age 7 months systolic blood pressure was higher in offspring of dams on low salt compared to medium (116±5) salt intake and remained higher until 12 months of age.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

TH-PO478

Superior Size and Volume Charts for Fetal Kidney Development Derived From Longitudinal US Measurements

Methods: We performed in vitro experiments with MDCK cells under various osmotic culture conditions. We previously established reference curves for sonographic size and volume of the fetal kidney and renal pelvis starting from the 15th week of gestation.

Results: In a prospective longitudinal study the length and anteroposterior and transverse diameters of both kidneys and the anteroposterior and transverse diameters of the renal pelvises of 102 consecutive fetuses were measured by 4-weekly ultrasound examinations. We carried out multilevel statistical analysis for comparison of kidney dimensions with gestational age and femur length. Tests for interaction were non-significant.

Conclusions: Size charts for fetal kidney dimensions, kidney volume and renal pelvis were created and compared with previously published charts. These charts were significantly different from previous published charts by Chitty et al. (see figure).

Funding: Private Foundation Support

TH-PO480

The Expression of Prox1 Is Regulated by the Osmolality in Mouse Renal Medulla

Methods: We performed in vitro experiments with MDCK cells under various osmotic culture conditions. We previously established reference curves for sonographic size and volume of the fetal kidney and renal pelvis starting from the 15th week of gestation.

Results: In a prospective longitudinal study the length and anteroposterior and transverse diameters of both kidneys and the anteroposterior and transverse diameters of the renal pelvises of 102 consecutive fetuses were measured by 4-weekly ultrasound examinations. We carried out multilevel statistical analysis for comparison of kidney dimensions with gestational age and femur length.

Conclusions: Size charts for fetal kidney dimensions, kidney volume and renal pelvis were created and compared with previously published charts. These charts were significantly different from previous published charts by Chitty et al. (see figure).

Funding: Government Support - Non-U.S.

TH-PO477

The Expression of Prox1 Is Regulated by the Osmolality in Mouse Renal Medulla

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Results: In a prospective longitudinal study the length and anteroposterior and transverse diameters of both kidneys and the anteroposterior and transverse diameters of the renal pelvises of 102 consecutive fetuses were measured by 4-weekly ultrasound examinations. We carried out multilevel statistical analysis for comparison of kidney dimensions with gestational age and femur length. Tests for interaction were non-significant.

Conclusions: Size charts for fetal kidney dimensions, kidney volume and renal pelvis were created and compared with previously published charts. These charts were significantly different from previous published charts by Chitty et al. (see figure).

Funding: Private Foundation Support
The Role of Ubiquitin Specific Protease 40 in the Glomerular Endothelial Cells

Further, our charts showed a high correlation between kidney and femur length ($r = 0.940$), and between the kidney volume and the calculated volume based on femur length ($r = 0.941$).

Conclusions: Longitudinal measurements have produced growth charts that differ from previous ones in that renal volume does not level off before birth, which is consistent with continued immediate postnatal growth. The added correlation charts with femur length will be useful to identify deviations in fetuses small or large for gestational age.

TH-PO481

The Role of Ubiquitin Specific Protease 40 in the Glomerular Endothelial Cells

Hiroshi Takagi,1 Michael P. Madario,2 Kunimasa Yan,1 Pediatrics, Kyorin University School of Medicine, Mitaka, Tokyo, Japan; 1Nephrology and Kidney Transplantation Section, Medical College of Georgia, Augusta, GA.

Background: The vascular endothelium is an essential structure of the glomerular tuft; however, the regulatory mechanism of vascularization in the developing glomerulus remains elusive. The ubiquitination pathway is a highly dynamic and coordinated process that regulates degradation of proteins within a cell, in which ubiquitin specific protease 40 (USP40) plays a role.

Methods: Mouse USP40 cDNA was cloned from kidney cDNA library and used for generation of recombinant protein and establishment of stably expressing cell line. Polyclonal antibody was generated by using recombinant USP40. RT-PCR was done to determine expression of USP40 mRNA. Immunohistochemistry and immunoelectron microscopy were done to examine localization of USP40 in the mature and fetal kidney.

Results: USP40 mRNA was abundantly expressed in the glomeruli compared with the rest of kidney. USP40 was specifically localized at microvascular endothelial cells intensely in the glomerulus, and moderately in the interstitium of mouse, rat, and human mature kidney. The subcellular localization of USP40 in the stable cell line and mouse glomerular endothelial cell line was observed in the cytoplasm, not in the specific organelas. Molecular migration of USP40 of cell lines, isolated glomeruli and the rest of kidney was revealed to be a 150-kDa. In the fetal kidney, USP40 was already recognized in the comma-shaped glomerulus. USP40 siRNA resulted in the drastic decrease of podocalyxin expression in the immortalized mouse endothelial cells.

Conclusions: The present data suggest that USP40 may play a crucial role in the glomerular development through interacting with ubiquitin, thereby regulating degradation of specific proteins of the microvascular endothelial cells including podocalyxin.

Funding: Government Support - Non-U.S.

TH-PO482

Gender Differences in Chronic Kidney Disease and Progression in Type 2 Diabetes

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Background: The effect of gender on chronic kidney disease (CKD) and its progression in diabetes is not well defined. The objective of this study is to evaluate gender differences in CKD and CKD risk factors in type 2 diabetes.

Methods: The Pathways Study is a prospective cohort of ambulatory, diabetic patients from a large managed care system in Seattle, WA. Chi-square tests, two-sample t-tests, and logistic regression were used to determine gender differences in baseline CKD stage, risk factors, and progression. Risk of mortality was evaluated by Cox-proportional hazards modeling.

Results: Among 4800 enrollees, 4749 patients met entry criteria (48.6% women, 51.4% men). Women had a higher baseline prevalence of hypertension (45.0% vs 41.0%, p=0.008) and mean LDL (115.9 vs 107.8 mg/dL, p=0.001) than men. Fewer women than men had their LDL checked (60.1% vs 67.5%, p=0.001) or were prescribed a statin (26.5% vs 35.4%, p=0.001). Men had a higher prevalence of baseline microalbuminuria (16.5% vs 14.5%, p=0.032) and number of end organ complications (p=0.001). Women had a higher prevalence of moderate/severe CKD (GFR<60 ml/min, 35.9% vs 31.6%, p=0.001). In logistic regression models, women had a greater odds of progressing to a higher CKD stage (OR=1.34, 95% CI=1.13-1.60) but there was no difference in progression to ESRD at 5 years (p=0.451). Men had greater all-cause mortality at 5 years (HR=1.30, 95% CI=1.05-1.60) but other adjustment for diabetic complications the difference was no longer significant (HR=1.14, 95% CI=0.93-1.41).

Conclusions: Diabetic women had a higher prevalence of hypertension and hyperlipidemia. They were less likely than men to have their cholesterol checked or to have a statin prescribed. Women were more likely to progress to a higher CKD stage but there was no difference in progression to ESRD at 5 years. Men had a higher 5-year mortality which seems related to their higher number of end organ complications. Further studies on the effect of gender on CKD progression in diabetes are warranted.

Funding: NIDDK Support, Other NIH Support - NIMH, Veterans Administration Support.

TH-PO483

Investigation of Trajectories of Renal Function Decline in Subjects with Type 1 Diabetes Mellitus and Proteinuria

Ian Slapak,1 Adam Smiles, Marcus G. Pezzolesi, Monika A. Niewczas, Robert C. Stanton, James Warram, Andrzej S. Krolevski. Joslin Diabetes Center and Harvard Medical School, Boston, MA.

Background: The modern-day natural history of renal function decline in patients with type 1 diabetes mellitus (T1D) and nephropathy remains unknown.

Methods: We identified 244 patients under care of the Joslin Clinic who had T1D and proteinuria, with normal renal function. These patients were followed for 5 to 18 years (98% until 2008). The multiple measurements of serum creatinine obtained during follow-up and eGFR (estimated glomerular filtration rate) values calculated with CKD-EPI formula were used to determine long-term trajectories of renal function changes.

Results: In one-half of the cohort, renal function was stable or decreased slowly (non-decliners); in the other half, renal function decreased rapidly (-3.5 to -71 ml/min/1.73m2/ year) with almost 100% risk of ESRD (decliners). The observed trajectories according to three periods of enrollment into the study are summarized in the Table.

<table>
<thead>
<tr>
<th>Patterns of trajectories</th>
<th>Calendar year of enrolment</th>
<th>Percent</th>
<th>Decliners</th>
<th>Non-decliners</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decliners</td>
<td>1990-1995</td>
<td>35.1</td>
<td>35.3</td>
<td>64.7</td>
</tr>
<tr>
<td>Non-decliners</td>
<td>1996-2000</td>
<td>48.6</td>
<td>53.7</td>
<td>46.3</td>
</tr>
<tr>
<td>Total</td>
<td>2001-2004</td>
<td>56.1</td>
<td>62.9</td>
<td>37.1</td>
</tr>
</tbody>
</table>

Most decliners had linear trajectory of eGFR loss. Transitions to slower rate of decline (deceleration) were rare. The risk of development of rapid decline in subjects with stable renal function was 28% in the sub-cohort with the longest follow-up.

Conclusions: Almost half of the patients with proteinuria had stable renal function and minimal risk of ESRD (end-stage renal disease) during their lifetime. The other half of the patients are at risk of ESRD, but time to onset varied between 2 and 20 years due to very variable rate of renal function decline, which was constant in the majority of the decliners. Infrequent decelerating trajectories suggest that the current therapies are ineffective in delaying renal function loss in decliners. It may be hypothesized that the risk of ESRD is primarily determined by currently non-modifiable, early, constitutional (possibly genetic) factors.

Funding: Other NIH Support - DK41526, Private Foundation Support.
Nondiabetic, nonalbuminuric CKD is more predominant in women and non-Hispanic Whites. The relationship between age and decreased eGFR in nondiabetic but not diabetic populations. CKD is greater in diabetic than nondiabetic people. Albuminuria is an effect modifier of the remission of albuminuria was related to the progression to macroalbuminuria (HR; 0.97, 95%CI 0.87-1.08, p = 0.29). In microalbuminuric patients, lower eGFR at the beginning of the episode of remission of albuminuria was related to the progression to macroalbuminuria (HR; 0.97, 95%CI 0.87-1.08, p = 0.03). Correlations between (1) 2hPPG and dglucose and (2) F Scr and 2hPP Scr are statistically significant (-0.49 < r < -0.44; P < 0.03 in every case). Scr levels were at the normal range and decreased with glycemic control. No correlation was found between F Scr and HbA1c (r = -0.012; P value = 0.45). 2hPPG and dglucose are strong predictors of renal preservation in DM. The background of diabetes mellitus (DM) and renal dysfunction may individually or simultaneously have a negative prognostic effect on patients with acute myocardial infarction (AMI). However, few studies are assessed the role of renal insufficiency and its association with DM in the context of AMI. The aim of this study was to investigate the interaction between renal dysfunction and DM in patients with AMI. We have shown that 2 hour postprandial glucose (2hPPG) and dglucose are strong predictors of renal preservation in DM. 2) HbA1c is not a dependable predictor of renal function change in DM.
Obese individuals. This relationship appears to be stronger in non-Hispanic Blacks and common among individuals with reduced eGFR. Retinopathy is predictive of low eGFR and UACR. Covariates included age, gender, HbA1c, systolic and diastolic blood pressures. Analyses were stratified to evaluate ethnicity/race and body mass index (BMI) as effect modifiers of this relationship.

Results: Of 269 participants with decreased eGFR, 39% had no microalbuminuria and no retinopathy; 16% had retinopathy with no microalbuminuria; 27% had microalbuminuria and no retinopathy and 22% had both microalbuminuria and retinopathy. Multivariate logistic regression analyses stratified by ethnicity demonstrated retinopathy to be more significantly predictive of decreased eGFR in non-Hispanic Blacks than non-Hispanic Whites (χ² = 11.9, P = 0.024). Multivariate logistic regression analyses stratified by BMI demonstrated retinopathy to be more predictive of decreased eGFR in participants with a BMI ≥ 30 (OR = 2.62; 95% CI 1.26, 5.46). Analyses of albuminuria showed no association with retinopathy and no effect modification by ethnicity or BMI.

Conclusions: In older onset diabetes the absence of albuminuria and retinopathy is common among individuals with reduced eGFR. Retinopathy is predictive of low eGFR but not albuminuria. This relationship appears to be stronger in non-Hispanic Blacks and obese individuals.

Prevalence of CKD in Type 2 Diabetes – First Results of a Nationwide Study in German Primary Care Physicians

Methods: The population was divided according to the CIMT in 2 groups: G1 (n=25) – CIMT ≥ 0.9 mm and G2 (n=25) – CIMT <0.9 mm. We performed descriptive statistics. Student t and Chi-Square tests were used for comparison between groups. We also used a single regression model to evaluate the relation between the CIMT with the FGF-23 and 25(OH)D3 levels.

Results: G1 showed higher malonaldehyde (3.7±2.3 µmol/g, p = 0.013) and lower vitamin D (24.9±8.6 vs 31.4±9.5, p = 0.037) levels. There were no differences regarding the other parameters, such as total cholesterol, phosphorus, HgA1c, HO-MA1r and eGFR. Furthermore, we found in the regression model, that FGF-23 (β=0.77, p=0.001) and 25(OH)D3 (β = 0.43, p=0.002) were significantly related with CIMT.

Conclusions: We conclude that diabetes patients with early stages of CKD there is a relationship between the mineral abnormalities and the CIMT, a known marker of subclinical atherosclerosis. Further prospective analysis are required to validate the clinical significance role of these new markers of cardiovascular in our renal patients. 

Funding: NIDDK Support
Recurrence of Diabetic Kidney Disease in Type 1 and Type 2 Diabetic Patients after Kidney Transplantation

Izumi Nyumura,1 Tetsuya Babazono,1 Kazuho Honda,2 Yasuko Uchigata.1

Patients after Kidney Transplantation

Izumi Nyumura,1 Tetsuya Babazono,1 Kazuho Honda,2 Yasuko Uchigata.1

Recurrence of Diabetic Kidney Disease in Type 1 and Type 2 Diabetic Patients after Kidney Transplantation

Izumi Nyumura,1 Tetsuya Babazono,1 Kazuho Honda,2 Yasuko Uchigata.1

Conclusions:

Several clinical factors were identified as predictive factors of higher incidence of hard renal outcomes in diabetic patients with CKD stage 4-5. Whether age, sex, urinary albumin-to-creatinine ratio (ACR) and eGFR at baseline, mean values of systolic and diastolic blood pressure, hemoglobin, glycated hemoglobin, serum albumin, uric acid, low- and high-density lipoproteins during the follow-up period on renal outcome were examined using the Cox proportional hazard model. Results:
The median follow-up period was 19 months (range: 6-137 months). Among 131 patients, 88 patients started dialysis and 10 patients died before initiation of dialysis.

Conclusions:

Several clinical factors were identified as predictive factors of higher incidence of hard renal outcomes in diabetic patients with CKD stage 4-5. Whether modification of these factors may diminish the risk should be determined.

Funding: Government Support - Non-U.S.

TH-PO493

Recurrence of Diabetic Kidney Disease in Type 1 and Type 2 Diabetic Patients after Kidney Transplantation

Izumi Nyumura,1 Tetsuya Babazono,1 Kazuho Honda,2 Yasuko Uchigata.1

Background:

Renal lesions are more heterogeneous in type 2 than type 1 diabetic patients due to modification by aging and concomitant obesity, hypertension and lipid disorders. However, information is scarce regarding whether severity of recurrent diabetic kidney disease (DKD) in the renal allograft may differ in type 1 and type 2 diabetic patients. We therefore conducted this study to highlight the relationship between type of diabetes and clinical or histological parameters associated with the recurrence of DKD in renal allograft.

Methods:

We studied 34 diabetic renal allograft recipients, 17 with type 1 and 17 with type 2 diabetes who underwent renal biopsy both in the intraoperative period and at 3-16 years after transplantation. Glomerular morphometrical analysis including mesangial area, capillary tuft number and capillary tuft area was performed by a computer-assisted image analyzer. The thickness of glomerular basement membrane (GBM) was evaluated by an electron microscopy. The rate of decline in estimated glomerular filtration rate (eGFR) and albuminuria were evaluated as clinical parameters.

Results:

Recipient’s age and body mass index (BMI) were significantly higher in type 2 than type 1 diabetes. There were no significant differences in terms of donor’s age, posttransplant observational period, blood pressure, HbA1c and lipid parameters. Increase in mesangial glomerular areas and thickness of GBM were significantly greater in type 2 diabetes (p<0.032 and p<0.005), however, after adjustment for covariates including recipient’s age, BMI, HbA1c, blood pressure and lipid parameters by covariance analysis, the statistical significance disappeared. Glomerular capillary number and area, the grade of tubulo-interstitial lesions, and vascular lesions, as well as the rate of decline in eGFR and albuminuria were not different in the two groups.

Conclusions:

Type of diabetes is less likely to be associated with the histological and clinical parameters of the recurrence of DKD in diabetic transplanted patients.

TH-PO494

Predictive Factors for Progression to End-Stage Renal Disease in Diabetic Patients with Chronic Kidney Disease Stage 4-5

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Background:

Although several factors have been shown to be associated with higher risk of the onset and progression of CKD in diabetic patients; predictive factors for ESRD in diabetic patients with later stages of CKD have never been determined. We therefore conducted this observational cohort study to identify clinical factors associated with higher risk of ESRD in diabetic patients with CKD stage 4-5.

Methods:

We studied a total of 131 diabetic patients with CKD stage 4-5, 58 and 73 patients with stage 4 and 5, respectively. There were 39 women and 92 men and the mean age at the start of follow-up was 63 ± 12 years (range: 32-88 years). The primary endpoint was the composite of the start of RRT and death. Patients were observed at least 6 months. Effects of age, sex, urinary albumin-to-creatinine ratio (ACR) and eGFR at baseline, mean values of systolic and diastolic blood pressure, hemoglobin, glycated hemoglobin, serum albumin, uric acid, low- and high-density lipoproteins during the follow-up period on renal outcome were examined using the Cox proportional hazard model.

Results:

The median follow-up period was 19 months (range: 6-137 months). Among 131 patients, 88 patients started dialysis and 10 patients died before initiation of dialysis. Five-year cumulative incidence of reaching the endpoint was 83.4% and the median follow-up period was 24.8 months. Among the covariates, higher urinary ACR and diastolic blood pressure, lower eGFR, serum albumin, and hemoglobin levels were significantly associated with higher risk of reaching the endpoints (p<0.05). Higher glycated hemoglobin and lower low-density lipoprotein levels had marginal effects (p=0.2).

Conclusions:

Several clinical factors were identified as predictive factors of higher incidence of hard renal outcomes in diabetic patients with CKD stage 4-5. Whether modification of these factors may diminish the risk should be determined.
Clinical Indications for Renal Biopsy in Diabetic Glomerulosclerosis with Superimposed Non-Diabetic Renal Disease Jon H. Steuernagle,1 LaTonya J. Hickson,2 Axel Pfueger,2 Ziad El-Zoghby,2 Sanjeev Sethi,3 Mary E. Fidler,3 Samih H. Nasr,3 Lynn D. Cornell,1 Internal Medicine, Mayo Clinic; Nephrology and Hypertension, Mayo Clinic; Anatomic Pathology, Mayo Clinic, Rochester, MN.

Background: Patients with diabetes have been found to have non-diabetic renal disease (NDRD) on biopsy. The clinical reason for performing such biopsies is not well known from large sample studies.

Objective: To examine clinical indications for renal biopsies having diabetic glomerulosclerosis (DGS) and concomitant NDRD.

Methods: A database was queried for DGS from 1994 to 2010 followed by review of each DGS report for NDRD. Renal pathologist comments on clinical indications for renal biopsy were recorded.

Results: Of 3735 biopsies with DGS, concomitant NDRD was present in 967 (27%). The most common NDRD was tubulointerstitial (69%), glomerular (29%) and arteriomegolic vascular (2%). Tubulointerstitial NDRD included acute tubular necrosis (60%), interstitial nephritis (36%), and others (4%). Glomerular-NDRD included IgA (22%), pauci-immune crescentic (17%), membranous glomerulonephritis (16%), post-inflammatory (16%), membranoproliferative(8%), thrombotic microangiopathy (5%) and others (16%). Mean age was 59.14 years, 54% were male, and creatinine was 4.1±2.5 mg/dl at biopsy. Biopsy reasons included acute renal failure with or without proteinuria (74%), nephrotic range proteinuria (15%), proteinuria (2%), hematuria (1%), rapidly progressive renal failure (1%), and unknown (7%). Acute renal failure (79%) and nephrotic range proteinuria (11%) were primary biopsy reasons in tubulointerstitial NDRD similar to glomerular NDRDs such as IgA (58% and 25%, respectively).

Conclusions: NDRD is common among diabetic patients undergoing renal biopsy. NDRD is oftentimes treatable beyond conventional therapies utilized for DGS. Acute renal failure and nephrotic range proteinuria with or without other features help identify a potential need for biopsy in this group.

Urinary Sulphate Excretion Is a Predictor of Progression of Diabetic Nephropathy Gudbjörg Andrésdóttir,1 Stephan J.L. Bakker,2 Henrik Post Hansen,1 Hans-Henrik Parving,2 Peter Rossing.1 Steno Diabetes Center, Gentofte, Denmark; 2Department of Internal Medicine, University Medical Center Groningen, Groningen, Netherlands; 3Rigshospitalet, Copenhagen, Denmark; 4Herlev Hospital, Denmark.

Background: Hydrogen sulphide (H2S) mediates smooth muscle relaxation and vasodilatation. Diabetes-related endothelial dysfunction may reduce its biosynthesis. H2S may be an early indicator of vascular disease (VMD) with a high C-statistic and Integrated Discrimination Improvement (IDI) in T2DM and marginal in HT (table 1).

Methods: We conducted a nested case-control study in the PREVEND-study. Progression of albuminuria (cases) was defined as transition from normo- to micro- or micro- to macro-albuminuria. Controls had non-progressive albuminuria during follow-up and were pair-matched for age, gender, and DM duration. We included 34 case/control pairs with T2DM and 75 pairs with hypertension (HT). The markers growth-differentiating factor-15 (GDF-15) and high-sensitivity Troponin T (hs-TNT) were measured at baseline and their contribution in predicting progression of albuminuria during follow-up (median 2.8 years) was assessed.

Results: GDF-15 was higher in cases than in controls in T2DM (332±799 vs. 909±381 pg/ml, P<0.001) but not in HT. GDF-15 was independently associated with progression in albuminuria in T2DM (adjusted OR 5.4[1.2-25.0]) and doubling of GFR in HT (2.94[1.20-7.20]) and contributed significantly to prediction of progression of albuminuria with a higher C-statistic and Integrated Discrimination Improvement (IDI) in T2DM and marginally in HT (table 1).

Conclusions: We identified GDF-15 and hs-TnT as promising markers for progression of albuminuria in T2DM and HT, with GDF-15 superior in T2DM and hs-TnT superior in HT.

C-statistics and IIDs

<table>
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<tr>
<th>T2DM</th>
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<td>Baseline Model*</td>
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<tr>
<td>Baseline Model + hsTNT</td>
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<tr>
<td>Baseline Model + GDF-15 + hsTNT</td>
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Sodium Bicarbonate and Cardiac Autonomic Neurotransmission in Chronic Kidney Disease Patients Fang Liu, Ping Fu. Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.

Background: Autonomic dysfunction (AS) is the most common complication of diabetes, which affects the morbidity and mortality of cardiovascular events. This study is to investigate whether sodium bicarbonate (SB) treatment could improve autonomic nervous system (ANS) function in patients with diabetes and chronic kidney disease (CKD) and whether this could provide additional information for the risk assessment of cardiovascular disease in patients with type 2 diabetes (T2DM).

Methods: The relationship between serum Cys C and the severity of CAS was investigated in 633 patients met the inclusion/exclusion criteria. Carotid intimal medial thickness (IMT) and the location and size of AS plaque were evaluated by Doppler ultrasonography. Based on glomerular filtration rate (GFR) estimated by simplified MDRD filtration rate (eGFR) and urinary albumin and/or protein excretion were determined for classification of diabetic nephropathy. Flow-mediated dilatation (FMD), intima-media thickness (IMT) and calcification of carotid artery were examined for atherosclerosis.

Results: Serum FGF-23 levels were 35.7±1.1 pg/ml, ranging from 10.0 to 148.0 pg/ml. Serum 25(OH) Vitamin D levels were 86.1±2.5 mmol/l, ranging from 28.3 to 239.3 mmol/l. We divided the diabetic subjects into two groups by the median level of FGF-23 (31.6 pg/ml). In the subjects of serum FGF-23 level more than 31.6 pg/ml (high FGF-23; n=124), BMI was significantly higher (25.4±3.9 vs. 23.6±3.5, P<0.001) and eGFR was significantly less (72.2±2.2 vs. 82.2±1.7 ml/min/1.73m2, P<0.001) than those in low FGF-23 group. In single regression analysis, serum FGF-23 levels had a positive correlation with BMI (r=0.25, P<0.001), and a negative correlation with eGFR (r=-0.30, P<0.001). Serum FGF-23 levels were gradually increased according to the progression of diabetic nephropathy (p value in trend test: <0.001). Its elevation was significantly greater in the diabetic subjects with nephrotic range proteinuria (OR= 2.69, 95%CI 1.6-4.6, P<0.001) than those with stages 1, 2 and 3A. There were no differences in FMD, IMT and vascular calcification between the two groups of high and low FGF-23. Also, we divided the subjects by median of 25(OH)Vitamin D (79.2 mmol/l), and evaluated the differences between the two groups. However, there was no difference in any parameter at all.

Conclusions: These findings indicate that serum FGF-23 levels increase in association with progression of diabetic nephropathy, but not with atherosclerosis, in type 2 diabetic subjects.
result, the patients were divided into those with chronic kidney disease (DM-CKD) and without CKD (DM-NCKD). The relationship of serum Cys C with the severity of CAS was examined.

**Results:** Serum Cys C was closely correlated with GFR in all subjects and in DM-CKD patients, but not in DM-NCKD patients. In 396 DM-NCKD patients (62.5%) with the eGFR ≥60 ml•min−1•1.73 m−2, 210 patients (210/396, 53%) with a significant large number of carotid AS plaques had a higher serum concentration of Cys C than those (186/396, 47%) without AS plaques formation (1.05±0.27mg/L vs 0.89±0.22mg/L). Moreover, the serum concentration of Cys C was closely correlated with the severity of CAS (r=0.338, P<0.001), even after adjusting for confounding factors (r=0.14±0.05), such as age and duration of diabetes. Multiple linear regression analysis also showed closely correlation of Cys C with the severity of CAS.

**Conclusions:** Cys C is more than a sensitive marker of renal function, but a marker of CAS severity in T2DM patients without CKD. Besides DM-CKD, it might serve as an indicator of morbidity and mortality of cardiovascular events.

**TH-PO502**

**Urinary Excretion of Markers of Metalloproteinases System and Their Association with Reduced Renal Function in Subjects with T1D Diabetes and Proteinuria**

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**Background:** Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) are thought to regulate extracellular matrix turnover contributing to renal fibrosis and to progression of chronic kidney disease (CKD). The aim of this study was to investigate profile of urinary excretion of MMPs/TIMPs system in subjects with type 1 diabetes (T1D), proteinuria and different CKD stages.

**Methods:** Glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease study equation. MMPs (MMP-1, 2, 7, 9, 12) and TIMPs (TIMP-1-4) were measured on the Luminex platform and adjusted with urinary creatinine for analyses.

**Results:** This study included 302 patients with T1D and overt proteinuria, 85 (28%) were in CKD stage 3, 49 (16%) were in stage 4, and the others were in stage 1 or 2. The median albumin to creatinine ratio (ACR) was 781 (455-1644) mg/g Cr. Among detectable markers, urinary excretion levels of MMP-2, TIMP-1, and TIMP-2 were increased proportionally with more advanced CKD stages, whereas urinary excretion of MMP-7 and MMP-9 did not differ by CKD stages. The proportions of subjects with the highest tertile of biomarkers distribution stratified by CKD stages are presented in the figure.

**Conclusions:** Urinary MMPs and TIMPs were closely correlated among each other. In multiple linear regression analysis, urinary excretion of MMP-2 (β = 0.0088, P<0.001), TIMP-1 (β = −0.0641, P<0.0001), and TIMP-2 (β = 0.0037, P<0.0001), but not of MMP7 and MMP9, were correlated with GFR inversely and independently from ACR.

**TH-PO503**

**Urinary Transcriptome Analysis of Diabetic Kidney Disease**

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**Background:** Urinary transcript levels have been suggested as a noninvasive marker of renal damage in patients with various renal diseases. However, genome-wide transcriptome analysis of control and DKD urines have not been performed. Here we analyzed mRNA expression profiles of urinary sediments using Affymetrix microarray to determine the feasibility of this approach.

**Methods:** Random urine were collected from healthy controls and patients with DKD. Patients with DKD had a mean of 1.8 g/day of proteinuria, and significantly decreased GFR (mean 32.8 µl/min). Total RNA was extracted from 30 ml of urine using Trizol and QiaGen kit after centrifugation with 3000 RPM for 15 min. The quality and integrity of RNA were determined by using the Agilent Bioanalyzer. Total RNA was amplified using the Affymetrix 3’ IVT kit and hybridized onto Affymetrix U133 expression arrays. Normalized gene expression data were analyzed using the GeneSpring GX and Ingenuity Pathway Analysis Softwares. Transcripts with statistically significant differential expression were determined using the Benjamini-Hochberg corrected p<0.05 and fold changes ≥ 2.0 parameters. In addition, we also examined individual gene expression levels using quantitative RT-PCR with primers specific for different renal cell types.

**Results:** Using statistical methods we identified 931 probesets with at least 2-fold change in their expression between control and DKD groups. The top 6 transcripts with the highest fold changes were ALB, ALDOB, BBOX1, DDAH1, GATM, and HPD. All these genes showed reduced expression in the DKD group. Network analysis indicated disturbances in protein trafficking, protein synthesis, cellular assembly and organization as the top differently regulated pathways in DKD urine samples.

**Conclusions:** The urinary transcriptome analysis may be a useful non-invasive tool to evaluate diabetic nephropathy. Further prospective studies will be needed to elucidate the validity of these transcription levels in diabetes and chronic kidney disease.

**Funding:** NIDDK Support, Private Foundation Support

**TH-PO504**

**Exercise-Induced Increases in Serum Retinol-Binding Protein 4 and Endothelial Progenitor Cells in Diabetic Subjects**

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**Background:** Retinol-binding protein 4 (RPB4) is an adipokine as well as primary carrier for retinol in plasma, and synthesized in liver, adipose tissues and muscular tissues. Endothelial progenitor cells (EPCs) derived from bone marrow are involved in vascular endothelial cell repair. We determined whether acute exercise load changes serum RBP4 and EPCs were determined by FACScan.

**Results:** Serum RBP4 levels, but not the numbers of EPCs, were increased according to the progression of diabetic nephropathy (p value in trend=0.043). In the 30 subjects without nephropathy (stage 1), an exercise load promptly increased serum RBP4 from 48.2±23.5 to 54.3±23.1 µg/mL (p=0.0006). In the 32 subjects with nephropathy (stage 2, 3, and 4), the exercise load did not alter serum RBP4. By contrary, an exercise load significantly increased the numbers of EPCs in both the stages. In contrast, the number of EPCs with stage 1 (42.9±8.3 to 11.4±9.1, 104.4 cells/100µL, p=0.0003), and stage 2, 3, and 4 (63.0±6.84 to 78.5±6.68 cells/100µL, p=0.005). The alteration in serum RBP4 and EPCs during the exercise had positive correlations with serum RBP4 levels (the numbers of EPCs; r=0.402, p=0.006; ΔEPCs; r=0.310, p=0.04).

**Conclusions:** These findings indicate that acute exercise promptly increases serum RBP4 levels and the number of EPCs in diabetic subjects without nephropathy, and that there may be some interaction of RBP4 with the induction of EPCs under acute exercise.

**TH-PO505**

**Association of Serum Leptin Levels with Progression of Diabetic Kidney Disease in Patients with Type 2 Diabetes**

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**Background:** The association of leptin with diabetic kidney disease (DKD) remains controversial, and furthermore previous reports were limited by cross-sectional design. We therefore conducted this longitudinal study to clarify the association of serum leptin levels with progression of DKD in patients with type 2 diabetes (T2D).

**Methods:** This was a simple longitudinal cohort study: This was a simple longitudinal cohort study. The mean follow-up period of 4.2±1.2 years, the mean rate of change in eGFR was -1.66±3.69 ml/min/1.73 m2/year in the eGFR cohort. Patients with low or high leptin levels had a significantly steeper eGFR decline than those with mid-range leptin levels in both the univariate and the multivariate model (all p values < 0.05 vs. patients with mid-range leptin levels). In the ACR cohort, 34 patients showed progression of albuminuria during the mean follow-up period of 3.2±1.6 years. Negative graded relationships between the hazard ratio for progression of albuminuria and 3 groups, based on leptin levels, were recognized in both the univariate and the multivariate model. Patients with the low leptin levels had a significantly elevated risk of progression of albuminuria without high leptin levels in both the univariate and the multivariate model (both p values < 0.05).

**Conclusions:** In this study of patients with T2D, both low and high serum leptin levels were risk factors for kidney function decline. Meanwhile, lower serum leptin levels were associated with progression of albuminuria.

**TH-PO506**

**The Good and the Bad: The Relationships between 25(OH) D and FGF-23 Levels with Cardiovascular Disease in Type 2 Diabetic CKD Patients**

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**Background:** Cardiovascular disease is the main risk factor of morbidity and mortality in chronic kidney disease (CKD) patients and its relevance increases when diabetes is the etiology of the renal disease. Carotid wall thickness, left ventricular hypertrophy and vascular calcification are commonly used surrogates of cardiovascular disease in these patients. Recently, the pathophysiologic mechanisms of the mineral metabolism were also related to cardiovascular disease, even in the early stages of CKD.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

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The aim of this study was to evaluate the relationships between vitamin D and FGF-23 levels with cardiovascular risk factors in a group of diabetic CKD patients. 

Methods: In a cross-sectional study, we included 50 type 2 diabetic patients (f=18, m=32) with CKD stages 3 and 4. The mean age was 60.4 years and the mean estimated (MDRD) GFR was 50 ml/min. Exclusion criteria were uncontrolled hypertension, known primary cardiovascular disease, diabetes mellitus type 1, hypothyroidism, or any neoplasm. Cardiac troponins I, D, and B, and C-reactive protein were measured. Relationships between FGF-23 and cardiovascular risk factors were evaluated using Pearson's correlation coefficient. 

Results: There was a significant positive correlation between FGF-23 and systolic blood pressure (SBP) (r = 0.317, p = 0.007) and Aix (r = 0.715, p = 0.001), an inverse correlation between 25 (OH)D levels and LVMi (r = -0.633, p = 0.001), CMAI (r = -0.332, p = 0.014) and Aix (r = -0.570, p = 0.01). 

Conclusions: In our study, we found that the FGF-23 and the 25 (OH)D levels, known risk markers of cardiovascular disease in CKD patients, were related with some cardiovascular parameters commonly used in the clinical practice. Further studies are needed to better understand the pathophysiology of these relationships and to ascertain if the correction of the FGF-23 and the 25 (OH)D levels improves the outcomes of our patients. 

Funding: NIDDK Support

TH-PO507

Total Serum Free Light Chains Are an Important Prognostic Marker in Patients with Type 2 Diabetes Mellitus

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Philip Young,

Richard Hughes,

Colin A. Hutchison.

The Binding Site Group Ltd, United Kingdom; Renal Institute of Birmingham, United Kingdom.

Background: Polyclonal serum free light chains (FLCs) have recently been shown to be prognostic of overall survival and cardiac events in patients with chronic kidney disease (CKD). High FLC levels represent both degree of renal impairment and severity of inflammation. FLCs have also been reported to be elevated in diabetes, which is a major cause of CKD and cardiovascular disease. The purpose of this study was to determine if serum FLCs are associated with a shorter overall survival (OS) in type 2 diabetic patients.

Methods: A total of 527 patients (263 females) were included. Baseline patient characteristics were: median (IQR) - systolic BP: 136 (26), diastolic BP: 82 (14), age: 54 (20), total cholesterol: 4.5 (1), HDL: 1.18 (0), LDL: 2.21 (1), ACR: 1.90 (6), BMI: 28 (7), eGFR: 75.6 (18.7), HBA1C: 6.6 (2), triglycerides: 2.3 (2) and total FLCs: 41.10 (21.6). The prognostic value of each variable was assessed using Kaplan-Meier and Cox-regression analysis.

Results: A total of 166 (80 males and 86 females) had elevated FLC levels (normal range: 9-48 mg/L). By 4.5 years 17/527 (3.2%) patients had died. Total FLC levels were significantly higher in patients who had died (52.8 mg/L) compared with those who were still alive (20.9 mg/L) (p=0.026). Patients with elevated FLC levels (>50 mg/L) had a significantly shorter OS than patients with FLCs <50mg/L (p=0.014). The following variables were included as part of the univariate analysis: systolic BP, diastolic BP, age, gender, creatinine, smoker status, total cholesterol, HDL, LDL, ACR, BMI, eGFR, HBA1C, triglycerides and total FLCs. Only raised FLCs (HR=3.2, p=0.002) and age (HR=3.6, p=0.002) were significantly associated with their outcomes when models were adjusted for age, sex, estimated GFR, serum albumin, hemoglobin A1c, and diabetes duration. In the final model, YKL-40 in the third tertile was associated with significant CAD, however when adjusting for sex and age these associations were no longer significant. 

Conclusions: We found that the A-megalin assay targets were primarily in the soluble fraction, whereas the C-megalin assay targets were in both soluble and insoluble fractions. In 52 T2DM patients, urinary C-megalin levels tended to be elevated in line with increased albuminuria and were associated positively with plasma inorganic phosphate (Pi) and negatively with hemoglobin (Hb) levels, independently of age and sex. Other urinary biomarkers such as albumin, NAG, α1-microglobulin, β2-microglobulin, L-FABP, NGAL and type IV collagen did not show such a combined association with Pi and Hb levels. In contrast, urinary A-megalin levels were significantly associated with normo-, micro- and macroalbuminuria but not in those with macroalbuminuria. In addition, urinary A-megalin levels were negatively associated with fractional excretion of Na.

Conclusions: Urinary full-length megalin expression as measured by the C-megalin assay appears to be linked to chronic progression of DN, Pi dysregulation, and anemia, whereas urinary excretion of megalin ectodomain as measured by the A-megalin assay may be associated with RPR-related distinctive mechanisms involved in renal Na handling in T2DM patients.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

TH-PO510

YKL-40 Levels Are Associated with Coronary Calcium Score in Type 2 Diabetic Patients with Albuminuria but without Known Cardiovascular Disease


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Background: Type 2 diabetic patients (T2D) with albuminuria have a poor prognosis, primarily due to increased morbidity and mortality from cardiovascular disease (CVD). Early detection of asymptomatic CVD is essential to improve treatment and prognosis. In this cross-sectional study we investigated the inflammatory biomarker YKL-40, which seems to reflect early atherosclerosis, in relation to asymptomatic significant coronary artery disease in T2D patients with elevated urinary albumin excretion rate and the association with non-proBNP and coronary calcium scores (CCS).

Methods: A cohort of 202 T2D patients with elevated urinary albumin excretion rate (micro- or macroalbuminuria), but normal plasma creatinine and without a history of CVD were investigated. Patients with plasma NT-proBNP levels above the median and/or coronary calcium scores (CCS) > 400 (n=133), were examined by myocardial scintigraphy and coronary angiography. YKL-40 levels were measured in all patients with a commercial ELISA assay (Quidel, USA).

Results: Median (IQR) (ng/mL) of YKL-40 was 89 (55-142) ng/mL. YKL-40 was higher in patients with significant CAD (110 ng/mL vs. 82 ng/mL, p<0.005). YKL-40 was associated with plasma NT-proBNP (R=0.2, p=0.001) and CCS (R=0.22, p=0.002). Only weak associations (R=0.3) were found between YKL-40 and traditional risk factors as age, known diabetes duration, vibration threshold, BMI and plasma creatinine. In a logistic regression model, YKL-40 in the third tertile was associated with significant CAD, however when adjusting for sex and age these associations were no longer significant.

Conclusions: In patients with T2D and albuminuria but without known CVD, YKL-40 levels are associated with CCS, a non invasive marker of coronary calcification, but not independently associated with CVD. This could prove helpful in future risk assessment.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Phosphorous as an Early Marker of Morbidity and Mortality in Type 2 CKD Diabetic Patients

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Background: Disturbances of the mineral metabolism, namely the phosphorous (P) level, have been related to increased morbidity and mortality in the general as well as in the chronic kidney population. However, studies conducted to assess the impact of P in stages 3 and 4 of renal disease are scarce. The purpose of this study was to evaluate the impact of P levels in a homogeneous population of type 2 chronic kidney disease (CKD) patients.

Methods: ± 25.1 ml/min. Exclusion criteria were: previous cardiovascular disease, undiagnosed hypertension, neoplastic or infectious diseases. The mean follow-up was 47.6 months. We divided the population in 3 groups, according to the P tertiles. GI (n=39) - P <3.60 mg/dl; G2 (n=39) - 3.70 < P < 4.60 mg/dl; G3 (n=41) - P ≥ 4.60 mg/dl

Several laboratory parameters were analyzed: albumin, hemoglobin, estimated glomerular filtration rate (MDRD), markers of inflammation (interleukin 6, TNFα, IL resistance (HOMA-IR), the left ventricular mass index (LVMI) and the hospitalization days caused by cardiovascular events.

In the analysis we used descriptive statistics, ANOVA for comparison among groups and the Kaplan-Meier method to compare the survival of the 3 groups.

Results: Patients in the higher P tertile (G3), showed lower estimated GFR (p = 0.0001), and hemoglobin (p = 0.030) levels; they also showed higher HOMA-IR (p = 0.0001), LVMI (p = 0.0001), IL-6 (p = 0.0001), TNF (p = 0.0001) and more hospitalization days (p = 0.0001) compared with other groups. Using the Kaplan-Meier analysis we found that the survival of the G1, G2 and G3 at 24 months was respectively: 93%, 86% and 71% (log-rank = 6.88 p=0.032).

Conclusions: In conclusion, we found in a group of type 2 diabetic CKD patients (stages 3 and 4) that higher P levels, even under the limits of therapeutic intervention, were associated with increased morbidity and mortality. Further studies are needed to verify if an earlier intervention regarding the P metabolism will be associated with improved outcomes in our patients.

Funding: NIDDK Support

Removing Efficacy of an L/N-Type Calcium Channel Blocker in Early Diabetic Nephropathy Complicated by Hypertension

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Background: The renoprotective efficacy of cilnidipine, an L/N-type calcium channel blocker (CCB), in hypertensive patients with chronic kidney disease has been reported, but the effect in early diabetic nephropathy compared with a L-type CCB has not been examined. We examined differences in efficiency in efficacy in L-N and type L CCBs for early nephropathy.

Methods: The present study was a multicenter crossover study pre-registered as a CLEARED study. It involved 90 patients (57 males and 33 females; age, 65±9 y) with early nephropathy complicated by hypertension. Their urinary albumin excretion (UAE) was 50±42 mg/g, and cGFR was 73±19 ml/min. Forty-nine patients were being treated with inhibitors of the renin-angiotensin system (RAS). For the group A (n=69) patients with switching to cilnidipine after administration of an L-type CCB for more than 6 months and the group with switching from cilnidipine to an L-type CCB (group B: n=21), we investigated changes in UAE 6 months after switching, at the time of switching, and 6 months after switching.

Results: Groups A and B each exhibited significant change in neither blood pressure (BP) nor HbA1c level after switching. Group A had a significant increase in UAE during L-type CCB (52.8 ± 92.1 mg/g to 92.1 mg/g, p<0.01), but a significant decrease in UAE in 6 months after switching to cilnidipine (~ 50.10 mg/g, p<0.01). Group B exhibited no significant change in UAE during cilnidipine, and no significant change in UAE in 6 months after switching to an L-type CCB. Factors related to increase in UAE in both groups were examined by logistic regression analysis. Cilnidipine was found to be a significant factor reducing the risk (OR: 0.246, 95% CI, 0.074-0.823, p<0.05) independent of age, sex, BP, RAS inhibitors, and HbA1c.

Conclusions: The L-N-type CCB cilnidipine exhibited excellent renoprotective efficacy in patients with early diabetic nephropathy compared with L-type CCBs, and switching from an L-type CCB to cilnidipine had beneficial effects.

Kidney Function and Albuminuria in Patients with Type 2 Diabetes Treated with Exenatide BID vs. Insulin Glargine

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Background: Albuminuria reduction and optimal blood pressure (BP) control are of utmost importance for patients with type 2 diabetes (T2DM) and nephropathy. Endothelin-1 receptor antagonism reduces BP and albuminuria, but fluid retention frequently occurs. No study has investigated the antihypertensive and antiproteinuric effects of the combined endothelin-converting enzyme/neutral endopeptidase (ECE/NEP) inhibitor daglutril.

Methods: A randomized, double-blind, placeo-controlled cross-over trial, 42 micro- or macroalbuminuric patients with T2DM and stable BP control were randomized to treatment with daglutril (300 mg/day) followed by placebo or the reverse. Each treatment lasted 8 weeks and was given on top of losartan (100 mg/day). Change in 24-hour albuminuria was the primary outcome. Secondary outcomes were changes in office and ambulatory BP, renal hemodynamics and glomerular size-selectivity.

Results: Mean±SD 8-week changes for daglutril vs placebo in daytime systolic and diastolic BP were -2.3±8.0 versus 3.2±9.4 mmHg (P=0.03) and 0.3±4.7 versus 1.8±5.5 mmHg (P=0.19), respectively. Corresponding changes in night-time systolic and diastolic

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

230A
TH-PO516

Effects of Lipid-Lowering Treatment on Platelet Reactivity and Platelet-Leukocyte Aggregation in Diabetic Patients with Chronic Kidney Disease – A Randomized Trial

Tora C. Almqvist,1,2 Stefan H. Jacobson,2 Per-Eric Lins,3 Richard W. Farndale,1 Paul Hjemdahl,1 Karolinska Institutet, Dept. Medicine, Clinical Pharmacology Unit, Karolinska University Hospital, Solna, Stockholm, Sweden; 2Karolinska Institutet, Dept. Clinical Sciences, Div. Nephrology, Danderyd Hospital, Stockholm, Sweden; 3Karolinska Institutet, Dept. Clinical Sciences, Div. Diabetology, Danderyd Hospital, Stockholm, Sweden; 4Dept. Biochemistry, University of Cambridge, United Kingdom.

Background: Diabetes mellitus (DM) is associated with hyperreactive platelets and increased platelet-leukocyte aggregation (PLA), but the impact of concomitant chronic kidney disease (CKD) has been much less studied. Platelet- and leukocyte activation may contribute to the high incidence of atherosclerotic cardiovascular disease in patients with DM and chronic kidney disease. Lipid-lowering treatment (LLT) with statins may have favorable effects on platelet activation and inflammation, and ezetimibe co-treatment provides additional cholesterol-lowering.

Methods: After a placebo run-in period, the effects of simvastatin alone (S) or simvastatin+ezetimibe (S+E) were compared in a randomized, double-blind, cross-over study on platelet reactivity, PLA formation, and inflammatory parameters. 18 DM patients with estimated glomerular filtration rate (eGFR) 15-59 ml/min x 1.73 m² (CKD stages 3-4) (DM-CKD) and 21 DM patients with eGFR >75 ml/min (DM only) were included.

Results: PLAs were elevated at baseline in DM-CKD compared to DM only patients (p=0.001). S+E reduced PLAs among total leukocytes and neutrophils in DM-CKD patients (p=0.01 for both). Platelet reactivity did not differ between patient groups or with LLT. Plasma sCD40L (p=0.001), elastase (p=0.01) and vWF (p=0.01) were elevated in DM-CKD compared to DM only patients. S+E tended to reduce sCD40L in DM-CKD patients, but LLT did not influence vWF or elastase.

Conclusions: In conclusion, DM patients with CKD stages 3-4 had increased platelet-leukocyte aggregation and inflammatory activity compared to DM patients with normal GFR. PLAs were elevated in patients with concomitant CKD, but did not influence inflammatory parameters in either patient group.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-PO517

Effect of Thiazides on GFR Decline in Diabetes Mellitus Type 2

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Background: Poor glycemic control and the use of diuretics in DM2 patients can both induce relative volume depletion. The combined effects on volume depletion could potentially induce intermittent acute tubular necrotic episodes and result in acceleration of kidney function decline over time. The current study examines the effect of hydrochlorothiazide (HCTZ), a mild diuretic, on the annual percentage of change in estimated glomerular filtration rate (eGFR) in DM2 patients stratified by glycemic control and blood pressure.

Methods: This is a retrospective study involving record retrieval of patients with DM2 and hypertension evaluated for any reason at UCLA-OVMC during 2009-2010. Data retrieved include age, gender, use of thiazides and inhibitors of the renin angiotensin system, eGFR, hemoglobin A1C (HbA1C), blood pressure, and albuminuria to creatinine ratio (ACR). Patients were stratified based on systolic blood pressure <= or > 140 mmHg and HbA1C <= or > 7%. Differences in the annual percentage change in eGFR during the duration of follow-up between any 2 groups comparing those receiving thiazides vs. not could potentially induce intermittent acute tubular necrotic episodes and result in acceleration of kidney function decline over time. The current study examines the effect of hydrochlorothiazide (HCTZ), a mild diuretic, on the annual percentage of change in estimated glomerular filtration rate (eGFR) in DM2 patients stratified by glycemic control and blood pressure.

Results: There were 497 patients' records were included. There were 71.8% female, with mean age 65 years, duration of DM 4 years, duration of follow-up 6.7 years, and eGFR 75 ml/min. P-values vs. PBO

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P-values vs. PBO</th>
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<tr>
<td>1.0.134</td>
<td>2.0.087</td>
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Conclusions: Subjects in the 0.75 mg and 1.75 mg groups had numerically greater % changes in UACR compared with PBO, and subjects in the 0.75 mg group also had a numerically greater reduction in systolic blood pressure (SBP) and eGFR, indicating that atrasentan could be useful for subjects with residual albuminuria while taking max-RAAS.

Funding: Pharmaceutical Company Support

TH-PO518

Effect of Atrasentan in Subjects Taking Maximum Doses of Renin Angiotensin-Aldosterone System Inhibitors (max-RAAS) for Diabetic Nephropathy (DN)

Blai Coll,1 Shihua Wen,1 Yiti Pritchett,2 Mark E. Molitch,2 John J. Brennan,1 Donald E. Kohan,3 Dennis L. Andress.11 Abbott; 2Northwestern U Feinberg Sch of Med; ‘U of Utah.

Background: RAAS inhibitors reduce albuminuria in subjects with DN, though >50% taking max-RAAS have residual albuminuria. Low dose atrasentan, a highly selective endothelin A receptor antagonist, reduces residual albuminuria. These analyses evaluated the efficacy and safety of atrasentan + max-RAAS in subjects with DN.

Methods: This was a randomized, double blind trial of subjects with type 2 diabetes and albuminuria on stable doses of RAAS inhibitors, an eGFR >20ml/min/1.73m², a UACR of 100-3000mg/g and a NT-proBNP level <500pg/ml who were allocated to placebo (PBO), or atrasentan 0.25 mg, 0.75 mg, or 1.25 mg QD for 8 weeks. This abstract is focused on 33 subjects taking max-RAAS out of 89 participating in the trial. Treatment-by-subgroup interaction was tested using ITT population.

Results: The treatment-by-subgroup interaction for change from baseline (BL) to final log UACR was not significant (P=0.816), suggesting subjects taking max-RAAS or taking lower than max-RAAS responded similarly to atrasentan. BL demographics were similar between the groups. Results are shown in the table. The small sample sizes prevented robust statistical comparisons.

Funding: Pharmaceutical Company Support

TH-PO519

Abstract Withdrawn
TPOS20
Aliskiren Added to Losartan Has Beneficial Effects across Different Levels of Albuminuria in Patients with Type 2 Diabetes and Nephropathy: The AVOID Study

Study: Frederik I. Persson, 1 Julia Lewis, 2 Edmund J. Lewis, 3 Peter Rossing, 1 Hans-Henrik Parving. 57 Steno Diabetes Center, Gentofte, Denmark; 1 School of Medicine, Vanderbilt University, Nashville, TN; 2 Rich V. University Medical Center, Chicago, IL; 3 Dept. of Medical Endocrinology, University Hospital of Copenhagen, Copenhagen, Denmark; 4 Faculty of Health Sciences, Aarhus University, Aarhus, Denmark.

Background: Aliskiren 300 mg once daily added to standard treatment with losartan 100 mg once daily and optimal antihypertensive therapy has antiproteinuric effect in patients with type 2 diabetes and nephropathy, however, the beneficial impact may depend on the level of baseline albuminuria. Consequently we performed a post-hoc analysis of the AVOID study to determine which group of patients benefits the most from the addition of direct renin inhibition.

Methods: In AVOID, 599 patients (eGFR>30 ml/min/1.73m2) with type 2 diabetes, hypertension and nephropathy received 6 months’ aliskiren (150 mg titrated to 300 mg after 3 months) or placebo added to losartan 100 mg and optimal antihypertensive therapy. The primary endpoint, changes in early morning urinary albumin creatinine ratio (UACR), was assessed by tertiles of baseline UACR levels.

Results: Patients were divided into tertiles of baseline UACR (<340 mg/g, ≥340 mg/g – 788 mg/g and >788 mg/g respectively). The antiproteinuric effect of aliskiren treatment was consistent across subgroups of baseline UACR with a between-treatment ratio of 0.78 (95% CI 0.62, 0.98) p=0.032 in the lowest tertile; 0.79 (0.63, 0.99) p=0.039 in the middle tertile and 0.84 (0.67, 1.05) p=0.15 in the highest tertile, independent of baseline BP.

Conclusions: This post-hoc analysis of the AVOID study suggests that renin inhibition with aliskiren 300 mg once daily added to losartan 100 mg once daily plus optimal antihypertensive therapy provides reductions in UACR of equal magnitude across different levels of baseline albuminuria.

Funding: Pharmaceutical Company Support

TPOS21
Cholecalciferol Reduces Urinary Angiotensinogen in Early Diabetic Kidney Disease

Christopher K. Johnson, 1 Alexandra V. Flynn, 1 Hirokouy Kobori, 2 Bryan R. Kestenbaum, 1 Ian H. de Boer. 1

Background: The renin-angiotensin system (RAS) is an important therapeutic target in diabetic kidney disease. Tissue and urinary levels of angiotensigen (AGT) reflect intrarenal RAS activation and oxidative stress. In animal models, vitamin D receptor agonists downregulate the RAS and reduce tissue and urinary AGT. We investigated whether treatment with cholecalciferol (vitamin D3) decreases urinary AGT in early human diabetic kidney disease.

Methods: In a randomized, double-blind, clinical trial, 22 participants, 6 were assigned to treatment with cholecalciferol 2000 IU daily or matching placebo for one year. All participants were randomized to cholecalciferol 2000 IU daily or matching placebo for one year. Underline represents presenting author. All participants were randomized to cholecalciferol 2000 IU daily or matching placebo for one year. Underline represents presenting author.

Results: On treatment, participants in the cholecalciferol group had a 22% decline in urinary ACR (95% CI -50%, -39%, 16%), while patients in the placebo group had a 1% decline (95% CI -28%, +36%). The treatment group showed a 15% decline (95% CI -51%, +47%); between group difference 58% (95% CI -83%, +6%; p=0.005) in the lowest tertile; 0.79 (0.63, 0.99) p=0.039 in the middle tertile and 0.84 (0.67, 1.05) p=0.15 in the highest tertile, independent of baseline BP.

Conclusions: This post-hoc analysis of the AVOID study suggests that renin inhibition with aliskiren 300 mg once daily added to losartan 100 mg once daily plus optimal antihypertensive therapy provides reductions in UACR of equal magnitude across different levels of baseline albuminuria.

Funding: Pharmaceutical Company Support

TPOS22
Analysis of Peripheral Edema in Subjects Taking Atrasentan for Diabetic Nephropathy (DP)

Blai Coll, 1 Shihua Wen, 1 Yili Pritchett, 1 Mark E. Molitch, 2

Background: Low dose atrasentan, a highly selective endothelin A receptor antagonist, reduces residual albuminuria in subjects with DN. Peripheral edema is the most frequently reported adverse event (AE) associated with endothelin A receptor blockade. The goal of these analyses was to characterize edema events in subjects taking atrasentan.

Methods: This was a randomized, double blind trial of subjects with type 2 diabetes on stable doses of RAAS inhibitors having an eGFR >20mL/min/1.73m2 and albuminuria of 100-3000mg/g (UACR, who were allocated to placebo (PBO), 0.25mg, 0.75mg, or 1.75mg atrasentan daily for 8 weeks. The protocol was later amended to have serum NT-pro-BNP<500pg/mL as an exclusion.

Results: 89 subjects were randomized: 23 to PBO and 22 to each atrasentan dose group. Baseline (BL) demographics were similar among groups. UACR lowering was different from PBO in the 0.75mg and 1.75mg groups. 21 subjects reported treatment-emergent edema. Of those 21, 13 had >3 of these analyses. UACR lowering was stable doses of RAAS inhibitors having an eGFR >20mL/min/1.73m2 and albuminuria of 100-3000mg/g (UACR, who were allocated to placebo (PBO), 0.25mg, 0.75mg, or 1.75mg atrasentan daily for 8 weeks. The protocol was later amended to have serum NT-pro-BNP<500pg/mL as an exclusion.

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Results: 89 subjects were randomized: 23 to PBO and 22 to each atrasentan dose group. Baseline (BL) demographics were similar among groups. UACR lowering was different from PBO in the 0.75mg and 1.75mg groups. 21 subjects reported treatment-emergent edema. Of those 21, 13 had >3 of these analyses. UACR lowering was different from PBO in the 0.75mg and 1.75mg groups. 21 subjects reported treatment-emergent edema. Of those 21, 13 had >3 of these analyses. UACR lowering was different from PBO in the 0.75mg and 1.75mg groups. 21 subjects reported treatment-emergent edema. Of those 21, 13 had >3 of these analyses.
magnesium were slightly increased with DAPA, but remained in the normal range. Serum uric acid decreased and parathyroid hormone increased with DAPA vs PBO.

Conclusions: In summary, DAPA did not improve HbA1c in pts with T2D and moderate renal impairment. Further research is needed to determine if DAPA exerts a renoprotective effect; however potential benefits must be balanced against possible risks in this population.

Results: Single dose pharmacokinetics of DAPA were similar in healthy subjects and patients with T2D and normal renal function while exposed Cmax and AUC of DAGO were incrementally higher with decreasing renal function. Compared to patients with T2DM with normal renal function, the steady-state geometric mean Cmax values for DAPA (D3OOG) were 4% [20%], 6% [37%], and 9% [52%] higher in subjects with mild, moderate, and severe renal impairment, respectively, and the corresponding geometric mean values for DAPA AUC-t >0 were 32% [54%], 60% [110%], and 87% [169%] higher, respectively. Isolated human kidney microsomes showed a high activity of UGT1A9 and a higher rate of D3OOG formation relative to human liver and intestinal microsomes (3- and 109-fold higher, respectively). These results suggest that DAPA may have reduced efficacy in patients with higher systemic DAPA exposure, due to a decreased GFR and subsequent decrease in filtered glomerular load. Further research is needed to determine the role of Nox5 in DN and ROS induced podocyte damage in response to classic diabetic stimuli.

Methods: In vitro, Nox5 protein expression was compared by immunofluorescence in human kidney biopsies obtained from non-diabetic and diabetic/albimunic individuals. In vitro, a conditionally immortalized human podocyte line (hPOD) was exposed to various diabetic stimuli including TGF-beta (1-10 ng/ml), advanced glycation end-products (AGE), high glucose (25 mmol/L), or equimolar mechanical stretch (10%, 60 min). Nox5 expression was determined by quantitative RT-PCR (qPCR), and western blotting. Results: Biopsies from diabetic individuals with confirmed albuminuria revealed higher immunodetectable Nox5 expression in glomerular structures vs non-diabetic controls. RT-PCR verified that Nox5p is the predominant isoform present in hPODs while qPCR demonstrated that Nox5 mRNA expression is significantly upregulated in hPODs in response to high-glucose/stretch (>2-fold), TGF-beta (>2 fold), and AGE (>4 fold). As expected Nox5 protein levels are also higher in hPODs in response to diabetic stimuli.

Conclusions: Upregulation of Nox5 in diabetic kidney occurs in response to classic diabetic stimuli. This may in part be responsible for ROS-induced podocyte damage and filtration barrier dysfunction observed in diabetic kidney disease. Mouse models with podocyte targeted Nox5 expression may elucidate the role of Nox5 in the development and progression of DN.

Funding: Government Support - Non-U.S.

Results: The PK effects of DAPA are reduced as renal function decreases, despite higher systemic DAPA exposure, due to a decreased GFR and subsequent decrease in filtered glucose load. These results suggest that DAPA may have reduced efficacy in patients with moderate to severe renal dysfunction.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represents presenting author.
Fusion of Bone Marrow-Derived Cells with Renal Tubules Contributes to Renal Dysfunction in Diabetic Nephropathy  
Tomohisa Yamashita, 1 Mineko Fujiyama, 2 Satoshi Yamamoto, 3 Masayuki Koyama, 1 Yusuke Okazaki, 1 Shutaro Ishimura, 1 Mareno Tanaka, 1 Masato Furushashi, 1 Hideaki Yoshioka, 1 Tetsuji Miura. 1

Background: We previously reported that in diabetes proinsulin-producing bone marrow-derived cells (BMDCs) appear in various organs and these cells undergo fusion with hepatocytes and neurons in the dorsal root ganglia. Fusion cells are polyplody and produce tumor necrosis factor (TNF)-alpha, suggesting that diabetes reprograms gene expression in BMDCs by turning on “inappropriate” genes, ultimately causing diabetic complications.

Methods: We performed bone marrow transplant (BMT) from green fluorescent protein transgenic mice to C57BL/6J mice and produced diabetes by streptozotocin (STZ) or feeding a high-fat diet. Immunofluorescence staining and fluorescence in situ hybridization for Y chromosome were performed and observed by confocal laser scanning microscopy.

Results: In the diabetic mice, massive infiltration of BMDCs occurred in the tubular interstitium, and tubular epithelial cell loss and basement membrane abnormality were prominent. The damaged tubular epithelial cells and BMDCs were positively stained with proinsulin and TNF-alpha. Cell fusion between BMDCs and renal tubules is confirmed by the presence of Y chromosome in female mice that received BMT from male mice. 15.4% of tubular epithelial cells contain Y chromosome in STZ-diabetic mice, 8.6% in HFHi-diabetic mice, but only 1.5% in non-diabetic mice. In addition, some nuclei contain two Y chromosomes or a large single Y chromosome in STZ-diabetic mice.

Conclusions: This study demonstrated that diabetes causes nuclear fusion between BMDCs and renal tubular epithelial cells at more than 10-fold higher frequency than nondiabetic mice. Chromosomal abnormality induced by the cell fusion may play a crucial role in DN.

Inhibition of the p66 Longevity Gene Prevents Diabetic Nephropathy in Mutant Akita Diabetic Mice  
Himanshu Vashistha, 1 Lisa A. B. Konrad, 1 Zane V. McRae, 1 Traci K. Brown, 1 SamaraV. Smith, 1 Leonard G. Meggs. 1

Background: The absence of an experimental model that faithfully mimics diabetic nephropathy (DN) in humans, has been a major limitation in dissecting the pathobiology of DN and in identifying novel molecular targets for therapeutic intervention. The recent development of Akita (Ins2−/−) diabetic mice, lacking the bradykinin-2 receptor (B2R)−/− is a significant advance, in that hyperglycemia (HG) is more durable and sustained, with inbred strains requiring streptozotocin injection, with a disease phenotype that more closely approximates DN in humans.

Methods: The ability of KIM-1 to mediate uptake of advanced glycosylation end products (AGEs) was determined in kidney epithelial cells in culture. To study the effect of mouse KIM-1-mediated endocytosis on DN disease progression, diabetes was induced in normal mice and mice which contain a targeted mutation of the extracellular mucin domain which prevents endocytosis. DN was induced by combining streptozotocin injection, unilateral nephrectomy and high fat diet.

Results: High glucose levels enhanced KIM-1 expression in cultured kidney epithelial cells via a process that was reactive oxygen species dependent. KIM-1 augmented tubule cell uptake of AGES and oxidized lipids, two components of the proximal tubular fluid in patients with diabetes mellitus. Mutant mice rendered diabetic developed less albuminuria, brush border loss and fewer interstitial myofibroblasts than normal mice. DN-associated glomerular enlargement, GBM thickening and sclerosis were also diminished by KIM-1 mutation. KIM-1 dependent ingestion of oxidized lipids and AGES and enhanced caspase-3 activity was inhibited in primary tubule cells from KIM-1 mutant animals.

Conclusions: KIM-1 expression facilitates tubular injury in early DN by endocytic processes which trigger a proinflammatory response leading to interstitial fibrosis. These observations suggest KIM-1 as a novel drug target for prevention and treatment of DN.

Inhibition of the p66 Longevity Gene Prevents Diabetic Nephropathy in Mutant Akita Diabetic Mice  
Himanshu Vashistha, 1 Pravin C. Singhal, 1 Ashwani Malhotra, 1 Surya V. Seshan, 1 Krzysztof Reiss, 1 Leonard G. Meggs. 1

Background: Inhibition of the p66 longevity gene prevents diabetic nephropathy in mutant Akita diabetic mice. However, the mechanism underlying this has yet to be elucidated. Abnormally elevated hepatic gluconeogenesis can cause hyperglycemia and hyperinsulinemia, secondary to upregulation of key gluconeogenic genes in particular phosphoenolpyruvate carboxykinase (PCK1), leading to elevated hepatic glucose production. Methods: 11b-hydroxysteroid dehydrogenase type 1 (11HSD1) catalyses intracellular conversion of the inactive glucocorticoid cortisone to active cortisol and promotes hepatic gluconeogenesis. We investigated the role of elevated hepatic 11HSD1 in uromia-induced IR.

Results: Uromia rats displayed hyperinsulinemia and abnormal glucose and insulin tolerance. This was associated with upregulation of 11HSD1 and PCK1. Additionally, levels of the gluconeogenic coactivator PPAR co-activator 1alpha (PGC1a) were increased. Uromia was also associated with increased concentrations of plasma triglycerides, non-esterified fatty acids and cholesterol, along with elevated expression of key lipogenic genes; fatty acid synthase (FAS), acetyl CoA carboxylase (ACC), steroid-regulatory elements and protein-1c and nuclear receptor 5a-reductase. Treatment with CX for two weeks corrected hyperinsulinemia, improved glucose tolerance and insulin sensitivity and reduced expression of PCK1 and PG1a along with correction of dyslipidemia and expression of ACC, SREBP1 and FAS accordingly.

Conclusions: Our results indicate that elevated expression of 11HSD1 contributes to IR and dyslipidemia in uromia, and confirms 11HSD1 inhibitors as a novel therapeutic target for management of IR in patients with CKD.

Uromia-Induced Increases in Renal Hepatic 11bHSD1 and Associated Enhanced Gluconeogenesis in Urinary Glucoses to Prevent Diabetic Chronic Kidney Disease  
Ananda Changde, 1 Julius Edward Kiewsc, 1 Steven Michael Harwood, 2 Martin J. Raffery, 3 Magdi Yaqobi. 1

Background: Insulin resistance (IR) is common in chronic kidney disease (CKD). However the mechanism underlying this has yet to be elucidated. Abnormally elevated hepatic gluconeogenesis can cause hyperglycemia and hyperinsulinemia, secondary to upregulation of key gluconeogenic genes in particular phosphoenolpyruvate carboxykinase (PCK1), leading to elevated hepatic glucose production.

Results: We performed bone marrow transplant (BMT) from green fluorescent protein transgenic mice to C57BL/6J mice and produced diabetes by streptozotocin (STZ) or feeding a high-fat diet. Immunofluorescence staining and fluorescence in situ hybridization for Y chromosome were performed and observed by confocal laser scanning microscopy.

Methods: We performed bone marrow transplant (BMT) from green fluorescent protein transgenic mice to C57BL/6J mice and produced diabetes by streptozotocin (STZ) or feeding a high-fat diet. Immunofluorescence staining and fluorescence in situ hybridization for Y chromosome were performed and observed by confocal laser scanning microscopy.

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Results: We performed bone marrow transplant (BMT) from green fluorescent protein transgenic mice to C57BL/6J mice and produced diabetes by streptozotocin (STZ) or feeding a high-fat diet. Immunofluorescence staining and fluorescence in situ hybridization for Y chromosome were performed and observed by confocal laser scanning microscopy.
Results: Triple mutant Akita (p66-/-/Im2+/C96Y/B2R-/-) mice at 11-12 mo of age, showed a pronounced increase in mesangial area and tubular injury, with a striking reduction in urinary albumin excretion. Podocytes/glomeruli did not differ from non-diabetic wild type (wt) control mice, with minimum evidence of foot process effacement. In vitro studies, with human podocytes expressing p66shRNA show inhibition of HGF-induced ROS production, local angiotensin II generation and apoptosis.

Conclusions: We speculate p66 is a target for therapeutic intervention in glomerular diseases, where ROS have been implicated in metabolic, inflammatory and toxic injury.

Funding: NIDDK Support

TH-PO534
Macrophage Liver X Receptor α Ameliorates Renal Lesions in Hyperlipidemic Diabetic Mice

Eva Kiss, Mahnaz Bonrouhi, Katja Wagenblass, Hermann-Josef Groene. German Cancer Research Center (DKFZ) Heidelberg.

Background: Abnormal lipid metabolism and renal accumulation of lipids are pathophysiological cofactors of diabetic nephropathy. Liver X receptors (LXR) α and β are nuclear receptors activated by oxidized LDL. LXR α ligands indicate that regulated genes linked to lipid and carbohydrate homeostasis and inhibit inflammatory gene expression in macrophages.

Methods: In this study the effects of systemic and macrophage specific LXR activation were analyzed on renal damage in hyperlipidemic diabetic mice. Diabetes was induced by streptozotocin in LDLR-deficient (LDLR-/-) mice on standard chow or western diet with or without the nonselective synthetic LXR-agonist, GW3965 for 20 weeks. The role of macrophage-LXRα mediated effects were analyzed in mice with macrophage overexpression of LXRα.

Results: Hyperglycemia and hypolipidemia acted synergistically in inducing renal injury (mesangial matrix increase, podocyte damage, foam cell formation) and altering renal function. LXR activation by GW3965 inhibited significantly impairment of renal function by a 60% higher creatinine clearance, reduced glomerular and interstitial mononuclear cell infiltration (Mac-2+, F4/80, CD3) and the number of interstitial myofibroblasts (α-smooth muscle actin by a 60% higher creatinine clearance, reduced glomerular and interstitial mononuclear cell infiltration (Mac-2+, F4/80, CD3) and the number of interstitial myofibroblasts (α-smooth muscle actin

Conclusions: LXR activation attenuated diabetic and hyperlipidemic renal lesions attesting to the potent regulatory role of these nuclear receptors in metabolic diseases.

Funding: Government Support - Non-U.S.

TH-PO535
Renal Effects of Inhibition of Toll like Receptor Signaling in Type-2 Diabetic Mice

Jim Joo Cha, Young Sun Kang, Young Youl Hyun, Jung Eun Kim, Deok Hwa Nam, Hye Kyung Song, Ji Eun Lee, Hyunwoo Kim, Sungje Cho, Kyoungsoo Han, Dae R. Cha. Internal Medicine, Korea University Medical College, Ansan, Kyeonggido, Republic of Korea; Internal medicine, Wonkang University Medical College, Gupo, Kyeonggido, Republic of Korea; Internal Medicine, Inje University Medical College, Koyang, Kyeonggido, Republic of Korea.

Background: Chronic inflammation caused by high concentration of glucose and free fatty acids (FFAs) is one of the major pathogenesis of type2 DM. Recent evidences suggest that the activation of toll-like receptor(TLR) signaling, which is involved in various innate responses, induces peripheral insulin resistance and mediates central insulin and leptin resistance. Present study was performed to investigate the renal effects of TLR signaling blockade in the diabetic mouse.

Methods: Eight-week old db/db mice were treated for 12 weeks with intraperitoneal GT27, which targets the function of macrophages through inhibition of TLR4 and TLR2/6 mediated signaling pathways, at a dose of 20mg/kg/day. Another group of db/db mice were treated with control vehicle.

Results: GT27 treated db/db mice showed decreased in HbA1c at 3months, improved glomerular filtration rate, attenuation of mesangial sclerosis and tubular injury, with a striking reduction in urinary albumin excretion. Podocytes/glomeruli did not differ from non-diabetic wild type (wt) control mice, with minimum evidence of foot process effacement. In vitro studies, with human podocytes expressing p66shRNA show inhibition of HGF-induced ROS production, local angiotensin II generation and apoptosis.

Conclusions: In summary, GT27 treatment improved insulin resistance, dyslipidemia and proteinuria in type 2 diabetic mice. These results suggest TLR pathway inhibition might have a direct protective role in diabetic kidney disease.

Funding: Pharmaceutical Company Support

TH-PO536
Resveratrol Prevents Diabetic Nephropathy Via Activating the AMPK-SIRT1-PPARα Pathway in db/db Mice

Sungjin Chung, Min-Young Kim, Ji Hee Lim, Hoon Suk Park, Byung Ha Chung, Hyun Wha Chung, Seok Joon Shin, Hyung Wook Kim, Yong-Soo Kim, Yoon-Sik Chang, Cheol Whee Park. Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Background: AMPK and SIRT1 play key roles in the regulation of lipid and glucose homeostasis and for controlling oxidative stress. Many of resveratrol’s effects are consistent with the activation of SIRT1-AMPK-PPAR-α and the modulation of their targets, including PARPs and FoxOs. In this study, we sought to uncover the mechanism by which resveratrol affects diabetic nephropathy.

Methods: The db/db and db/m mice were treated with or without resveratrol starting at 8 weeks of age for 12 weeks.

Results: The db/db mice treated with resveratrol had decreased albuminuria, and resveratrol ameliorated glomerular matrix expansion compared to the control db/db mice even under the same degree of hyperglycemia. Resveratrol decreased inflammatory cell infiltration and the intrarenal free fatty acid and triglycerides contents associated with increased phospho-Thr172 AMPK-SIRT1- PPARα signaling and activation of its key downstream effectors PPARα ERR-α expressions. Furthermore, resveratrol decreased the activity of PI3K-Akt phosphorylation and FoxO3a phosphorylation, which resulted in a decrease of proapoptotic Bax and increases in the anti-apoptotic Bcl-2 and anti-oxidant SOD1 and SOD2 expressions in the db/db mice. Consequently, resveratrol reversed the diabetes-induced lipid accumulation in the kidney and the increased renal apoptotic cells and oxidative stress that is reflected by the serum 8-hydroxydeoxyguanosine (8-OHG) and urinary 8-OHG and isoprostane levels.

Conclusions: Our results suggest that resveratrol prevents diabetic nephropathy in db/db mice by activation of AMPK-SIRT1-PPARα signaling, which results in prevention of lipotoxicity, apoptosis and oxidative stress in the kidney.

TH-PO537
CCR2 Inhibition in Diabetic Mice Results in a Rapid and Robust Improvement of Renal Function


Background: Diabetic Nephropathy represents one of the major consequences of uncontrolled diabetes. The chemokine receptor CCR2 has been implicated in the recruitment of blood monocytes into kidney in response to hypertension and hyperglycemia. In addition, parenchymal renal cells are thought to upregulate CCR2 under those pathological conditions. We set out to assess the therapeutic benefit of CCR2 antagonism in a mouse model of diabetic nephropathy.

Methods: CCX417 (a small molecule CCR2 antagonist, analog of the clinical compound CCX140-B) was dosed daily to male db/db mice (age 12-19 weeks). Weekly assessments included body weight, fasting plasma glucose, serum clinical chemistry, and 24 hr urinary volume and output of albumin, creatinine and glucose.

Results: Treatment with CCX417 significantly reduced urinary albumin excretion (UAER) and albumin:creatinine ratio (ACR). Statistically significant improvements in UAER and urinary ACR were noted as early as 48 hours after initiation of CCX417 treatment. Serum markers of renal function were also improved after 14 days of CCX417 treatment: serum creatinine and blood urea nitrogen. The benefits seen on renal function preceded significantly reduced fasting plasma glucose levels. Significant reductions in urinary output were also seen with CCR2 antagonism.

Conclusions: Robust and rapid improvements of albuminuria, serum markers of renal function, and hyperglycemia were seen following pharmacological intervention with a small-molecule CCR2 antagonist in a rodent model of diabetic nephropathy. These results support the clinical evaluation of CCR2 antagonists, such as CCX4140-B, for the treatment of diabetic nephropathy.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

235A
TH-PO538

A Novel Role for Liver X Receptors in Diabetic Nephropathy. Xiaoxin Wang,1 Monika Patel,2 Hannah Danielle Santamaria,3 Weidong Wang,2 Nathaniell L. Solis,4 Liu Qiu,4 Carolyn L. Cumnings,5 Moshe Levy.1 1University of Colorado Denver; 2University of Toronto.

Background: The liver X receptor (LXR) belongs to the nuclear receptor superfamily of ligand-activated transcription factors. Oxysterols and oxidized derivatives of cholesterol are endogenous ligands for LXR. Activation of LXR induces the transcription of genes involved in reverse cholesterol transport and inflammation. We hypothesized that these properties of LXR agonists would make them useful in the modulation of diabetic nephropathy. However, certain LXR agonists also induce de novo fatty acid synthesis which may limit their efficacy. Recently, a novel LXR agonist N,N-dimethyl-3beta-hydroxy-cholesterol (DMCHCA) has been developed that does not have undesirable lipogenic effects and therefore facilitates the exploration of LXR function in the diabetic kidney.

Methods: In this study we induced type 1 diabetes in western diet fed DBA/2J mice using multiple low dose streptozotocin injections and treated mice for 10-wk after diabetes was confirmed with the LXR agonist DMCHCA at a dose of 80mg/kg body weight/day.

Results: We found that LXR agonist treatment improves proteinuria (DBA/Con: 123±26mg/mg; DBA/STZ: 286±41mg/mg; DBA/STZ/DMAHC: 137±22mg/mg; p<0.01), podocyte loss, mesangial expansion, and tubulointerstitial fibrosis. In addition LXR treatment prevented renal lipid accumulation, macrophage infiltration, inflammation, and oxidative stress. In contrast induction of diabetes in the LXR a/b double knockout mice resulted in exaggerated diabetic nephropathy characterized by increased urinary albumin and nephrin excretion, and increased inflammation and lipid accumulation.

Conclusions: These results therefore indicate a novel and an important role for LXR in modulation of diabetic nephropathy.

TH-PO539

Effect of Physical Exercise on Urinary Albumin and N-acetyl-beta-glucosaminidase Activity and Podocyte Numbers in Diabetic KK-A’- Mice Yuji Ishikawa, Tomohito Gohda, Mitsuo Tanimoto, Keisuke Omote, Saori Yamaguchi, Masako Furukawa, Maki Murakoshi, Shinji Hagisawa, Yasuhiro Tomino. Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan.

Background: Exercise is generally recommended for the management of type 2 diabetes, but its effects on diabetic nephropathy are still unknown. We hypothesized that appropriate physical exercise improves early diabetic nephropathy via attenuation of chronic inflammation and oxidative stress.

Methods: Type 2 diabetic KK-A’- mice, a spontaneous diabetic nephropathy model, underwent different kinds of exercise for one hour five days a week (moderate intensity) and 30 minutes three days a week (low intensity) for 8 weeks. Sedentary mice underwent two different kinds of exercise for one hour five days a week (moderate-intensity) and 30 minutes three days a week (low intensity) for 8 weeks. Sedentary mice or those undergoing exercise regimens causing no significant body weight loss were used. We examined the urinary excretion of albumin and N-acetyl-beta-glucosaminidase (NAG), number of podocytes in glomeruli, renal expressions of hypoxia inducible factor (HIF)-1α and monocyte chemoattractant protein (MCP)-1, and biomarkers of oxidative stress such as urinary 8-hydroxydeoxyguanosine (8-OHdG) and serum superoxide dismutase (SOD).

Results: Exercise reduced urinary albumin excretion and NAG, and also maintained the number of podocytes in the exercised KK-A’- mice independently of improvements of body weight and hyperglycemic status. However, the moderate-intensity exercise increased expression of HIF-1α in the kidneys. Sedentary KK-A’- mice showed increased expression of MCP-1 in the tubules, urinary 8-OHdG levels, and decreased serum SOD levels compared with exercised KK-A’- mice.

Conclusions: On the whole, low-intensity exercise attenuates progression of early diabetic nephropathy via decreasing renal inflammation, improving albumin excretion, changing NAG and maintained podocyte numbers, with parallel improvements in oxidative damage and chronic inflammation are related to the beneficial effects of exercise in diabetic kidney disease.

TH-PO540

Fractalkine/CX3CR1 Mediates Extracellular Matrix Accumulation in Diabetic Kidney Kyung Hee Song, Jebyun Park, Hunjo Ho. Department of Biopsied Science, College of Pharmacy, Ewha Womans University, Seoul, Republic of Korea.

Background: Fractalkine (FKN, CX3CL1) functions not only as a chemokine but also as an adhesion molecule and plays an important role in the recruitment of macrophages into the kidney through binding to its receptor CX3CR1. Previous studies have demonstrated that FKN/CX3CR1 play a role in ischemic and protein-overload renal injury. However, their role in diabetic renal injury and the mechanism involved in have not been clearly understood. This study examined whether FKN/CX3CR1 mediates diabetic stimuli-induced extracellular matrix accumulation in the kidney.

Methods: Streptozotocin (STZ; 50 mg/kg/day) was intraperitoneally administered for 5 days in male C57BL/6 male mice. Mouse mesangial cells (MMCs) transfected with siRNA for CX3CR1 were used to further elucidate the direct effects of FKN/CX3CR1 mRNA and protein expression on ECM synthesis.

Results: At 12 weeks after the induction of diabetes, equivalent hyperglycemia was observed in diabetic WT and CX3CR1 null mice. However, parameters of diabetic renal injury including increased plasma creatinine, kidney to body weight ratio, glomerular volume, fractional mesangial area, urinary protein excretion, and accumulation of renal fibronectin, collagen, and macrophage were much more severe in diabetic CX3CR1 null mice compared with diabetic WT mice. In MMCs, 30 mM high D-glucose, 100 µM oleic acid, 10 ng/ml of transforming growth factor-β1 upregulated FKN and CX3CR1 mRNA and protein expression along with ECM expression, which was significantly attenuated by CX3CR1 siRNA. More importantly, FKN itself increased ECM synthesis in MMCs, and CX3CR1 siRNA abrogated FKN-induced ECM synthesis.

Conclusions: These results demonstrated that FKN/CX3CR1 may play an important role in diabetic renal injury through ECM upregulation, apart from macrophage infiltration, and suggest that FKN/CX3CR1 system may become an effective therapeutic target for the prevention of diabetic renal injury.

TH-PO541

Resveratrol Attenuates Diabetic Nephropathy by Modulating Angiogenic Factors Donghai Wen, Xinzhong Huang, Chuan-Ming Hao. Division of Nephrology, Huashan Hospital, Fudan University, Shanghai, China.

Background: Angiogenesis plays an important role in the pathogenesis and progression of diabetic nephropathy (DN). In the present study, we investigated the therapeutic potential of resveratrol, a natural polyphenol with anti-angiogenic activity, in DN.

Methods: In a type 1 diabetic rat model induced by streptozocin, urinary albumin excretion (13.81±1.25 vs 15.33±0.38 mg/24h), kidney weight (2.26±0.11 vs 1.94±0.04 g) and creatinine clearance rate (0.32±0.05 vs 0.09±0.02 ml/min/100 g body weight) were increased, and were suppressed by eight weeks treatment of resveratrol (6.55±0.72 vs 4.2±0.91; p=0.07 vs 0.18±0.03 ml/min/100 g body weight, respectively). Morphological studies revealed increased glomerular diameter, mesangium expansion, and glomerular basement membrane thickness in diabetic rats (121±14.05 vs 158±33±6.69μm), which were also reduced by resveratrol treatment (179±67±7.64μm). In addition, treatment of the diabetic rats with resveratrol significantly alleviated renal fibrosis detected by increased expression of fibronectin, PAI-1, CTGF, type IV collagen and TGF-β1. In the diabetic kidney, increased expression of VEGF, Flk-1 and angiopoietin 2, and reduced expression of Tie-2 were observed. These changes in angiogenic growth hormones and their associated receptors were attenuated by resveratrol treatment. No change in angiopoietin 1 expression was detected among each group of rats. In vitro, resveratrol significantly down-regulated high glucose-induced VEGF and Flk-1 expression in cultured mouse glomerular podocytes and endothelial cells, respectively. However, these effects of resveratrol were blocked by knocking-down silent information regulator 1 (Sir1) using RNA interference, consistent with a role of Sir1 in mediating the effect of resveratrol. On the other hand, up-regulation of Sir1 in cultured endothelial cells by transfecting the cells with Sir1 plasmid reduced Flk-1 expression. Increased permeability and cellular junction disruption of cultured endothelial cells caused by VEGF were also inhibited by pretreatment with resveratrol.

Conclusions: Resveratrol may attenuate DN via modulating the angiogenic system.

Funding: Government Support - Non-U.S.

TH-PO542

mTOR Regulates Nox4-Mediated Podocyte Loss in Diabetic Renal Injury Assaad Antoine Eid, Bridget M. Ford, Jeffrey L. Barnes, Yves C. Gorin, Goutam Ghosh-Choudhury, Hanna E. Abboud. Medicine/Nephrology, University of Texas Health Science Center at San Antonio, TX.

Background: Glomerular podocyte apoptosis represents a critical mechanism for excessive loss of urinary albumin that eventuates in kidney fibrosis. Pharmacological doses of the mTOR inhibitor rapamycin reduce albuminuria in diabetes by unknown mechanism.

Methods: We explored the hypothesis that mTOR mediates podocyte injury in diabetes.

Results: High glucose (HG) induces apoptosis of cultured podocytes and increases the levels of Nox4 and NADPH oxidase activity. HG also inhibits the phosphorylation of AMPK on the activating site Thr172, increases the phosphorylation of tuberin on its inactivating sites Thr192, HG also activates mTOR and enhances the phosphorylation of its substrate S6 kinase. Inhibition of mTOR by low doses of rapamycin prevents HG–induced expression of Nox4, NADPH oxidase activity and podocyte apoptosis. Inhibition of mTOR had no effect on AMPK or tuberin phosphorylation indicating that mTOR is downstream of these two signaling molecules. In isolated glomeruli of OVE26 type 1 diabetic mice, there is similar decrease in the phosphorylation/activation of RAF of urinary albumin change and urinary NAG, and maintained podocyte numbers, with parallel improvements in oxidative damage and chronic inflammation are related to the beneficial effects of exercise in diabetic kidney disease.

Conclusions: Our data provide evidence for understanding a novel function of mTOR in Nox4-derived ROS generation and podocyte apoptosis that contribute to urinary albumin excretion in type 1 diabetes. Thus mTOR inhibition may represent a therapeutic modality of diabetic kidney disease.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Increased Plasma Glucose and Reduced Albuminuria in AMP-Activated Protein Kinase (AMPK) Null Mice with Streptozotocin-Induced Type 1 Diabetes

Type 1 diabetes was induced by 5 i.p. injections of streptozotocin (STZ). Mice were sacrificed at 4 weeks post-injection.

Results: There was a significant increase in plasma glucose in AMPK−/− mice at days 28 compared with WT controls (e.g. day 28, 31.1±8.0 vs 20.8±8.3 mmol/L, p<0.05). Insulin concentrations were similar in both groups. Plasma adiponectin was reduced in AMPK−/− mice compared with WT (p=0.05), but was similar in the diabetic mice groups. Despite the difference in plasma glucose, diabetic AMPK−/− mice had less albumin excretion than WT at 28 days (ACR 64.7±11.8 vs 34.2±9.5 mg/mmol, p<0.001). Creatinine clearance was unchanged. Kidneys from diabetic AMPK−/− mice also had less α-smooth muscle actin accumulation than diabetic wild type controls by Western blot (p=0.01) but no difference in TGF-β1 mRNA expression. Administration of the AMPK activator metformin 600 mg/kg to diabetic C57Bl/6 mice for the last 7 days of the STZ model was associated with significantly increased albumin excretion (ACR 63.4±20.6 vs 94.8±26.4 mg/mmol, p<0.05) but no change in plasma glucose.

Conclusions: These studies confirm that AMPK contributes to glucose homeostasis independent of any effect on insulin signaling. Within the kidney, reduced AMPK signaling was associated with less albuminuria and markers of fibrosis. The data suggest that anti-diabetic drugs which activate AMPK, such as metformin and the glitazones, may have deleterious effects on kidney survival in diabetes.

Funding: Government Support - Non-U.S.

Angiotensin II Type II Receptor (AT2R) Deficiency Accelerates the Development of Nephropathy in Type 1 Diabetes Via Oxidative Stress and ACE2

Angiotensin II (Ang II) binds to two distinct subtypes of the AT1 receptor, AT1A and AT1B. AT1A receptor activation mediates most of the harmful effects of Ang II, whereas AT1B receptor activation has been suggested to protect the kidney from injury. However, the role of AT1B receptor in the development of diabetic nephropathy remains unclear.

Methods: We induced diabetes with low-dose streptozotocin (STZ) in both ATA and AT2RKO mice and wild type (WT) male mice aged 12 weeks and followed them for 4 weeks. Three subgroups [non-diabetic, diabetic and insulin-treated diabetic (Rx insulin)] of each type (WT) mice by injection of STZ. Animals injected with citrate buffer only served as controls. At 14 weeks, blood and urine samples were collected and mice were sacrificed to obtain tissues for histological study.

Results: In contrast to animals only injected with citrate buffer, mice that received STZ developed hyperglycemia. No differences in the degree of hyperglycemia and blood pressure were seen between diabetic KO and WT mice. Albuminuria and mean glomerular volume were significantly increased in diabetic KO mice compared to diabetic WT mice. Lack of endostatin led to increased macrophage accumulation, expression of MCP-1 and TNF-α, but not of VEGF, and number of CD31+ blood vessels in the kidney of diabetic mice. CD31/CD34+ cells were also observed in the glomeruli from diabetic KO mice.

Conclusions: Physiological levels of endostatin can slow the progression of diabetic nephropathy in vivo, but attenuated in insulin-treated diabetic WT and AT2RKO mice. The renal changes noted above were significantly enhanced in diabetic AT2RKO mice, but attenuated in insulin-treated diabetic WT and AT2RKO mice. The renal changes noted above were significantly increased in diabetic AT2RKO mice, but partially attenuated in the insulin-treated diabetic WT and AT2RKO mice.

Funding: Pharmaceutical Company Support, Clinical Research Support
The Protective Role of miR-29 in Diabetic Kidney Disease: Mechanism and Therapeutic Potential
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Background: TGF-β/Smad signaling plays a vital role in renal fibrosis including diabetic nephropathy (DN). However, general blockade of TGF-β signaling may promote renal inflammation. Thus, identification of TGF-β/Smad3-dependent miRNAs related to fibrosis may be the key step towards to develop specific therapy for DN. We have previously shown that miR-29 is TGF-β/Smad3-dependent miRNA in renal fibrosis, thus, we further demonstrated the role of miR-29 for diabetic kidney disease.

Methods: miR-29 expression and diabetic kidney injury was examined in db/db mice. The therapeutic effect of miR-29 on diabetic kidney disease was determined by delivering a Dox-inducible miR-29 to 10-week db/db mice using an ultrasound-microbubble-mediated technique. The regulating mechanisms of miR-29 under diabetic conditions was investigated in tubular cells lacking Smad2 or Smad3 and in a stable mesangial cell line with Dox-inducible miR-29 overexpression and knockdown.

Results: In db/db mice, miR-29 was reduced by 60% over the 20 week time, which was associated with a marked increase in TGF-β/Smad3 signaling, renal fibrosis (collagen I, IV), and microalbuminuria (all p<0.01). Ultrasound-mediated gene therapy restored normal levels of miR-29 in db/db mice, resulting in inhibition of diabetic kidney injury as described above (all p<0.01). In vitro, addition of AGES induced a loss of miR-29 (60%+4%) and increased collagen I & IV (p<0.01), which was further significantly enhanced in miR-29 knockdown mesangial cells, but blocked in cells overexpressing miR-29. Moreover, we found that AGES-downregulated miR-29 expression was Smad3-dependent, but not Smad2.

Conclusions: miR-29 is lost in the diabetic kidney of db/db mice and is mediated by TGF-β/Smad3. Overexpression of miR-29 is able to attenuate diabetic kidney disease in db/db mice and in vitro under diabetic conditions. Thus, miR-29 may represent a novel therapy for diabetic kidney complication.

Funding: Government Support - Non-U.S.

TH-PO548
Spironolactone Diminishes Urinary Albumin Excretion in Type 1 Diabetic Patients with Microalbuminuria: A Randomized Placebo-Controlled Clinical Trial
Takeshi Futami,1 Katrine A. Pedersen,2 Dietmar Walter Zdunek,2 Anthony Carter,2 Chris R. Kennedy.2

Background: Recent clinical and animal studies have reported high glucose media as contributing to direct podocyte injury and/or abnormal podocyte niche progression of renal impairment in diabetic nephropathy (DN). However, the direct role of macrophages and/or macrophage/podocyte interaction in DN is not known. We hypothesize that macrophages contribute to direct podocyte injury and/or abnormal podocyte niche leading to DN.

Methods: Experiments were conducted in CD11b-DTR mice treated with diphtheria (DT) toxin (VEH) or vehicular (DT) following streptozotocin (STZ) induced diabetes.

Results: We first established a dose, route and time response curves of DT in CD11b-DTR and mice and we were able to chronically deplete kidney macrophages but not B cells, T cells or neutrophils. We further demonstrated that macrophage depletion using DT in diabetic CD11b-DTR mice significantly attenuated diabetic albuminuria to normal range (5-fold reduction; p<0.05) compared to diabetic CD11b-DTR mice treated with vehicle despite comparable blood glucose levels after 6 weeks of diabetes.

Vehicle-treated diabetic CD11b-DTR mice showed significant increases in kidney macrophages (p<0.05) compared with control mice using Mac-2 staining and flow cytometry and was associated with an early phase of increased M1/M2 macrophage populations followed by a late phase of increased M2/M1 macrophages. In contrast, kidney macrophages in DT-treated diabetic CD11b-DTR mice were similar to control mice.

In vitro, we demonstrate that podocytes grown on high glucose media are associated with significant (p<0.005) in macrophage migration from those grown in normal glucose media using transwell migration assay and this effect was completely blocked with the addition of anti-MCP-1 antibody. In addition, classically activated M1 macrophages (CD11b+LY6C+LY6G Tnf-α+Il1β+Ilcd+260+), but not alternatively activated M2 macrophages (CD11b+LY6C+LY6G Tnf-α+Il1β-Il6+Il10+260+) induced podocyte plasticity and cell death in vitro.

Conclusions: These findings provide evidence that macrophages directly induce diabetic renal injury; mainly by altering podocyte permeability through the pro-inflammatory M1 but not the anti-inflammatory M2 subsets.

Funding: NIDDK Support

TH-PO550
Fenofibrate Ameliorates Diabetic Nephropathy Via Inhibiting the Canonic Wnt Pathway
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Background: One such receptor—the PGE2 EP1 receptor, contributes to the pathogenesis of albuminuria in DN.

Methods: To test this hypothesis, gene-targeted EP1-null male mice (Ep1−/−), on an FVB background, were co-treated with high glucose and different concentrations of 1 diabetes (5 day i.p., 50 mg/kg bw; Na-citrate as vehicle) and studied for 16 weeks. Blood pressures were obtained by tail cuff plethysmography while glomerular filtration rate was measured by FITC-imulin clearance. Albuminuria was determined by ELISA of 24 h urine collections. Kidney cortical LRP6 and cubilin expression were assessed by immunoblot and immunohistochemistry.

Results: The onset of diabetes had no impact on systolic blood pressure, yet both WT-stz and Ep1−/−stz groups became equivalently hyperglycemic (>35 mM) and were hyperfiltrating in a similar degree to the non-diabetic groups (WT-stz, 26.1 ± 2.3 mL/min/1.73 m2 vs. WT, 10.3 ± 2.3 mL/min/1.73 m2, p>0.2 for all comparisons), and GFR decreased from 78(8) to 72(6) mL/min/1.73 m2 placebo (p=0.01). Blood pressure (24h) did not change during spironolactone treatment but not Smad2.

Background: Recent clinical and animal studies have reported that Fenofibrate, a pleiotropic agent, and the PPARα agonist, might play a role in diabetic nephropathy. However, the underlying mechanism is not clear. Recently, we have demonstrated that alteration of the canonical Wnt pathway plays a role in diabetic nephropathy and nephropathy. The objective of the present study is to investigate whether Fenofibrate ameliorates diabetic nephropathy by inhibiting the canonical Wnt pathway.

Methods: Diabetes was induced in rats by one single intraperitoneal injection of Streptozotocin. Diabetic rats were fed with standard chow diet or chow diet containing Fenofibrate. Renal function was evaluated by measuring the urine albumin level and levels of collagenIV, ICAM-1, Tnf-α and CTGF. Primary human renal proximal tubular epithelial cells (HRPTCs) were cultured with high glucose and different concentrations of Fenofibrate. The levels of LRPs, a key co-receptor of Wnt ligands, β-catenin, an essential effector of the Wnt pathway, and CTGF, a target gene of the Wnt pathway, were determined by Western blot analysis.

Results: One such receptor—the PGE2 EP1 receptor, contributes to the pathogenesis of albuminuria in DN.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.
Results: Fenofibrate treatment reduced albuminuria, decreased collagen IV accumulation, diminished ROS and TxnIP over expression in the kidney of db/db mice (all p<0.05). Without affecting blood glucose and blood pressure, EOCs not only attenuated both mesangial and peritubular matrix expansion, as well as tubular apoptosis, but also effective. Effective in normal controls. In contrast, urinary TIMP-1 mRNA was significantly decreased in DN samples. In addition, oral anti-CD3 antibody treatment has a significant decrease in urine protein excretion and the preservation of renal histologic changes in the treated animals.

Conclusions: Thus, our results suggest that oral anti-CD3 antibody treatment has a protective role in progression of diabetic kidney disease possibly via regulatory T cell modulation.

TH-PO556
Early Kidney Response to Diabetes Mellitus in Mice Lacking Slt2a

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
thereby lowering blood glucose levels. We studied gene-targeted mice lacking Sglt2 (−/−) to elucidate the role where SGLT2-mediated glucose reabsorption and to glomerular hyperfiltration by lowering NaCl delivery to the macula densa.

Methods: Diabetes was induced by low-dose streptozocin (STZ: 50 mg/kg ip on 5 days). GFR was determined by FITC-imulin kinetics in awake mice.

Results: In STZ-induced diabetic mice, Sglt2−/− mice have significantly decreased in the glomeruli of db/db:ROCK1−/− compared with WT mice consistent with lower renal glucose reabsorption in the absence of SGLT2. Induction of STZ-diabetes resulted in similar plasma concentrations of Na+ , K+ , and aldosterone, and a modestly greater hematocrit in Sglt2−/− vs. WT (45±3.0 vs. 42.3±0.5 %; n=9–10; P<0.05). The STZ-induced increase in GFR observed in WT (291±11 (in non-diabetics) to 385±8 (n=15, P<0.01) and WT (344/6 to 397±15 mg, n=12; P<0.01).

Conclusions: We conclude that lack of SGLT2 lowers blood glucose levels in early STZ-diabetes mellitus and prevents glomerular hyperfiltration but not the increase in kidney weight.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO557
The ZDSD Rat, a Translational Model of Obesity, Metabolic Syndrome and Diabetes That Expresses the Characteristics of Human Diabetic Nephropathy
Richard G. Peterson, 1 Karen M. Zimmerman, 2 Vincent H. Gattoone. 3 PreClinOmics, Indianapolis, IN; 4Anatomy, Indiana University School of Medicine, Indianapolis, IN.

Background: Most previously available rodent models of type II diabetes have leptin/leptin receptor mutations. The ZDSD rat was selectively inbred over a period of 10 years to be a model of type 2 diabetes without these defects. This model is also an excellent model of pre-diabetes/metabolic syndrome.

Methods: The ZDSD rat was developed by crossing lean ZDF rats to polygenic obese CD rats. Selective inbreeding has resulted in a consistent, translational model of metabolic syndrome and type 2 diabetes.

Results: During the pre-diabetic state, ZDSD male rats are obese with: hypertension, glucose intolerance, insulin resistance, hyperlipidemia, hyperglycemia, elevated glycated Hb and increased cardiovascular and inflammatory biomarkers. In the overtly diabetic state, the model exhibits complications very much like the human condition such as delayed wound healing, osteoporosis and nephropathy. Findings include increased kidney biomarkers: urinary: albumin, beta-2 microglobulin, cystatin-C, KIM-1, clusterin, osteopontin, GSTY b-1 and RPA-1; and serum: NGAL, beta-2 microglobulin, GST alpha, and von Willebrand factor. Morphologically, light and electron (transmission and scanning) microscopy revealed mesangial expansion with sclerosis (Kimmelstiel-Wilson-like nodules), thickened glomerular basement membranes, podocyte effacement and glomerulosclerosis. These markers and conditions become discernable within reasonable times after diabetes develops.

By 8-12 weeks of diabetes there are significant elevations in most of the kidney biomarkers and conditions become discernable within reasonable times after diabetes develops.

Funding: NIDDK Support

TH-PO560
The Effect of Oral Anti-CD3 Antibody Treatment on High Fat Induced Obesity and Nephropathy
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Background: Cellular sodium and water transport are dysregulated in diabetes mellitus. We have demonstrated that EGFr regulates high glucose-induced Na reabsorption via the sodium-hydrogen exchanger-3 (NHE3) in human proximal tubule cells (PTCs). PPARγ agonists, such as Pioglitazone, are used in patients with diabetes, but their use is limited by fluid retention. We have shown that PPARγ agonist induces NHE3 and the water channel AQP1 in PTCs. The role of EGFr in PPARγ agonists mediated increase of sodium and water retention in the proximal tubule are not known.

Methods: PTCs were exposed to 5mM, 25mM glucose (HG) or 3µM Pioglitazone. P-EGFr, NHE3, PPARγ and AQP1 protein expression were measured by Western blot. The specificity of EGFr fluorescence was elucidated using the EGFr tyrosine kinase inhibitor PKI166. The specific role of PPARγ in HG mediated increase of P-EGFr, NHE3 and AQP1 was determined by effectively silencing PPARγ using siRNA technique. The expression of P-EGFr and P-PPARγ was determined in diabetic ren2 rats with and without pioglitazone. The interaction between PPARγ and EGFr was determined by CHIP assay and the effect of TZDs on EGFr activation by luciferase assay.

Results: Exposure of PTCs to both HG and pioglitazone increased protein expression of P-EGFr, NHE3, AQP1 and PPARγ (P<0.05). EGFr and PPARγ are increased in diabetic rats and the increase is increased in the presence of pioglitazone. HG induced increase of NHE3 and AQP1 was abolished with PKI166 (P<0.05). HG induced increase of P-EGFr, NHE3 and AQP1 was significantly decreased with pioglitazone siRNA (P<0.05). Pioglitazone induced PPARγ binding to EGFr promoter and subsequent downstream activation.

Conclusions: Our data suggest a role of EGFr in mediating PPARγ induced Na reabosption via NHE3 and water reabosption via AQP1 channels in the proximal tubule. This suggest that EGFr inhibition may have the potential to direct future therapeutic targets in DN, as well as limiting salt and water retention which currently restricts the use of PPARγ agonists.

Funding: Government Support - Non-U.S.
Peroxisomal Dysfunction Mediates Renal Injury in Diabetic Catalase Null Mice

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Background: Overproduction of reactive oxygen species (ROS) under hyperglycemia plays an important role in diabetic vascular complications, including diabetic nephropathy (DN). Plasma free fatty acid (FFA) as well as glucose is increased under the diabetic milieu and peroxisome and mitochondria participate in cellular FFA oxidation; therefore, we investigated whether deficiency of catalase, a major antioxidant enzyme in peroxisome, determines accelerated diabetic renal injury through peroxisomal dysfunction and abnormal renal FFA metabolism associated with increased ROS.

Methods: Experimental diabetes was induced by multiple injections of low dose streptozotocin into catalase knock-out (CKO) and wild-type (WT) C57BL/6 mice. Mesangial cells transfected with siRNA for catalase were used to further elucidate the role of endogenous catalase in peroxisomal and mitochondrial function regulating FFA oxidation.

Results: At four weeks after the induction of diabetes, equivalent high plasma glucose and FFA levels were observed in diabetic WT and diabetic CKO mice. However, parameters of DN including urinary albumin excretion, glomerular hypertrophy, and accumulation of extracellular matrix (ECM) proteins along with markers of oxidative stress were much more severe in diabetic CKO mice than diabetic WT mice. Catalase deficiency in CKO mice and mesangial cells induced defects in peroxisomal and mitochondrial biogenesis and FFA oxidation leading to renal lipid accumulation. Catalase deficiency in mesangial cells also increased mitochondrial ROS.

Conclusions: The present data provide unprecedented evidence that FFA-induced peroxisomal dysfunction exacerbates diabetic renal injury, and that endogenous catalase is an important antioxidant that protects the kidney through peroxisomal and mitochondrial functions under diabetic stress.

TH-PO562

Deficiency of Matrix Metalloproteinase-2 Accelerated Renal Injury in Streptozotocin-Induced Diabetic Mice

Nana Ohara, Kei Fukami, Sho-Ichi Yamagishi, Yoshimi Takamjya, Yusuke Kaida, Seiji Ueda, Seiya Okuda.

1Division of Nephrology, Department of Medicine, Kurume University School of Medicine, Kurume, Fukuoka, Japan; 2Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Fukuoka, Japan.

Background: Matrix metalloproteinase-2 (MMP-2) is one of the potent metalloproteinases. Accumulation of ECM in the late stage diabetic kidney are, at least in part, by down-regulation of MMP-2. However, whether MMP-2 could affect in early stage of diabetic nephropathy has not been elucidated. Therefore, we investigated the effects of MMP-2 deficiency on renal injury in early stage of diabetic mice.

Methods: Diabetes was induced by streptozotocin (STZ) (50mg/kg) in male MMP-2 knockout mice (MMP-2 KO) and C57BL/6 mice (Ctrl). After 16 weeks, cortical MMP-2 expression was determined using immunohistochemistry and real-time PCR. Control and MMP-2 knockout animals were used to determine renal FFA oxidation and accumulation of extracellular matrix (ECM) proteins along with markers of oxidative stress.

Results: Plasma levels of glucose and HbA1c were increased by about 2.3 folds in 16 week diabetic (DM) mice compared with non-DM Ctrl mice (plasma glucose; 490.3±7.5 mg/dl, HbA1c 9.91±0.22 % in 16 week diabetic mice). MMP-2 expression and activity in diabetic kidney cortex of diabetic mice were significantly increased. Serum levels of BUN and creatinine (Cr) were significantly increased in the diabetic mice. MMP-2 KO DM mice showed decreased renal injury as evaluated by PAS and Masson-trichrome stain, respectively.

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Conclusions: Deficiency of MMP-2 accelerated renal injury in early stage of diabetic kidney in the streptozotocin-induced diabetic mouse model. MMP-2 KO DM mice showed decreased renal injury as evaluated by PAS and Masson-trichrome stain, respectively.

TH-PO563

VEGF165b Modifies Glomerular Permeability and Slows Progression of Albuminuria in Animal Models of Diabetic Nephropathy


1Microvascular Research Laboratories, University of Bristol, United Kingdom; 2Academic Renal Unit, University of Bristol, United Kingdom.

Background: VEGF165b, an alternatively-spliced isoform of VEGF, decreases glomerular permeability to water (LpA/Vi) when directly administered to isolated intact glomeruli ex vivo, or transgenically over-expressed in podocytes in vivo [1]. We investigated whether VEGF165b decreases both glomerular permeability to water and albuminuria in animal models of diabetic nephropathy.

Methods: All animals were made diabetic using standard streptozotocin protocols. Glomerular permeability to water (LpA/Vi) was determined as previously described (Salmon et al, J Physiol 2006). uACr ratio was determined from spot urine collection. (p<0.05 vs control animals; p<0.05 vs diabetic WT animals; p<0.05 vs sham-treated diabetic animals; all ANOVA)

Results: VEGF165b increased albuminuria (2-fold)* six days after diabetes induction with streptozotocin (STZ) (single 45mg/kg injection i.p.). Elevated glomerular permeability to water in diabetic glomeruli (1.7-fold vs sham*) was blocked by 1hr incubation in 1nM VEGF165b (0.9-fold vs sham#).

Conclusions: These results support the hypothesis that in diabetes obesity the kidney is an active participant in complement mediated renal injury by upregulating key complement genes. These critical injury pathways, acting in situ, may amplify inflammation and contribute to chronic renal injury.

TH-PO564

Activation of Renal Genes Encoding Complement Components in Diabetic Nephropathy: Implications for Hypothesis Generation By RNAseq Discovery

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Background: We have demonstrated accelerated progression of nephropathy in obese, diabetic Zs (F1 hybrids of Zucker and spontaneously hypertensive heart failure) rats following a single episode of renal ischemia.

Methods: We now examined the nature of renal inflammation in diabetic nephropathy using whole transcriptome RNA sequencing to evaluate transcript expression in three groups: lean, obese-diabetic/sham surgery, obese-diabetic/postischemia (2 weeks).

Results: The nephropathy in this model is characterized by increased serum creatinine (1.8 ± 0.1 in obese-diabetic/postischemia vs 0.4 ± 0.4 mg/dl in lean rats), proteinuria, and increased renal inflammation with fibrosis. An average of 43M (50 nt) reads were detected from each sample and the total number of unique and validated gene transcripts was 16536. Components of the classical (C1q, C2, C3, C4) complement pathway were significantly increased, 1.5 to 2.5 fold, in the obese-diabetic/sham group when compared to the lean group. The alternate complement component 6 was increased 4 fold in the obese-diabetic/ sham group. Expression was further increased in the obese-diabetic/postischemia group: 2-5 fold for components of the classical pathway and 8.8 fold for C6. In addition, regulators of the complement system were significantly downregulated in the diabetic kidneys. These include CDS5, “decay accelerating factor,” and CD59 which inhibits the membrane attack complex. Complement receptors were increased (for example 42 fold increase in CR2 in obese-diabetic/sham group compared to lean) consistent with the inflammatory phenotype observed. Although complement components have long been described in diabetic nephropathy, this comprehensive gene expression analysis has been very illuminating.

Conclusions: These results support the hypothesis that in diabetes obesity the kidney is an active participant in complement mediated renal injury by upregulating key complement genes. These critical injury pathways, acting in situ, may amplify inflammation and contribute to chronic renal injury.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support

TH-PO565

Systems Genomics Studies Implicate Xanthine Oxidase in Diabetes-Induced Podocyte Depletion

Haiping Qi, Ilse S. Duenh, Erwin P. Bottinger. Medicine, Mount Sinai School of Medicine, New York, NY.

Background: Diabetic nephropathy (DN) risk in diabetics is controlled by genetic susceptibility. Podocyte depletion is a hallmark of experimental murine and human DN. Inbred C57BL/6(B6) are resistant and DBA/2(D2) are susceptible to diabetes-induced glomerular lesions [Qi, et al. Diabetes,2005],including podocyte depletion [Qi H, et al. ASN 2009].

Methods: Bkd recombinant inbred strains and parental B6 and D2 strains were made diabetic using streptozotocin (STZ) low-dose protocol and analyzed for QTL mapping using GeneNetworks tools. In a 2nd experiment, diabetes was induced in 8 week-old D2 mice in the absence or presence of xanthine oxidase inhibitor(XO) allpurinol,administered with mouse chow.

Results: The QTL mapping study identified two loci on Chr 13 and Chr 17, respectively, that were significantly associated with diabetes-induced podocyte depletion(DIP). Xanthine dehydrogenase(Xdh) gene was localized within the peak of Chr 17 QTL. Xdh gene expression was strongly increased by diabetes in glomeruli of D2, but not B6. Xdh and xanthine oxidase(XO) are interchangeable forms of the same enzyme encoded by the Xdh gene. Circulating XO activities were not different between diabetic B6 and diabetic B2 and D2 mice, but significantly(65%) elevated in diabetic D2 mice. Thus, Xdh is a candidate gene for DN susceptibility in D2 mice. Next, we treated non-diabetic and diabetic D2(D2T) with XO. XOHad no effect on time of onset and levels of hyperglycemia.
at 6 or 12 wk of diabetes. XOI mediated significant protection against diabetes-induced albuminuria in D2 mice. The podocyte number per glomerular section and ACR ratio

<table>
<thead>
<tr>
<th>N=5/group</th>
<th>Podocytes/glom Section</th>
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<th>ACR (mg/mg)</th>
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<tbody>
<tr>
<td>D2 ctr</td>
<td>11.4±0.81</td>
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<td>11.16±0.17</td>
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*compared with D2 ctr, compared with D2 STZ, p<0.05

Conclusions: We propose that XDH/XO may mediate DN susceptibility. Future studies will test XDH locus variants in human DN and evaluate the therapeutic/preventive potential for XOI in human DN.

Funding: NIDDK Support

TH-PO566

Metallothionein Overexpression May Underlie Tubular Injury in Type 2 Diabetic Nephropathy Yumi Takivama, Nanami Kobayashi, Yukihiko Fuyuta, Yasutaka Takeda, Jun Honjo, Tsuyoshi Yanagimachi, Hiroshi Kusumoto, Hisakatsu Sakagami, Yuichi Makino, Masakazu Haneda. Department of Medicine, Asahikawa Medical University, Asahikawa, Hokkaido, Japan.

Background: Oxidative stress is thought to play an important role in the onset and progression of diabetic nephropathy (DN). On the other hand, chronic hypoxia and TGF-β1 contribute to the development of renal fibrosis in DN.

Methods: For investigation of whether other key factors contribute to the pathogenic mechanism of DN, we analyzed microarray data using Affymetrix GeneChip (Rat Gene 2.0 ST Array) with microdissected juxamedullary proximal tubules in a rat model of type 2 diabetes, Zucker Diabetic Fatty (ZDF) rats.

Results: A total of 27,342 transcripts were analyzed, among them, 47 were upregulated (>2-fold) in the ZDF rat compared with the lean control rat. One of them which were upregulated in diabetic renal tubules was metallothionein (MT)-1. MT is a cysteine-rich protein with low molecular weight, and act as an antioxidant against the toxicity of metals, and is associated with the significant serial alterations of renal tubular MT expression in response to hypoxia, insulin or profibrotic TGF-β1, and that enhanced expression of MT may be an early pathogenic event reflecting abnormalities of antioxidant systems in DN.

Funding: Japan Society for the Promotion of Science

TH-PO567

SGLT1 Can Reabsorb 70% of the Filtered Glucose Load in Mice Lacking SGLT2 David Powell, Christopher M. Davacosta, Robert Read, Brian Zambrowicz, Melanie K. Shadom. Lexicon Pharmaceuticals, Inc, The Woodlands, TX.

Background: SGLT2 (SZ) reportedly reabsorbs 90% of the filtered glucose (G) load. Inhibiting S2 to increase urinary G excretion (UGE) is a promising approach for improving glycemic control in diabetic patients, but clinical trials suggest that SGLT inhibitors highly selective for S2 over S1 (SGLT1) block G reabsorption by only 16-50%. We studied the role of S1 in renal G reabsorption in the absence of S2.

Methods: We generated S1 knockout (KO), S2 KO, S1 & S2 double KO (DKO), and wildtype littermate (WT) mice. Mice fed a high fat diet with fructose as the only carbohydrate (G-free diet) had 24 hr UGE measured; S2 KO and DKO mice were studied with an oral G tolerance test (oGTT), or were fed G-containing diet, exposed to vehicle (Veh) or streptozotocin (STZ, to induce diabetes), and then followed for blood G and HbA1c levels.

Results: All mice appeared healthy on G-free diet. Table 1 shows male UGE data; WT (336 ± 89, n=22) relative to WT (442 ± 112, n=21) mice (p<0.01); females showed the same pattern. All data are presented as mean ± SD.

<table>
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<tr>
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Conclusions: We propose that XDH/XO may mediate DN susceptibility. Future studies will test XDH locus variants in human DN and evaluate the therapeutic/preventive potential for XOI in human DN.

Funding: NIDDK Support

TH-PO568

Intradiabetical Parenteral Nutrition (IDPN) Increases Serum Prealbumin (PA) Levels in Malignourined Hemodialysis (HD) Patients – A Prospective, Multicenter, Open, Phase-IV-Study Tobias A. Marsen, Roman Fiedler, Helmut Mann. DPN Study Group, Germany.

Background: Malnutrition (MA) is increasingly becoming a clinical problem in maintenance HD patients. Since PA is a nutritional parameter whose increase was shown to be positively correlated with patient survival and decreased morbidity, it was the goal of this study to assess PA and other nutritional parameters during 16 weeks (w) of IDPN. A total of 76 patients were enrolled; 40 patients on high fat diet (HFD) (male: 35±5 years, PA=55±10 mg/l, phase angle <5°; with health check up) and 36 patients on a regular diet (Fargl) (male: 40±5 years, PA=50±10 mg/l, phase angle <5°). All patients were followed in 3 months of nutritional support provides improved survival (Cano: JASN 2007) as a positive marker for patient prognosis. Our data demonstrate a positive influence on PA after 16 w with IDPN, thus favouring IDPN as a beneficial option of therapy. Due to small patient numbers there is a lack of statistical power to evaluate responsiveness of secondary endpoints in this study.

Funding: Pharmaceutical Company Support

TH-PO569

A Reduced Appendicular Skeletal Muscle Mass Is Associated with Total Mortality in Male Hemodialysis Patients Akihiko Kato,1 Takako Takita,2 Mitsuyoshi Furuhashi,1 Hideo Yasuda,1 Yukitoshi Sakano,2 Akira Hishida.3 Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; 2Division of Nephrology, Maryaway Hospital, Hamamatsu, Shizuoka, Japan; 3First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

Background: Decreased skeletal muscle mass and increased abdominal adiposity are often observed in dialysis patients. However, it remains unclear whether either sarcopenia or abdominal adiposity is associated with cardio-metabolic outcomes.

Methods: We measured appendicular skeletal muscle mass (ASM), a sum of the muscle mass in the four limbs, and trunk fat mass (TFM) from a dual energy X-ray absorptiometry, and determined a skeletal mass index (SMI) as ASM/height2 (kg/m2) in hemodialysis (HD) patients aged from 41 to 75 years old (male/female=16/53, time on HD: 10.7±1.6 years). We also followed all patients for the 5 years, and examined the impact of changes of body composition on total mortality.

Results: SMI was positively correlated with serum creatinine (r=0.37, p<0.01) and albumin (r=-0.21, p=0.01), while negatively with age (r=-0.25, p<0.01) in male patients. In contrast, no association was found between SMI and clinical parameters in female patients. TFM was significantly correlated with total cholesterol (r=0.05) and triglyceride (p<0.05) in both sexes. In male patients, there was also a significant association of TFM with hemoglobin (r=-0.23, p<0.01) and carotid artery intima-media thickness (CA-IMT) (r=0.21, p<0.05). During the follow-up, 27 male and 20 female patients had expired. Cox-hazards analysis after the adjustment for co-morbid risk factors revealed that male patients with the lowest tertile of SMI (≤6.5 kg/m2) (n=48) had a significantly higher risk for total mortality (RR: 8.31 folds [95%CI, 1.45-47.51], p<0.02) when compared with those with the highest tertile of SMI (>8.3 kg/m2) (n=49). In contrast, SMI did not associate with 5-year mortality in female patients. BMI also did not relate to total death in both sexes.

Conclusions: These findings suggest that SMI less than 6.5 kg/m2 was a significant determinant of total mortality in male HD patients.
**TH-PO570**

Prognostic Value of Geriatric Nutritional Risk Index for Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patients on Chronic Hemodialysis

Kanor Yasuda,1 Shoichi Maruyama,1 Hiroaki Kasugai,2 Tomoki Kosugi,1 Naotake Tsuibo,2 Waichi Sato,1 Yashukiko Ito,1 Enyu Imai,1 Seichei Matsu,1 1Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; 2Department of Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Aichi, Japan.

**Background:** Malnutrition is a prevalent complication in end-stage renal disease (ESRD) patients on hemodialysis (HD), and is associated with cardiovascular (CV) morbidity or poor survival. Geriatric nutritional risk index (GNRI) has been recently developed as a more accurate tool for malnutrition status. We investigated the prognostic value of the GNRI at start of HD therapy for mortality in ESRD patients.

**Methods:** A total of 1,568 ESRD patients who started HD therapy were examined. The GNRI was calculated as follows; GNRI = (14.89 × albumin) + [41.7 × (body weight / body weight at BMI of 22)] Thereafter, the patients were divided into quartiles according to GNRI levels; quartile 1 (Q1): < 84.9, Q2: 85.0-91.1, Q3: 91.2-97.2 and Q4: > 97.3, and were followed up for up to 10 years.

**Results:** During follow-up period (mean 63.4±42 months), 363 patients died including 180 CV deaths. Kaplan-Meier survival rates for 10 years were 57.9, 73.3, 80.8 and 89.2% for CV mortality, and were 32.1, 51.9, 61.3 and 73.8% for all-cause mortality in Q1, Q2, Q3 and Q4, respectively (both p<0.0001). Even after adjustment for other risk factors, GNRI was an independent predictor for both mortality.

**GNRI and mortality**

<table>
<thead>
<tr>
<th>Cardiovascular mortality</th>
<th>All-cause mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (95%CI)</td>
<td>P for trend</td>
</tr>
<tr>
<td>GNRI &lt; 97.3 (Reference)</td>
<td>0.0001</td>
</tr>
<tr>
<td>91.2 - 97.2</td>
<td>1.56 (0.87-2.79)</td>
</tr>
<tr>
<td>85.0 - 91.1</td>
<td>1.90 (1.07-3.30)</td>
</tr>
<tr>
<td>&lt; 84.9</td>
<td>3.37 (1.96-5.80)</td>
</tr>
</tbody>
</table>

Upon receiver operating characteristic (ROC) analysis, area under (AUC) for CV mortality was larger in GNRI (0.71) compared to albumin (0.64) and BMI (0.63) alone. AUC for all-cause mortality was also 0.70, 0.63 and 0.63 in GNRI, albumin and BMI, respectively.

**Conclusions:** GNRI at starting HD therapy could strongly predict CV and all-cause mortality in ESRD patients. This simple marker might be clinically useful for the assessment of malnutrition status in HD patients.

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**TH-PO571**

Body Composition and Mortality in Prevalent Hemodialysis (HD) Patients: HEMO Study

Rebecca Filipowicz,1 Tom H. Greene,3 Guo Wei,1 Srinivasan Beddu.1,2,4 VA,1 Univ Utah.

**Background:** Both higher body size (as indicated by body mass index- BMI) and higher muscle mass (as indicated by serum creatinine-SCr) are associated with better survival in HD patients (pts), but the relative importance of muscle vs. body size is not established.

**Methods:** The HEMO Study was a 2 X 2 factorial design study conducted by NIDDK to evaluate the effects of flux and dialysis dose on outcomes in amniotic HD pts. Details of HEMO Study have been published elsewhere. In the current study, the associations of Scr, BMI and the ratio of Scr to BMI with time to death were examined in Cox proportional hazards models.

**Results:** The mean age, Scr, and BMI were 58 ± 14 yrs, 10.2 ± 2.9 mg/dl, 25.5 ± 5.3 kg/m², respectively. 56% were men, 65% black, and 45% had DM. There were 860 (47%) deaths over an average 2.85 years of follow-up.

**Conclusions:** Both Scr and BMI were associated with lower risk of death in HD pts. However, in the HEMO Study, the magnitude of the association of Scr with mortality was stronger than the magnitude of the association of BMI with mortality, and higher Scr/BMI ratio was also associated with lower risk of death. Interventions targeting muscle mass at any level of BMI may improve survival in HD pts.

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**TH-PO572**

Novel Lipoprotein Subtraction and Size Measurements in Prediction of Mortality in Maintenance Hemodialysis Patients

Nazarun Noor,1 Michael Caulfield,2 Susanne B. Nicholas,3 Miklos Z. Molnar,1 Csaba P. Kovesdy,4 Kamyar Kalantar-Zadeh,5,6 Harold Simmons Center; 7Quest Diagnostics Nichols Institute; 8David Geffen School of Medicine at UCLA; 9Salem VA Medical Center.

**Background:** Studies on CKD patients (pts) are often limited to conventionally measured total cholesterol and LDL-C. What remains to be determined is what measures of lipoprotein help better identify high risk dialysis pts & whether LDL-"altering" strategies are more effective in this population than LDL-lowering medications.

**Methods:** Mortality-predictability of LDL particle diameter and lipoprotein subtraction concentration, as measured by novel ion mobility, was examined in a cohort of 235 MHD pts who were followed for up to 6 years using Cox models with incremental levels of multivariate adjustment: (A) Case-mix variables. (B) Lipids included LDL and HDL-C and TG concentrations. (C) Malnutrition-inflammation complex syndrome (MICS) variables included serum or blood levels of phosphorus, albumin, creatinine, calcium, ferritin, hemoglobin, nPNA, & BMI. (D) Adjustment for CRP, IL-6, & TNFα. 

**Results:** Over 6 yrs, 71 pts (31%) died. Conventional lipid profile was not associated with mortality. The death hazard ratio (HR, 95% confidence interval) of the highest versus lowest quartiles of very small and large LDL-particle concentrations were 2.14 (1.00-4.62) and 0.47 (0.20-0.99), respectively. As figure shows, across increasing quartiles of the LDL particle diameter, death HRs were 1.0, 0.93 (0.46-1.87), 0.43 (0.21-0.89), and 0.45 (0.31-1.00), respectively.

**Conclusions:** Whereas conventional lipid profile cannot predict mortality in MHD, novel lipoprotein measures such as larger novel LDL particle diameter or higher large LDL particle concentrations appear predictive of greater survival, while higher very small LDL particle concentration is associated with higher death risk.

**Funding:** NIDDK Support

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**TH-PO573**

The Predictive Value of the Changes of Nutritional and Anthropometric Parameters for Survival and Hospitalization Outcome in the Hemodialysis (HEMO) Study

Chi-Ting Su,1,2 Francis Pike,3 Mark L. Unruh,4 Nephrology, National Taiwan University Hospital, Yan-Lin, Taiwan; 5GSPH, Genetics, U. Pittsburgh; 6Biostatistics; 7Renal-Electrolyte Division, University of Pittsburgh Medical Center.

**Background:** While previous works have focused on relationships between baseline measures and all-cause mortality in end-stage renal disease, markers of nutrition vary over time and may affect mortality due to either infections or cardiovascular disease. We conducted the study to examine whether changes in nutritional status,anthropometric parameters and Karnofsky performance index would predict cause-specific mortality in hemodialysis patients.

**Methods:** Anthropometric assessments were measured in HEMO study and functional status was measured by Karnofsky index. Index of coexistent diseases score,baseline demographics,blood studies were covariates.The definition of cause-specific mortality in the HEMO study has been described elsewhere.Cox-regression model was used to examine patient survival along with the change of anthropometric parameters,and performance status on mortality.

**Results:** There were 1404 patients,in HEMO study,with at least one follow up anthropometric measurements. The difference of mid-arm muscular circumference(MAMC) between baseline and the data in the first visit was significant(p<0.01).Lower serum albumin and MAMC were associated with increased mortality(p<0.001) and cardiac mortality. Patients with higher Karnofsky index had less all-cause mortality from congestive heart failure(p=0.01).The decline of MAMC was associated with all-cause mortality,death from infection(p=0.01)and time to 1st cardiac hospitalization or cardiac death(p=0.03). Adjusted survival analysis revealed declining MAMC is an independent predictor for...
all-cause mortality (HR:1.46, p=0.05) and time to first cardiovascular hospitalization (HR:1.25, p=0.002).

Conclusions: In patients undergoing maintenance hemodialysis, nutritional status and functional performance status are associated to survival. The decline of MAMC is a predictor for patient mortality. Serum albumin, MAMC and Karnofsky index are key modifiable predictors to consider as targets for improving patient outcomes.

TH-PO574

Global Variation of Nutritional Status in Children Undergoing Peritoneal Dialysis


Background: Despite the availability of clinical guidelines, it remains unknown if nutritional practices and outcomes vary globally for children receiving peritoneal dialysis (PD).

Methods: We examined the nutritional status of 1466 children from 70 centers in 30 countries participating in the International Pediatric Peritoneal Dialysis Network (IPPN). Body Mass Index (BMI) and height measurements were normalized to WHO (2000) and CDC (2000) standards for children < and ≥ age 5, respectively (table).

Results: Enteral feeding practices for patients < age 5 varied widely with the highest rate seen in North America. Analysis of BMI distribution by age showed high rates of BMI >85% and unfavorable outcomes in pediatric PD patients.

Conclusions: These findings demonstrate considerable global variation in nutritional status and suggest provision of calories alone does not correct growth failure in children receiving chronic PD. Future studies are needed to further examine nutritional practice patterns including underutilization as well as excessive use of enteral feeding in some age groups/regions, and to determine what, if any, association exists between a BMI <5% or >85% and unfavorable outcomes in pediatric PD patients.

Funding: Pharmaceutical Company Support

TH-PO575

Validation of Measurement of Hydration of Fat Free Mass in Volume Expanding Dialysis Patients

Alessio Mollino,1,2 Birl R. Don,3 George A. Kayser.1 1Nephrology, UC Davis, Davis, CA; 2Clinical Nutrition, University of Rome; 3Clinical Medicine, Sapienza University of Rome, Italy.

Background: Fat mass (FM) is measured with dual-energy X-ray absorptiometry (DXA), but is expensive and not portable. Multifrequency bioimpedance spectroscopy (BIS) measures total body water (TBW) and intracellular and extracellular water (ICW and ECW). Adiposity is calculated by subtracting Fat Free Mass (FFM) from weight assuming fractional hydration of FFM of 0.73. Hemodialysis patients (HD), however, have non-physiologic expansion of ECW.

Methods: We estimated the hydration of FFM in healthy adult subjects (C) and HD with BIS using a formula that allows ECW and ICW to vary (TBW/FFM = (1 + ECW/ICW)). Adiposity is calculated by subtracting Fat Free Mass (FFM) from weight assuming fractional hydration of FFM of 0.73. Hemodialysis patients (HD), however, have non-physiologic expansion of ECW.

Conclusions: In patients undergoing maintenance hemodialysis, nutritional status and functional performance status are associated to survival. The decline of MAMC is a predictor for patient mortality. Serum albumin, MAMC and Karnofsky index are key modifiable predictors to consider as targets for improving patient outcomes.

Funding: Pharmaceutical Company Support

TH-PO576

Nutrition Status Prior to Starting Hemodialysis Is Strongly Associated with One-Year Mortality in Hemodialysis Patients

Eiichiro Kanda,1 Jenna O. Krisher,2 William M. McClellan,3 1Tokyo Kyosai Hospital, Japan; 2ESRD Network 6; 3Emory University.

Background: Malnutrition is a common complication in hemodialysis patients. We examined nutritional status prior to starting hemodialysis to determine whether it was independently correlated with one-year mortality among incident hemodialysis patients.

Methods: We enrolled 6304 incident hemodialysis patients in North Carolina, South Carolina and Georgia (ESRD Network 6 area) and followed up these individuals for up to one year. Prehemodialysis nutritional status was assessed using data from the Centers for Medicare & Medicaid Services (CMS) 2728 Form. Nutritional status was evaluated on the basis of body mass index (BMI), serum albumin level, and gastrointestinal nutritional risk index (GNRI) (calculated from height, weight and serum albumin levels). Patients were categorized into quartile groups by GNIR. Mortality was evaluated using a fully adjusted Cox proportional hazard model.

Results: The mean age (SD) was 61.3 (15.0) years old; female 47.9%; diabetes 46.2%; mean BMI 29.1 (9.0) kg/m²; mean serum albumin 3.02 (0.72) mg/dL; 5.9% under the care of a dietitian prior to dialysis; GNRI median 62.7 (IQR 50.8-90.7). Nutrition status prior to hemodialysis was correlated with mortality: low BMI (less than 23) hazard ratio (HR) 1.88 (95% CI confidence interval 1.15-2.52), hypoalbuminemia (less than 3.8 g/dL), 2.09 (1.59-2.75). The group with the lowest GNRI, Group 4 (<50.8), had an HR of 1.41 (1.16-1.71) relative to Group 1 (GNRI>90).

Conclusions: Patients who were under the care of dietitians had higher serum albumin levels (t-test, P<0.001) and lower mortality, HR 0.74 (0.55-0.99) than patients who were not. Patients who were under the care of dietitians had higher serum albumin levels (t-test, P<0.001) and lower mortality, HR 0.74 (0.55-0.99) than patients who were not.

Conclusions: In dialysis patients, nutritional status prior to starting hemodialysis is associated with early mortality risk. Moreover results suggest that the control of nutritional status may decrease the risk.

TH-PO577

Quality of Sleep and Daytime Sleepiness in Chronic Hemodialysis – A Study of 400 Patients

Sonia Maria Holanda Almeida Araujo,1 Andre Pantaroto,2 Nicole Araujo,1 Constance Almeida de Alencar Araújo,1 Gilson Almeida,1 Elizabeth De Francesco Daher,1 Pedro Bruni,1 Veralice Meireles Sales Bruni,1 1Medicina, Universidade Federal do Ceará, Fortaleza, Ceara, Brazil; 2Medicina, Faculdade de Medicina de Jundiaí, Jundiaí, Sao Paulo, Brazil; 3Medicina, Faculdade Christus, Fortaleza, Ceara, Brazil.

Background: Impaired sleep has potential health consequences in chronic hemodialysis patients. To date, this issue has not been examined in studies involving a large number of subjects. We aimed to identify factors associated with poor sleep quality and excessive daytime sleepiness (EDS) in dialysis patients.

Methods: This is a cross-sectional observational study, involving 400 patients (59% male) from three hemodialysis centers (SD-HEMOFOR). Quality of sleep was evaluated by the Pittsburgh Sleep Quality Index (PSQI); EDS by the Epworth Sleepiness Scale (ESS); risk of obstructive sleep apnea (OSA) by the Berlin questionnaire and comorbidity severity by the Charlson Comorbidity Index (CCI).

Results: Poor sleep quality (PSQI>5) was found in 227 individuals (57%) and was associated with older age (p=0.001), diabetes (p=0.03), heart failure (p<0.005), hypoaebuminaemia (p=0.01), low transferrin saturation (TSAT) (p=0.009), higher CCI score (p=0.005). Independent factors were older age, heart failure, low TSAT and depressive symptoms. Daytime somnolence was present in 108 patients (27%) and was independently associated with stroke (OR=2.84 CI= 1.03-7.76), lower hemoglobin concentration (OR=2.45 CI= 0.93-6.30) and high risk of OSA (OR=1.65 CI= 1.03-2.63). High risk of OSA (N=120; 30%), was associated with hypertension (p<0.001), overweight/ obesity (p=0.001), older age (p=0.003) and symptoms of depression (p=0.01).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
Conclusions: Poor sleep quality and EDS are prevalent on chronic hemodialysis. Heart failure, low TSAT and depressive symptoms are independently associated with poor sleep quality. Stroke, anemia and high risk of OSA are independently associated with EDS. These results provide new insight into possible treatment strategies.

Funding: Government Support - Non-U.S.

TH-PO578
High Dietary Fiber Intake Associates with Lower Indoxyl Sulfate Concentrations in Chronic Kidney Disease

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Background: Mounting in vitro and clinical evidence indicate that indoxyl sulfate (IndS), a protein-bound uremic toxin originating from bacterial protein fermentation in the colon, is involved in the pathogenesis of accelerated cardiovascular (CV) disease in chronic kidney disease (CKD) patients. High dietary fiber intake suppresses protein fermentation and is associated with decreased CV morbidity and mortality in both CKD and non-CKD populations.

Methods: We performed a cross-sectional observational study to investigate associations between dietary fiber intake and serum IndS levels. Fasting blood samples were collected and analyzed for routine biochemistry and IndS (HPLC) in 195 stable CKD patients (105 male; age 65±16 year). Dietary intake was estimated through a dietary history and nutrient content was calculated using Bence® Nutritional Software.

Results: Demographics and biochemistry are summarized in table 1.

<table>
<thead>
<tr>
<th>CKD1-2</th>
<th>CKD3</th>
<th>CKD4-5</th>
<th>CKD5D</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>28</td>
<td>55</td>
<td>64</td>
<td>82</td>
</tr>
<tr>
<td>Age (y)</td>
<td>44</td>
<td>68</td>
<td>69</td>
<td>70</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26</td>
<td>18</td>
<td>18</td>
<td>18</td>
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<tr>
<td>Total kcal/day</td>
<td>2162</td>
<td>1808</td>
<td>1647</td>
<td>1717</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>84</td>
<td>78</td>
<td>69</td>
<td>71</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>269</td>
<td>259</td>
<td>207</td>
<td>215</td>
</tr>
<tr>
<td>Fiber (g/d)</td>
<td>24</td>
<td>22</td>
<td>20</td>
<td>13</td>
</tr>
</tbody>
</table>

Median values are shown

Total energy intake, as well as intake of dietary protein, carbohydrate, fat and fiber intake were lower in more advanced CKD stages. Of all macronutrients, only dietary fiber intake was associated with Inds levels (β=-0.06; p<0.0001), independent of eGFR, age, sex and BMI.

Conclusions: Dietary fiber intake is inversely associated with indoxyl sulfate concentrations in CKD patients, independent of eGFR. Suppressing indoxyl sulfate serum levels might be one of the mechanisms through which dietary fiber could decrease CV morbidity and mortality.

TH-PO579
Olfactory Dysfunction of ESRD Patients Was Recovered by Initiation of Dialysis

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Olfactory dysfunction is considered an important factor in the quality of life in patients with ESRD. However, there are no studies that have investigated whether olfactory function is impaired in chronic kidney disease patients. In this prospective study, we aimed to determine whether olfactory function is impaired in ESRD patients who initiate hemodialysis (HD) or peritoneal dialysis (PD).

Conclusions: The present study showed that olfactory function of ESRD patients did not differ from those of healthy controls. However, there was no prospective study following initiation of dialysis on olfactory function in ESRD patients. This prospective study was designed to clarify the effect of dialysis on olfactory function of ESRD patients who initiate hemodialysis (HD) or peritoneal dialysis (PD).

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245A

TH-PO580
Increased Levels of Serum Parathyroid Hormone and Fibroblast Growth Factor-23 Are the Main Factors Associated with the Progression of Vascular Calcification in Long-Hemodialysis Patients

Guillaume Jean, Charles Chazot. Nephrology, NEPHROCARE, Tassin, France.

Background: Vascular calcifications (VCs) are frequently observed in hemodialysis (HD) patients and have been associated with poor outcomes. The aim of the present study was to assess the frequency and the factors associated with the progression of VCs using a semi-quantitative X-ray score.

Methods: We included all prevalent HD patients between January 2006 and January 2007, with initial radiological scores ranging from 0 to 3 according to the severity and extent of the VCs (abdominal aorta, iliac, femoral, popliteal, and radial arteries). Patients were classified as non-progressors or progressors according to the change in their VC score after a 3-year follow-up. Patients classification was not modified and VCs were infrequently observed. Calcium analogs (38%), cinacalcet (15%), dialysate calcium (mean 1.48 mmol/L), and calcium-10% (30%) and non-calcium based phosphate binders (38%) were prescribed on an individual basis.

Results: Among the 85 patients still alive after 3 years, 47 were classified as non-progressors and 38 as progressors (44.7%). No regression in VC score was observed. Among all the studied parameters, only serum PTH and fibroblast growth factor (FGF)-23 levels were increased in the progressor group. On evaluating the association between patients with higher serum PTH and FGF-23 levels and VC progression, we found that only exhibiting high levels of both parameters is associated with the risk for VC progression (odds ratio, 5.8, 1.7–19.9, P = 0.004). Hyperphosphatemia (<10%), and especially, hypercalcemia (1%), and hyperparathyroidism (HPT >; 585 pg/ml = 0%) were infrequently observed. Calciotrol analogs (38%), cinacalcet (15%), dialysate calcium (mean 1.48 mmol/L), and calcium-10% and non-calcium based phosphate binders (38%) were prescribed on an individual basis.

Conclusions: After 3 years, VC progression was observed in 44.7% of prevalent HD patients using long-dialysis sessions and an individual therapeutic strategy. The main factor associated with VC progression was the association of serum PTH and FGF-23 levels. It remains to be seen whether patients should be treated to lower their PTH value, even in the target range, using calcitriol analogs, calcimimetics, parathyroidectomy, or by modifying the Klotho-FGF-23 axis.

TH-PO581
Greater Reductions in Mortality over Time for Obese and Extremely Obese Patients Compared to Normal or Underweight Patients Who Begin Dialysis Therapy: A Cohort Study from 1995-2005

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Background: Prior studies have identified an inverse correlation between body mass index (BMI) and survival with obese patients experiencing the greatest benefit. The relationship between changing trends in body size and associated mortality risks among new dialysis patients has not been previously explored.

Methods: We compared trends in mortality among underweight, normal, overweight, obese, and extremely obese patients who began dialysis therapy between 1995-2005 using data from the US Renal Data System. National incidence data were available on new patients (N=851,480) between May 1995 and December 2005 and all patients were classified as non-progressors or progressors according to the change in their VC score after a 3-year follow-up. Patients classification was not modified and VCs were infrequently observed. Calcium analogs (38%), cinacalcet (15%), dialysate calcium (mean 1.48 mmol/L), and calcium-10% and non-calcium based phosphate binders (38%) were prescribed on an individual basis.

Conclusions: After 3 years, VC progression was observed in 44.7% of prevalent HD patients using long-dialysis sessions and an individual therapeutic strategy. The main factor associated with VC progression was the association of serum PTH and FGF-23 levels. It remains to be seen whether patients should be treated to lower their PTH value, even in the target range, using calcitriol analogs, calcimimetics, parathyroidectomy, or by modifying the Klotho-FGF-23 axis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
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**TH-POS82**

Effect of Oral Nutritional Supplements on Mortality in Hemodialysis Patients

**Eduardo K. Lacson, Weiling Wang, Barbara L. Zebrowski, J. Michael Lazarus, Raymond M. Hakim.**

**University of Warsaw; 3National Research Institute of Mother and Child.**

**Background:** We evaluated whether supervised oral nutritional supplement (ONS) at every treatment impacts survival in hemodialysis (HD) patients.

**Methods:** All chronic in-center HD patients in Fresenius Medical Care North America facilities, with albumin ≤3.5 g/dL in Q4-2009 without oral supplements in the prior 90-days were eligible for ONS until albumin ≥4.0 g/dL. Patients prescribed ONS in Q4-2009 were study patients (supplement start date defined as Day 1 of follow-up), the rest were controls (date of 1st albumin ≤3.5 g/dL defined as Day 1 of follow-up). Case-mix data as of Day 1 and lab values for hemoglobin, phosphorus and eKt/V from the prior 90-days were recorded. Mortality was tracked for 1 year up to 12/31/2010. Cox models were used to compare one-to-one propensity score and geographic region-matched groups. The propensity score used age, gender, race, diabetes, vintage, BMI, cause of ESRD, hospitalization in the prior 30 days, albumin, hemoglobin, phosphorus, eKt/V, access type and an indicator for incident patients (i.e. vintage ≥590 days).

**Results:** Propensity score plus region matched patients (N=4,354 pairs) had similar 90-days were recorded. Mortality was tracked for 1 year up to 12/31/2010. Cox models were used to compare one-to-one propensity score and geographic region-matched groups. The propensity score used age, gender, race, diabetes, vintage, BMI, cause of ESRD, hospitalization in the prior 30 days, albumin, hemoglobin, phosphorus, eKt/V, access type and an indicator for incident patients (i.e. vintage ≥590 days).

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**Conclusions:** The increased prevalence of overweight, obesity and extreme obesity was associated with a trend of falling mortality risks from 1995-2005. The larger the BMI category, the proportionally greater the reduction in relative mortality risks over time with the survival of underweight patients unchanged. Increasing BMI among successive incident dialysis cohorts is associated with temporal improvements in overall survival.

**TH-POS83**

Leptin, NPY and Cortisol Concentration Changes in 4-Hour Hunger Test in Patients with Renal Failure

**Staslaw Niemczyk, 1 Katarzyna Romejko-Ciepielewska, 1 Ewa Paklerska, 1 Longin Niemczyk, 2 Katarzyna Samotulka, 3 Joanna Matuszko-Z ROWowska, 2 **Military Institute of Medicine; 2 **Medical University of Warsaw; 3 **National Research Institute of Mother and Child.

**Background:** Malnutrition is a common finding in end-stage renal disease. Weight loss is present in approximately 40% of dialysis patients. Hormone disturbances in end-stage renal disease may be responsible for malnutrition. High leptin concentration in patients with renal failure inhibits food intake and may lead to the loss of weight. Cortisol increases leptin concentration. NPY is an orexigenic peptide. Leptin inhibits NPY synthesis in hypothalamus.

**Methods:** The study involved 87 pts (37 HD pts and 50 controls). Blood samples for leptin and cortisol determination were taken twice (at 8 a.m. and after a 12-hour fast, and at noon, after 4 hours of prolonged fasting).

**Results:** During a 4-hour fast, we observed a decrease in leptin levels in all HD patients, with statistically significant differences in HD patients with normal body weight (p<0.006). We also observed high leptin concentration in fasting state in obese patients (0.14-225) and obese (0.275) patients. NPY levels were slightly reduced in undernourished HD patients. We observed a fall of cortisol concentration in 4-hour hunger test in all HD patients, with statistically significant differences in HD obese (p=0.034) and normal weight patients (p=0.013). Cortisol composition in fasting stage were higher in all HD patients in comparison with the control group, we observed the statistically significant differences between malnourished (p=0.010) and obese (p=0.018) pts.

**Conclusions:** High fasting leptin concentration and no significant reduction in these concentrations during prolonged fasting is an indicator of a lack of association between leptin and the development of malnutrition. This association may result from reduced NPY levels. High fasting leptin concentration in HD obese patients is a result of leptin resistance in this group of patients. High cortisol concentration in HD malnourished patients may be responsible for inhibition of food intake and the loss of weight.

**TH-POS84**

Site and Size of Vascular Calcifications Are Different in Dialysis Patients with Various Underlying Diseases

**Hiromichi Suzuki, Tsutomu Inoue, Hirokazu Okada, Tsunoe Takenaka. Department of Nephrology, Saitama Medical University, Iruma gun, Saitama, Japan.**

**Background:** It is well known that vascular calcification (VC) contributes to increased cardiovascular disease in dialysis patients and is used as a marker of the severity of vascular disease. However, VC occurs in vessels of various diameters and no definitive studies have determined the significance of VC in different vessels. The aim of this work was to learn if there was any association between the site of VC in the arteries and the underlying disease of dialysis patients.

**Methods:** This was an observational and cross-sectional study that included 78 dialysis patients. Using computed tomography (CT) scans, the total volume of VC in the thoracic and abdominal aorta and in the arteries of the lower limbs with a density more than 130 Hounsfield Units were semi-quantitatively determined as the sum of all voxels. Clinical characteristics and laboratory variables were determined by cardiac echocardiography, dual-energy x-ray absorptiometry and pulse wave velocity.

**Results:** The patients (66% men, 40% diabetic) had median age and dialysis period of 67.3 yr and 76.7 months, respectively. VC in the thoracic aorta was present in 92%, in the abdominal aorta in 90% and in the lower limbs in 90% of the patients. All three lesions correlated significantly with each other. Stepwise regression was applied in which the independent variables were identified from the univariate analyses. Significant associations were seen for the following: the prevalence of calcification in the thoracic aorta with age, presence of diabetes, and calcium supplement; the abdominal aorta with period of dialysis, elevations of both systolic and diastolic blood pressure and levels of serum albumin; arteries of the lower limbs with presence of diabetes mellitus, use of sevelamer and cinacalcet and serum levels of intact parathyroid hormone and albumin.

**Conclusions:** The presence and extension of VC in thoracic and abdominal aortas and in arteries of lower limbs might be regulated in a complex manner and the use of these variables as a marker of the severity of vascular disease should proceed with caution.

**TH-POS85**

Impact of High Flux Membrane Dialysis on Clearance of Cardiac Troponin T in Asymptomatic Hemodialysis Patients

**Azharuddin Mohammed, Simon Fletcher, Daniel Zehnder.**

**Department of Nephrology, University Hospitals Coventry, Coventry, United Kingdom.**

**Background:** Cardiac troponin T (or I) level at baseline and 6-12 hours later is used to diagnose acute myocardial injury in HD patients. It is not uncommon for the 1st or the 2nd troponin sample collection timing to coincide when the patient is on dialysis. This questions whether collecting a timed troponin sample during dialysis offers reasonable diagnostic accuracy, and if dialysis impacts their clearance significantly.Aim of this study is to assess the impact of dialysis on clearance of troponin T.

**Methods:** 111 asymptomatic maintenance HD patients using Nipro high flux dialysis membranes and dialysing in centers, had undergone midweek cardiac troponin T testing predialysis. Of these 111 patients, 94 had both pre and post dialysis troponin T checked using Roche E170 immunoassay. Using local troponin T cut off range we divided patients into 3 groups. Troponin T level of <0.01 ng/L are classed as normal(A), 0.01-0.10 ng/L are classed as high(B) and a troponin T of >0.1 ng/L are classed as very high(C).A change in troponin T concentration and a class change post dialysis is then analysed.

**Results:** Prevalence of troponin T in the 3 groups were 22.5 % (25) in group C, 74.7% (83) in group B and 2.7% (3) in group A (n=111). We then analysed those who had both pre and post dialysis troponin T checked (n=94). Mean troponin T in group B was 0.045 ng/L (0.1-0.10) and 0.26 ng/L (0.11-1.69) in group C. Percentage of patients in the three groups pre and post dialysis were 3.2% Vs 4.25%, 76.5% Vs 80.8% and 20.2% Vs 14.9% respectively for group A, B and C. Mean troponin T change after dialysis in group B was 0.075 ng/L and 0.03 ng/L in group C (p=0.296). A sub analysis of group C (n=19) showed none of the patients changed to group A after dialysis, but 5 patients changed to group B; p=0.318. In group B (n=72), 1 patient changed class to group A.

**Conclusions:** Dialysis using high flux membrane has shown a minimal clearance of troponin T which is not statistically significant. Scheduled timely serial measurement of troponin T can be undertaken with a reasonable diagnostic accuracy to evaluate for acute myocardial injury in HD patients even if they are on dialysis.

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**246A**
Concentration is 0.5 mEq/L. Higher concentration should be considered in order to prevent
concentration in PD pts should be investigated in more detail. Current dialysate magnesium
independent predictor of long-term survival in PD pts. Factors affecting serum magnesium
of mortality in this model.

p=0.04) and C-reactive protein (Relative risk=1.055, p=0.04) were also significant predictors
on dialysis at enrollment (Relative risk:1.022, p=0.002), albumin (Relative risk=0.131,
survival (Kaplan Meier) than that of pts with magnesium<1.6 mEq/L (p=0.02). In Cox's
observation, pts with enrollment magnesium >1.6 mEq/L had significantly better cumulative
Pts were stratified into 2 groups by enrollment serum magnesium. Upon 10.46 years of
serum magnesium compared to those who did not survive (1.76 vs. 1.50 mEq/L, p=0.028).
Current dialysate magnesium concentration is 0.5 mEq/L. Higher concentration should be considered in order to prevent magnesium depletion.

Funding: Private Foundation Support

TH-PO587
Bone Mineral Density in Patients Receiving Hemodialysis Therapy for More Than 30 Years
Shigeru Otsubo,1 Takashi Akiba,2 Kosaku Nitta.3
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2Department of Blood Purification, Kidney Center, Tokyo Women’s Medical University, Tokyo, Japan;
3Department of Medicine, Kidney Center, Tokyo Women’s Medical University, Tokyo, Japan.

Background: Chronic kidney disease-mineral and bone disorder is a regular complication of hemodialysis patients. On the other hand, the number of patients receiving hemodialysis therapy for more than 30 years has been increasing. No information seems to be presently available concerning bone mineral density (BMD) in such patients. We investigated tartrate-resistant acid phosphatase (TRACP) 5b and the BMD, of extremely long-term hemodialysis patients.

Methods: Ninety-three outpatients receiving maintenance hemodialysis at our Hospital were enrolled. TRACP-5b was measured using a novel fragments-absorbed immunoenzyme enzyme immunoassay and two monoclonal antibodies. BMD was assessed using dual-energy X-ray absorptiometry scans. The absolute BMD values for the 1/3 distal radius on the side not containing the vascular access were reported. We classified the patients according to the duration of hemodialysis therapy (less than 10 years (n=57), 10-20 years (n=10), 20-30 years (n=9), or more than 30 years (n=18)) and compared them.

Results: The TRACP 5b level was higher in more than 30 years group (1192.4 ± 484.1 mU/dL), compared with each of the other groups (479.6 ± 253.3 mU/dL, in less than 10 years group (p=0.0001), 456.7 ± 296.4 mU/dL in 10-20 years group (p=0.0001), 509.6 ± 556.3 mU/dL in 20-30 years group (p=0.0001)). The BMD and Z score was 0.59 ± 0.17 g/cm² and -0.62 ± 1.42 in less than 10 years group, 0.59 ± 0.16 g/cm² and -1.50 ± 2.33 in 10-20 years group, 0.45 ± 0.12 g/cm² and -2.23 ± 1.16 in 20-30 years group and 0.43 ± 0.13 g/cm² and -3.21 ± 1.86 in more than 30 years group. The BMD was lower in more than 30 years group than in less than 10 years group (p=0.019) and 10-20 years group (p=0.013). The Z score was reduced gradually according to the duration of hemodialysis and was significantly lower in more than 30 years group than in less than 10 years group (p=0.0001) and 10-20 years group (p=0.009).

Conclusions: In patients receiving more than 30 years hemodialysis therapy, the BMD was reduced.

TH-PO588
Endogenous Glucocorticoid and Insulin Resistance Induced Muscle Wasting in Hemodialysis Patients
Huilin Wang Division of Nephrology, Jinhui hospital, Shanghai, China.

Background: Patients undergoing advanced chronic kidney disease often present muscle wasting, to causes of poor physical function and living quality. The endogenous glucocorticoids (eGC) may trigger skeletal muscle atrophy by interaction with insulin-resistance. However, the relationship between eGC lever or insulin-resistance and muscle wasting still need systematically addressed in clinic

Methods: We retrospectively evaluated 102 patients undergoing maintenance hemodialysis (MHD) without diabetes, and presenting malnutrition status evaluated by subjective global assessment (SGA); 30 healthy adults as control. Magnetic resonance imaging (MRI) of lower leg was determined the cross-sectional area (CSA) of muscle and fat. The serum corticosterone, fasting insulin and insulin resistance (HOMA-IR) was measured.

Results: The results showed there were no significant differences in gender and blood glucose. The bodyweight and body mass index, serum albumin were decreased in MHD. The serum corticosterone and the fasting insulin increased 4-5 times, the HOMA-IR was increased significantly compared with control. The MRI results showed the length of thighbone was no difference between MHD patients and the control group. The total cross-sectional area (CSA) of leg was significantly reduced in MHD. The morphologic observation showed muscle atrophy in MHD, the muscle CSA/total CSA ratio was lower, but the fat CSA/muscle CSA ratio was higher in MHD. The Spearman correlation analysis showed the eGC, fasting insulin levels and HOMA-IR was positively correlated to the MQSGA and MRI fat CSA; fat/muscle ratio; but negatively correlated with MRI muscle CSA and muscle /total ratio

Funding: Private Foundation Support

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TH-PO590

Body Composition Monitoring in Chronic Hemodialysis and Kidney Transplant Patients Sylvie Sulkova, Roman Safranek, Michael Kubisova, Lydie Habanova, Petr Moucka, Katerina Petranova, Miroslav Merta. Department of Nephrology, Gerontology and Metabolic Care, Medical Faculty and Teaching Hospital, Hradec Králové, Czech Republic.

Background: Chronic hemodialysis (HD) patients are at increased risk of malnutrition. Simple assessment, e.g. BMI calculation are not suitable for HD patients. The aim of our work was to assess one-year monitoring of body composition of HD patients and compare it with kidney transplant patients.

Methods: Body composition was assessed in 63 HD patients (22 females, 64 (54; 71) years) in two-month interval for one year. Bioimpedance spectroscopy (Body Composition Monitor) was used to estimate LTI (lean tissue index, kg/m²), FTH (fat tissue index, kg/m²). BMI (body mass index, kg/m²) was calculated. Body composition of HD patients was compared with 100 kidney transplant patients (40 females, 57 (51; 70) years, 53 (20; 99) months after kidney transplant, serum creatinine 123 (94; 156)µmol/l). Data are given as median (lower; upper quartile).

Results: BMI, LTI, and FTH at the beginning of the study were 28.8 (26.2; 33.8)kg/m², 13 (11.4-14.8)kg/m², and 15.5 (10.8; 18.3)kg/m², in HD patients and 28.3 (25; 31.2)kg/m², 14.9 (12; 17.2)kg/m², 12.8 (8.7; 16.8)kg/m² in kidney transplant patients. Compared to reference range for normal population, we observed low LTI in 44% and 33% of HD and kidney transplant patients and high FTH in 65% and 60% of HD and kidney transplant patients, respectively. We observed no change in BMI during the course of the study in the group of HD patients, but changes in lean and fat tissue index in individual patients.

Conclusions: Bioimpedance spectroscopy shows low lean and high fat tissue index in HD patients. Similar results we obtained in a group of kidney transplant patients, which indicates profound defect in tissue composition regulation both in HD as well as in transplant status. We observed no influence of kidney graft function and time from kidney transplant on body composition. Body composition monitor is a useful tool for monitoring of fat and lean tissue mass in both HD and transplant patients and reveals changes of body composition in individual patients that may help in management of the patients.

Funding: Government Support - Non-U.S.

TH-PO592

Plasma Obestatin Levels Are Negatively Correlated with Appetite in Hemodialysis Patients Cristiane Moragas,1 Julie Lobo,2 Milena Barcaz Stockler-Pinto,2 Amanda Barros,3 Denis Fougue,2 Denise Mafra.1 Department of Clinical Nutrition, Nutrition Faculty, Federal University Fluminense, Niterói, Rio de Janeiro, Brazil; 1Institute of Biophysics Carlos Chagas Filho, Health Science Centre, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 2Department of Nephrology, Hôpital E.Herriot, Univ Lyon, Lyon, France.

Background: Obestatin, an appetite suppressant which seems to be up regulated in hemodialysis (HD) patients, appears of interest in chronic kidney disease since the majority of these patients suffer of protein-energy wasting (PEW). The purpose of this study was to analyze plasma obestatin levels in HD patients and its correlation with appetite and biochemical parameters.

Methods: Thirty-two HD patients (64.7% men, 46.6 ± 13.5 yr, BMI, 23.2 ± 4.0 kg/m², 30.5 ± 6.7% body fat, 57.0 ± 36.0 months on dialysis) were studied. Obestatin levels were measured using the enzyme immunometric assay methods. Anthropometric parameters were recorded and, the appetite was assessed with Appetite and Diet Assessment Tool (ADAT) where the higher score means lower appetite.

Results: Obestatin levels (3.0 ± 0.1pg/ml) and ADAT values did not present differences between gender or BMI groups (cutoff point of BMI 25.0 kg/m²). Obestatin levels were positively correlated with ADAT values (r=0.5; p=0.004, Figure 1) and negatively correlated creatinine (r=0.4; p=0.019). ADAT and creatinine were negatively correlated (r=-0.4; p=0.019).

Conclusions: Obestatin levels are increased in HD patients with poor appetite and these patients present loss of muscle mass according to creatinine. Therefore high plasma obestatin could be related to PEW.

Funding: Government Support - Non-U.S.

TH-PO591

Body Composition and Physical Performance in Maintenance Hemodialysis (MHD) Patients Jun Chul Kim,1,2 Bryan B. Shapiro,2,3 Kaymarr Kalantar-Zadeh,1,2,3 Usama Feroze,1 Janos Porszasz,1 Rachelle Bross,1 Joel D. Kopple.

Background: MHD patients often display protein-energy wasting, sarcopenia and diminished physical performance. This study was undertaken to assess the relationship between body composition and physical performance in MHD patients.

Methods: Body composition, assessed by dual-energy x-ray absorptiometry and body mass index (BMI), were compared to three measures of physical performance: 6-minute walking distance, sit-to-stand testing and stair climbing. At present, 28 patients undergoing MHD for ≥6 months have been examined in this ongoing study.

Results: Patients were 53.1±13SD years, 36% female; 36% diabetic; dialysis vintage was 53±48 months. Unadjusted analyses indicated that 6-minute walking distance correlated with lean body mass (LBM) (r=0.358; p=0.031), LBM index (LBMI) (kg of LBM/m²) height; r=0.322; p=0.047) and % body fat (r=0.435; p=0.010); stair climb correlated with LBMI (r=0.341; p=0.038), LBMI (r=0.366; p=0.028) and possibly with LBMI of both legs combined (r=0.318; p=0.050) (Figures A and B). Six-to-st punch did not correlate with any body composition measure. There were no associations between BMI (range, 19.8-44.2 kg/m²) and physical performance.

Conclusions: These preliminary findings indicate that in MHD patients body composition, and especially LBMI, was associated with certain measures of physical performance, and particularly with 6-minute walking distance and stair climbing.

TH-PO593

Association of Adipokines with Cardiovascular Mortality in Patients on Hemodialysis Shoichi Maruyama,1 Kaoru Yasuda,1 Hirotake Kasuga,2 Yoshinari Yasuda,1 Tomoki Kosugi,1 Wachi Satoh,1 Naotake Tsuboi,1 Yasuhiako Ito,1 Enyu Imai,1 Seichi Matsuoc1 1Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; 2Department of Nutrition, Nagoya Kyoritsu Hospital, Nagoya, Aichi, Japan.

Background: The role of adipokines for cardiovascular disease remains controversial in chronic hemodialysis (HD) patients. We prospectively investigated the association of adipokine with cardiovascular mortality in patients on HD.

Methods: Plasma adiponectin, leptin, resistin and tumor necrotic factor-a (TNF-a) were measured in pre-dialysis blood sample in 203 patients on HD (age: 65±11 years, diabetes: 49%, duration of HD: 8.1±7.1 years). Geriatric nutrition risk index (GNRI) as a parameter of nutrition status was also calculated from individual serum albumin levels and body mass index. Patients were followed up for 5 years.

Results: During follow-up period (mean 54±14 months), 27 patients (13.3%) died due to cardiovascular disease. On Cox analysis, only adiponectin (HR: 1.07, 95%CI: 1.02-1.12, P=0.01) and leptin (HR: 0.94, 95%CI: 0.88-0.99, P=0.021) were significantly associated with cardiovascular mortality, respectively. Based on cut-off levels using ROC analysis (1.71µg/ml, AUC=0.72 for adiponectin, and 3.54ng/ml, AUC=0.74 for leptin, respectively), 5-year freedom from cardiovascular mortality was lowest in chronic adiponectin group than in the low adiponectin group (77.3% vs. 94.1%, P=0.0006), and was lower in the low leptin group than in the high leptin group (72.3% vs. 93.7%, P=0.0001), respectively. After adjustment for other risk factors, high adiponectin and low leptin were independent risk factors of cardiovascular mortality (HR: 2.54, 95%CI: 1.01-6.59, P=0.045, and HR:2.82, 95%CI:1.12-7.14, P=0.029, respectively). Adiponectin levels were negatively correlated with GNRI levels (r=0.36, P=0.001), and leptin levels were positively correlated with GNRI (r=0.49, P<0.0001).

Conclusions: Obestatin levels are increased in HD patients with poor appetite and these patients present loss of muscle mass according to creatinine. Therefore high plasma obestatin could be related to PEW.

Funding: Government Support - Non-U.S.
Conclusions: In contrast to general population, high adiponectin and low leptin were associated with an increased risk of cardiovascular mortality in patients on HD. These paradoxical associations were hypothesized to be affected from prevalent malnutrition status in HD patients.

**TH-PO594**

Phase Angle, a Bioimpedence Analysis (BIA)-Derived Parameter of Body Composition and Cellular Health, Predicts Long-Term Survival in Hemodialysis (HD) Patients (Pts) Neal Mittman, Brinda Desiraju, Jyotiprakas Chattopadhyay, Morrell M. Avram. Avram Division of Nephrology, SUNY Downstate University Hospital at LIHC, Brooklyn, NY.

**Background:** BIA has been validated as a useful tool to measure body composition in HD pts. Phase angle (PA), a BIA-derived parameter, has been associated with cellular health in dialysis pts. The objective of this study was to explore the relationship between PA, clinical, and biochemical characteristics in HD pts.

**Methods:** Fifty-eight HD pts in our urban center were enrolled from 2000, clinical data was recorded, and pts were followed over time.

**Results:** Mean age was 61 yrs. Fifty-seven percent were women, 37% were diabetic, and 79% were of African descent (79%). Mean dialysis vintage was 81 mo. Mean PA was 5.17 degrees. PA decreases with age, and men had significantly higher PA (6.17 v. 4.42 degrees, p = 0.001). Pts with PA<26 degrees had better cumulative survival compared to those with lower PA (p = 0.005). In Cox’s multivariate analysis, after adjusting for age, gender, diabetes, race, and disease status, PA was an independent predictor of mortality (RR = 0.617, p = 0.012). Therefore, for each degree higher, the relative mortality decreases by 38%. The correlates of PA are shown below:

<table>
<thead>
<tr>
<th>Variables</th>
<th>Correlation Coefficient</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>-0.35</td>
<td>0.007</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>0.36</td>
<td>0.019</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.51</td>
<td>0.001</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>0.32</td>
<td>0.037</td>
</tr>
<tr>
<td>Months on dialysis</td>
<td>-0.24</td>
<td>0.078</td>
</tr>
<tr>
<td>Survival period (years)</td>
<td>0.39</td>
<td>0.004</td>
</tr>
</tbody>
</table>

**Conclusions:** As previously noted, PA is positively correlated with survival, and negatively correlated with age. Higher PA also correlated with arkers of somatic (creatinine) and visceral (albumin) protein stores, serum potassium and hematocrit. In summary, PA is an independent predictor of mortality risk, likely related to its reflection of cellular health and nutritional status in dialysis pts, and therefore may be a useful prognostic tool in this population. The use of serial BIA measurements to assess risk and impact survival, ideally in association with aggressive management of protein calorie malnutrition, should be evaluated in prospective trials.

**Funding:** Private Foundation Support

**TH-PO595**

Optimizing Oxalate Removal in Primary Hyperoxaluria by Hemodialysis Colin A. Hutchison1, Richard T. Keir,1 Anne Bevins,2 Neil D. Evans,3 Paul Cotterill4, 1Renal Unit, University Hospital Birmingham, United Kingdom; 2Division of Nephrology, Birmingham, United Kingdom; 3University of Warwick, United Kingdom; 4The Binding Site Group Ltd, Birmingham, United Kingdom.

**Background:** Primary hyperoxaluria is frequently associated with end stage renal disease which in turn is complicated by early cardiovascular disease secondary to oxalosis. It is therefore necessary to optimize the removal of oxalate by hemodialysis to minimize this complication. The purpose of this study was to evaluate the removal of oxalate by different dialysis schedules in a patient recently returned to dialysis following a failed kidney transplant.

**Methods:** During a three month period the clearance of oxalate was studied with the following dialysis modalities and schedules: high flux hemodialysis (HD), 2 hrs 6x week, and 4 hrs or 8 hrs 3x week; hemodialfiltration (HDF), 4 hrs 3x week; and high cut-off (HCO) hemodialysis, 4 hrs 3x week. A two compartment mathematical model was used to simulate oxalate clearance.

**Results:** The absolute clearance rate of oxalate with HDF was significantly higher compared with HD alone. However the simulations of the different modalities determined that the greatest reduction in area under the curve (oxalate exposure per week) was achieved with 8 hrs of HD three times per week.

**Conclusions:** In contrast to general population, high adiponectin and low leptin were associated with an increased risk of cardiovascular mortality in patients on HD. These paradoxical associations were hypothesized to be affected from prevalent malnutrition status in HD patients.

**TH-PO596**

**Metabolic Profiling of Frailty in Hemodialysis Patients**

Quinlyn A. Soltow1, Rebecca H. Zhang,2 Dean P. Jones,3 Nancy G. Kutner,1 1Department of Medicine, Emory University; 2Department of Rehabilitation Medicine, Emory University, Atlanta, GA.

**Background:** The phenotype of frailty provides criteria that define individuals who lack functional reserve and are at risk for functional decline. An ongoing special USRDS study (ACTIVE/ADPOSE) is focusing on identifying components and progression of frailty dimensions in prevalent hemodialysis (HD) patients. As an adjunct to this study, our group is using metabolomics to investigate specific metabolites and metabolic patterns unique to frailty.

**Methods:** A subset (n = 29) of prevalent patients undergoing HD in Atlanta GA dialysis clinics and participating in ACTIVE/ADPOSE were age- and sex-matched with an equal number of healthy controls. All were assessed for frailty characteristics and supplied plasma samples, which were compared using a dual chromatography-Fourier-transform mass spectrometry (DC-FTMS) method developed at Emory to generate global metabolic profiles.

**Results:** Mean (sd) age of HD patients = 54.8 (13.1); mean (sd) age of healthy controls = 53.7 (14.7); 62% of each cohort were male. Frailty status was defined by the Fried criteria of recent weight loss, exhaustion, low physical activity, slow walk time, and low grip strength. The DC-FTMS-based metabolomics method detected over 10,000 metabolic features in plasma samples from 58 subjects. Using the following data reduction techniques, Principal Component Analysis separated metabolic patterns unique to HD and frailty status, while False Discovery Rate identified 120 metabolic features that differed significantly by frailty status in HD patients. Among these significant features, many nutritionally relevant metabolites matched to metabolomics databases, including various amino acid dipeptides and tripeptides, lipids, and others.

**Conclusions:** Metabolic patterns detected by DC-FTMS are altered in HD patients and change according to frailty status, and metabolic features relating to nutritional status and protein metabolism can be identified that change with frailty progression. Metabolic profiling is a useful tool for detection of potential biomarkers or patterns of metabolites to predict frailty in hemodialysis patients and to evaluate interventions that delay functional decline.

**Funding:** NIDDK Support, Other NIH Support - NIA, NIEHS

**TH-PO597**

Changes in Pre-Dialysis Weight Relate to Key Laboratory Parameters in Hemodialysis Patients Richard Amerling1, Penny Faith Palmiero,2 James F. Winchester1, Len A. Usiyat3, Nathan W. Levin1, Peter Kotanko2,1 Division of Nephrology and Hypertension, Beth Israel Medical Center, New York, NY; 2Renal Research Institute, New York, NY.

**Background:** Low hemoglobin (Hb) and serum albumin (SA) levels are predictors of poor outcome in hemodialysis (HD) patients (pts). Fluid overload expands intravascular volume and lowers Hgb and SA. Given that these parameters are measured pre-HD, we hypothesized that fluid overload lowers SA and Hgb and confounds their use as prognostic indicators.

**Methods:** We performed a retrospective record review in 5510 in-center HD pts treated in RRI clinics in February and March 2011. For each pt we identified the first date in February and March 2011 when Hb, SA, and pre-HD weights were recorded. Simple linear regression analysis was used to assess the relationship of ApGo-weight (ΔPW) [March pre-HD weight – February pre-HD weight] versus ΔHb [March Hb – February Hb] and ΔSA [March SA – February SA]. Pts were divided into quintiles of ΔPW.

This represented the importance of clearing the extra-vascular compartment by time alone. This was a 43% reduction from the exposure when HD was just 4 hrs three times per week. In comparison there was only a 10% reduction in the area under curve for HDF (4hrs, 3x week). HCO membranes provide clearance rates that were equivalent but not superior to standard HD.

**Conclusions:** To provide optimal removal of oxalate in a chronic dialysis patient the duration of dialysis was the most significant factor.
**Results:** We found a weak yet significant inverse correlation between ΔPW and ΔHgb (r = -0.15; P < 0.001) and ΔPW and ΔASA (r = -0.12; P < 0.001). A 1 kg decrease in pre-HD weight was associated with a Hb rise of 0.08 g/dL and an albumin rise of 0.012 g/dL. Dividing patients into quintiles of ΔPW suggests a dose response relationship between ΔPW and ΔASA and Hb (ANOVA with post-hoc Tukey test demonstrated significant differences between all quintiles of ΔPW). Month to month weight change vs. ΔASA and ΔHb

<table>
<thead>
<tr>
<th>Weight change quintile (range)</th>
<th>Mean ΔASA (g/dL)</th>
<th>Mean ΔHb (g/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (&lt;1.3)</td>
<td>0.043</td>
<td>0.35</td>
</tr>
<tr>
<td>2 (1.3 to 4.4)</td>
<td>0.333</td>
<td>0.144</td>
</tr>
<tr>
<td>3 (4.03 to 0.4)</td>
<td>0.01</td>
<td>-0.02</td>
</tr>
<tr>
<td>4 (0.4 to 1.3)</td>
<td>-0.02</td>
<td>-0.048</td>
</tr>
<tr>
<td>5 (&gt;1.3)</td>
<td>0.042</td>
<td>-0.262</td>
</tr>
</tbody>
</table>

ΔPW remained a significant predictor of ΔASA and ΔHgb using simple linear regression models adjusted for Pre-HD weight, gender, race, vintage, and age.

**Conclusions:** We demonstrate that short-term changes in pre-HD weight, which are likely to reflect differences in extracellular volume, are significantly and inversely correlated with changes in Hbf and SA. Volume status should be considered when interpreting Hbf and SA. Volume overload may partially explain association of low Hb and SA with poor outcomes in HD patients.

**Funding:** Private Foundation Support

**TH-PO598**

Impact of Membrane Flux on Growth and Mineral Metabolism in Children with End Stage Renal Disease on Hemodialysis

Nataliya Chorny,1 Gail Prado,1 Sreevidya Kasuma,2 Amrit Bhangoo,2 Anil K. Mongia.1

**Background:** Growth retardation in children with ESRD is attributed to factors including efficacy of hemodialysis, proinflammatory state and bone disease. High flux dialyzers are efficient in removal of both small non-protein bound and middle molecules like β2-microglobulin and may reduce inflammation.

**Methods:** We hypothesized that children on high flux membranes will show better growth and improved mineral metabolism. Retrospective review of 21 children on long term hemodialysis from 2006-2011. Patients divided into two group based on high flux (Optiflux and Polyflux) or low flux dialyzers (DZ). One patient in high flux group received rGH, but was discontinued. We measured height, weight and weight Z scores, change in weight and weight Z scores and laboratory variables including serum albumin, CO2, Hemoglobin, serum ferritin, calcium, phosphorus, PTH, urea reduction rate (URR), kT/V. Statistical analysis was done using t-test

**Results:** Ages at onset ranged from 2 years to 21 years, 10 were males. 14 were AA, 2 were white, race and DM, compared to the highest tertile of Scr/Wt, the lowest tertile was associated with a 46 min walk distance (β -61, 95% CI -94 to -29 m). Similarly, compared to the highest tertile, the lowest tertile was associated with a 71% increased risk of CRP. Longitudinal changes in daily energy intake (P<0.001) and with a 0.0135 g/kg/day reduction in mean Hb (P=0.015).

**Conclusions:** Both, MIS and GNRI are valid tools for the longitudinal assessment of nutritional status of hemodialysis patients, whereas the MIS has lower inter-observer reproducibility, but is more comprehensive than GNRI.

**TH-PO600**

Serum Creatinine to Body Weight Ratio – A Simple Measure of Body Composition? Rebecca Filipowicz,1 Talat Alp Ikizler,2 Glen Morrell,1 Guo Wei,1 Tom H. Greene,1 2 Shirin Azizi Bedhuy.1 2 VA; 1 Univ Utah; 1 Vandersl

**Background:** Body composition is not routinely measured in clinical practice in HD pts. Therefore, we examined whether Scr (a marker of muscle mass in HD pts) to weight (Wt) ratio is associated with measures of body composition, inflammation and functional ability.

**Methods:** This study is a secondary analysis of an ongoing longitudinal study of nutritional status in HD pts. 116 pts who underwent at least one DEXA scan for measurement of body composition were included. Ht, wt and 6-min walk distance were measured on a non-HD day. Intra-abdominal fat at the L4-L5 level and muscle area at mid thigh level were measured with MRI. hsCRP was measured using Roche MODULAR P analyzer. hsCRP > 10 mg/L was defined as ↑ CRP. Generalized estimating equations (GEE) were used to fit a pooled cross-sectional regression model relating outcomes to concurrently measured Scr/Wt across 4 study visits.

**Results:** Baseline characteristics by Scr/Wt tertiles

<table>
<thead>
<tr>
<th>Scr/Wt</th>
<th>n</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.14</td>
<td>33</td>
<td>1.8 ± 0.3</td>
</tr>
<tr>
<td>0.14-0.16</td>
<td>37</td>
<td>2.1 ± 0.5</td>
</tr>
<tr>
<td>&gt;0.16</td>
<td>34</td>
<td>2.8 ± 1.3</td>
</tr>
</tbody>
</table>

**Conclusions:** In children on chronic dialysis, growth may be improved by using High Flux dialysis. This may be related to better urycin toxin removal, acidosis improvement and reduced dialysis induced inflammation.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

**250A**

Background: Patients with chronic kidney disease have impaired glucose tolerance, a known cardiovascular risk factor, irrespective of etiology. Serum fructosamines (SF), including glycated albumin, have been evaluated as superior markers of glycemic control to HbA1c (A1c) in this population. We have reported that SF, but not A1c, is associated with infection and hospitalization in DM HD pts (KI, 2010). We are unaware of any published work associating degree of glucose intolerance in NDM HD pts with outcomes.

Methods: We enrolled sixty NDM (A1c≤5.6%, no history DM), measured SF (corrected for albumin, AlbSF), and followed them for up to 6 years.

Results: Mean age was 54 yr, and they were evenly divided by gender. The majority (78%) were of African descent. Mean A1c was 5.1% (range, 4.4-5.6%); 75% had SF above normal range (normal≤26±6 µmol/L; mean, 285; range 187-378). There was no association of A1c with morbidity or mortality during 2 and 5 years of observation. AlbSF, on the other hand, was correlated with frequency (p<0.001) and duration of hospitalization (p<0.03) over both periods of observation. In addition, univariate Cox regression analysis revealed that AlbSF was associated with increased mortality risk. In Cox’s multivariate analysis, after adjusting for age, gender, and dialysis vintage, AlbSF remained a significant independent mortality predictor (See Table).

Conclusions: In conclusion, severity of glucose intolerance in NDM HD pts, as measured by AlbSF, is highly associated with long-term outcomes. These results need to be confirmed in large prospective trials, including therapeutic interventions aiming to normalize or reduce SF levels.

Funding: Private Foundation Support

Risk Factors for Depressive Symptoms in a Large Population of Chronic Hemodialysis Sonia Maria Holanda Almeida Araujo, 1 Nicole Araujo, 2 Andre Pantanoto, 1 Constance Almeida de Alencar Araujo, 1 Gilson Almeida, 1 Pedro Bruin, 1 Veralice Meireles Sales Bruin, 1 Elizabeth De Francesco Daher. 1Medicina, Universidade Federal do Ceará, Fortaleza, Ceará, Brazil; 2Medicina, Faculdade de Medicina de Jundiaí, Jundiaí, São Paulo, Brazil; 3Medicina, Faculdade de Medicina Christus, Fortaleza, Ceará, Brazil.

Background: Despite significant effect in quality of life, depressive symptoms have not been sufficiently evaluated as an important parameter in hemodialysis patients. We aimed to identify depressive symptoms, and study risk factors in a large group of individuals with end-stage renal disease (ESRD) on chronic hemodialysis.

Methods: Cases were analyzed according to the presence/absence of depressive symptoms. Individuals were identified by interview and all variables were measured concurrently. Depressive symptoms were evaluated by the Beck Depression Inventory (BDI-II:16) and poor sleep quality by the Pittsburgh Sleep Quality Index (PSQI:<5).

Results: In 400 patients (59% male), depressive symptoms were present in 77 (19.3%). Depressive symptoms were more common in women and were independently associated with poor sleep quality (p<0.005), unemployment (p<0.005), diabetes (p<0.005), hypoalbuminemia (p=0.01), heart failure (p=0.01) and pruritus (p<0.03).

Conclusions: Women with ESRD on chronic hemodialysis are at increased risk of depression. Furthermore, unemployment and the presence of diabetes, hypoalbuminemia, heart failure and pruritus should raise suspicion for this diagnosis. Depressive symptoms are also independently associated with poor quality sleep and studies about the effects of sleep hygiene therapy on depressive symptoms are warranted.

Funding: Government Support - Non-U.S.

Correlation of Nutritional Status with Depression and Cognitive Function in Hemodialysis Patients Young Kim Song, Jwa-Kyung Kim, Jung-Woo Noh, Ja-Ryong Koo. Division of Nephrology and Kidney Research Institute, Hallym University Sacred Heart Hospital, Anyang, Kyunggi-do, Korea.

Background: Malnutrition, depression and cognitive impairment are in themselves challenging problems in hemodialysis patients, but diagnosis and treatment are often suboptimal. Nutritional status and its association with cognitive function and depression remains uncertain

Methods: Seventy seven patients with maintenance hemodialysis were enrolled as part of this cross-sectional study. Depression assessment was undertaken with a b-informant interview and Alzheimer’s disease assessment procedure, the Body Composition Monitoring (BCM), handgrip strength (HGS), a seven-point subjective global assessment (SGA) and serum biomarkers (albumin, prealbumin, cholesterol, CRP). HGS was measured before and after HD sessions on the non-fistula side with dynamometer (Jamar) and the highest value was used for analysis. The total score for mental function (MF) was defined as less than the 10th percentile of population-based reference in Korea. Neuropsychological test was performed by Mini-Mental State Examination in the Korean version of CERAD assessment packer (MMSE-KC) and Beck Depression Inventory (BDI) during the 1st hour of dialysis.

Results: The mean age was 59.2 years and the median dialysis duration was 39.5 months. 56.6% were men, 58 % had diabetes. The Mean MMSE and BDI score were 27.1 ± 3.1 and 20.5 ± 10.8, respectively. 68.9 % had MF loss and 51.3 % had moderate to severe depression. In the multivariate analysis, BDI score correlated with handgrip strength (r=0.34, p<0.003), SGA score (r=0.226, p=0.050) and Lean tissue index (r=0.247, p=0.035). MMSE score correlated with age (r=0.337, p=0.003), handgrip strength (r=0.259, p=0.026), lean tissue index (r=0.273, p=0.019), fat tissue index (r=0.345, p=0.003), albumin (r=0.283, p=0.013). After adjustment for other clinical parameters, Patients with MF loss were more likely to be depressed (adjusted OR=3.78, 95% CI 1.2-12.7).

Conclusions: Handgrip strength was associated with depression, independently of age, sex, albumin, prealbumin and CRP levels. MMSE score was not associated with nutritional status.

Funding: Private Foundation Support

Early Time-Dependent Changes in Serum Albumin Predict 2-Year Survival in Maintenance Hemodialysis Jorge P. Stroffregen-de-Matos, 1 Giselly R. Pereira, 1 Jorge Reis-Almeida, 1 Adriano Marcos Guimbs, 1 Cristina Marelli, 1 Ana Beatriz Lesques, 1 Marcos Sandro Fernandes de Vasconcelos, 1 Eufronio D’Almeida, 1 Frederico Razany, 1 Jocerin R. Lugon. 1Divisão de Nefrologia, Universidade Federal Fluminense, Niteroi, Brazil; 2Fresenius Medical Care, Buenos Aires, Argentina; 3Fresenius Medical Care, Rio de Janeiro, Brazil.

Background: Our aim was to assess the predictive value of early time-dependent changes in serum albumin (sAlb) on late outcome in hemodialysis (HD) patients.

Methods: In this observational study, 1,775 incident patients on HD from 25 dialysis facilities had the sAlb measured at admission and 3 months later. The time-dependent change in sAlb was calculated as the ratio of 3-month sAlb/initial sAlb, and expressed as percentage. Patients were split into two groups according to initial sAlb (below or above the median) and followed-up for 2 years. The risk of death associated with the early change in sAlb was calculated by a Cox regression model with adjustment by age, gender and diabetes.

Results: Patients were 56% females, 50% 16 years old, and a median a sAlb of 38 g/dL. The 2-year survival was significantly higher in the group with initial sAlb above the median (94.3% vs. 85.5%, P<0.0001). The adjusted hazard ratio (HR) of death was 0.88 (95% confidence interval [CI] 0.78 to 0.99; P=0.04) for each 10% reduction in sAlb among patients in the group with initial sAlb below the median. Conversely, the adjusted HR of death was 1.35 (95% CI 1.21 to 1.50; P<0.0001) for each 10% reduction in sAlb in the group with initial sAlb above the median.

Conclusions: Early-time dependent sAlb changes have a significant impact on 2-year survival rate. Patients with initial low sAlb can have good outcomes if an early elevation in sAlb is seen, whereas those with initial satisfactory levels can switch to a bad prognosis in case of early reduction in sAlb.


Background: Metabolic and inflammatory pathways are closely interrelated with an impaired sequestration of nutrients. No study has investigated impact of anti-oxidants on the postprandial metabolic and inflammatory response in end-stage renal disease patients (pts).

Methods: A randomized, double-blind study comparing fasting and postprandial circulating markers of glucose and lipid homeostasis and inflammation was conducted in 5 non-diabetic hemodialysis (HD) pts and 9 matched controls assessed at 30, 60, 120 and 240 min after a standardized meal consisting of 75 g of milk fat, 80 g of carbohydrates and 6 g of proteins. Subjects were tested 4 times: at baseline, and after 7 days treatments of N-acetylcysteine (MP665, 240 mg/day), N-acetylcysteine (NAC, 600 mg/day) and placebo (lactose, 200 mg/day) respectively.

Results: NAC resulted in increased plasma NAC levels (17.2±4.1 mmol/L) and decreased circulating homocysteine levels (14.1±3.0 mmol/L after placebo vs 10.9±1.5 mmol/L after NAC, p<0.05). Following the meal, glucose increased in a similar manner in the two groups, while insulin and C-peptide increased more in HD-pts. After NAC treatment, a smaller area under the curve of change during the 4 hour postprandial period (AUCGlu) of insulin was observed compared to baseline (p=0.03) and placebo treatment (p=0.005). No effects of anti-oxidants. HDL- and LDL- cholesterol marginally decreased in both groups with no effects of the treatments. Unexpectedly, IL-6 slightly decreased over time in pts (AUCIL-6 = -3.3±1.2 hr pg/ml) in contrast to controls (AUCIL-6 = 0.8±1.2 hr pg/ml, p<0.001). No effects of the treatment on AUCIL-6 of IL-10 INF-γ were detected in pts and control subjects.

Conclusions: The postprandial state in non-diabetic HD-pts is characterized by impaired insulin sensitivity and slight decrease in circulating proinflammatory cytokines. Treatment with anti-oxidants had no impact on selected postprandial metabolic and inflammatory markers in HD-pts.

Funding: Private Foundation Support

Background: The technical data of high permeability dialyzers in pre-dilutional hemodialfiltration (HDF) show a high clearance of the cobalamin. The cobalamin is a vitamin not produced by the human body and its serum rate depends on dietary intake. It's well reported that an overdose of cobalamin may increase risk of neoplasia. In chronic HDF, intravenously or orally cobalamin supplementation is not frequent.

Methods: A retrospective study followed by a prospective study, were performed on patients of our HDF centers (C1) and (C2). Retrospective study over a period of one year. From 2009-June 2010, analyzed cobalamin serum level among 50 patients in C1 (28 women, 22 men) and 36 patients in C2 (15 women, 21 men). Patients of C1 had received a weekly injection of cobalamin intravenously; the patients of C2 did not receive any supplementation since June 2009. The prospective analysis was conducted over a period of 6 months, using monthly intravenous cobalamin injection for patients of C1, C2 patients constituting the control group without supplementation.

Results: RETROSPECTIVE ANALYSIS shows that C1 patients, all had an overdose of cobalamin. Patients in the C2, initially overdosed in cobalamin, had presented a significant decrease in serum after 6 months (p = 0.0002) while remaining in the normal range, and this rate remained stable after one year (p = 0.3). The prospective analysis showed decreased but remaining high normal serum cobalamin in patients of C1, and stability in normal in patients of C2.

Conclusions: In vitro studies of high permeability dialyzers with pores up to 30 kDa, showed a very high clearance of cobalamin (1,335 KDA). Mean daily intakes of cobalamin (in order of 4 mg) cannot compensate per-dialytic clearance. In this study, serum level of cobalamin may remains normal without supplementation, and cobalamin binding to high molecular weight protein could be an explanation.

In pre-dilutional HDF, quarterly or biannual cobalamin supplementation seems to be sufficient. A prospective study is underway in our centers, using quarterly cobalamin , intravenous (C1, n = 130) or orally (C2, n = 72) supplementation.

A Pilot Study of Nuclear Magnetic Resonance (NMR) Lipid Profiling in End Stage Renal Disease Hanis Kassem, Manish P. Ponda. Nephrology, New York University School of Medicine, New York, NY.

Background: Lipoproteins can predict coronary heart disease risk. Hemodialysis (HD) patients suffer from accelerated cardiovascular disease. They also tend to have atherogenic changes in the lipid profile, including hypertriglyceridemia and low HDL. However, conventional therapy has not been sufficiently powerful in lowering lipids appropriate to this population. Nuclear Magnetic Resonance (NMR) lipid analysis can determine lipoprotein particle size and number, and can complement the standard lipid profile. NMR lipid analysis has been previously studied in the dialysis population, but not in comparison to matched controls.

Methods: We sought to assess, using NMR analysis, lipoprotein particle properties in patients on hemodialysis as compared to matched controls without kidney disease.

After obtaining informed consent, fasting plasma samples were collected from 21 patients (C1, n=15) undergoing in-center intermittent hemodialysis and from 13 controls (without kidney disease) matched for gender, age, and gender and presence of diabetes. Both routine lipid analysis and NMR spectroscopy were performed by Liposcience (Raleigh, NC). Means of different lipoprotein particle numbers and size were compared between both groups.

Results: Both groups were well matched for age, gender and presence of diabetes. There was a statistically non-significant higher frequency in control in terms of obesity. Subjects on HD had significantly lower total cholesterol, HDL, and LDL cholesterol levels. Using NMR analysis, HD subjects had lower number of LDL and HDL particles. The percentage of small LDL particles in relation to total LDL particles was not different between groups. The mean size of VLDL, LDL, and HDL particles was not different between groups.

Conclusions: The dyslipidemia of hemodialysis patients is largely captured by the standard lipid profile. These patients have similar particle profiles as compared to matched controls without kidney disease. Larger studies are required to detect more subtle changes that may impact cardiovascular risk.
percentage of LTM (r = −0.241, p = 0.07). OH is common, with 72.1% having OH of >1 litre, while 20.5% had OH >5 litres. OH is more common in male patients, and in diabetic patients.

OH has a significant positive relationship with blood pressure, body mass index, body weight and pulse wave velocity of the carotid-femoral territory. Degree of comorbidty assessed using Charlson’s score, and serum albumin, has a significant positive correlation with parameters of OH and a significant inverse correlation with LTM. OH has a modest positive correlation with periocular transport characteristics and dialysis adequacy, though not with residual renal function.

After a mean follow-up of 12 months, 7 patients died, 2 were converted to hemodialysis, and 2 had transplants.

Conclusions: Fluid overload is common in PD patients. Markers of fluid overload had good correlation with the degree of comorbidity, systolic BP, serum albumin and pulse wave velocity. Longer follow-up would be useful in assessing the relationship of OH with clinical outcomes.

TH-P0611
Serum Nutritional Markers, Body Composition and Mortality in Peritoneal Dialysis (PD) Patients

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Background: Malnutrition and abnormal levels of some body composition parameters are important predictors of mortality. In this study we have investigated the relationships between visceral and somatic protein stores such as albumin, creatinine and body composition and outcomes in PD pts.

Methods: We enrolled 62 PD pts in this study between November 2000 to May 2008. On enrollment, demographic, clinical and biochemical data were recorded. Pts were followed to May 2011. Body composition was determined by bioelectrical impedance analysis (BIA).

Results: Mean age was 54 years. At enrollment, the mean (±SD) serum albumin and creatinine were 3.71±0.59 g/dL and 11.38±4.2 mg/dL respectively. Mean phase angle analysis (BIA).

Conclusions: Compared with LFHD, HFHD and OL-HDF may be more efficient in removal of uremic toxins, resulting in improvement of appetite and protein intake. However HFHD and OL-HDF may have negative protein balance probably because of more protein loss during dialysis.

TH-P0613
Association between Metabolic Alkalosis and Malnutrition-Inflammation Complex Syndrome in Maintenance Hemodialysis Patients

Kawin Tangs danakanon, Daranne Chewaprong, Eric J. Bloom, Rasib Raja. Department of Nephrology, Albert Einstein Medical Center, Philadelphia, PA.

Background: Metabolic alkalosis is a common finding in maintenance hemodialysis patients partly due to the use of high-bicarbonate dialysate bath and/or malnutrition. Malnutrition-Inflammation Complex Syndrome (MICS) is generally associated with poor dialysis outcomes including increased mortality. However, there is limited and conflicting evidence regarding the association between metabolic alkalosis and MICS in hemodialysis patients.

Methods: We conducted a retrospective analysis on 94 hemodialysis patients at an outpatient hemodialysis unit. All patients received maintenance hemodialysis 3 times per week with 35 mmol/L bicarbonate bath. Demographic and laboratory data including various markers of inflammation and nutritional status were analyzed and compared between 2 stratified groups based on predialysis serum bicarbonate level: the higher bicarbonate group (bicarbonate ≥26 mmol/L, n=51) and the lower bicarbonate group (bicarbonate <26 mmol/L, n=43).

Results: Lower mean predialysis BUN (mg/dL) (53.16 ± 13.38, 60.84 ± 16.20, p=0.04) and lower normalized protein nitrogen appearance or pNPA (0.89 ± 0.22, 1.00 ± 0.27, p=0.031) were shown in the higher bicarbonate group. However, other markers of nutritional status and inflammation including serum albumin, prealbumin, creatinine, ferritin, as well as lipid profiles were not statistically different between the 2 groups. Urea reduction ratio and Kt/V were also similar between the 2 groups. Even though not significant, mean C-reactive protein (mg/L) was lower in the higher bicarbonate group (21.34 ± 38.05, 30.55 ± 77.36, p=0.52).

Conclusions: Our study suggested that metabolic alkalosis in hemodialysis patients might be associated with poor dietary nutrition rather than chronic inflammation. A large prospective study with measurement of various nutritional and inflammatory markers is warranted for further investigation.

TH-P0614
Changes in Plasma Ghrelin Concentrations and Feelings of Hunger or Satiety in Hemodialysis and Continuous Peritoneal Dialysis Patients with Chronic Renal Failure in Post- and Pre-Prandial Periods

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Background: Ghrelin (G) is known as an appetite-stimulating hormone. However, little is known about the changes in plasma G level and their association with feelings of hunger or satiety in hemodialysis (HD) or continuous peritoneal dialysis (CAPD) patients.

Methods: We measured the plasma concentration of G and assessed the hunger feelings in 51 chronic dialysis patients (36 HD, 15 CAPD), and in 10 healthy controls for comparison. Feeling of hunger or satiety was assessed using a hunger-satiety score (H-S score) which were rated as grade 5. Blood samples and H-S scores were obtained both postprandially (1 hour after breakfast) and preprandially (5 hours after breakfast, just before lunch) on the same day. In the HD patients, 4-hour HD treatment was performed between the postprandial and preprandial periods. In CAPD patients, a 2.5% glucose PD solution was dwelled between the postprandial and preprandial periods.

Results: The mean plasma G levels (ng/ml) in postprandial period was not significantly different among the patient groups. In the preprandial period, mean plasma G levels were significantly lower (p=0.05) in the HD patients than in the other patient groups: 12.6±12.2 (HD patients), 24.5±24.5 (CAPD patients), 26.9±13.3 (HD patients on non-HD day), 38.3±22.8 (controls). Compared with the plasma G level in the postprandial period,
that in the preprandial period in the HD patients was significantly lower (p<0.007), while those in the other groups were significantly higher (p<0.05). The mean H-S score (points) in the preprandial period were significantly higher than those in the postprandial period in each patient group, with no significant differences among the patient groups.

**Conclusions:** Plasma G levels are reduced by HD even in fasting patients. Feelings of hunger or satiety synchronize with plasma G levels except patients receiving HD treatment.

**TH-P0615**

**Nutritional Intervention Strategy in Malnourish Chronic Hemodialysis Patients:** Results of a 412-Months Prospective Study

**Ignace Mpio, Christine Clauud, Walid Arkouche, Jean-Christophe Szela, Nouredine Boumedjel, Carlos Cardozo, Alejandra Lenz, Denis Fouque, Maurice Laville.**

**AURAL, Lyon, France.**

**Background:** Undernutrition (UN) in chronic hemodialysis (CHD) is a major clinical problem because it affects negatively the survival of patients (pts). Several therapeutic and dietary approaches are described. This work aimed to evaluate the effects of nutritional strategy in our hemodialysis-pts

**Methods:** The study was conducted during 12 months in 132 CHD (49 women, 83 men, mean age was 66 ± 16 years (median 71 years) dialysis vintage 72 ± 74 months (48 months)). The nutritional care (NC) was based on the action of a multidisciplinary team (nephrologists, dietetician, nurses) in order to improve a better compliance. The protocol consist of an optimization of dietary protein intake and energy with, if required, oral supplementation at home and especially during dialysis session (20 g of protein, 400 kcal).

We analyzed serum albumin (ALB), CRP, pNa, and K, at V at initiation (D0), at 6 month and at endpoint (M12). Undernutrition was defined as severe with ALB<35g/L, mild with ALB between 35 and 38g/L.

**Results:** At the end of the study the nutritional status of pts is reversed. The frequency of patients with optimal nutritional status was 68% at month 12 while 59% of subjects were malnourished at D0. A M12 the ALB was > 38 g / L, under the effect of 3 potentials factors: the improvement of the inflammatory condition, Kt/V, and a protein intake > 1 g / kg / d. The inflammatory status was determining because ALB was higher in pts with CRP <10mg/L vs CRP ≥10 mg/L (40.2 vs. 37.7 g / L, p = 0.002 *), there was an inverse correlation between CRP and ALB (R^2 = 0.080, p = 0.002 **). (Mann-Whitney test, ** ANOVA test) Five pts died at 1 year.

**Conclusions:** Our study showed an improvement of the nutritional status of patients. The success of the NC in CHD malnourished pts can be obtained with a multidisciplinary involvement, under an effective dialysis dose and absence of an inflammatory state.

**TH-P0616**

**Prospective Comparison on the Progression of Abdominal Aortic Calcification According to Dialysis Modality in End-Stage Renal Disease Patients**

**Moon-Jae Kim. Division of Nephrology & Hypertension, Inha University School of Medicine, Incheon City, Korea.**

**Background:** In end-stage renal disease patients on dialysis, the prevalence, degree and progression of arterial calcifications are greater than in general population. However, there have been few reports whether the progression of abdominal aortic calcifications (AAC) is different or not according to dialysis modality. The purpose of this study was to compare the incidence of AAC progression between hemodialysis (HD) and peritoneal dialysis (PD) patients.

**Methods:** HD and PD patients who had been on dialysis for ≥ 3 years were included in this observational, prospective 1-year study. AAC was assessed with plain lateral lumbar spine x-ray using anterior-posterior abdominal aortic calcification score at the baseline and 12 month. AAC progression was defined as the increase in the length of linear calcification or new appearance of calcification.

**Results:** Three patients were progressed (Group 1) and 68 were unchanged (Group 2) in AAC. In group 1, 11 were HD and 20 were PD patients. In group 1, the AAC scores at baseline and after 1 year were unchanged in HD (6.3±7.0) and PD (6.6±7.2). There were no differences in the mean AAC scores at baseline and after 1 year were unchanged in HD (6.3±7.0) and PD (6.6±7.2)

**Conclusions:** Dietary modality seems not to affect differently on the progression of abdominal aortic calcification.

**TH-P0617**

**Enteral Nutrition Is Superior Than Intraluminal Parenteral Nutrition in Hemodialysis Children**

**Yolanda Fuentes, Georgia Toussaint, Saul Valverde, Ana M. Hernandez, Maria J. Garcia-Roca, Mara Medeiros. Hospital Infantil de Mexico Federico Gomez, Mexico, DF, Mexico.**

**Background:** Protein-energy wasting syndrome is present in 30 to 60% of the patients with chronic kidney disease. The etiology is complex and multifactorial. The intraluminal parenteral nutrition (IPN) has been used in adults with an improvement of nutritional status, reduction in mortality rates and regression of the net negative whole body muscle protein balance. There is scarce experience regarding IPD in children; the intervention is expensive with no studies comparing oral supplementation with IPDN.

The aim of this study was to examine the effectiveness of IPDN compared to oral supplementation to improve the nutritional status in children on hemodialysis.

**Methods:** Prospective, crossover, randomized trial in children (6 - 7 years) on hemodialysis program, with an ABN (anephropeptic and bioelectric impedance analysis) score < 10.33. During dialysis procedure they received 3 months the intervention A (oral supplementation) or B (IPDN), designed to provide a third of the required daily caloric intake. Protein RDA for age plus 0.5 mg/kg/day. There was no washout period and patients were switched to the other arm to complete the crossover design. Nutritional status was evaluated monthly using the Anthropometry-Bioimpedance-Nutritional-score (ABN) Score.

**Results:** Twenty one patients completed 6 months of treatment. Eleven started with IPDN and 10 with enteral supplementation. The ABN score improved after 6 months of nutritional intervention from 8 ± 1.6 to 9.3 ± 1.7 (p<0.001). After 3 months of enteral supplementation the ABN improved from 8.2 ± 1.8 to 9.04 ± 1.5 (p<0.04), and after 3 months of IPDN the ABN score improved from 8.6 ± 1.5 to 9.2 ± 1.8 (p=0.17)

**Conclusions:** Nutritional intervention (oral and IPDN) is safe and well tolerated in children with ESRD in hemodialysis. The improvement is significant after enteral intervention but not after IPDN.

**Funding:** Pharmaceutical Company Support

**TH-P0618**

**Changes in Concentration of Thyroid Hormones, RT3, Conversion Ratios and Ratios of Thyroid Hormones to Carrier Proteins in a Single Hemodialysis and Hemodiafiltration Longin Niemczyk, 1 Stanislaw Niemczyk, 1 Katarzyna Szamotulka, 1 Wieslaw Klato, 2 Zbigniew Bartoszewicz, 1 Ivanna Dubach, 2 Malgorzata Gomolka, 1 Urszula Syta, 1 Janusz Wyzgal, 1 Joanna Matuszkiewicz-Rowinska. 1 Medical University of Warsaw; 2Military Institute of Medicine; National Research Institute of Mother and Child; 1Hospital, Ciechanow, Poland.

**Background:** Previous studies have not explained influence of a single HD and HDF on thyroid function.

**AIMS:** To assess changes in concentration of TSH, thyroid hormones (TH), conversion ratios (CR) and ratios of thyroid hormones to carrier proteins (TH/P) immediately after and 6h after HD and HDF.

**Methods:** Patients aged 56±15 years, undergoing HD (n=23) or HDF treatment (n=11) were investigated. Concentrations of TSH, FT4, TT4, TT3, rT3 and TBG were measured before, immediately after and 6 hours after single dialysis. General linear model for repeated measurements was applied in statistical analysis. Non-normally distributed variables were log-transformed.

**Results:** Concentration of TSH, TH, rT3, CR and TH/P before dialysis (means ± SD) as well as values of observed trend is shown in the table (\(\cup\) - increase, \(\cap\) - decrease). There were no significant differences in the shape of the changes between HD and HDF group, except deeper transient reduction in TSH concentration in HDF.

**Conclusions:** Nutritional intervention causes important temporal changes in concentration of TSH, TH, rT3, FT3, FT4, and CR. The character of these changes is similar in HD and HDF, apart from TSH concentration.
Uremic Retention Solutes in Hemodialysis Patients

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Results: Twelve solutes except indoxylacetyl glycine and phenylacetyl glycine were detected in serum of HD patients and healthy subjects. Serum levels of 11 solutes except 4-ethylphenyl sulfate (4EtPhS) were significantly higher in HD patients than in healthy subjects.

Indoxyl sulfate (IS), p-cresol sulfate (PCS), phenyl sulfate (PhS), indoleacetic acid, CMPF and 4EtPhS showed protein binding ratios more than 90%. Ratios in serum level of HD patients to healthy subjects were much higher in free fraction than in total fraction in most solutes such as IS, PCS, PhS.

Conclusions: We confirmed serum levels of 11 uremic retention solutes were significantly higher in HD patients, as well as in CKD rats. A significant relationship was observed between protein binding ratios and reduction rates by IS, PCS, CMPF, 4EtPhS showed reduction rate less than 30%, with more than 90% protein binding ratios.

Funding: Pharmaceutical Company Support

TH-P0620

The Effects of On-Line Hemodiafiltration on Inflammatory Markers and other Clinical Findings: Crossover Study

Won Min Hwang, Sung Ro Yun.

Department of Nephrology, Konkuk University Hospital, Daejeon, Republic of Korea.

Results: Twelve solutes except indoxylacetyl glycine and phenylacetyl glycine were detected in serum of HD patients and healthy subjects. Serum levels of 11 solutes except 4-ethylphenyl sulfate (4EtPhS) were significantly higher in HD patients than in healthy subjects.

Indoxyl sulfate (IS), p-cresol sulfate (PCS), phenyl sulfate (PhS), indoleacetic acid, CMPF and 4EtPhS showed protein binding ratios more than 90%. Ratios in serum level of HD patients to healthy subjects were much higher in free fraction than in total fraction in most solutes such as IS, PCS, PhS.

Conclusions: We confirmed serum levels of 11 uremic retention solutes were significantly higher in HD patients, as well as in CKD rats. A significant relationship was observed between protein binding ratios and reduction rates by IS, PCS, CMPF, 4EtPhS showed reduction rate less than 30%, with more than 90% protein binding ratios.

Funding: Pharmaceutical Company Support

TH-P0621

Effect of Insulin Infusion on Liver Protein Synthesis during Hemodialysis

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Background: Hemodialysis (HD) is a catabolic procedure that may contribute to the high frequency of protein-energy wasting among patients on maintenance HD. The aim was to investigate the effect of insulin infusion on liver protein synthesis during HD.

Methods: In a randomized cross-over study 10 non-diabetic, HD patients (M/F=7/3; median age 59 years, range 33-79) received 1 treatment (NT), 2 glucose infusion (G) 10% glucose (G10) 2.5 ml/kg/h), 3 insulin infusion (I) (10 U/kg/h added 30 units of NovoRapid®/liter 2.5 ml/kg/h) during a standardized 4 h HD. During insulin blood glucose (BG) was maintained at 8.0-10.0 mmol/L by additional glucose infusion. Glucose and insulin infusion infusions were commenced 2 h prior to HD and continued throughout the HD. Fasting blood samples were collected before infusions were started (baseline) and followed by a meal. After HD start, blood samples were collected every hour for hormones and every second hour for inflammatory markers until 2 hours post-HD.

Results: Data presented as mean±SD. BG concentrations were comparable at baseline in the three treatment groups (p=0.57). BG levels from baseline to end of dialysis were significantly different only in the G10 group compared with the other two groups (p=0.04). During all three treatments there was an overall increase in serum albumin (37.3±2.0 to 40.5±2.9 g/L, p<0.001 for each treatment) and fibrinogen (11.3±1.7 to 12.8±2.0 µg/mL, p<0.001 for each treatment), but no significant differences between treatments over time for either albumin (p=0.24) or fibrinogen (p=0.14). Preliminary data on 4 patients showed a postprandial increase in bioactive insulin-like growth factor I (IGF-I) 3 h after baseline (0.83±0.34 to 1.32±0.51 µg/L, p=0.001 for each treatment). We also evaluated changes in the metabolic syndrome status and other clinical endpoints.

Conclusions: Neither glucose nor insulin-glucose infusion appear to add to the anabolic effects of a meal on liver protein synthesis.

Funding: Pharmaceutical Company Support, Private Foundation Support

TH-P0622

Effects of Six Versus Three Times Per Week Hemodialysis on Depressive Affect and Mental Health: Frequent Hemodialysis Network (FHN) Trials


Background: Patients undergoing maintenance hemodialysis have a significant burden of poor mental health. More frequent hemodialysis has been associated with gains in general mental health and improved depressive affect in observational studies. We studied the effects of frequent in-center and nocturnal dialysis on depression and mental health in randomized trials.

Methods: A total of 332 patients were randomized to frequent (six times per week) as compared with conventional (three times per week) hemodialysis in the Frequent Hemodialysis Network (FHN) Trials: Frequent Hemodialysis Network Daily (n=245) and Nocturnal (n=87) Trials. Adjusted change in scores over 12 months on the Beck Depression Inventory (BDI), RAND Short-form health survey 36-item (SF-36) self-reported mental health composite score (MHC) and emotional subscale.

Results: Frequent in-center dialysis resulted in significant improvements in MHC (3.7 ± 0.9 vs. 0.2 ± 1.0; P<0.01) and emotional subscale. In the Daily Trial, subjects randomized to frequent as compared with conventional in-center hemodialysis demonstrated significant changes in BDI (adjusted mean change of −2.0 ± 0.7 vs. −0.4 ± 0.7; P=0.10), but experienced clinically significant improvements in MHC (5.7 ± 0.9 vs. 0.2 ± 1.0; P=0.01) and emotional subscale (5.5 ± 1.7 vs. 1.7 ± 1.7; P<0.01).

Conclusions: Daily in-center hemodialysis did not appreciably differ according to age, sex, race, or education status. In the Nocturnal Trial, there were no significant changes among subjects randomized to nocturnal as compared with conventional hemodialysis in BDI (adjusted mean change of −2.00 ± 1.2 vs. −0.3 ± 2.0; P=0.30), MHC (3.1 ± 1.6 vs. −0.8 ± 1.7; P=0.08) or emotional subscale (3.5 ± 2.7 vs. 2.2 ± 2.7; P=0.13).

Funding: NIDDK Support
Chairside Cognitive Behavioral Treatment of Depression in Hemodialyzed Patients Is Effective: A Randomized Controlled Trial

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Background: Depression is common, and associated with morbidity and mortality in ESRD patients, but there have been few treatment studies. Non-drug psychotherapeutic treatments, which are effective in the general population, have been under-studied in HD samples.

Methods: We designed an individual standardized intervention for depression in ESRD HD patients. The ten week, once weekly psychosocial intervention (CBT) was administered chairside during dialysis. It taught both cognitive and behavioral skills focused on reducing depressive affect. Subjects randomly assigned to the control group waited for 3 months before getting the intervention. Assessments were at baseline, 3 and 6 months.

Results: The sample included 36 participants (18 cases, 18 controls), 97% African / Caribbean American. 60% were born outside the US. At baseline the population was moderately depressed according to both self-reported (Beck Depression Inventory [BDI] mean = 23.2 ± 8.8) and clinician-administered scales (Hamilton Depression Scale [HAM] mean = 13.5 ± 5.5). Subjects assigned to the treatment condition showed mood improvement (BDI 15.6 ± 9.0, HAM 11.2 ± 5.2) at 3 months when compared to the wait-list controls (BDI 9.6 ± 9.0, HAM 11.2 ± 3.2). There was a significant interaction effect between group and time, indicating the intervention group improved significantly more than controls by both self report (p=0.08) and clinician administered (p=0.008) depression measures. The intervention group mean HAM scores (7) reflect remission.

Conclusions: A non-drug depression intervention in ethnic minority HD patients is feasible and effective. CBT may be a cost-effective, clinically applicable approach to reducing HD patient depression which has been associated with increased morbidity and mortality.

Funding: NIDDK Support

Neurocognitive Function and the KDQOL Cognitive Function (CF) Subscale

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Background: Cognitive impairment is common and underdiagnosed in ESRD. Prior work suggests that poor performance on the KDQOL cognitive function (CF) subscale can help identify cognitive impairment in ESRD. We tested this hypothesis using a detailed neurocognitive screening assessing multiple cognitive domains.

Methods: The 3-question KDQOL-CF asks patients to quantify on a 6-point scale if they react slowly to things said and done, have difficulty concentrating or thinking, or become confused. KDQOL-CF results were converted to a 100-point scale; a higher score is consistent with better performance. Cognitive tests were administered at the start of a dialysis session. In primary analyses, cognitive outcomes were quantified using principal components analysis as memory and executive function domains. Regression models adjusted for age, sex, race, education and ESRD cause. Consistent with prior work, performance of a KDQOL-CF score ≤50 also was also assessed.

Results: Of 168 patients, 144 had complete data. Age was 62±17 years; 51% were men, 30% black, 90% high school graduates, and 33% had diabetes causing ESRD. KDQOL-CF mean = 23.2 ± 8.8, HAM 11.2 ± 5.2. There was a significant interaction effect between group and time, indicating the intervention group improved significantly more than controls by both self report (p=0.08) and clinician administered (p=0.008) depression measures. The intervention group mean HAM scores (7) reflect remission.

Conclusions: There is a high prevalence of cognitive impairment in HD patients, particularly as it relates to subspecialty function. We hypothesized that the latter may be due to a higher prevalence of clinical and subclinical cerebrovascular disease in HD patients.

Funding: NIDDK Support

Comparison of Cognitive Function across ESRD Treatment Modalities: A Systematic Review

Deidra C. Crews,1 Priscilla Auguste,1 Tanjala S. Purnell,1 Raquel Greer,2 Temitope Olufade,3 Julio Lamprea,1 Johanna Shue,1 Patti Ephraim,4 Hamid Rabb,4 Neil R. Powe,4 L. Ebony Boulware.1 Johns Hopkins University,1 Harvard University,2 San Francisco General Hospital.

Background: Patients with ESRD are encouraged to make informed decisions about their choice of renal replacement treatment (RRT). Limitations in cognitive function are common among ESRD patients. However, the quality and quantity of evidence regarding differences in cognitive function among patients treated with different RRTs is unknown.

Methods: We performed a systematic review of published studies describing differences in patients’ cognitive function across different RRTs (hemodialysis-HD, peritoneal dialysis-PD, kidney transplant-Tx). We identified relevant articles (English, published after 1987) from PubMed and hand-searched bibliographies. We abstracted data on 5 domains of cognitive function. Two independent reviewers assessed studies’ quality (e.g. selection bias and validation of outcome assessment). We calculated standardized effect sizes (Cohen’s d) of associations between each RRT and outcome.

Results: Of 110 potentially relevant studies, 15 reported RRT comparisons, with 26 comparisons of relevant outcomes (Table). Most studies were from outside the U.S. (80%) and all were observational (11 cross-sectional, 2 cohort and 2 pre-post designs). Studies ranged from very low to moderate quality (only 4 adjusted for important confounders

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
such as educational level) and suggested better or no different cognitive outcomes among patients on PD and TX compared to patients on HD.

**Results:** Increasing age, history of cancer, diabetes and peripheral vascular disease, as non-modifiable factors, and use of anticoagulant therapy were associated with an increased risk of mortality. Lower BMI was associated with a higher mortality risk, the inverse of the relationship observed in community-based datasets. Mortality risk was higher when Hb was <10 g/dL and lower when Hb was >12 g/dL. PTH values <150 pg/mL indicated higher mortality risk. Use of vitamin D was associated with a slightly lower risk. The similarity of the difference between the development and the validation datasets was assessed by the c-statistic which reached 0.69 (0.67, 0.70). The resulting clinical additive score [range -9 to +20] conveyed a 2-year mortality risk of 2% to >90%. This allowed building up a simple heat map for risk assessment in an individual patient.

**Conclusions:** A predictive clinical score for 1- and 2-year mortality risk developed in a large European HD database is now available as a simple clinical tool. An external validation and refinement of the score is currently being conducted in a 12000 incident HD patient database.

**Funding:** Pharmaceutical Company Support

**TH-PO630**

**Mortality and Race in Pediatric End-Stage Renal Disease: Who Is Dying before Transplant?**

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2Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA;
3Rehabilitation Medicine, Emory University, Atlanta, GA.

**Background:** Children with End-Stage Renal Disease (ESRD) have increased risk of morbidity and mortality. Kidney transplantation may mitigate these risks and prolong patient survival and quality of life. Yet, some children with ESRD never proceed to transplantation due to premature death. Little is reported about racial differences in mortality risk after incident ESRD.

**Methods:** We performed a retrospective cohort study of all incident ESRD patients < 21 years of age from January 2000 through Sept. 2008, followed through Sept. 2009. We examined rate of death among dialysis patients by race/ethnicity using multivariable-adjusted Cox survival models. We censored patients at death at death or end of follow-up and excluded patients who received a transplant. We considered neighborhood poverty and patient’s health insurance at incident ESRD as measures of socioeconomic status.

**Results:** Among 8,146 pediatric ESRD patients, 896 (9.7%) deaths occurred. 735 (82%) died before waitinglist and 161 (18%) died on the waiting list. Median time to death after incident ESRD was 463 days; mean age at death was 14.9 yrs. Compared to the incident ESRD population, a greater proportion of patients who died were black (vs. white or Hispanic). In adjusted analyses, the effect of race on death was significantly modified by health insurance, with Hispanics experiencing lower rate of death across all levels of insurance status. This was most striking in Hispanics with no health insurance who had 64% lower rate of death (HR=0.36; 95% CI: 0.18-0.71) compared to whites with no insurance, whereas black patients with no health insurance had 59% higher (HR=1.59; 95% CI: 0.95-2.57) rate of death compared to whites.

**Conclusions:** We found that blacks with no health insurance had a greater rate of death after incident ESRD compared with whites, while Hispanics had significantly lower rate of death after incident ESRD vs. the other racial groups regardless of insurance status. Further studies are needed to elucidate why these racial differences in mortality exist.

**TH-PO631**

**In-Center Daily Hemodialysis and Patient Survival: A Multinational Cohort Study**

**Rita Sun1, Robert M. Lindsay,2 Brian Bieber,2 Ronald L. Pisoni,3 Amit X. Garg,4 Peter Austin,5 Louise M. Moist,6 Bruce M. Robinson,6 Yun Li,4 Carl J. Stevens,4 Eduardo K. Lacson,5 Deborah Lynn Zimmerman,6 Gihad E. Nesrallah.1
1Pediatrics, Emory University, Atlanta, GA; 2Epidemiology, Rollins School of Public Health, Emory University, Atlanta, GA; 3Rehabilitation Medicine, Emory University, Atlanta, GA.

**Background:** Increasing hemodialysis frequency from 3 to 6 times per week improves left ventricular mass and health-related quality of life. However, the effects of increased frequency on survival remain uncertain.

**Methods:** We identified 556 patients from France, the United States, and Canada in the International Quotidian Dialysis Registry, who received daily hemodialysis ≥5 times per week from January 2001 to August 2010. Using propensity-score based matching techniques, we matched 318 of these patients to 575 contemporaneous patients receiving conventional, 3 times weekly; hemodialysis in the Dialysis Outcomes and Practice Patterns Study. All patients had session times of less than 5 hours, and received hemodialysis in the clinic or hospital setting. We compared mortality rates between groups using Cox proportional hazards regression.

**Results:** Mean dialysis frequency in the daily group was 5.8±0.6 sessions/week. Mean weekly treatment time was 15.7±4.3 hours for daily patients and 11.9±1.0 hours for conventional patients. During 1382 patient-years of follow-up, 170/893 patients died. Patients receiving daily hemodialysis had a significantly higher mortality rate than patients receiving conventional hemodialysis (1.56 vs. 10.9 deaths per 100 patient-years; hazard ratio 1.6; 95% CI 1.1-2.3; p=0.021). Similar results were observed in pre-specified subgroup and sensitivity analyses.

**Conclusions:** In contrast to previous studies, in-center daily hemodialysis was not associated with any appreciable mortality benefit. As we cannot exclude the possibility of residual confounding, these findings were from an adequately powered, randomized trial. Until then, decisions to undertake daily hemodialysis should not be based on assertions of improved survival.
TH-PO632

Relationship of Missed and Shortened Hemodialysis [HD] Treatments to Hospitalization and Mortality
Chamberlain I. Obialo,1 W. Hunt,2 Khalid Bashir,1 Philip Zager.2
1Renal Section, Dept of Medicine, Morehouse School of Medicine, Atlanta, GA; 2Dialysis Clinic, Inc., Albuquerque, NM.

Background: Missed and shortened HD treatments are more common in US than in Europe and Japan. The factors that predict these events and their relationships to clinical outcomes have not been previously studied in a nationally representative sample of US HD patients. The present study was conducted to explore: the frequency of missed and shortened treatments by race, treatment schedule, geographic region; and the effect of these events on mortality and hospitalization.

Methods: We studied a prevalent cohort of 15,340 HD patients treated in facilities operated by Dialysis Clinic Inc (DCI) between January 2007 and June 2008. The cohort consisted of 48% non-Hispanic whites [NHW], 41% African Americans [AA], 6% Hispanics, 2% Native Americans [NA], 2% Asians and 1% unknown.

Results: The percent of missed and shortened treatments differed significantly by race.

<table>
<thead>
<tr>
<th>Race/Ethnicity</th>
<th>AA</th>
<th>NA</th>
<th>Hispanic</th>
<th>NHW</th>
<th>Asian</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missed HD (%)</td>
<td>3.1*</td>
<td>2.9*</td>
<td>2.8*</td>
<td>1.8</td>
<td>0.9*</td>
</tr>
<tr>
<td>Shortened HD (%)</td>
<td>18.4*</td>
<td>15.0*</td>
<td>13.0*</td>
<td>12.6</td>
<td>8.9*</td>
</tr>
</tbody>
</table>

*M p<0.001 vs. NHW

Missed and shortened treatments were more frequent among patients treated on the Tuesday, Thursday, Saturday vs. the Monday, Wednesday, Friday schedule (2.9% vs. 2.1% missed and 16.4% vs. 14.9% shortened, both p<0.0001). The frequency of missed and shortened treatments differed slightly by geographic region and was lowest in the northeast. Missed and shortened treatments were associated with increased mortality.

Funding: Clinical Revenue Support

TH-PO633

Robert N. Foley,1,2 David T. Gilbertson,1 Arcef Ishani,1 Allan J. Colline,1,2 USRDS Coordinating Center; Minneapolis, MN; Medicine, University of MN, Minneapolis, MN; Minneapolis VAMC, Minneapolis, MN.

Background: Substantial reductions in mortality have occurred in US dialysis patients, in spite of increasing burdens of comorbid illness and successive failures of large, multicenter trials to define salutary interventions. Hence, we set out to address the hypothesis that mortality reductions in dialysis populations reflect general-population phenomena. We performed a Kaplan-Meier analysis of the cohort and used multivariate Cox regression analysis to identify significant predictors of survival.

Results: Of the 22,394 dialysis patients studied, 303 (1.35%) had SLE. Patients with SLE were predominantly women and younger than the other patients (30 ± 13 years vs. 59.4 ± 14.2 years, p < 0.001). The Kaplan-Meier survival curves for dialysis patients with and without SLE are illustrated.

The 1- and 5-year cumulative survival rates were 97.6% and 84.8% in patients with SLE, and 92.7% and 62.2% in patients without SLE. After adjusting for age, gender, dialysis modality, and comorbidities, we did not find a significant survival difference between the two patient groups after 8 years of follow-up. Multivariate analysis showed that older age (≥65), male gender, and the presence of diabetes were independent predictors of mortality (p<0.05) among SLE patients.

Conclusions: The long-term survival outcome was similar between the SLE and non-SLE patients undergoing dialysis. The factors affecting patient mortality were not all identical in these two groups.

TH-PO634

Long-Term Outcomes and Predictors for Mortality among SLE Dialysis Patients: National Cohort Study in Taiwan Chi-Hai-Chiang Chien. Departments of Nephrology, Chi-Mei Medical Center, Tainan, Taiwan.

Background: To compare the prognosis of patients with systemic lupus erythematosus (SLE) receiving dialysis and non-SLE patients receiving dialysis and determine the factors that affect survival after dialysis.

Methods: We used the National Health Insurance Research Database and collected data on patients who started maintenance dialysis from 2001 to 2003. The patients were followed from initiation of dialysis until death, discontinuing dialysis, or the end of 2008. We performed a Kaplan-Meier analysis of the cohort and used multivariate Cox regression analysis to identify significant predictors of survival.

Results: Of the 22,394 dialysis patients studied, 303 (1.35%) had SLE. Patients with SLE were predominantly women and younger than the other patients (39.4 ± 15.3 years vs. 59.4 ± 14.2 years, p < 0.001). The Kaplan-Meier survival curves for dialysis patients with and without SLE are illustrated.

The 1- and 5-year cumulative survival rates were 97.6% and 84.8% in patients with SLE, and 92.7% and 62.2% in patients without SLE. After adjusting for age, gender, dialysis modality, and comorbidities, we did not find a significant survival difference between the two patient groups after 8 years of follow-up. Multivariate analysis showed that older age (≥65), male gender, and the presence of diabetes were independent predictors of mortality (p<0.05) among SLE patients.

Conclusions: The long-term survival outcome was similar between the SLE and non-SLE patients undergoing dialysis. The factors affecting patient mortality were not all identical in these two groups.

TH-PO635

Single Use Versus Reprocessed Dialyzers and Mortality Daniel E. Weiner, Hocine Tighiouart, Klemens B. Meyet. Tufts Medical Center, Boston, MA.

Background: There is limited overall evidence either supporting or discouraging dialyzer reprocessing.

Methods: To explore the long-term medical utility of dialyzer reuse, we compiled patient-level data for all prevalent hemodialysis patients with Medicare coverage treated at a DCI facility on January 1, 2005, merging DCI data with USRDS data to better define comorbidity and outcomes based on medical claims. Reuse status was determined by patient dialyzer prescription. The primary outcome was time to all-cause mortality through July 2009, ascertained with both DCI and USRDS data; sensitivity analyses examined transplant and PD-censored mortality. Primary analyses used Cox regression with extensive comorbidity adjustment while secondary analyses matched patients based on their propensity to reuse. A sandwich estimator accounted for center effect.

Results: There were 9,051 Medicare patients included; 5735 were reuse patients in reuse facilities and, of 3316 non-reuse patients, 2641 were in non-reuse facilities. Reuse patients were more likely to be African American, Hepatitis C negative, and had more grafts and fewer catheters. Dialysis vintage was 3 months longer, blood pressure slightly higher and heparin use substantially lower in non-reuse patients. Propensity score matching successfully paired 2533 patients in each groups. Results of primary and propensity matched analyses were similar and revealed no significant difference in outcomes by reuse status (Figure), with a hazard ratio for reuse of 1.01 (0.96, 1.07) and 0.99 (0.92, 1.06) in multivariable adjusted non-matched and propensity-matched analyses, respectively. Analyses censoring at modality change were similar to those in the figure.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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Conclusions: Dialyzer reuse is not associated with mortality when compared to single use. The decision to reuse therefore should be based on other factors, including resource utilization and cost and environmental concerns.

Funding: Private Foundation Support

TH-PO636

Early Start of Renal Replacement Therapy in Critically Ill Patients Improves Renal Outcome but Not Survival

Anne Lerner-Gräber, Timo Speer, Jochen G. Raimann, Len A. Usvyat, Peter Kotanko, Nathan W. Levin

Background: To assess the relationship between serum creatinine and urea levels at the beginning of renal replacement therapy (RRT) on mortality and need for RRT after discharge in critically ill patients.

Methods: Observational study of all patients requiring RRT in 2 internal medical and 4 surgical intensive care units (ICUs) of a tertiary hospital between 03/2007 and 08/2009. Patients being on chronic RRT before admission to the hospital were excluded. Serum creatinine and urea levels at the start of RRT, mortality and recovery of kidney function during hospitalization and after discharge (mean follow-up period 511 days) were assessed.

Results: In total, 583 patients and 5694 RRTs were included for analysis. The in-hospital mortality was 68.4% (n = 399), whereas the mortality rate in survivors after discharge who were not lost to follow-up (n = 140, 24.0%) was 25.7%. There was no statistically significant difference of serum creatinine and urea levels at initiation of RRT between those patients who died or who survived. After discharge, in 20 survivors no recovery of renal function after acute kidney injury could be observed and they required RRT due to persistent renal insufficiency. In these patients serum creatinine (261 ± 151 vs. 475 ± 282 µmol/L, p<0.000) as well as serum urea levels (20.8 ± 11 vs. 30.8 ± 16.5 mmol/l, p=0.007) were assessed.

Conclusions: In critically ill patients with kidney injury requiring RRT on the ICU, lower serum creatinine and urea levels at the start of RRT signalize better renal outcome after hospital discharge, while mortality is not improved.

TH-PO637

Predictors of Hospitalizations in Maintenance Hemodialysis (HD) Patients

Jochen G. Raimann, Len A. Usvyat, Peter Kotanko, Nathan W. Levin

Background: Despite technological advances in hemodialysis (HD) technology, mortality and hospitalization rates remain high in these patients (pts) (USRDS 2010). This is of substantial clinical and economic impact. This analysis attempts to identify significant predictors of hospitalization in hemodialysis pts.

Methods: Pts receiving HD in RRT clinics for more than 365 days from Jan 1, 2008 to Dec 31, 2010 were included. Clinical parameters, hospital days and admissions during the observation period were averaged per patient for the entire observation period. Two multivariate regression models were developed to predict hospital days (Model 1) and number of admissions (Model 2), respectively, during the observation period. Both models were adjusted for age, gender, race, HD vintage, diabetes (DM), HD access, pre HD systolic and diastolic blood pressure (SBP, DBP), pre HD temperature (temp), interdialytic weight gain (IDWG), post HD weight (wt), difference of post HD wt and prescribed target wt (Δ TW), enPCR, Kt/V, albumin, total calcium, ferritin, hemoglobin (Hgb), potassium, phosphorus, transferrin saturation (TSAT) and treatment time.

Results: 5758 incident and prevalent HD patients (age 60.5±15.4 years, 55% male, 51% blacks, BMI 28.7±12.8 kg/m²), with an average of 1.74 hospital admissions lasting 11.8 days per year, were studied. Model 1 (adjusted R² 0.25, P<0.05) significantly predicted hospital days and Model 2 (adjusted R² 0.25, P<0.05) hospital admissions. Table 1 shows significant predictors and unstandardized coefficients.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Table 1: Predictors of Hospitalization

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.08</td>
<td>0.85</td>
</tr>
<tr>
<td>Age [yrs]</td>
<td>0.97</td>
<td>0.80</td>
</tr>
<tr>
<td>Male [♂]</td>
<td>0.54</td>
<td>0.44</td>
</tr>
<tr>
<td>HD Vintage [yrs]</td>
<td>0.30</td>
<td>0.21</td>
</tr>
<tr>
<td>Diabetes [♂]</td>
<td>0.36</td>
<td>0.31</td>
</tr>
<tr>
<td>eKt/V</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>Δ TW [kg]</td>
<td>0.24</td>
<td>0.12</td>
</tr>
<tr>
<td>Hgb [g/dL]</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>TSAT [%]</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Tx [time]</td>
<td>0.07</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Conclusions: This analysis showed a significant relationship between hospitalization rates and potential modifiable indicators (fluid overload (IDWG, Δ TW), anemia management (Hgb, TSAT) and vascular access (catheter). Knowledge of predictive parameters may aid the identification of pts at risk and thus help to prevent hospital admissions.
TH-PO639

Health Literacy and Outcomes in Hemodialysis Patients: Jamie Green, 1 Maria K. Mor, 2 Anne-Marie Shields, 2 Mary Ann Sevick, 1, 3 Robert M. Arnold, 1 Paul M. Palevsky, 1, 2 Michael J. Fine, 1, 3 Steven D. Weisbord, 1, 2 Medicine, University of Pittsburgh, PA; 4VA Pittsburgh Healthcare System, Pittsburgh, PA.

Background: Although limited health literacy is common among chronic hemodialysis patients, its associations with patient outcomes are not well characterized. We sought to evaluate the associations of limited health literacy with dialysis treatment adherence, hospitalizations, and mortality.

Methods: Using the Rapid Estimate of Adult Literacy in Medicine (REALM), we measured the baseline health literacy of 260 chronic hemodialysis patients enrolled in a randomized clinical trial of symptom management strategies. We used Poisson regression to evaluate the independent associations of limited health literacy (REALM score <61) with dialysis adherence (abbreviated and missed treatments) and hospitalizations, and Cox regression to characterize the independent association of limited health literacy with mortality over a follow-up period of up to 24 months. The analyses adjusted for potential confounders and used multiple imputation to account for missing data.

Results: Overall, 41 of 260 patients (16%) demonstrated limited health literacy. Limited health literacy was independently associated with a greater number of abbreviated dialysis treatments (9.1% of sessions vs. 8.9% of sessions; incident rate ratio = 1.2; 95% CI 1.1-1.3) and missed treatments (3.6% of treatments vs. 1.7% of treatments; incident rate ratio = 1.4; 95% CI 1.1-1.7). There was a non-statistically significant trend towards a higher risk of hospitalization among patients with limited health literacy (2.7 hospitalizations per year vs. 2.2 hospitalizations per year; incident rate ratio = 1.2; 95% CI 1.0-1.4). Limited health literacy was not associated with a higher risk of mortality (hazard ratio = 1.3; 95% CI 0.6-2.9).

Conclusions: Patients receiving chronic hemodialysis with limited health literacy are more likely to shorten and miss dialysis treatments and may be at greater risk for hospitalization. Interventions to address limited health literacy may increase adherence and reduce health resource utilization in this chronically ill patient population.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO640

Relationship between Outpatient “AKI”, Proteinuria, Diagnosis Diabeties, Race and Rate of Decline in Renal Function, Nine Years Prior to Dialysis Initiation: Steven J. Rosansky, 1, 2 James W. Hardin, 1, 3 Frankie Richards, 1, 3 Kathlyn Sue Haddock, 1Ann M. O’Hare, 1 William F. Clark. 1 Research, Dorn Research Institute, Columbia, SC; 2 Biostatistics, University of SC School of Public Health, Columbia, SC; 3 Nephrology, University of Western Ontario, London, ON, Canada; 4 Nephrology, VA Seattle, Seattle, WA.

Background: AKI has been examined as a discharge coded, inpatient phenomenon. Predialysis creatinine based e GFR is commonly used in the decision to initiate dialysis, which may not account for AKI episodes and temporarily higher serum creatinine . Proteinuria is a primary determinant of renal function trajectory.

Methods: The current study examines the change in MDRD e GFR (ml/min/1.73m2/yr) using initial and final serum creatinine, to determine the change of renal function by: AKI; diagnosis diabetes; urine proteinuria and race. Growth curve analysis examined the effect of covariates on renal function decline. We studied 212 chronic dialysis patients with at least: 3 creatinine values over 3 years. The method of creatinine determination ≥ 1.4; 95% CI 1.1-1.7). There was a non-statistically significant trend towards a higher risk of hospitalization among patients with limited health literacy (2.7 hospitalizations per year vs. 2.2 hospitalizations per year; incident rate ratio = 1.2; 95% CI 1.0-1.4). Limited health literacy was not associated with a higher risk of mortality (hazard ratio = 1.3; 95% CI 0.6-2.9).

Conclusions: Patients receiving chronic hemodialysis with limited health literacy are more likely to shorten and miss dialysis treatments and may be at greater risk for hospitalization. Interventions to address limited health literacy may increase adherence and reduce health resource utilization in this chronically ill patient population.

Funding: Veterans Administration Support, Private Foundation Support

TH-PO641

Serum Albumin Dynamics before Death in Chronic Hemodialysis (HD) Patients – Results of an International Study: Michael Etter, 1 Yuedong Wang, 2 Claudia Barth, 3 Mathias Schaller, 3 Adrian Marcos Guinigu, 3 Daniele Marcelli, 1 Cristina Marcelli, 1 Adam Tashman, 2 Len A. Usyvath, 4 Nathan W. Levin, 5 Peter Kotanko, 6 FMC Asia Pacific, Hong Kong, Hong Kong; 7 University of California - Santa Barbara, Santa Barbara, CA; 8 Kuratorium für Dialyse und Nierentransplantation, Cologne, Germany; 9 FMC Latin America, Buenos Aires, Argentina; 10 FMC Europe, Bad Homburg, Germany; 9 RRI, NY, NY; 11 University of Cologne Medical Center, Germany.

Background: HD patients (pts) experience mortality rate between 14-20% per year. In a US HD cohort a decline of serum albumin (SAib) before death was described (Kotanko 2009). Here, we investigate if the SAib decline holds for a globally diverse HD population.

Methods: HD databases from FMC clinics in Europe, Asia, Latin America, RRI clinics in US, and K/H in Germany were queried. SAib was standardized to the BCG. Pre-death SAib dynamics were analyzed by estimating the mean SAib level before death and its 1st derivative using quintic splines.

Results: 27807 HD pts from 23 countries were studied (Europe 17 [N=12333]; age 71.7, 59% males]; South-East Asia 4 [N=1484]; age 68; 53% males]; Argentina [N=10517]; age 63.1; 58% males]; USA [N=3473]; age 69.9; 56% males]). SAib levels prior to death [mean (SD); g/L] were 3.53 (0.63); 3.45 (0.59); 3.63 (0.54); 3.3 (0.62); same order as above. In all regions, SAib levels dropped between 0.24 (South-East Asia) and 0.44 (USA) mg/dL in the 2 years preceding death in female pts (left).

Conclusions: The rate of SAib decline accelerated before death and was similar in all databases (right). The results were identical in male pts and in pts from K/H (not shown).

Funding: This international study corroborates previous findings in US HD pts. Insights into the pre-death biology are key to the development of alert systems to facilitate timely diagnostic and therapeutic interventions.

TH-PO642

Frequency of Pulmonary Embolism and Associated Mortality in Patients with Chronic Kidney Disease and End Stage Renal Disease: Arik Kotnik Komn, Gigli, Kumar, Puneet Sood, Rahul S. Nanchal, Aaron T. Dall. Medicine, Medical College of Wisconsin, Milwaukee, WI.

Background: Chronic Kidney Disease (CKD) and End Stage Renal Disease (ESRD) are growing problems in the United States. Studies are not clear with respect to frequency of Pulmonary Embolism (PE) in these patients.

Methods: We analyzed the data from the Nationwide Inpatient Sample database from the year 2007. Adult patients with ESRD, CKD and PE were identified using appropriate ICD-9-CM codes as previously used. The primary outcomes measured were frequency of PE in patients with CKD and ESRD. Secondary outcome was all cause in-hospital mortality.

Results: Of 32,759,253 estimated adult discharges, 30,119,186 had normal kidney function, 1,799,785 had CKD and 840,282 had a discharge diagnosis of ESRD. The unadjusted frequency of PE was 0.95% in patients with CKD and 0.59% in patients with ESRD in comparison to 0.83% in patients with normal renal function (p < 0.001). On multivariate regression analysis, patients with CKD are more likely to have a PE (OR 1.27; 95% CI: 1.14 - 1.42) and those with ESRD are less likely to have a PE (OR 0.69; 95% CI: 0.61 - 0.78) when compared to patients with normal renal function. The all cause in-hospital mortality in patients with PE increased with decline in renal function. 7.2% of patients with normal renal function who had a PE died, this number rose to 12.6% for those with CKD and 17.2% for those with ESRD (p<0.0001).

Conclusions: Patients with CKD have a higher frequency of PE whereas patients with ESRD have a lower frequency of PE in comparison to those with normal renal function. All cause in-hospital mortality in patients with PE is higher in those with CKD in comparison to those with normal renal function and is highest in those with ESRD.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

260A
The Effect of Censoring for Transplantation When Analyzing Survival on Dialysis: Applying Competing Risk Analysis to ERA-EDTA Registry Data Marlies Noordzij, Karlijn J. Van Stralen, Kitty J. Jager. ERA-EDTA Registry, Department of Medical Informatics, Academic Medical Center, Amsterdam, Netherlands.

Background: When studying patient survival on dialysis treatment, kidney transplantation (TX) is often considered a censored observation, while it is in fact an event (vascular access at death). Kaplan-Meier (KM), a conventional method for unadjusted survival analysis, can handle only one outcome and may therefore be inappropriate in the presence of competing risks. We therefore compared the results of KM with those of the Cumulative Incidence Competing Risk (CICR) method; an alternative method specifically designed for analyzing competing risks.

Methods: Based on data of incident dialysis patients from countries participating in the ERA-EDTA Registry we studied 1, 2, and 5 years patient survival of patients starting dialysis between 1999 and 2003. The probabilities of separate events, i.e. death, TX and event-free survival (EFS) from day 91 after the start of dialysis onwards were calculated using both the standard KM and the CICR methods.

Results: Data analysis was based on 69,081 patients from Europe. When studying 1-year patient survival on dialysis, both methods yielded similar results, i.e. probabilities of death, TX and EFS of 17%, 7%, and 76%, respectively, with an expected total of 100%. For 2-years survival the KM method yielded probabilities of 31% for death, 16% for TX and 58% EFS (total 105%). These probabilities were 29%, 13% and 58%, respectively, for the CICR method (total 100%). Finally, after 5-years of follow-up, the difference between the methods was even more pronounced. The probabilities of death (61%) and TX (34%) were overestimated by KM (EFS 25%) to a total of 120%, while the KM method yielded lower probabilities of 51%, 24% and 25%, respectively, adding up to 100%.

Conclusions: The KM method overestimates the risk of both mortality and TX with percentages as high as 10% after 10 years after the start of dialysis Therefore, this method is inappropriate to analyze patient survival in the presence of competing risks and using the CICR method is recommended.

TH-PO644
Time to Death from Cessation of Dialysis in an Elderly Veteran Population Rizwan Badar,1,2 Xiao Wan,3 Seydi-Al Sadjadi,3 Navin Jainapal,3 Ruth Peeters.1 1Nephrology, VA Loma Linda Healthcare System, Loma Linda, CA; 2Nephrology, Loma Linda University Medical Center, Loma Linda, CA.

Background: An extensive review shows a paucity of literature on time to death after cessation of dialysis in end stage renal disease (ESRD) patients. A common question nephrologists often face from patients is the length of time before death after dialysis is withdrawn. In a report by Murray et al in 2006 using the United States Renal Data System (USRDS) data from 2001 to 2002, the time to death after stopping dialysis was not looked at.

Methods: We conducted a single center retrospective chart review of 174 adult veteran dialysis patients between January 2002 and May 2011 to determine length of time until death after withdrawal from dialysis. We also looked at DNR status, place of death, and vascular access at death.

Results: The 174 patients had a mean age of 69.0±10.9 years and the following characteristics: 97.1% male, 68.4% diabetic, 93.7% hypertensive, and 59.8% had coronary artery disease. Diabetes and hypertension were the most common cause of renal failure and accounted for 65% of the patients. Mean duration on dialysis was 3.25 years, and vascular access at death was catheter (43.7%) or fistula (48.8%). 74.7% of patients had a DNR, of which only 1.7% did not have it honored. 37.9% of patients withdrew from dialysis. Mean time to death after stopping dialysis was 10.7 days with a standard deviation of 15.5 days. Place of death was 48.3% hospital, 33% hospice, 6.9% nursing home, 5.2% home, 4.6% dialysis unit.

Conclusions: Our results show that withdrawal from dialysis (37.9%) is much more common than that reported by the USRDS. This could be due to the characteristics of our cohort, an elderly veteran population. Mean time to death after cessation of dialysis (10.7 days) is also slightly higher than that reported in the literature. Further studies regarding withdrawal of dialysis as well as patient and family satisfaction with the dying process are needed.

TH-PO645
Cause of Death in a Veteran Dialysis Population: Death Certificate vs. Chart Review Rizwan Badar,1 Xiao Wan,3 Seydi-Al Sadjadi,3 Navin Jainapal,3 Ruth Peeters.1 1Nephrology, VA Loma Linda Healthcare System, Loma Linda, CA; 2Nephrology, Loma Linda University Medical Center, Loma Linda, CA.

Background: According to USRDS, “other” is the most common cause of death (COD) in dialysis patients with ESRD, followed by cardiac death. This information is gathered from the ERSD Death Notification Form 2746, which is completed by nephrologists, other physicians, or non-physicians. Often times the death certificate (DC), which is frequently inaccurate, is used to obtain this information. Determining the COD is difficult due to co-morbidities such as anemia, poor general health (GH), and lack of physician training.

Methods: We conducted a single center retrospective chart review (CR) of 174 adult veteran dialysis patients between January 2002 and May 2011 to determine the correlation between CR and DC COD. A panel of trained physicians determined the CR COD and extracted the DC COD, and the two were categorized and compared by calculating the Pearson correlation coefficient (r). Statistical analysis was performed using SPSS, version 17.0.

Results: The 174 patients had a mean age of 69.0±10.9 years and the following characteristics: 97.1% male, 68.4% diabetic, 93.7% hypertensive, and 59.8% had coronary artery disease (CAD). Mean duration on dialysis was 3.25 years, and vascular access at death was 43.7% catheter vs. 48.8% arteriovenous. The most common categorized COD by CR and DC are shown in Table 1. The correlation between CR and DC COD was statistically significant (p<0.001) but weak (r=0.42).

Table 1. Cause of Death* by Death Certificate vs. Chart Review

<table>
<thead>
<tr>
<th>DC COD</th>
<th>CR COD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac, n(%)</td>
<td>11(9.2)</td>
</tr>
<tr>
<td>Infectious, n(%)</td>
<td>31(30.8)</td>
</tr>
<tr>
<td>Traumatic, n(%)</td>
<td>15(13.5)</td>
</tr>
<tr>
<td>Malignancy, n(%)</td>
<td>45(37.5)</td>
</tr>
</tbody>
</table>

144 patients with missing DC were excluded

Conclusions: Our findings demonstrate poor correlation between COD by CR and DC. In addition, infection and not “other” or CAD, was the leading cause of death in this elderly veteran dialysis population. We speculate that death certificates are often inaccurate due to lack of physician training and bias in coding, and that ESRD death notification form shortcomings limit the ability to properly report the accurate cause of death.
patients in their high seventh or eighth life decade exhibit a yearly mortality of about 20%. From such epidemiological reasoning, stable long-term RRT in the elderly is a satisfying, life-prolonging and adequate treatment modality.

TH-PO648  
Estimated Glomerular Filtration Rate (eGFR) at Initiation of Dialysis and Mortality in Patients with Advanced Chronic Kidney Disease (CKD). A Meta-Analysis  
Paweena Susantitaphong, Sarah Altamimi, Motaz Ashkar, Bertrand L. Jaber. Medicine, St. Elizabeth’s Medical Center, Boston, MA.

Background: The number of patients with advanced CKD initiating dialysis is on the rise worldwide. Recent data suggest that initiation of dialysis at higher eGFR may be associated with worse clinical outcomes. We conducted a meta-analysis to examine the association of eGFR at initiation of dialysis with mortality in patients with advanced CKD.

Methods: We searched for randomized controlled trials, prospective and retrospective cohort studies in MEDLINE through March 2011. We selected studies that focused on early versus late initiation of dialysis (defined by eGFR or creatinine clearance) and mortality among patients with advanced CKD. Using random-effects model meta-analysis, we computed the adjusted hazard ratio of eGFR at initiation of dialysis and all-cause mortality.

Results: Sixteen observational studies and one randomized controlled trial were identified (n = 3,365,933). By meta-analysis, which was restricted to the 14 cohort studies with retrievable data (n = 468,188), higher eGFR (per 1 mL/min/1.73 m² increase) at initiation of dialysis was associated with a higher pooled adjusted hazard ratio (HR) for all-cause mortality (1.038; 95% CI 1.026, 1.050; P<0.001; Figure). Among the 7 studies that adjusted analytically for nutritional variables, higher eGFR remained associated with all-cause mortality (adjusted HR 1.028; 95% CI 1.013, 1.044; P<0.001) compared to those that did not (adjusted HR 1.053; 95% CI 1.034, 1.072; P<0.001).

Conclusions: Hospitalizations are common in older incident hemodialysis patients and are often recurrent. Location of dialysis initiation and patient preparedness for therapy may provide insight into degrees of risk for recurrent hospitalizations.

TH-PO650  
Socioeconomic Gradients of ESKD Incidence Vary with Age and Primary Renal Disease  

Background: An inverse relationship between socioeconomic status (SES) and the incidence of renal replacement therapy (RRT) has been shown in several settings. In Australia, public hospitals provide access to RRT under a government-funded scheme without a cost barrier. However, previous studies have suggested an inverse relationship exists, not explained by higher benefits among Indigenous people in Australia. We examined relationships between SES and RRT incidence across various groups, including age and primary renal disease.

Methods: Residential postcodes of non-Indigenous people who started RRT in Australia from 1999 to 2009 (N=19,411) were mapped to SES using standard concordance files from the Australian Bureau of Statistics. RRT incidence rates were compared across SES deciles using Poisson regression to adjust for age, gender and year.

Results: The overall incidence rate of RRT decreased with increasing SES advantage (RR per decile 0.96 [95% CI 0.96 – 0.97]). A more marked gradient was noted for ESKD attributed to diabetic nephropathy (in contrast there was lesser or no gradient observed for reno-vascular or genetic diseases).

Trends across the SES spectrum were similar for males and females. However, the SES gradient was more marked among people aged 40-69 years (RR 0.94 [0.94 – 0.95] per decile) than older people (RR per decile 1.00 [1.00 – 1.01]). The gradient was also more marked among those who lived in major cities (RR per decile 0.90 [0.88 – 0.92]) compared with more remote locations (RR per decile 0.98 [0.92 – 1.02]).

Conclusions: A clear, inverse relationship exists in Australia between rates of RRT and SES. The differential is most marked among those with diabetic nephropathy: either the incidence of diabetes is higher among people with lower SES, or diabetic nephropathy progresses more rapidly. The SES effect is most marked among people of working age, and attenuated among older people.

*Funding: Government Support - Non-U.S.
TH-PO651

Lessons Learnt from Consecutive Health-Related Quality of Life (KDQOL-SF) Surveys in a Mayo Clinic Stable Hemodialysis Patient Population

Macaulay A. Onuigbo,1,2 *College of Medicine, Mayo Clinic, Rochester, MN; 1Nephrology, Mayo Clinic Health System, Eau Claire, WI.

Background: The Kidney Disease Quality of Life Short Form (KDQOL-SF), a well validated epidemiological research tool, is a short-form health survey that measures ESRD patients’ self-assessment of functioning and well-being as determined by 3 component scores of physical component summary (PCS), mental component summary (MCS) and kidney disease component summary. More recently, there is increasing application of KDQOL-SF scores as a measure of the ESRD patients’ health-related quality of life (HRQOL).

Methods: A prospective ongoing administration of KDQOL-SF assessments by certified and trained Dialysis Social Workers as part of ongoing established KDQOL care of HD patients was repeated in 56 patients between October 2008 and September 2010, at least six months between the two consecutive assessments.

Results: A total of 112 administrations of the KDQOL-SF assessments to the 56 HD patients were analyzed; they were administered a mean of 13 (6-22) months apart. PCS mean score increased from 32 to 34 and mean of burden score increased from 47 to 49. Overall, all the indices showed a trend towards improved higher scores at the second testing. When fortuitously, the second testing was administered within days of an acute illness or hospitalization, patient scores were reduced, albeit transiently.

Conclusions: In general, HRQOL scores in stable HD patients tend to get better with more time on dialysis. More of such studies are warranted to enhance the use of this research tool in the improved care of ESRD patients on maintenance HD. Such surveys must be avoided within 30 days of acute illnesses and hospitalizations.

TH-PO652

Usefulness of Assessing Dialysis-Related Symptom by Serum Level of β2-Microglobulin as a New Surrogate Marker Evaluating the Quality of Life Among Dialysis Patients

Atsuhiko Kanno, Ikuto Masakane, Satoko Ito, Minoru Ito, Kiyotaka Yabuki. Yabuki Hospital.

Background: The level of β2-microglobulin (β2-M) is strongly associated with the presence of carpal tunnel syndrome and dialysis-related amyloidosis in chronic dialysis patients. However, it is not fully clarified whether serum level of β2-M is related to the presence of other common problems among dialysis patients, though history of dialysis is now considered a long-term.

Methods: A total of 342 patients performed with chronic maintenance hemodialysis (HD) or hemodiagnosis [HDf] in our three facilities were participated and they were evaluated by the self-rating score questionnaire based on the five graded range scales from 0 (none) to 5 (very strong) according to the severity of symptoms. They include 20 physical and psychological common dialysis-related symptoms. Odds ratio for each of moderate to severe (score = 2, 3, 4) or severe (score = 3, 4) levels of these complaints were calculated using a multiple logistic regression model adjusted for confounding factors including age, gender, years of dialysis, history of diabetes mellitus, K/V, normalized protein catabolic rate, body mass index, prediagnostic values of hemoglobin, albumin, β2-M, sodium, potassium, phosphate.

Results: Among participants, 146 (42.7%) patients were treated with HDF and the overall mean values were 66.7 years for age and 19.3% of participants had a history of dialysis for more than 10 years (median 5.4 years). There was a significant relationship between β2-M and moderate to severe length (odds ratio [OR] 1.02, P = 0.024), loss of interest (OR 1.05, P = 0.0028), reduction of blood pressure during dialysis (OR 1.06, P = 0.0056) and a sense of thirst (OR 1.06, P = 0.0028).

Conclusions: In our study, it was shown that predialysic serum level of β2-M was significantly associated with various dialysis-related symptoms. There is a possibility that β2-M would be a useful clue for the presence of common complaints, though history of dialysis was not necessarily a long-term. It is considered to be verified that an effort to diminish the level of β2-M, as a surrogate marker estimating the quality of HD, would contribute to relieve these symptoms.

TH-PO653

Frailty Is Associated with Earlier Dialysis Initiation in End-Stage Renal Disease

Yeran Bao,1 Lorien S. Dalrymple,2 Glenn M. Chertow,2 George A. Kaysen,2 Kirsten L. Johansen.1 *Medicine, UCSF, San Francisco, CA; 1Medicine, UC Davis, Davis, CA; 2Medicine, Stanford University, Palo Alto, CA.

Background: Over the last decade, patients have been started on dialysis at progressively higher levels of residual kidney function (early start). Some have suggested higher mortality with early start, while others argue that this could reflect a tendency for sicker patients to start early. We hypothesized that frailty, a predictor of adverse outcomes in end-stage renal disease (ESRD), is associated with dialysis initiation at a higher level of kidney function.

Methods: We undertook a cross-sectional study using data from the Comprehensive Dialysis Study (CDS), a United States Renal Data System (USRDS) special study. We examined the baseline prevalence of frailty, its associated clinical characteristics, and its relationship with early start of dialysis. Frailty was defined using a modification of Fried’s criteria. Early start was defined as starting dialysis at an estimated glomerular filtration rate (eGFR) >10 ml/min/1.73 m². We used multivariate logistic regression to model the relationship between clinical characteristics and frailty and the association between frailty and early start.

Results: Among a total of 1,576 CDS participants included in the study, the prevalence of frailty was 73.3%. Female gender, being on Medicaid, higher disease burden, and higher eGFR were associated with higher odds of frailty. Each 1 ml/min/1.73 m² increase in eGFR at dialysis initiation was associated with an 8% higher odds of being frail (OR 1.08, 95% CI 1.05-1.11, p<0.001). When the association between frailty and early start was examined, frail patients were nearly twice as likely to start dialysis at an eGFR >10 ml/min/1.73 m² (OR 1.91, 95% CI 1.50-2.44, p<0.001) after adjusting for age, gender, race, Medicaid status, tobacco use, serum albumin, serum hemoglobin, early nephrology referral, erythropoietin use and disease burden.

Conclusions: In light of the recent trend of early start and the lack of evidence to support it, our study highlights that frailty may be an important clinical measure as we assess the timing and effectiveness of renal replacement therapy.

Funding: NIDDK Support, Veterans Administration Support

TH-PO654


Paul L. Kimmel, Dmitry Vishniakov, Paul W. Eggers. NIDDK, NIH, Bethesda, MD.

Background: Access to highly active antiretroviral therapy (HAART) since 1996 has changed HIV infection from a fatal to a chronic illness. Previous ESRD hemodialysis patient studies showed incidence rates plateaued in the 1990’s. We report updated trends in incidence, hospitalizations and prevalence of ESRD HIV infection.

Methods: A retrospective cohort study of HIV infected ESRD patients was performed, using the CMS Medical Evidence Form where HIV was listed as a primary cause of ESRD and/or Medicare billing data where ESRD HD patients had at least 1 hospitalization or 2 outpatient encounters with an HIV/AIDS diagnosis.

Results: The number of incident cases of ESRD HD HIV infection was relatively stable (mean 816±45 per year), but the incidence rate decreased from 3.0 (1995) to 2.7 per million US population (2007), and from 1.4 to 0.67% of incident ESRD patients (1995/2007). Mean incident age increased from 39.0±0.3 to 44.7±0.4 y, black patients decreased from 90.4 to 86.2%, and women increased from 25.3 to 36.0% of the incident population. Median survival tripled from 0.9 (1995) to 2.7 y (2005). In contrast, prevalent HIV ESRD increased linearly from 1.971 to 6.741 cases. Prevalence rate increased from 7 to 12 HIV infected per 1000 ESRD patients from 1995 to 2007 respectively (0.3 to 0.6% of the ESRD HD population). The ESRD program hospitalization rate was 0.218 in 2007, while in HIV infected patients it was 22.4% higher (0.267). There was an approximately 2.5 fold increase in the number of hospitalizations for infection in HIV patients compared to uninfected patients, a disparity not seen in other diagnostic groups.

Conclusions: While the incidence of HIV infection has remained numerically stable, and has decreased in comparison to the increase in the total ESRD incident population, this does not explain the program prevalence of HIV infection. Survival has increased dramatically, leading to doubling in the albeit low prevalence of HIV infection in the ESRD population over the past decade. While access to HAART in the US has resulted in a decrease in incidence, the number and age of HIV infected patients has grown as patient survival improves. HIV infection in HD patients will remain a concern for the foreseeable future.

Funding: NIDDK Support

TH-PO655

Risk of Infection-Related Hospitalizations by Dialysis Modality: A Propensity-Score Matched Cohort

Jean-Philippe Laffranchie,1 Elham Rahme,2 Sameena Z. Iqbal,2 *Michel Vallee.1,3 1Université de Montréal, Canada; 2McGill University, Canada; 3Centre de Recherche Hôpital Maisonneuve-Rosemont, Canada.

Background: Infection is the second leading cause of admission after cardiovascular disease among patients receiving maintenance dialysis. How peritoneal dialysis (PD) compares to hemodialysis (HD) in terms of overall infection-related hospitalization risk is not known.

Methods: We conducted a retrospective cohort study using a database linking administrative databases and a dialysis registry in Quebec, Canada, between January

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

Background: Pre-HD body temperature (BT) is important routine indicator, however, only BT typically below or above normal range is usually considered relevant. Since little is known about the significance of BT changes over time in HD patients (pts), this study aimed to explore this area.

Methods: Data of all RRI HD pts beginning HD b-n Jan 1, 2000 and Feb 28, 2010 were analyzed. Pts’ baseline pre-HD BT (BTBL) was computed as an average of the first 30 days on HD; the slope of BT change (BTSLICE) was computed for 1st yr. All BTBL values were well balanced in each group including: Mean age (PD: 57.8 ± 15.6 vs HD: 57.9 ± 15.0), sex (PD: 52.6% vs HD: 48.0%), and diabetes (PD: 40.6% vs HD: 40.0%). During the study period, 338 patients were hospitalized once for an infection, 119 twice, and 101 at least three times. PD was associated with an increased risk of infection-related hospitalizations compared to HD (unadjusted hazard ratio: 1.65, 95% confidence interval: 1.43, 1.89).

Conclusions: PD pts are at higher risk of infection-related hospitalizations than HD pts. Better knowledge of epidemiology of infection-related hospitalizations may help to prevent this high-burden morbidity among chronic dialysis patients.

TH-PO658

Hemoglobin A1C Levels among Diabetic ESRD Patients with Possible Relationship to a-Erythropoietin Therapy and Inflammation

Background: The objective of this study was to determine if Erythropoietin (a-Erythropoietin) therapy and inflammation affected A1C levels among Diabetic End Stage Renal Disease (ESRD) patients.

Methods: All subjects with ESRD at the three free standing dialysis units on Guam, a U.S. territory in Micronesia, were identified. Data from their medical records were collected and patient questionnaires from all subjects were completed.

Results: Out of 410 total subjects on dialysis, 341 participated in the study. 267 (78%) is caused by diabetes, 43 (13%) is caused by hypertension, 16 (5%) by Glomerulonephritis, 12 (3%) were other causes. The average A1C level among diabetic subjects was 7.0 (ranging from 2 to 12.7). The average random blood sugar on three different occasions was 189 g/dL (ranging from 60 to 637). Out of 341 subjects, 247 (72%) were on Erythropoietin Therapy (500-20,000 units/treatment). Out of 247, 159 were diabetic subjects. Out of 159 diabetic subjects, 101 (67%) had high Ferritin levels (greater than 500 units). The average Ferritin level was 831 units (ranging from 16 to 1,971). Cox proportional hazards models adjusted for demographics, labs, and multiple comorbidities were constructed to assess the relationship b-n BT and BTSLOPE and survival in the 1st and 2nd year on HD.

Conclusions: Our results indicate that A1C levels may be affected by Erythropoietin Therapy and acute or chronic inflammation (established by high Ferritin levels). A1C readings alone may not be a good method for the management of diabetic dialysis patients, nor is it a good indicator of true glucose control among patients who have inflammation or are on Erythropoietin Therapy. We recommend combination of A1C and random blood sugars for better management of diabetes for patients who are on dialysis. Clinicians who care for diabetic dialysis patients should take into consideration the evaluation of patients for Erythropoietin dosage and inflammatory assays for better and more accurate management of their diabetes.

Funding: Private Foundation Support

TH-PO659

Low Serum Bicarbonate Is Associated with an Increased Risk of Death in the US Population

Background: Low serum bicarbonate levels are associated with a higher risk of CKD progression and death in people with CKD, however, the association between low serum bicarbonate levels and death in the general population is unknown.

Methods: Adult NHANES III participants were grouped into one of four serum bicarbonate categories according to the baseline value: ≤20, 21-25, 26-30, and >30mM. The hazard of death for each group, compared to the 26-30mM group, was determined after adjusting for age, gender, race, CKD, albuminuria, CHF, lung disease, and diuretic use.

Results: Those with serum bicarbonate ≤20mM were more likely to be female, African-American, and have CHF, CKD, and higher albuminuria. Characteristics of NHANES III participants by serum bicarbonate

<table>
<thead>
<tr>
<th>Variable</th>
<th>≤20</th>
<th>21-25</th>
<th>26-30</th>
<th>&gt;30</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum HCO3 (mM)</td>
<td>≤20</td>
<td>21-25</td>
<td>26-30</td>
<td>&gt;30</td>
<td>p</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>44.7 (20.1)</td>
<td>42.0 (16.7)</td>
<td>45.2 (16.8)</td>
<td>46.6 (17.7)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Men(%)</td>
<td>51.3 (21.0)</td>
<td>51.5 (16.5)</td>
<td>49.4 (19.2)</td>
<td>51.3 (15.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>African-American Race(%)</td>
<td>15.5 (9.2, 24.9)</td>
<td>13.3 (10.3, 14.6)</td>
<td>10.6 (9.3, 12.0)</td>
<td>9.4 (7.7, 11.5)</td>
<td>0.396</td>
</tr>
<tr>
<td>Albumin:Cr (mg/gm)</td>
<td>1.70 (0.8, 3.0)</td>
<td>1.74 (0.7, 4.6)</td>
<td>1.54 (4.6, 3.1)</td>
<td>1.52 (4.7, 3.2)</td>
<td>0.004</td>
</tr>
<tr>
<td>Diabetic use(%)</td>
<td>8.5 (5.6, 19.0)</td>
<td>4.3 (3.3, 5.5)</td>
<td>7.2 (4.6, 8.1)</td>
<td>10.5 (8.0, 12.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Albumin/Cr (mg/mg)</td>
<td>111.4 (69.0)</td>
<td>30.2 (217.3)</td>
<td>23.9 (171.1)</td>
<td>22.9 (172.0)</td>
<td>0.106</td>
</tr>
</tbody>
</table>

Percentages shown as percent (95% C.I.); Continuous measures shown as mean (standard deviation).

Conclusions: Compared to the reference group, those with serum bicarbonate ≤20mM had a two-fold increased hazard of death (HR 1.98, 95%CI 0.97-4.06), whereas no difference was detected in the 21-25mM and >30mM groups.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: Serum bicarbonate levels ≥20 mM appear to be associated with an increased risk of death in the general population even after adjusting for demographic factors, CKD and other possible confounders. Funding: NIDDK Support, Other NIH Support - National Institutes of Health and the National Cancer Institute grant 1K12CA156723

TH-PO660

Time-Dependent Effects of Dietary Sodium Bicarbonate on Renal Disease Progression in 5/6 Nephrectomized Rats Sejong Kim,1 Nam Ju Heo,2 Yun Kyu Oh,3 Ki Young Na,2 Kwon Wook Jo,2 Jin Suk Han,2 1Internal Medicine, Gachon University of Medicine and Science, Korea; 2Internal Medicine, Seoul National University College of Medicine, Korea.

Background: Sodium bicarbonate therapy ameliorated metabolic acidosis (MA) and the decrease in glomerular filtration rate (GFR) only after the control of blood pressure (BP) in 5/6 nephrectomized rats with casein diet. Recent clinical trials, however, showed beneficial effect of sodium bicarbonate in patients with early and late stage chronic kidney disease. We evaluated time-dependent beneficial effect of sodium bicarbonate to prevent the decline in GFR and correct MA in rats with a remnant kidney.

Methods: Sprague-Dawley rats ate dietary sodium bicarbonate (NaHCO3) or sodium chloride (NaCl) with 20% casein without any antihypertensive medication for 12 weeks after 5/6 nephrectomy.

Results: After treating casein diet, alkali-treated group had higher levels of serum bicarbonate than control group (20.3 ± 0.55 vs. 15.6 ± 0.61 mM/L at week 4, 25.0 ± 1.73 vs. 13.1 ± 1.29 mM/L at week 10, and 31.3 ± 3.38 vs. 17.6 ± 0.93 mM/L at week 12). After week 4, systolic blood pressure in the two groups was over 160 mm Hg, and there was no difference between the groups. At week 4, glomerular filtration rate (GFR) in NaHCO3 group was higher than in NaCl group (0.36 ± 0.09 vs. 0.15 ± 0.01 mL/min/100g BW, P<0.01). After week 10, there were no differences in GFR between the two groups (0.14 ± 0.03 vs. 0.15 ± 0.04 mL/min/100g BW at week 10, and 0.07 ± 0.03 vs. 0.07 ± 0.01 mL/min/100g BW at week 12). At week 4 and week 10, glomerulosclerosis (GS) and tubulointerstitial damage indices (TI) in NaHCO3 group were less severe than in controls. In contrast, at week 12, GS in the alkali-treated group was more profound than in control group, and there was no difference in TI damage indices between the two groups.

Conclusions: Dietary sodium bicarbonate had short-term beneficial effects in ameliorating metabolic acidosis and preventing the decrease in GFR without correction of blood pressure. However, these effects did not sustain after 10 weeks.

TH-PO661

Dietary Determinants of Urinary Citrate Excretion Ernest I. Mandel,1,2 Eric N. Taylor,2 Gary C. Curhan,2 1Renal Division, Department of Medicine, Brigham and Women’s Hospital, Boston, MA; 2Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital, Boston, MA.

Background: General strategies for prevention of kidney stones include restricting animal protein intake and increasing fruit and vegetable intake. While these strategies may increase citrate excretion by altering dietary acid-base balance, the independent dietary predictors of urinary citrate excretion have not yet been explored.

Methods: We conducted a cross-sectional study within a sub-cohort of 2612 individuals from the Health Professionals Follow-up Study (older men), Nurses’ Health Study I (older women), and Nurses’ Health Study II (younger women) who provided two 24-hour urine collections for previous studies of nephrolithiasis. Dietary data were ascertained from the 131-item Willett food frequency questionnaire. Lifestyle and clinical data were culled from responses to biennial questionnaires. Multivariable linear regression was used to quantify the predictors of urinary citrate excretion.

Results: After adjusting for dietary, lifestyle, and clinical factors, those in the highest quintile of non-dairy animal protein intake had urinary citrate excretion 148 mg/d (95% confidence interval, 70.9, 224.5), 76 mg/d (12.3, 139.0), and 57 mg/d (8.3, 122.3) lower than those in the lowest quintile for older men, older women, and younger women respectively. Conversely, those in the highest quintile of dietary potassium intake had citrate excretion 155 mg/d (79.3, 231.4), 121 mg/d (51.7, 189.3), and 56 mg/d (-16.4, 127.7) higher than those in the lowest quintile. Among older men, those with daily vitamin C intake of 1000 mg/d or more had citrate excretion 146 mg/d (47.7, 244.4) lower versus those consuming less than 90 mg/d. Both dairy protein intake and urinary sodium excretion, as a marker of sodium intake, were associated with higher citrate excretion.

Conclusions: In addition to non-dairy animal protein and potassium intake, dietary intake of vitamin C, dairy protein, and sodium are significant independent predictors of urinary citrate excretion in certain populations. Funding: NIDDK Support

TH-PO662

Alkali Therapy from Prenatal Period Is Superior to Postnatal Alkalization in NBC1 W516X Knock-In Mice Featuring Severe Metabolic Acidosis and Early Lethality Yu-Wei Fang,1,2 Sung-Sen Yang,3 Min-Hua Tseng,1 Shih-Hua P. Lin1,3 1Graduate Institute of Medical Science, National Defense Medical Center; 2Division of Nephrology, Department of Medicine, Shin-Kong Wu-Ho-Su Memorial Hospital, Taipei, Taiwan; 3Division of Nephrology, Department of Medicine, Tri-Service General Hospital, Taipei, Taiwan.

Background: We have created the NaHCO3/cotransporter (NBC1) W516X knock-in mice as model of isolated proximal renal tubular acidosis (Ki 2011). These mice had early lethality associated with severe metabolic acidosis and may be used for evaluating the deleterious effects of metabolic acidosis on cell and organ dysfunction.

Methods: NBC1W516X/W516X mice were divided into 3 groups: (1) non-alkali treatment controls, (2) prenatal alkalization since post-coitus by feeding with 280 mM sodium bicarbonate (NaHCO3) in drinking water and 7.5% NaHCO3 100 µL/g intraperitoneally since day 6 after birth (Pre-Tx) and (3) The same alkalization since day 6 after birth (Post-Tx). Blood acid-base status, renal function, organ abnormalities, and survival rate were assessed. Results: NBC1W516X/W516X mice with severe metabolic acidosis and associated anemia, volume depletion, prerenal azotemia, several organ abnormalities (kidney atrophy, splanomegaly, reduced bone density) exhibited early lethality before weaning (21 days). Both Pre-Tx and Post-Tx alkali administration in NBC1 W516X/W516X mice markedly prolonged the survival well beyond weaning, dramatically decreased protein catabolism, and attenuated several organ abnormalities. Pre-Tx group achieved a significantly higher bicarbonate (15.5±1.4 mM/L in Pre-Tx, n=7 vs. 7.3±1.7 mM/L in Post-Tx, n=12, p<0.05) and pH (7.29±0.03 in Pre-Tx, n=7 vs. 7.14±0.05 in Post-Tx, n=12, p<0.05) accompanied by a significantly less splanomegaly and higher body weight than Post-Tx group. On day 60 after birth, the survival rate was significantly higher in Pre-Tx than Post-Tx group (57 % vs 33%, P<0.05).

Conclusions: Alkali therapy from prenatal period is superior to postnatal alkalization in treatment of severe metabolic acidosis in NBC1 W516X/W516X mice. Prenatal alkali therapy can also apply to some inherited diseases featuring metabolic acidosis before birth.

TH-PO663

Deletion of Glutamine/Neutral Amino Acid Transporter ASC2T (ATB0) Does Not Alter Acid-Base Regulation under Baseline Condition or in Response to Acid Loading Faraaz Siddiqui,1 Manoocher Soleimani, Hassane Amlal. 1Internal Medicine, University of Cincinnati, OH.

Background: Glutamine metabolism through ammoniagenesis plays an important role in the regulation of acid-base homeostasis. Several glutamine/neutral amino acid transporters are expressed in the proximal tubule cells but their role in glutamine transport is not well understood. An amino acid transport system ASC2T (ATB0) is reported to transport glutamine and is expressed in the brush border membranes (BBM) of kidney proximal tubule and large intestine, however, its role in glutamine metabolism and acid excretion is unknown.

Methods: To understand the role of ASC2T in glutamine metabolism and acid excretion, mice with genetic deletion of ASC2T (KO) were generated. KO and wild-type(WT) mice were placed in metabolic cages and subjected to 280 mM NH4Cl lodging for 6 days to induce metabolic acidosis.

Results: he ASC2T mutant mice (KO) showed complete absence of ASC2T expression in both kidney and colon. They are fertile, have normal growth and did not exhibit any acid-base abnormalities at steady-state. urinary NH4+ excretion increased from 67 to 753 µmole/day in KO and from 62 to 751 µmole/day in WT mice after 6 days of acid loading, indicating that the capacity of KO mice to excrete daily acid load is not hampered by the deletion of ASC2T. In the cortex of acid-loaded animals, the expression of apical glutamine transporter BoAT1 was not altered in KO vs. WT mice, whereas the expression of BBM gamma glutamyl transferase (GGT), which converts glutamine to glutamate, was significantly increased (+38%, P<0.02) in KO vs. WT mice. Interestingly, the expression of basolateral glutamine transporter SN1 was reduced by 46% (P<0.04) in KO vs. WT animals.

Conclusions: These studies indicate that the deletion of ASC2T results in compensatory activation of other mechanisms including GGT, which lead to increased ammoniagenesis and enhanced daily net acid excretion in response to acid loading. Funding: Clinical Revenue Support
TH-PO664
Angiotensin II-Stimulated Ammonia Production in Cultured Mouse Proximal Tubule Cells Is Modulated by Type 2 Angiotensin II Receptors: Effect of pH


Background: Angiotensin II is a hormone that activates ion transporters in the renal proximal tubule. This study investigated the role of Type 2 angiotensin II (AT2) receptors in regulating ammonia production.

Methods: Cultured mouse proximal tubule cells were incubated with angiotensin II and either PD123319 (PD) or b) AT2R cell surface expression levels.

Results: After exposure to pH 7.4, cells acutely treated with Ang II (10-9M) + PD (10-6M) displayed higher TAP compared to cells treated with Ang II alone (7.0±0.2 vs 7.0±0.2 mmol/min/mg prot, N=6, p<0.05). After exposure to pH 7.0 for 2h, cells treated with Ang II+PD displayed TAP that was similar to cells exposed to Ang II alone (8.0±0.4 vs 8.0±0.4 mmol/min/mg prot, N=6). AT2R cell surface protein expression was 2.2±0.3-fold higher in cells pre-incubated for 2h at pH 7.4 vs cells incubated at pH 7.0 (p<0.05, N=5). The reduction in expression levels with low pH was blocked by echinocandin.

Conclusions: Although we previously reported that low pH enhanced the stimulatory effect of Ang II on TAP in proximal tubule cells by increasing AT1R expression, the present results suggest that Ang II-stimulated TAP can be inhibited by AT2R activation and that the colchicine-sensitive reduction in AT2R expression with low pH may also contribute to the enhanced stimulatory effect of Ang II on TAP.

Funding: Veterans Administration Support

TH-PO665
Rescuing Kidney Anion Exchanger I Trafficking Mutants Emmanuelle Cordi, Carmen Chu, R. Todd Alexander.

Physiology, University of Alberta, Edmonton, AB, Canada.

Background: The anion exchanger I (AE1) is a membrane glycoprotein that exchanges chloride for bicarbonate. It is found in erythrocytes (eAE1), and in the basolateral membrane of alpha intercalated cells (kAE1). Mutations in the gene encoding the anion exchanger I can cause hereditary spherocytosis and/or distal renal tubular acidosis (dRTA). Some dRTA mutants, although functional in red blood cells or when expressed in Xenopus oocytes, are retained intracellularly in tubular epithelial cells. We investigated whether these mutants can be rescued to the cell surface, where they may be functional.

Methods: We tested chemical treatments and lower temperature incubations on two recessive dRTA mutations, G701D and C479W, and a dominant mutation, R589H, of kAE1. We tested chemical treatments and lower temperature incubations on these mutations.

Results: Our experiments showed that glycerol and DMSO can rescue the trafficking of kAE1 mutants G701D and R589H, but not C479W. Inhibition of protein synthesis with cycloheximide caused the rescued G701D and R589H mutants to return to the cytoplasm. These results indicate that the introduction of either a large structural change (L522I) or a positive charge (L522Q) in the critical domain of the NBCe1-A dimeric interface significantly affects the stability of the NBCe1-A dimer.

Conclusions: These results indicate that the introduction of either a large structural change (L522I) or a positive charge (L522Q) in the critical domain of the NBCe1-A dimeric interface significantly affects the stability of the NBCe1-A dimer.

Funding: NIDDK Support

TH-PO666
The Cytosolic Mutant L522P of NBCe1 Has a Dominant Negative Effect Osamu Yamazaki, Hidomi Yamada, Masashi Suzuki, Shoko Hori, Motonobu Nakamura, George Seki, Toshiro Fujita.

Internal Medicine, Tokyo University, Tokyo, Japan.

Background: Homozygous mutations in the electrogenic Na-HCO3 cotransporter NBCe1 cause proximal renal tubulocystic disease (pRTA) and ocular abnormalities. The defective membrane expression of NBCe1 causes familial miliary (PNAS 107,15963,2010). While the homozygous A656R mutation causes pRTA, the full-length ocular abnormalities, and hemiplegia, the heterozygous A656P mutation, which forms hetero-oligomer complexes with Wild-type NBCe1 in oocytes and retinae through a dominant negative effect. In the present study, we examined whether the L522P mutant of NBCe1, a common variant with pRTA, and hemiplegia, has a similar dominant negative effect.

Methods: Two-electrode voltage-clamp method in Xenopus oocytes and cell measurement in HEK293 cells were used to determine the NBCe1 activity.

Results: L522P alone had no electrogenic activity, but decreased the NBCe1 current by 67% when co-expressed with WT in Xenopus oocytes. In HEK293 cells, L522P was predominantly expressed in cytoplasmic regions. When co-expressed with WT, L522P showed a decreased cytoplasmic overlapping with the diminished membrane expression of WT. Functional analysis confirmed that L522P alone showed no transport activity, but significantly reduced the net NBCe1 activity by 49% when co-expressed with WT. Co-immunoprecipitation analysis using myc- and GFP-tagged constructs confirmed the association between WT and L522P. To examine the role of L522P in more detail, we also examined the properties of artificial mutants L522I and L522Q in HEK293 cells. L522I was properly expressed in the plasma membrane, showing the transport activity comparable to WT. By contrast, L522Q was predominantly expressed in cytoplasmic regions, showing no transport activity. While the co-expression of L522I with WT increased the net NBCe1 activity compared with WT alone, the co-expression of L522Q with WT significantly reduced the net NBCe1 activity by 42%.

Conclusions: These results indicate that the introduction of either a large structural change (L522I) or a positive charge (L522Q) in the critical amino acid L522 impaired the proper expression of NBCe1, resulting in the requirement of dominant negative effect.

Funding: Government Support - Non-U.S.

TH-PO667
The N-Terminal of NBCe1-A TM1 Tightly Interacts with the Cytoplasmic Domain Quansheng Zhu, Liyo Kao, Debra Newman, Weixin Liu, Ira Kurtz. Department of Medicine, UCLA.

Background: NBCe1-A is expressed on the basolateral membrane of the renal proximal tubule where it plays a critical role in bicarbonate reabsorption. The transporter consists of 1035 amino acids and spans the lipid bilayer 14 times. We have recently determined that the transmembrane segment 1 (TM1) is involved in forming part of the NBCe1-A subunit subunit interaction interface; however, the actual size of TM1 in the lipid bilayer has remained elusive. The importance of TM1 in NBCe1-A is also exemplified by a TM1- S427L mutation that causes proximal RTA. NBCe1-A-TM1 was previously assumed to have a similar length to that of AE1-TM1 based on the sequence homology, where the N-terminus is connected to the cytoplasmic domain with a highly flexible loop. In this study, we analyzed the folding of the N-terminal cytoplasmic region (Cys389 - Gln424) of NBCe1-A-TM1 using the substituted cysteine accessibility method combined with extensive chemical peeling and functional transport assays. Our findings have uncovered that NBCe1-A-TM1 is structurally unique: 1) TM1 contains 33 residues that start at amino acid Ala410; 2) the region of Arg394 - Leu406 is inaccessible to aqueous media even after 3 M sodium carbonate stripping; 3) the region prior to Arg394 is increasingly accessible to the aqueous media; 4) substitution of any of the three charged residues, Asp505, Lys409 and Arg394, blocks NBCe1-ATM1 expression; 5) co-immunoprecipitation analysis using myc- and GFP-tagged constructs confirmed the association and function of residues in the Cys389 - Gln424 region affects transporter function only minimally. On the basis of the results, we conclude that the NBCe1-A-TM1 is significantly longer than AE1-TM1 and interacts with the cytoplasmic domain tightly.

Funding: NIDDK Support

TH-PO668
TM5 and 6 Play an Essential Role in Forming the NBCe1-A Dimer Interface Quansheng Zhu, Liyo Kao, Debra Newman, Weixin Liu, Ira Kurtz. Department of Medicine, UCLA.

Background: The basolateral electrogenic sodium bicarbonate cotransporter NBCe1-A mediates the absorption of the filtered bicarbonate load in the proximal tubule. We previously determined that NBCe1-A consists of 14 transmembrane (TM) helices and exists as a dimer in the lipid bilayer. The molecular mechanism underlying the formation of the NBCe1-A dimeric interface is currently unknown. To address this question we performed substituted cysteine scanning mutagenesis coupled with disulfide cross-linking studies on NBCe1-A dimers expressed in HEK-293 cells. The results show that: 1) individual cysteine substituted amino acids at the beginning (Try567-Ser568) and the end (Gln463-Asp464) of extraacellular loops 3 are strongly cross-linked to form NBCe1-A dimers in the plasma membrane; 2) the cross-linked dimers are sensitive to DTT treatment indicating disulfide bond formation; 3) NBCe1-A dimers are resistant to NEM pre-treatment suggesting the cross-linking is formed during protein synthesis. Biotin maleimide labeling analysis revealed that the extracellular aqueous/lipid interface of TM 5 is at amino acid Tyr566 and TM 6 is at Ile468, indicating that extracellular loop 3 consists of 82 amino acids. Our experiments have demonstrated for the first time that the NBCe1-A dimeric interface is formed by the self-association of TM 5 of each monomer and the self-association of TM 6 of each monomer.

Funding: NIDDK Support

TH-PO669
Interplay between Glycosylation and Disulfide Bonding Defines NBCe1-A Extracellular Topography Quansheng Zhu, Liyo Kao, Rustam Azimov, Debra Newman, Weixin Liu, Ira Kurtz. Department of Medicine, UCLA.

Background: A common feature among sodium-driven bicarbonate transporters within the SLC4 family is the presence of four cysteines (Cys) in the glycosylated extracellular loop 3 (EC-loop 3). We recently showed that EC-loop 3 is the largest highly exposed surface at the NBCe1-A and appears to reside at the apex. In the present study, we determined the significance of the unique pattern of disulfide bond formation and glycosylation in the extracellular region of NBCe1-A.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
**TH-P0670**

**Towards the High-Resolution Structure of hhNBCn1**  
Natalia Abuladze, Nathaniel Magilnick, Kirill Tsirlinukov, Alexander Pushkin.  
*Medicine, UCL, Los Angeles, CA.*

**Background:** Integral membrane proteins account for ~30% of all proteins, and human membrane proteins are the targets for ~50% of therapeutic drugs. Despite these facts, high-resolution three-dimensional (3D) structures of less than 300 unique membrane proteins have been solved at atomic resolution mostly by X-ray crystallography, of which eukaryotic membrane proteins account for less than 10%. Recent advances of cryo-electron microscopy (cryoEM) and single-particle reconstruction have solved the structures of eukaryotic membrane proteins at atomic resolution mostly by X-ray crystallography, of which eukaryotic membrane proteins account for less than 10%. Recent advances of cryo-electron microscopy (cryoEM) and single-particle reconstruction have solved the structures of eukaryotic membrane proteins at atomic resolution.

**Methods:** We are using cryoEM for structural study of the human heart electrotonic sodium-co-transporter NBC3 (hhNBCn1) that plays an important role in the studies of eukaryotic membrane proteins. To form a domain-like structure on the surface of the transporter resembling receptors such as G-protein coupled receptors.

**Funding:** NIDDK Support

**TH-P0671**

**Structural Study of AE1 Chloride-Bicarbonate Exchanger**  
Kirill Tsirlinukov, Nathaniel Magilnick, Natalia Abuladze, Alexander Pushkin, Ira Kurtz.  
*Department of Medicine, UCL, Los Angeles, CA.*

**Background:** In erythrocytes and the collecting duct, AE1 (SLC4A1) mediates 2 major functions: 1) Cl−/HCO3− exchange, and 2) support of the structural integrity of erythrocytes via interaction with cytoskeletal proteins. AE1 (~100 kDa) consists of ~55 kDa membrane domain responsible for anion-exchange, and a cytoplasmic domain, which functions as an anchoring site for membrane-associated proteins. We have recently generated a 3D model of full-length AE1 using electron tomography of negatively stained AE1 dimers purified from bovine erythrocytes. In the present study the model was refined using single particle 3D reconstruction of approximately 1,000 images of AE1 dimers negatively stained with uranyl formate were recorded on a FEI Tecnai F20 electron microscope operated at 200 kV and magnification 50,000X. Analysis of images suggested that the AE1 dimer consists of a larger and a smaller domain. 3D reconstruction from ~5,000 randomly selected dimer particle images agreed with the proposed 2-domain model but suggested that particles of more than one conformation are present. To separate AE1 dimers were negatively stained with uranyl formate were recorded on a FEI Tecnai F20 electron microscope operated at 200 kV and a magnification 50,000X. Analysis of images suggested that the AE1 dimer consists of a larger and a smaller domain. 3D reconstruction from ~5,000 randomly selected dimer particle images agreed with the proposed 2-domain model but suggested that particles of more than one conformation are present.

**Methods:** We were using cryoEM for structural study of the human heart electrotonic sodium-co-transporter NBC3 (hhNBCn1) that plays an important role in the studies of eukaryotic membrane proteins. To form a domain-like structure on the surface of the transporter resembling receptors such as G-protein coupled receptors.

**Funding:** Private Foundation Support

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**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**Underlines represent presenting author.**

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**TH-P0672**

**CO2/NH3 Selectivities and Inhibitor Sensitivities of Mammalian Aquaporins**  
*Physical and Biophysics, Case Western Reserve University, Cleveland; Physiol & Biophys, University of Sao Paulo, Brazil.*

**Background:** The 13 mammalian aquaporins (AQP0-12) have diverse localization patterns and can be divided into 2 major families: orthodox AQPs and aquaglyceroporins. Previously we expressed mammalian AQP1, AQP4-M23 and AQP5 in Xenopus oocytes and used video microscopy to compute osmotic water permeability (Pf), and used microelectrodes to record the transient rise in surface pH (ApH) caused by CO2 or NH3 influx. Subtracting the respective values for day-matched, H2O-injected control oocytes yielded the channel-specific values for Pf and ApH, (semiquantitative index of gas permeability). We had found that AQP1, AQP4-M23, and each has a characteristic CO2/NH3 and CO2/H2O permeability ratio. Also, we had shown that pCMBS (blocks 4 H2O pores) reduces AQP1’s (ApH)h2o by ~100% and reduces (ApH)h2o by ~40%. DDS (does not block H2O pores) has no effect on (ApH)h2o, but reduces (ApH)h2o by ~60%.

**Methods:** We, here extend our study to determine if other AQPs (0-9) exhibit CO2/NH3 selectivity, and use inhibitors to explore the CO2 and NH3 pathways through AQP6 and AQP9.

**Results:** (ApH)h2o differed significantly from 0 for AQP9, AQP1, AQP4-M23, AQP5, AQP6, AQP7, AQP8, and AQP9. Pf differed significantly from 0 for all AQPs tested except AQP6. The ratio (ApH)h2o/Pf fell in the sequence AQP6 (vsAQP5/AQP4-M23>AQP1>AQP9>AQP3>AQP8). The ratio (ApH)h2o/Pf fell in the sequence AQP6 (vsAQP5/AQP4-M23>AQP1>AQP9>AQP3>AQP8). Also, for AQP6, pCMBS (1 mM) increased Pf by ~70%, but has no effect on (ApH)h2o or (ApH)h2o. DDS (100 mM) has no effect on Pf or (ApH)h2o, but reduces (ApH)h2o by 50%. For AQP9, pCMBS reduces Pf by ~60%, (ApH)h2o by 100%, and (ApH)h2o by ~50%. DDS (100 mM) in effect on Pf or (ApH)h2o of AQP9.

**Conclusions:** In summary, we show that mammalian AQPs exhibit a diverse range of selectivities for CO2 vs NH3 vs H2O. Also, with AQP9—as with AQP1—H2O, NH3, and 50% of CO2 move through monomeric (pCMBS-sensitive) H2O pores, whereas 50% of CO2 takes another (DIDS-sensitive) pathway that is still unidentified.

**Funding:** Other U.S. Government Support, Government Support - Non-U.S.
Deletion of the Cl/\(\text{HCO}_3\)\(^{-}\) Exchanger Pendrin Downregulates Calcium-Absorbing Proteins in the Kidney and Causes Calcium Wasting

**TH-PO674**

Sharon L. Barone,1, 2 Jie Xu,1, 2 Hassane Amlal,1 Manoocher Soleimani,1, 2 Medicine, University of Cincinnati, OH; 1Research Services, Veterans Administration, Cincinnati, OH.

**Background:** The epithelial calcium channel ECaC (TRPV5) and the Cl/\(\text{HCO}_3\)\(^{-}\) exchanger pendrin (SLC26A4) are expressed on the apical membrane of tubular cells in the distal tubus and play essential roles in calcium reabsorption and bicarbonate secretion, respectively. ECaC works in tandem with the basolateral Na/CA exchange to reabsorb the filtered calcium in the distal nephron. ECaC expression and/or activity is downregulated in vitro by extracellular acidic pH. We hypothesized that the acidic urine in pendrin KO mice might affect the expression and/or activity of ECaC/cationic Na/CA exchange pathway and alter urine calcium excretion.

**Methods:** Northern hybridization, immunoblot analysis and immunofluorescent labeling were performed and urine calcium excretion rates were measured.

**Results:** Immunolocalization studies demonstrate that pendrin and the Na/CA exchanger are expressed by distinct cell types within the distal convoluted tubule and connecting tubules. In the collecting duct we observe the expression of pendrin but not the Na/CA exchange. Our results confirm that deletion of pendrin causes acute urinary pH 4.9 in vs. 5.9 in wt mice, p<0.03. We further observe that pendrin deletion downregulates the calcium-absorbing molecules ECaC and Na/CA exchange in the kidney. These changes were associated with a ~100% increase in 24 hr urine calcium excretion in pendrin null mice (~8.1 μmols/24 hrs in wt vs. 17.6 mols in pendrin KO mice, p<0.01). Subjecting the wild type and mutant mice to oral bicarbonate loading for 12 days increased the urine pH to ~9 in both genotypes, normalized the expression of ECaC and Na/CA exchange, and reduced the urine calcium excretion in pendrin null mice to levels in wt mice.

**Conclusions:** We propose that acidic urine caused by pendrin impairment can play an important role in the pathogenesis of calcium, uric acid and possibly cystine stones. Future studies should investigate whether single nucleotide polymorphisms in pendrin gene are associated with the currently unexplained acidic urine in humans with calcium or uric acid stone.

**Funding:** NIDDK Support, Veterans Administration Support

Expression of the Ammonia Transporter Family Members, RH B Glycoprotein and RH C Glycoprotein, in Foxi1 Null Mice

**TH-PO676**

Ki-Hwan Han,1 Hyun-Wook Lee,2,4 Alexandra F. Kovar,2 Sven Enerback,3 Jill W. Verlander,2 David Weiner,1

**1**Department of Anatomy, Ewha Womans University, Seoul, Korea; **2**Renal Division, University of Florida, Gainesville, FL; **3**Department of Biomedicine, University of Gothenburg, Sweden; **4**Nephrology Section, NF/SGVH, Gainesville, FL.

**Background:** Mice lacking the transcription factor Foxi1 have an intercalated cell differentiation defect and develop renal tubular acidosis. RH B glycoprotein (Rhbg) and RH C glycoprotein (Rhcg) are ammonia transporters critical for renal acid excretion. This study's purpose was to examine Rhbg and Rhcg expression in Foxi1 null mice.

**Methods:** Kidneys from Foxi1 null and wild-type mice processed for immunohistochemistry. Cell-specific expression was examined using double-immunolabel immunohistochemistry.

**Results:** In control mice, basolateral Rhbg was heterogeneous, strong in intercalated cells and weak in principal cells. In Foxi1 null mice, in cortical collecting duct (CCD) and outer medullary collecting duct (OMCD) Rhbg immunoreactivity was homogenous; cells with strong Rhbg label were not present. Rhcg in control mice was apical and basolateral, and greater in intercalated than in principal cells. In Foxi1 null mice, Rhcg was homogenous and no intensely labeled cells were present in CCD and OMCD. However, Rhbg and Rhcg expression in CCD and OMCD of Foxi1 null mice was similar to or greater than expression in principal cells in wt mice. In segmenting segment cells (CNT), both Rhbg and Rhcg expression was intense and unchanged by Foxi deletion. Finally, both AE1-positive and pendrin-positive intercalated cells in Foxi1 null mouse CNT, CCD and OMCD. CA II expression was present in most CNT and OMCD cells in Foxi1 null mouse, consistent with the known CA II expression in mouse principal cells.

**Conclusions:** Rhbg and Rhcg expression is regulated through two separate pathways; one downstream of Foxi1-dependent stimulation of A-type intercalated cell and non-A, non-B cell development and one independent of Foxi1 and primarily present in CNT and principal cells.

**Funding:** NIDDK Support, Government Support - Non-U.S.
Methods: 11 participants (8 females) on lithium therapy were matched with 6 healthy volunteers (3 females). They received an acute dose of amiloride (100 mg/kg body weight). Urine and blood samples were taken at the time and 2, 4, and 6 hours later. Rats maintained for 6 months on a lithium diet were also given an acute dose of ammonium chloride (100 mg/kg body weight). Urinary acidification was measured and the kidneys subsequently removed for immunohistochemistry and western blots for Treg expression.

Results: At baseline neither the humans nor rats had evidence of a metabolic acidosis. Humans on lithium showed a decreased ability to respond to an acute metabolic acid load and developed a more marked hyperchloremic metabolic acidosis and a less acidic urine. Similarly rats on chronic lithium treatment failed to respond as well to an acute acid load as did control rats. In controls urinary pH fell to 6.5 ± 0.1 and remained low for 6 hours whereas in the lithium rats urinary pH only fell to 6.7± 0.1 and returned to pH 7.0 after 2 hours. Ammonium transport in the kidney was significantly altered in lithium transporters. Rats in the outer medulla of rat kidneys showed an increase both with western blots (15 ± 4; control: 7 ± 4, p<0.001) and by immunohistochemistry.

Conclusions: Humans on long-term lithium therapy, demonstrated a reduced ability to excrete an exogenous acid load. This was also evident in our chronic lithium-exposed rat model. Immunohistochemical examination of renal kidneys following long term exposure to lithium revealed a substantially altered and increased expression of Treg. This was confirmed with western blots. We presume that this increased expression is a response to prevent the development of lithium-induced metabolic acidosis.

Funding: Government Support - Non-U.S.


Background: Dorsal root ganglion neurons with projections to the kidney exhibit a significantly higher portion of tonically firing neurons (repetitive firing) upon electrical stimulation than neurons with non-renal projections. As renal nerve endings are exposed to pH-changes in e.g. inflammation, we tested the hypothesis that tonically firing renal neurons exhibit currents upon acute stimulation significantly different from neurons with other firing patterns predominantly linked to non-renal sites.

Methods: Dorsal root ganglion (DRG) neurons from Sprague Dawley Rats (Th11-L2) were characterized according to firing response (tonic – sustained firing vs. phasic – single firing). Furthermore, inward currents due to acid stimulation (pH 5.3) consisting either of a large transient component and a smaller, sustained component or only sustained component were measured in voltage clamp recordings and analyzed in terms of the putatively involved channels (ASIC or TRPV1) by adding the respective blockers (Amiloride, Capsazepine).

Results: 40 DRG neurons were examined. Tonic firing pattern was found more frequently in renal DRG neurons than in non-renal neurons (47% vs.10%, p<0.05), as shown previously. There was no significant difference in cell size (Ø 40,8 vs. 40,3µm).

Conclusions: For the first time we could show that tonically firing neurons, which are found predominantly in renal afferent innervations, exhibit transient inward currents significantly different from neurons with other firing patterns mainly linked to non-renal sites.

TH-PO680 Different Modulation of Regulatory T Cells by Toll Like Receptor Activation in Primary IgA Nephropathy and in Henoch-Schoenlein Purpura Nephritis: A Factor Regulating the Different Clinical Outcome? Elisa Loizacana, Roberta Camilla, Valentina Dapra, Laura Morando, Rachele Gallo, Manuela Bianciotto, Francesca Maria Bosetti, Rosanna Coppo. 1Nephrology, Dialysis, Transplant, R. Margherita Hosp, Torino, Italy; 2Department of Pediatrics, R. Margherita Hosp, Torino, Italy.

Background: Primary IgA Nephropathy (IgAN) and IgA related to Henoch-Schoenlein Purpura (HSP) have common histological features but differ in clinical outcomes. Children mostly show remission in IgAN related to HSP vs progression in primary IgAN. In both cases a dysregulation of innate immunity results in failure of mucosal antigen elimination and/or altered IgA synthesis. Among the innate immunity actors, toll-like receptors (TLR) modulate regulatory T (Treg) cells that express the transcription factor Forkhead box P3(Foxp3) involved in suppressing pro-inflammatory responses. We had reported increased TLR expression in mononuclear cells(PBMC) of patients with IgAN.

Methods: We investigated 21 children with HSP (7.9±2 y.o; 10 with and 11 without urinary abnormalities) and 13 children with primary IgAN (10.7±3 y.o). TLR2, TLR4 and TLR9 mRNA, Foxp3 mRNA (Treg marker) and TGF mRNA (Th17 pro-inflammatory marker) were quantified in PBMC of these children and in healthy controls by real time PCR(Taqman), normalizing with Ab mRNA.

Results: We observed a significant inverse correlation between TLR4 and Foxp3 mRNAs in PBMC from children with primary IgAN (r = 0.45, p<0.05) while in cases of HSP TLR4 and Foxp3 mRNAs were directly correlated (r = 0.46, p<0.05).

Conclusions: These data suggest that in primary IgAN TLR4 hyperexpression and downregulation of Treg cells may turn into a pro-inflammatory and chronic outcome, while an enhanced Treg activity in front of a similar innate immunity engagement may favor the recovery in children with HSP.

Funding: Government Support - Non-U.S.

TH-PO681 Activation of Innate Immunity in Henoch-Schoenlein Purpura with and without Nephritis: Increased Toll-Like Receptors Expression in Circulating Lymphomononuclear Cells Roberto Camilla, Elisa Loizacana, Valentina Dapra, Laura Morando, Rachele Gallo, Manuela Bianciotto, Francesca Maria Bosetti, Licia Peruzzi, Alessandro Amore, Rosanna Coppo. 1Nephrology Dialysis Transplant, R. Margherita H., Torino, Italy; 2Pediatric Emergency, R. Margherita H., Torino, Italy.

Background: Innate immunity is involved in pathogenesis of primary IgA nephropathy (IgAN), where it has been reported an oversexpression of TLR4 in circulating mononuclear(PBMC) from patients with pIgAN. Henoch-Schoenlein purpura (HSP) is a systemic vasculitis with renal features of pIgAN.

Methods: TLR2, TLR4, and TLR9 expression was detected in BMMC of 28 HSP children (4-13 y.o., mean age 7.6 ±2y) at clinical onset: 13 had urinary abnormalities(hematuria, proteinuria > 0.250 mg/day in 9/13). Children control groups were 15 pIgAN (5-16 y.o., mean age 10±3y), 27 idiopathic nephrotic and 40 healthy subjects (HC). TLRs were detected by flow cytometry, expressed as MIF and mRNAs were quantified by real time PCR(Taqman).

Results: TLR2 expression with HSP showed, in comparison to HC, increased expression of TLR2 (4.2±1.5 vs 3.0±0.6 MIF, p=0.02), TLR4 (2.2±0.7 vs 1.7±0.4 MIF, p=0.02), and TLR9 mRNA (2.9±2.0 vs 0.7±0.4, p=0.005). No difference was found in HSP with or without renal involvement. TLR9 mRNA, higher levels of TLR9 and TLR4 mRNAs (TLR9: 2.9±2.0 vs 0.9±0.6, p=0.01; TLR4: 2.9±2.3 vs 1.6±1.2, p=0.004).

Conclusions: In conclusion, this study shows for the first time an activation of TLRs during HSP suggesting an activation of innate immunity similar to what found in pIgAN.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author. 269A
A Multi-Center Validation Study of the Oxford Classification of IgA Nephropathy in China: Working Group on Validation Study of the Oxford Classification of IgA Nephropathy in China

Cai-Hong Zeng,1 Wei-Bo Le,1 Shao-Shan Liang,1 Hong-Bing Shen,2 Agnes B. Fogo,2 Zhi-Hong Liu1,3
1Research Institute of Nephrology, Jilin University, Nanjing University School of Medicine; 2Nanjing, China; 3Department of Epidemiology and Biostatistics & Ministry of Education Key Lab for Modern Toxicology, School of Public Health, Nanjing Medical University, Nanjing, China; 4Renal/EM Division, Department of Pathology, Vanderbilt University Medical Center, Nashville.

Background: The Oxford classification of IgA nephropathy (IgAN) identified four pathologic lesions (MEST) for prediction of renal prognosis of IgAN independent of the clinical features. However, the limited number of patients and their heterogeneous origin necessitate validation studies in larger cohorts.

Methods: In this study, we validated the Oxford results using a cohort of 1026 adults with IgAN recruited from 18 centers in China.

Results: 31% of the patients in this cohort received immunosuppression and 27% prednisone, both similar to the Oxford cohort. The mean follow-up was 53 months. Compared with the Oxford cohort, the patients in the current study had slightly milder clinical course, as suggested by lower proteinuria (median, 1.3 vs 1.7 g/24h) at biopsy, and slower decline rate of eGFR (-1.5±10.5 vs -3.5±8.5 ml/min per 1.73m² per year) clinical course, as suggested by lower proteinuria (median, 1.3 vs 1.7 g/24h) at biopsy, and slower decline rate of eGFR (-1.5±10.5 vs -3.5±8.5 ml/min per 1.73 m² per year).

Conclusions: Compared with the Oxford cohort, the patients in the current study had slightly milder clinical features. However, the limited number of patients and their heterogeneous origin may contribute to the slight differences.

Clinical and histological Features

<table>
<thead>
<tr>
<th>Features</th>
<th>No FSGS Lesions (27)</th>
<th>FSGS Lesions (50)</th>
</tr>
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<tbody>
<tr>
<td>Age (y)</td>
<td>31 (23-42)</td>
<td>33 (25-50.2)</td>
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<tr>
<td>Female</td>
<td>15 (55.5%)</td>
<td>17 (56.6%)</td>
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<tr>
<td>eGFR (ml/min)</td>
<td>74 (50-91)</td>
<td>34 (25-65.5)</td>
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<td>Proteinuria (g/day)</td>
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<tr>
<td>eGFR (ml/min)</td>
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<tr>
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<td>10 (33.3%)</td>
</tr>
</tbody>
</table>

Results are shown as median ± IQR. *p < 0.05.

Finally, in univariate analysis FSGS lesions group showed a tendency to have a worse renal outcome at the end of follow-up (p=0.06) (OR 4.0 95% CI 1.3-12.8). We couldn’t analyze the impact of FSGS subtypes on outcome because the low number of patients did not allow us to run a multivariate analysis.

Conclusions: Our results underline the usefulness of monitoring all IgAN patients for FSGS, given their potential negative impact on renal outcome.

Funding: Government Support - Non-U.S.

**TH-PO685**

CD68 Positive Cells in Renal Biopsy Predict Long Term Prognosis in Proliferative Lupus Nephritis

Cristiane Bitencourt Dias,1 Patricia Malafaonde,1 Jin Lee,2 Aline Lázara Resende,1 Cilene Carlos Pinheiro,1 Denise Maria Avancini Costa Malheiros,1 Rui Toledo Barros,2 Victorika Woronik.

1Nefrologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil; 2Patologia, Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.

Background: No long-term assessment about macrophages in renal histology in lupus nephritis/LN is known. The aim was to describe relations of renal outcome with tecmaculal outcomes of CD68+ expressed in biopsy obtained on the diagnosis.

Methods: Thirty four female patients with proliferative LN were prospectively followed. Immunohistochemical performed with monoclonal antibody (MAB) CD68 in renal biopsy. Patients were stratified in two groups according to renal outcome: GFR ≤ 60 mL/min/1.73m² (n=14) and GFR > 60mL/min/1.73m² (n=20). Patients received prednisone and cyclophosphamide on induction.

Results: From the groups with better and worse renal outcome were not different between age (25.0±8.3 vs 26±11.5 years), activity index (5±1.6 vs 4±3.2), initial proteinuria (3.8±2.5 vs 5.4±2.7 g/day), systemic sediai (12.0(12.0-16.0) vs 12.0(8.0-12.0), and follow-up (3.5±0.6 vs 3.5 (2.7-4.0)years). The group with better renal outcome showed less chronicity index (1.3±0.5 vs 4.0±0.5), initial serum creatinine (1.4±1.2 vs 2.6±1 mg/dl, p<0.03) and CD68+ tubulointerstitial 6.4(5.5-10.5) vs 6.8±5.0 cells/field p=0.0006. Tubule-interstitial expression of CD68+ cells showed positive correlation with final serum creatinine (r=0.6, p<0.0001) and patients' CD68+ expression over 50 cells/field showed worse renal outcome p=0.003, with relative risk of 3.2.

Conclusions: CD68+ cells tubule interstitial expression in renal biopsies predict long term GFR. Values more than 50 CD68+ cells/field showed relative risk of 3.2 to develop worse renal outcome.

**TH-PO686**

Lupus Nephritis Versus “Lupus-Like” Glomerulonephritis: Are the Same Disease? 

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Background: Patients who present with typical histological lesions but do not fulfill the American Rheumatism Association criteria for the diagnosis of systemic lupus erythematosus (SLE) may represent a diagnostic problem. Our aim of this study was to compare the clinicopathologic features of patients with SLE and lupus-like GN.

Methods: We performed a retrospective study of 10 patients with lupus-like GN, defined by: (1) immunofluorescence microscopy(IF) staining for granular glomerular IgG, IgM, C3 and C1q≥1+ (0 to 4+ scale); (2) negative anti-nucleotide antibodies (ANA) 1:100 and negative anti-dsDNA antibodies; and (3) without extra-renal lupus activity. Cases were randomly matched with 20 patients with classical LN IV, according to baseline clearance (MDRD simplified formula) and time of follow-up. Treatment was decided by the clinical staff based on literature protocols.

Results: The clinical and histological features are summarized in Table 1.

Clinical and Histologic Features

<table>
<thead>
<tr>
<th>Features</th>
<th>Lupus-like GN</th>
<th>Class IV LN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(yr)</td>
<td>42 (35-47)*</td>
<td>27 (20-33)*</td>
</tr>
<tr>
<td>Female</td>
<td>60% (60/100)</td>
<td>100% (100/100)</td>
</tr>
<tr>
<td>Serum Creatinine</td>
<td>14(0-7.3)</td>
<td>16(0-12)</td>
</tr>
<tr>
<td>C4FRI</td>
<td>47 (29-98)</td>
<td>49 (25-98)</td>
</tr>
<tr>
<td>Serum Albumine</td>
<td>2.6(2-6.8)</td>
<td>2.6(2-6.3)</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>10 (8-12)</td>
<td>10 (8-12)</td>
</tr>
<tr>
<td>Hematuria</td>
<td>13 (6-55)</td>
<td>13 (6-55)</td>
</tr>
<tr>
<td>ANA</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>anti-ADNA</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>No. Glomerulos</td>
<td>25 (12-32)</td>
<td>25 (12-32)</td>
</tr>
<tr>
<td>Intensit fibrosis</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Crescents</td>
<td>11 (1)</td>
<td>11 (1)</td>
</tr>
<tr>
<td>Follow up</td>
<td>24 (11-57)</td>
<td>24 (11-57)</td>
</tr>
<tr>
<td>eGFR</td>
<td>75 (52-93)</td>
<td>72 (46-98)</td>
</tr>
<tr>
<td>eGFR at discharge</td>
<td>2.2(2-4.4)</td>
<td>2.2(2-4.4)</td>
</tr>
<tr>
<td>eGFR</td>
<td>1177 (73-145)</td>
<td>911 (76-161)</td>
</tr>
<tr>
<td>Proteinuria(g/day)</td>
<td>0.5(0-3.5-1.1)</td>
<td>1.0(0-4-1)</td>
</tr>
<tr>
<td>eGFR</td>
<td>0.75 (0.6 - 8.6)</td>
<td>1.5(23.5 - 45)</td>
</tr>
</tbody>
</table>

Results are shown as median ± IQR. *p < 0.05.

Finally, in univariate analysis FSGS lesions group showed a tendency to have a worse renal outcome at the end of follow-up (p=0.06) (OR 4.0 95% CI 1.3-12.8). We couldn’t analyze the impact of FSGS subtypes on outcome because the low number of patients did not allow us to run a multivariate analysis.

Conclusions: Our results underline the usefulness of monitoring all IgAN patients for FSGS, given their potential negative impact on renal outcome.

Funding: Government Support - Non-U.S.
The immunosuppressive therapy used did not differ between groups and included prednisolone, cyclophosphamide and mycophenolate. At the end of follow-up, one patient progressed to end stage renal disease in “Lupus-like” GN group and 2 patients in class IV LN group.

Conclusions: In our study, “Lupus-like” GN was very similar to LN regarding the clinical features, therapeutic response and outcome. Funding: Government Support - Non-US.

TH-PO687
Worse Renal Outcome with MPO-ANCA-Associated Nephritis Compared to PR3-ANCA-Associated Nephritis

Background: A Multi-Center Study on Renal Transplantation in ANCA-Associated Vasculitides (AAV). Renal involvement and high age at diagnosis have been shown to predict ESRD, however, more prognostic factors are needed.

Methods: In order to analyse the impact of serological specificity on renal outcome clinical and laboratory data at presentation were retrieved from all patients diagnosed during a 13-year period (1997-2009) within a defined population in southern Sweden. All patients were followed until the end of 2010 and renal survival among proteinase 3 (PR3)-ANCA+ and myeloperoxidase (MPO)-ANCA+ patients were studied by Kaplan-Meier analysis.

Results: A total of 121 patients with AAV, renal involvement and positive ANCA tests (PR3=62; MPO=59) were included in the study. The clinical and laboratory characteristics are listed in the table below. Patients with PR3-ANCA had significantly higher disease activity and larger number of organs involved at diagnosis. Among MPO-ANCA+ patients, 19 (32%) developed end-stage renal disease compared to 10 (16%) of the PR3-ANCA+ patients (p=0.032). These results were consistent after correction for sex, age and creatinine at diagnosis. There was no significant difference in the mortality rate between the two groups.

Conclusions: In this population-based cohort of AAV, MPO-ANCA+ had a doubled risk of developing ESRD compared with PR3-ANCA+ patients, despite similar renal function at diagnosis. These findings should be taken into consideration when prescribing treatment and stratifying patients in therapeutic trials.

TH-PO688
Histopathologic Classification of Glomerular Lesions in ANCA-Associated Glomerulonephritis Does Not Reliably Predict Clinical Outcome

Background: A recently published classification schema identified 4 classes of GN associated with antibodies to neutrophil cytoplasmic antigens (ANCA) based on the appearance of 100 glomeruli; focal (F), crescentic (C), mixed (M) and sclerotic (S) and found class to be an independent predictor of clinical outcomes in a European multicenter cohort of 100 subjects(1). We performed a ten year retrospective analysis of all 21 adults with ANCA-associated GN at a Northern New England center.

Methods: Biopsies were classified according to strict criteria of Berden(2). Statistical analysis was by ANOVA.

Results: Biopsies were readily categorized: 8 as class F; 4 C; 6 M and 3 S. Mean # glomeruli 11.1. Follow up 42.4 (2-122) mo. Mean age 68.1 yr with trend to older age in class M; 71.0 were male. Treatment did not differ between groups. Mean entry eGFR 24.4 ml/min. There was a trend to lower entry eGFR in the S class, but no significant difference between groups or in entry eGFR between the non-S classes. Mean changes in eGFR at one year and follow up were +8.24 and +18.75 ml/min respectively. There was a trend to greater improvement in class D and decline in function in class C, respectively in the data of Berden(3), which showed greatest improvement in F. 8 reached ESRD: 2 C; 3 F; 2 M and 1 S. 4 died: 1 C; 3 F.

Conclusions: We describe a ten year of ANCA-associated GN in 21 subjects in a population similar to the index study. We did not detect correlation of clinical outcomes with pathologic class. We did not confirm that subjects with F histology had higher eGFR at entry or greater improvement. We conclude that the schema proposed by Berden(2) is not consistently reliable for clinical prognostication in individual patients. Our conclusion is tempered by the small number of subjects (n=21) relative to the index study (100), and by wider variation in length of follow up. Our study points to a need for continuing collaborative data collection in this rare but important disease.

Berden et al JASN 2010 1626-38

Funding: Clinical Revenue Support

TH-PO690
Clinical Implications of the New Pathological Classification of Pauci-Immune Small Vessel Vasculitis of the Kidney

Background: We aimed to study the clinical correlation of the recent international working of renal pathologists (IWGRP) classification of small vessel renal vasculitides. Clinical and histological features are re-evaluated by two pathologists to assess the clinicopathologic correlates of eGFR at presentation, follow up and the IWGRP classification.

Results: Of the 12,200 renal biopsies, 107(0.9%) were pauci-immune renal vasculitis (mucin-deposited crescentic glomerulonephritis). The mean serum creatinine and proteinuria at presentation were 5.8±4.6mg/dl & 1.6±2.1g/d. ANCA was positive in 28% and pANCA in 34.6%. Predictors of eGFR at presentation<30ml/min/1.73sq.m., were glomerular neutrophilic infiltrates(0.005), eosinophilic infiltrates(0.035), karyorrhectic debrid(0.020), segmental or global sclerosis(0.0017) and interstitial fibrosis(0.001). Based on IWGRP classification of the 9.3% focal , 29.9% crescentic, 55.1% mixed and 5.6% sclerotic lesions, 9.1, 39.0,44.2 and 7.8% respectively had an eGFR<30ml/min/1.73sq.m at presentation (< 0.001). Among the 28 cases followed up , these groups showed a mean eGFR at 1 year after diagnosis of 49.8±28.2, 46.3±23.4, 71.6±27.9 and 5.3 respectively, with the highest increment in eGFR in the crescentic group. eGFR at 6 and 12 months were independently predicted by the IWGRP class (temporally increasing β) and eGFR at presentation (temporally decreasing β).

Conclusions: The IWGRP class and eGFR at presentation are independent predictors of outcome in renal small vessel vasculitis. As interstitial fibrosis is predictive of eGFR at presentation, it could be incorporated into the IWGRP classification.

TH-PO691
Histologic Classification of Pauci-Immune Glomerulonephritis: Outcomes Predictors

Background: Although renal biopsy is the gold standard for the diagnosis of pauci-immune glomerulonephritis, its pathologic classification remains a controversial issue. A recently developed classification proposes four general categories of lesions: focal, crescentic, mixed, sclerotic. The aim of our study is to access its prognostic value.

Methods: We analyzed 58 patients with biopsy-proven pauci-immune glomerulonephritis at our center from 2000 to 2010, 51 patients were included in analysis(5 excluded for missing
data and 1 for insufficient number of glomeruli on biopsy) Biopsies were reviewed and classified as new case at first biopsy, no ANCA, and multiple logistic regression analyses were performed as appropriate. The glomerular filtration rate (GFR) was estimated using modified MDRD simplified equation.

**Results:** At baseline, the mean age was 45 ± 20 yrs, and 37.2% of the patients were males. ANCA test was positive in 19 (37.3%), negative in 22 (43.1%) and missing in 10 (19.6%). Mean eGFR at entry was 19 ± 24 ml/min/1.73m². Regarding the histological features, cases were subdivided as shown in table.

<table>
<thead>
<tr>
<th>Renal outcome according to class</th>
<th>eGFR Entry</th>
<th>eGFR 12 Months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Focal</td>
<td>110 ± 41</td>
<td>125 ± 42</td>
</tr>
<tr>
<td>Crescent</td>
<td>8.4 ± 4.1</td>
<td>9</td>
</tr>
<tr>
<td>Mixed</td>
<td>23 ± 11</td>
<td>17</td>
</tr>
<tr>
<td>Sclerotic</td>
<td>12.7 ± 11.4</td>
<td>23</td>
</tr>
<tr>
<td>Total</td>
<td>51</td>
<td>19</td>
</tr>
</tbody>
</table>

Results are shown as means ± SD

As depicted in Table, the renal biopsy categories were correlated to the degree of renal failure at presentation (p<0.002), and showed a tendency at 1 year follow-up (p=0.08).

We couldn't analyze the impact of focal subtype on outcome because the low number of patients. Finally, logistic regression analysis showed that sclerotic class was significantly associated with a worse renal outcome after one year of follow-up, even after adjusting initial eGFR (OR 4.7, 95%CI 1.17-19.2; p=0.02).

**Conclusions:** In our study, the histologic classification was correlated with renal failure at baseline and was associated to ESRD at 1 year regardless of eGFR.

**Funding:** Government Support - Non-U.S.

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**TH-PO692**

**Clinical Characteristics of Japanese Patients Having Anti-Neutrophil Cytoplasmic Antibody-associated Glomerulonephritis with Immune Depots**

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**Background:** Anti-neutrophil cytoplasmic antibody-associated glomerulonephritis (ANCA-GN) is pathologically characterized as a pauci-immune type of necrotizing crescentic glomerulonephritis. However, several earlier studies investigating European/ American patients reported that ANCA-GN was often accompanied by the glomerular immune deposits. The deposition rate and the clinical significance of the immune deposits were controversial, and there are no data concerning these issues in Japanese patients with ANCA-GN.

**Methods:** We retrospectively investigated 27 Japanese patients with rapidly progressive kidney impairment who were positive for serum ANCA (MPO-ANCA 24 cases, PR-3 ANCA 3 cases) and exhibited biopsy-proven necrotizing crescentic glomerulonephritis. The immune deposits were confirmed by two methods, immunofluorescence microscopy, and electron microscopy. The pathological and clinical data were compared between immune-deposits-positive and negative groups.

**Results:** Among these 27 cases, 8 exhibited the glomerular immune deposits (30%). The clinical parameters at the time of admission, including serum creatinine (2.3 vs 2.5 mg/dl) and ANCA titer (298 vs 556 EU), did not differ between the immune-deposits-positive and negative groups, while massive proteinuria was significantly detected in the immune-deposits-positive (2.8 vs 1.1 g/day, P<0.029). However, there was no significant difference in kidney survival rate (75 vs 90%), survival rate (100 vs 84%), or the incidence of extrarenal organ injury (63 vs 53%), between the groups throughout the observation period (26 months).

**Conclusions:** Glomerular immune deposits were unexpectedly detected at a high rate in Japanese ANCA-GN patients. The clinical course of the ANCA-GN for 2 years after diagnosis was not altered, but the level of proteinuria was markedly enhanced by the glomerular immune deposits, suggesting that they had a potential effect as an aggravation factor of kidney dysfunction. This additional study investigating the long-term effects of the glomerular immune deposits in ANCA-GN is needed in the future.

**TH-PO693**

**Identification of Early Pathologic Features of Focal Segmental Glomerulosclerosis by Repeat Renal Biopsy**

Cai-Hong Zeng, Hong-Guang He, Feng Xu, Qing-Yan Zhang, Xi Tang, Zhi-Hong Liu.

Research Institute of Nephrology, Jinhua Hospital, Nanjing University School of Medicine, Nanjing, China.

**Background:** To explore the early pathologic features of focal segmental glomerulosclerosis (FSGS).

**Methods:** We analysed 10 patients (8 males and 2 females) whose first renal biopsies showed minimal change lesions while second biopsies indicated FSGS (3 cases classified as 0, 5 cellular, 2 tip, 1 perihilar, and 1 collapsing).

**Results:** The mean age at the time of the first biopsy was 22.9±6.7 years. The mean interval time of the two biopsy collections was 22.3±16.6 months. The proportion of segmental glomerulosclerosis was 11.4±7.2 (3.2-22.9%). At the first biopsy, there were no global glomerulosclerosis and mesangial proliferation. The splitting of Bowman’s basement membrane (BBM) at tubular pole were seen in 7 cases, inflammatory infiltration surrounding glomeruli in 6 cases, and tubular cell refluxing into Bowman’s capsule in 6 cases. These numbers increased to 16, 7 and 10, respectively, at the repeat biopsy. The presence of granular swelling lesions in the capillaries was 6 (60%) in the first biopsy, 7 (70%) in the second biopsy, 17 (79%) at first biopsy, and 17 (80%) at second biopsy.

**Conclusions:** In conclusion, splitting of BBM at tubular pole, inflammatory infiltration surrounding glomeruli, and tubular cell refluxing into Bowman’s capsule, ATIL, demarcation of foot process from GBM, and cytoplasm vacuolation are the early lesions of FSGS.

**Funding:** Government Support - Non-U.S.

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**TH-PO694**

**Low Birth-Weight-Related Nephropathy Has Similar Pathological Changes Both in Glomeruli and in Tubular Cells with Those of Mitochondrial Cytopathy**

Toshiyuki Imasawa, #1 Moritoshi Kadomura, #1 Takehiko Kawachi, #1 Yutaka Yamaguchi, #1 Hiroshi Kamita.

#1 Division of Nephrology, Chiba-East National Hospital, Chiba-City, Chiba, Japan; #2 Yamaguchi’s Pathology Laboratory, Matsudo-city, Chiba, Japan.

**Background:** Glomerular involvement in patients with mitochondrial cytopathy is a focal segmental glomerulosclerosis (FSGS). In addition, it was recently reported that granular swollen epithelial cells (GSECs), which include increasing damaged mitochondria, are specific pathological changes in distal tubuli or collecting ducts in patients with mitochondrial cytopathy. On the other hand, adults who were born with low birth weight (LBW) have high risk of kidney damages and sometimes showed FSGS lesions in their glomeruli. Here, we assessed their tubular changes and mitochondrial gene mutations.

**Methods:** Five adult patients with FSGS lesions who were born under 2500g, two mitochondrial cytopathy patients, and five obese-related FSGS patients who were born over 2500g, were analyzed in this study.

**Results:** In all five patients with LBW, glomeruli showed hypertrophy and FSGS perivascular lesions. Interestingly, in four of five patients, GSECs, which were previously reported in mitochondrial cytopathy, were also observed in collecting ducts. A part of these tubular cells were dropping out of tubular arrangement with chromatin condensation. Mitochondrial gene mutations in blood, urine sediments, and kidney specimens were checked by PCR-Luminex assay. Any mitochondrial gene mutations were not detected in these patients with LBW. Two mitochondrial cytopathy patients also had FSGS perivascular lesions in glomeruli and had GSECs in their tubuli. PCR-Luminex assay in all blood, urine sediments, and kidney specimens revealed mitochondrial gene mutations (3243 A to G) in all patients. Five obesity-related nephropathy patients had FSGS perivascular lesions, their tubular cells did not show GSECs.

**Conclusions:** These results suggest that mitochondria should be associated with the etiopathogenesis of LBW-related nephropathy probably by functional defects.

**Funding:** Government Support - Non-U.S.

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**TH-PO695**

**Bilirubin Attenuates Arteriolopathy and Tubular Proteinuria by Inhibition of NADPH Oxidase in Cyclosporine Induced Nephropathy**

Seung Oh, Ki Young Na, Su-Hyang Kwon, Ho Jun Chin.

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**Background:** Epidemiological studies have shown that patients with hyperbilirubinemia have a lower incidence of cardiovascular disease and strokes. In addition, bilirubin has been reported as an endogenous antioxidant by inhibition of reactive oxygen stress. We determined whether injection of bilirubin could attenuate vascular or tubular injury though inhibition of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits in cyclosporine (CsA) induced nephropathy.

**Methods:** Male Sprague Dawley rats were administered vehicle, CsA, CsA + bilirubin (BIL), and BIL. Bilirubin was injected ipanteropically three times for one week (60mg/kg). All groups kept on 0.05% low salt diet were treated with vehicle (VH) or CsA for 4 week (15mg/kg/day, subcutaneous). Physiologic and histologic changes were studied in addition to the concentration of urine Kidney injury molecule-1 (Kim-1), urine Neutrophil gelatinase associated lipocandin (NGAL) by ELISA, and the protein expression of NADPH oxidase (NOX) and p22phox, NADPH oxidase subunits western blot. Urine hydrogen peroxide was measured using 2',7'-dichloro-dihydrofluorescein diacetate. Urine hydrogen peroxide was measured using 2',7'-dichloro-dihydrofluorescein diacetate. Hydrogen peroxide was lowered in CsA + BIL group compared than CsA group (20.5± 3.4 % vs. 37.3± 9.0 %, P<0.004).

**Results:** There was no difference on renal function between CsA group and CsA + bilirubin group (1.69 ± 0.93 vs. 1.71 ± 0.35, P=0.967). Arteriolaropathy was improved in CsA + BIL group compared than CsA group (20.5± 3.4 % vs. 37.3± 9.0 %, P=0.004).

**Conclusions:** In conclusion, splitting of BBM at tubular pole, inflammatory infiltration surrounding glomeruli in 6 cases, and tubular cell refluxing into Bowman’s capsule in 6 cases. These numbers increased to 16, 7 and 10, respectively, at the repeat biopsy. The presence of granular swelling lesions in the capillaries was 6 (60%) in the first biopsy, 7 (70%) in the second biopsy, 17 (79%) at first biopsy, and 17 (80%) at second biopsy.

**Funding:** Government Support - Non-U.S.

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**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**
Results: The most prominent difference between IMN and sMN was subsets of CD8+ T cells. In the IMN group, CD8- T cells decreased, which was resulted from decrease in CD8- naive T cells. However, CD8 effector-memory T cells increased, and level of CD8 effector-memory T cells correlated with the levels of proteinuria. In contrast, in the sMN group, CD 28 null CD8- T cells increased, although level of those cells was not correlated with any of clinical parameters. Another difference was level of CD25 high CD4+ regulatory T cells, which decreased only in the IMN group.

Conclusions: These results indicate that although histological findings are similar, immunologic processes underlying the deposition of immune complex are distinct in idiopathic and in secondary membranous nephropathy, and specific therapeutic approach is required.

Funding: Pharmaceutical Company Support

TH-PO697

The Mechanism of the Development of Segmental Glomerular Sclerosis in Idiopathic Membranous Nephropathy

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Background: Membranous nephropathy (MN) with focal segmental sclerosis is associated with a poorer prognosis than MN without these lesions. The mechanism of the development of segmental sclerosis in MN is still uncertain.

Methods: We selected renal biopsy cases of idiopathic MN cases with segmental sclerosis (n=26 total 250), and assessed the development of segmental sclerosis, focusing on the glomerular endothelial cell injuries, organization and thickening of GBM, and VEGF expression in podocytes.

Results: The average age of these cases is 62.4±9.77 years. About 77% (n=20) of these cases developed nephrotic syndrome. eGFR (mean 57.36±18.1 mg/dl) was significantly lower compared to MN without segmental sclerosis. In histopathology, the most common pattern of segmental sclerosis in MN was characterized by glomerular capillary collapse with extracellular matrix accumulation. All MN cases with segmental sclerosis showed endothelial cell injuries that characterized by obliteration and loss of glomerular capillaries with disappearance of CD34+ endothelial cells. Interestingly, in all cases with segmental sclerosis, narrowing of glomerular capillaries with endothelial cell injuries was evident even in non-sclerotic areas. In deed, by computer assessed morphometric analysis, MN cases with segmental sclerosis had significantly to smaller glomerular capillaries and larger GBM thickness on podocytes isolated from urine sediment using magnetic beads coupled with anti-podocalyxin antibody. The cytological characteristics for podocytes was examined on the slide glass. The slide glass was processed to conventional IF procedure using anti-

PH-PO698

Increased Podocyte CD80 Expression

Serum Factor from Minimal Change Nephrotic Syndrome Patients

Takui Ishimoto, 1 Richard J. Johnson, 2 Eduardo H. Garin. 2 Division of Renal Diseases & Hypertension, University of Colorado Denver, Aurora, CO; 2Division of Pediatric Nephrology, University of Florida, Gainesville, FL.

Background: Proteinuria in Minimal Change (MC) patients is thought to be due to a circulating factor released by patients lymphocytes. In MC patients in relapse, glomerular expression of CD38 and urinary protein/creatinine ratio was increased. Because CD38 is known to mediate proteinuria, we have evaluated the effect of serum and supernatants of Peripheral Blood Mononuclear Cells (PBMC) cultures from MC patients on CD38 expression. Methods: Thirteen MC patients were studied in relapse and in remission. Five normal subjects served as controls. Immunized human podocytes were incubated with 15% of MC patients serum or with 15% of 10x concentrated supernatants of MC patients PBMC cultures from normal controls and from MC patients. PBMC (1 x 10^6/ml) were incubated with 15% FBS for 72 hr prior to obtain the supernatants. RNA was extracted at 6 hr, and QPCR for CD38 was done. Results: Serum albumin was 2.18 +/- 0.53 (g/dl) and urinary protein/creatinine ratio 11.2 +/- 5.12 (mg/mg) in relapse, and 4.1 +/- 0.73 (g/dl) and 0.19 +/- 0.12 (mg/mg) in remission respectively. MC patients in relapse serum significantly increased podocyte CD38 expression when compared to that seen in remission (p< 0.01) (Fig. left). No difference in CD38 expression among the groups was seen when podocytes were incubated with supernatants of PBMC cultures (Fig. right). Conclusions: (1) MC patients in relapse serum increases in vitro CD38 poodyte expression. This finding can explain, at least in part, the mechanism of proteinuria in these patients. (2) The lymphocyte is not involved in the CD38 mediated proteinuria in MCD patients.

Funding: NIDDK Support

PH-PO700

Routine Microscopic Examination of Urinary Sediment Can Identify Urinary Podocytes

Masaori Hara. Pediatrics, Yoshida Hospital, Tsubame, Niigata, Japan.

Background: Podocytopenia is involved in the progression of glomerular sclerosis. One of the causes for podocytopenia is a detachment of podocytes from the glomerular basement membrane into urine. To detect urinary podocytes has a diagnostic value to predict ongoing severe podocyte injury. In this study we evaluated the usefulness of routine microscopic examination of urine as a simple method compared to the previous immunofluorescent (IF) examination.

Methods: Urine samples from patients with various glomerular diseases such as IgA nephropathy, Schoenlein Henoch purpura nephritis, lupus nephritis (N=25) were used. Urine was centrifuged at 1800 rpm for 5 minutes and the sediment was mixed with Sternheimer staining solution and examined carefully for characteristics of the cells appeared in urine sediment using conventional light microscope. For IF study urine sediment was suspended in PBS and then the cells in the sediment were trapped using BD SurePath™ Pap Test on the slide glass. The slide glass was processed to conventional IF procedure using anti-podocalyxin antibody. The cytological characteristics for podocytes was examined on the podocytes isolated from urine sediment using magnetic beads coupled with anti-podocalyxin antibody and then stained by Sternheimer solution.

Conclusions: Cytological characteristics of urinary podocytes was as follow; 1) 15-30 μm in diameter, 2) round or oval shape, 3) the margin of cells is mostly smooth, sometimes having processes, 4) the nucleus is located mostly in the center or sometimes deviated slightly, 5) often binucleated, 6) high N/C ratio, 7) large and clear nuclear staining, and 8)
violet (C30M70Y0K0) by CMYK color model) cytoplasmic color. The cells matched for all of the above criteria were regarded as podocytes and the number was counted. The time consumed for whole procedure by routine microscopic examination is within 30 minutes, although that by IF examination is about 3 hours. The number of podocytes by routine microscopic examination was well correlated with that by IF examination (Spearman, N=32, r=0.0001).

Conclusions: Routine microscopic examination of urine sediment by Sternheimer staining is very simple but reliable on identifying urinary podocytes.

**TH-PO701**

ELISA Analysis of Urinary Sediment Podocin, Podocalyxin in the Patients with Different Glomerular Diseases  
**Bin Zhu, Yi Lin, Xiaoling Zhu, Sen Zhong, Caifeng Zhu, Ying Lu. Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Zhejiang University of Chinese Medicine, Hangzhou, Zhejiang, China.**

Background: To investigate the urinary sediment podocin and podocalyxin in the patients with biopsy proven glomerulonephritis using ELISA method.

Methods: Patients with biopsy proven glomerulonephritis were enrolled for the analysis of urinary sediment podocin and podocalyxin measured using ELISA.

Results: We collected totally 40 patients (15 Males and 25 females). There were 19 proliferative glomerulonephritis cases (IgAN: 10 cases; Crescentic glomerulonephritis: 2 cases; IgMN: 2 cases; Lupus nephritis IV(A/G): 5 cases);19 non-proliferative glomerulonephritis cases (MCD: 5 cases; FSGS: 8 cases; MN: 6 cases) and 2 patients with renal amyloidosis. 10 healthy persons were enrolled as the control. Results: Urinary podocin and podocalyxin in the healthy person was the lowest. There was no significant difference in the urinary podocin and podocalyxin between the proliferative glomerulonephritis group and the non-proliferative glomerulonephritis group(P>0.05). The urinary podocin and podocalyxin was increased significantly in the patients with crescentic glomerulonephritis and the non-proliferative glomerulonephritis group(P>0.05). The urinary podocin and podocalyxin between the proliferative glomerulonephritis group and the non-proliferative glomerulonephritis group compared with the patients with other renal disease (podocin: P<0.05; podocalyxin: P<0.01).

*Funding: Government Support - Non-U.S.*

**TH-PO702**

Clinical Implication of Detection of Urinary Podocyte-Associated mRNA Profile in Various Stages of Diabetic Nephropathy  
**Min Zhong, Linli Lv, Jie Ni, Kun Ling Ma, Bi-Cheng Liu. Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.**

Background: Podocyte injury and subsequent excretion in urine play a crucial role in the pathogenesis and progression of diabetic nephropathy (DN). Quantification of messenger RNA (mRNA) expression in urinary sediment by real-time PCR is emerging as a noninvasive method of screening DN-associated biomarkers. We hypothesized that the urinary mRNA profile of podocyte-associated molecules may provide important clinical insight into the different stages of diabetic nephropathy.

Methods: DN patients (N=51) and healthy controls (N=13) were enrolled in this study. DN patients were divided into a normoalbuminuria group (UAESmall=30 mg/g, n=17), a microalbuminuria group (UAESmall>300 mg/g, n=15), and a macroalbuminuria group (UAESmall>300 mg/g, n=19), according to their urinary albumin excretion (UAE). Urinary cell pellet was collected from each study participant. Total RNA was extracted and cDNA was synthesized. Relative mRNA abundance of synaptopodin, podocalyxin, CD2-AP, α-actin4, and podocin were quantified by real-time PCR technology, and correlations between target mRNAs and clinical parameters were examined.

Results: The urinary mRNA levels of all genes studied were significantly higher in the DI group compared with controls (P<0.05), and mRNA levels increased with DN progression. Urinary mRNA levels of all target genes positively correlated with both UAE and BUN. The expression of podocalyxin, CD2-AP, α-actin4, and podocin mRNA correlated with serum creatinine (r=0.457, P<0.001; r=0.329, P<0.01; r=0.286, P=0.021; r=0.157, P=0.006, respectively). Furthermore, podocalyxin mRNA was found to negatively correlate with eGFR (r=-0.349, P<0.01). The ROC curve analysis showed that all target genes were effectively able to discriminate between DN patients and normal controls, with an AUC above 0.5.

Conclusions: The urinary mRNA profiles of synaptopodin, podocalyxin, CD2-AP, α-actin4, and podocin were found to increase with the progression of DN, which suggested that quantification of podocyte-associated molecules will be useful biomarkers of DN.

*Funding: Government Support - Non-U.S.*

**TH-PO703**

Lectin Microarrays of Intact Urinary Exosomes Indicate Distinct Glycosylation Profiles  
**Jared Q. Gerlach,1 Anja Krüger,1 Shirley Hanley,1 Susan Gallogly,1 Marie C. Hogan,2 Christopher James Ward,2 Lokesh Joshi,1 Matthew D. Griffin,2 Regenerative Medicine Institute (REMDAD) and Glycoscience Engineering Group, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland; 2Dept. of Medicine, Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN.**

Background: Urine exosome-like vesicles (ELVs) contain many nephron-derived glycoproteins but characterization of their surface glycosylation is limited and may be compounded by Tamm-Horsfall protein (THP) contamination. Intact ELVs and purified THP were profiled by lectin microarray and flow cytometry (FCM) to compare and contrast their glycosylation profiles.

Methods: 43 lectins were spot-printed on Nexterion® hydrogel slides. ELVs, isolated from healthy adult urine by ultracentrifugation (UC) or ultrafiltration (UF) were labeled with lipophilic dyes (CM-DiI, PKH26), incubated with lectin arrays and laser-scanned at 5 μm resolution with fluorescence intensities generated for each lectin. Specificity was determined based on ≥50% inhibition with haptenic sugars. For FCM, ELVs were adsorbed to 4μm latex beads then incubated with biotinylated lectins and fluorochrome-labeled streptavidin prior to analysis on a FACScanto® cytometer. Identical protocols were applied to AF647-labeled THP.

Results: N-acetyl-glucosamine-binding lectins (LEL, WGA) had the highest fluorescence intensities with ELVs on microarrays and FCM suggesting high N-linked surface glycosylation. The α-l-fucose-specific lectin UEA-I exhibited negative or minimal binding to ELVs in either format. The galactose/N-acetyl-galactosamine-binding lectin SNA-II and the sialic acid-binding lectin SNA-I also bound ELVs on arrays, suggesting O-linked modifications and α2-6-linked terminal sialic acid, respectively. UC- and UF-prepared ELVs had closely comparable array profiles. THP and ELV profiles were similar but with distinct differences in relative intensities, e.g. β-galactose-binding lectin ABL (THP higher) and SNA-II (ELVs higher).

Conclusions: Microarrays and FCM allow rapid, ELV-specific glycosylation profiling, providing a basis to screen for protein glycosylation abnormalities in kidney disease and to facilitate urine ELV isolation.

*Funding: NIDDK, Support Government Support - Non-U.S.*

**TH-PO704**

Refinement and Technical Validation of Assay for Urinary microRNAs  
**Taro Horino, Takayuki Tsuji, Robert A. Star, Peter S.T. Yuen. NIDDK, NIH, Bethesda, MD.**

Background: We previously showed that miRNAs in the urine exosomal fraction are biomarkers for acute and chronic kidney disease, but our assay required large 4 ml samples. Non-exosomal urinary miRNAs from glomerular filtration, tubular secretion, and/or cell death, are also potential biomarkers. Therefore, we developed a more sensitive purification/assay system, then measured and validated the distribution of miR27b in different fractions of normal urine.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

Underline represents presenting author.
Methods: We centrifuged healthy human or rat urine at 17,000 x g for 15 min, retained the 17k pellet, then ultracentrifuged the 17k supernatant at 200,000 x g for 1 h to obtain the exosomal fraction (200k pellet). We purified miRNA by Qiaqual miRNeasy kit or TRIzol/miRNeasy. We measured miR27b or miR192 concentration by Taqman (RT-PCR) using the 17k pellet, then ultracentrifuged the 17k supernatant at 200,000 x g for 1 h to obtain the exosomal fraction (200k pellet). We purified miRNA by Qiaqual miRNeasy kit or TRIzol/miRNeasy. We measured miR27b or miR192 concentration by Taqman (RT-PCR) using synthetic mature miRNA (Dharmacon) standards. We validated assays by the Standard Addition Method (SAM; add 4 known increasing amounts of synthetic miRNA to an unknown sample, then extrapolate back to 0 addition).

Results: We readily detected miR27b in 100 µl of normal rat urine (30 amol/ml, validated by SAM). 20% of miR27b from normal rat urine was in the 17k pellet and 10% in the 200k pellet by direct measurement or SAM. The 200k supernatant contained 70% (direct measurement) or 80% (SAM) of urinary miR27b (Figure 1). In contrast, human urine 200k sap (but not the exosomal fraction) contained an endogenous inhibitor which caused the assay to underestimate [miR27b]. Recovery of pure miR27b purified by miRNeasy or TRIzol/miRNeasy was 70% and 90%, respectively; this recovery was inhibited 29% by 5 µl of human urine (n=4 p<0.05). EDTA treatment, gel filtration, or dialysis could not remove the endogenous inhibitor.

Conclusions: We developed and validated a sensitive assay for urinary miRNAs that needs only 100 µl of urine. A lower volume requirement will allow miRNA measurements on archival samples. Urinary miR27b was mainly in the 200k supernatant in normal rats. The endogenous inhibitor present in human urine may interfere with miRNA detection in healthy human urine fractions.

Funding: NIDDK Support

TH-P0705

Ultrastuctural Pathology in IgG4-Related Kidney Disease  Shinichi Nishi,1 Hideki Fujii,1 Yoko Takeda,1 Keiji Kono,1 Kentaro Nakai,1 Shunsuke Goto,1 Takako Sacki.2 1Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Hyogo, Japan; 2Department of Internal Medicine, Nagaoka Red Cross Hospital, Nagaoka, Niigata, Japan.

Background: Autoimmune or allergic disorders were suspected to be pathogenic mechanisms of IgG4-related kidney disease (IgG4KD) characterized by tubulointerstitial nephritis (TIN). In order to explore pathogenic mechanisms we evaluated the distribution of electron-dense deposits (EDDs) in the interstitium and glomeruli in seven cases with IgG4KD.

Methods: Subjective seven cases were diagnosed according to the criteria of IgG4KD working group in the Japanese Society of Nephrology. Routine light, immunofluorescence and electron microscopy, as well as immunohistochemical and indirect immunofluorescence studies for IgG subclasses were performed. A clinicopathological study was conducted in all the cases.

Results: The distribution of the EDDs was variable, with incidence of deposition being highest in the mesangium (71.4%) and tubular basement membrane (85.7%). EDDs were also observed in the interstitium between collagen fibres, with the deposition rate being 71.4%. Some cases showed no immunological abnormalities, hypocomplementemia or positive anti-nuclear antibody, but showed EDDs in their kidney. The histochemical and immunohistochemical studies for IgG4 did not indicate that the EDDs were immune complexes containing IgG4.

Conclusions: Although EDDs were found frequently in the interstitium and glomeruli of cases with IgG4KD, they appear to develop independently of autoimmune or allergic disorders. Further investigation is essential for EDDs to be found responsible in the development of IgG4KD from both immunological and pathological points of view.

TH-P0706

A Novel Case of Nephrotic Syndrome Caused by Immune-Mediated Severe LCAT Deficiency Satoshi Takahashi,1 Keiji Hiruorma,1 Mayuko Tsukida,1 Yoko Ohiishi,1 Hiroko Hamatani,1 Noriyuki Sakurai,1 Toru Sakairi,1 Hidekazu Ikouen,1 Yoshimi Kitamura,1 Takashi Kuroiwa,1 Michio Nagata,1 Yoshihisa Nojima,1 1Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan; 2Department of Pathology, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tsukuba, Ibaraki, Japan.

Background: Lecithin-cholesterol acyltransferase (LCAT) is a key enzyme in maintaining cholesterol homeostasis. In familial LCAT deficiency (FLD), abnormal lipid deposition causes renal injury and nephrotic syndrome, frequently leading to end-stage renal disease. We found a case of nephrotic syndrome due to acquired LCAT deficiency and examined the mechanism of inhibition of LCAT activity.

Methods: [Case report] A 63-year-old Japanese woman was admitted to our hospital because of edema. Laboratory data revealed U-Pro 4.1 g/dL, Albumin 2.5 g/dL, S-Cr 0.58 mg/dL, HDL-Chol 2 mg/dL, LDL 51 mg/dL and LCAT activity <50 U/renal biopsy specimen staining that marked accumulation of foam cells and lipid deposits in most glomeruli, together with depositions of immune complexes along the capillary wall. By the steroid treatment, LCAT activity and urinary protein level returned to normal within 2 month. The re-biopsy specimen at 5 month after the initiation of treatment showed disappearance of foam cells and marked reductions of lipid deposits and in immune complexes.

Results: The mixing test with the patient’s serum and healthy control serum revealed the existence of an inhibitory factor to LCAT activity in the patient serum. The purified IgG fraction from the patient’s serum also suppressed LCAT activity of normal serum. Co-immunoprecipitation study showed that protein G beads preincubated with the patient’s serum could capture LCAT of healthy control serum, demonstrating that the patient’s serum contained an antibody against LCAT.

Conclusions: This is the first report to describe the patient with an inhibitory antibody against LCAT activity and glomerular lesions comparable to those of FLD. This case would be important in considering the pathogenesis of glomerular lipid depositions and the possibility of treatment of renal injury in FLD with enzyme replacement therapy.

Funding: Other U.S. Government Support

TH-P0707

Renal Monoclonal Immunoglobulin Deposition Disease: A Report of 64 Cases from a Single Institution Samih H. Nasr,1 Anthony M. Valeri,2 Lynn D. Cornell,3 Mary E. Fidler,1 Sanjeev Sethi,1 Nelson Leung,1 1Pathology, Mayo Clinic, Rochester, MN; 2Nephrology, Columbia University, New York, NY; 3Nephrology, Mayo Clinic, Rochester, MN.

Background: Monoclonal immunoglobulin deposition disease (MIDD) is a rare dysproteinemica-related renal disease. In order to better define the disease’s clinical-pathological spectrum and provide updated information about the largest reported cohort of patients.

Methods: The characteristics of 64 MIDD patients who were seen at Mayo Clinic, Rochester, between 1992-2011, are provided.

Results: Of the 64 cases of MIDD included 51 cases of light chain deposition disease (LCDD), 10 cases of heavy chain deposition disease (HCDD), 2 cases of lambda light chain deposition disease (LCDD) and 1 case of kappa light chain deposition disease (LCDD) (Table 1). 20% of miR27b from normal rat urine was in the 17k pellet and 10% in the 200k pellet by direct measurement or SAM. The 200k supernatant contained 70% (direct measurement) or 80% (SAM) of urinary miR27b. The endogenous inhibitor present in human urine may interfere with miRNA detection in healthy human urine fractions.

Conclusions: Serum FLC ratio is abnormal in most of MIDD patients. Nodular sclerosing glomerulopathy is present in most but not all cases. NS is more common in HCDD than LCDD. The prognosis for MIDD is improving. The degree of renal impairment at diagnosis is a predictor of renal survival.

Funding: NIDDK Support

TH-P0708

Myeloproliferative Neoplasm-Related Glomerulopathy: Matthew M. Weinshenker, Jennifer Melville,1 Camilla Smith,1 Arthur E.素质教育, Rochester, MN; 2Nephrology, Mayo Clinic, Rochester, MN; 3Pathology, Mayo Clinic, Rochester, MN; 4Pathology, Columbia University, New York, NY; 5Pathology, Mayo Clinic, Rochester, MN.

Background: Myeloproliferative neoplasms (MPN) are clonal hematopoietic stem cell disorders affecting the erythroid, granulocytic and megakaryocytic lineages. The characteristics of MPN-related glomerulopathy are undefined.

Methods: We evaluated the features of 11 patients with MPN-related glomerulopathy who were identified by searching our nephropathology laboratory archives from 2000-2010.

Results: There were 8 men and 3 women with a mean age of 73 yr (range 60-87 yr) at biopsy. The type of MPN was primary myelofibrosis in 8 patients, chronic myelogenous leukemia in 1, polycythemia vera in 1 and essential thrombocythemia in 1. Indications for biopsy were nephrotic-range proteinuria (mean 6.8 g/day) and chronic renal insufficiency (mean 5.6 mg/dL). Nephrotic syndrome, hypoalbuminemia, edema, and hematuria were present in 4, 9, 6, and 3 patients, respectively. The mean time from diagnosis of MPN to biopsy was 7.2 yrs (range 1-17 yrs). Histologically, mesangial sclerosis and hypercellularity were seen in all 11 cases, segmental sclerosis in 8, features of chronic thrombotic microangiopathy (TMA) in 9, and intracapillary infiltrating hematopoietic cells in 4. On follow-up, 4 progressed to ESRD and the remaining 7 had persistent renal dysfunction, despite the individualized institution of RAS blockade, immunosuppression and treatment directed to the hematologic disorder.

Conclusions: We describe a novel and under-recognized form of glomerulopathy associated with MPN that enlarges the spectrum of glomerular diseases associated with hematologic neoplasms. MPN-related glomerulopathy appears to be a late complication of MPN, particularly primary myelofibrosis. It is characterized clinically by heavy proteinuria and hypercellularity, segmental sclerosis, TMA, and intracapillary hematopoietic cells.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
Prognosis is guarded. Greater awareness of this entity and larger studies are needed to define the optimal therapy.

**TH-PO709**

**Association of Pathological Features of Renal Biopsies with Clinical Manifestations in Patients with Immunoglobulin Light Chain Amyloidosis – An Analysis of 170 Cases in a Chinese Centre**

Ying Yao, Su-Xia Wang, Yongkang Zhang. Department of Nephrology, Peking University First Hospital, Beijing, China.

**Background:** This study aimed to investigate the association of pathological features of renal biopsies with clinical manifestations in patients with AL.

**Methods:** All patients with biopsy-proven renal amyloidosis were collected from our institute during 1988 and 2010(n=194). Immunohistochemistry with a panel of antibodies against Ig κ and λ, light chains, AA protein, transthyretin, lysozyme, apolipoprotein A-I, and fibrinogen were performed on paraffin sections of renal biopsy. The severity of amyloid deposition in glomeruli, vessels and tubulointerstitium were scored according to the histopathologic grading system proposed by Sait S(Arch Pathol Lab Med. 2010;134:532-544).

**Results:** AL was diagnosed in 170 cases (accounted for 87.6% of renal amyloidosis), with a ratio of λ, light chain in 87%. The others included 1 case of secondary amyloidosis and fibrinogen As-chain amyloidosis respectively, 22 cases unclassified. In AL patients, the mean age was 56.8 years (range 26-83 years) with 108(63.5%) males. Multiple myeloma was diagnosed in 12 cases; M protein was detected in 74.3% (55/74) patients by serum or urine IFE. The level of urine protein and incidence of renal insufficiency was significantly higher in patients with diffuse marked glomerular deposition (score 5) than in patients with lesser score(p=0.004, 0.000). Patients with marked vascular amyloid deposition (score ≥4 or overt interstitial amyloid deposition) scored 2) had a significant higher incidence of liver involvement than that with lesser scores(p=0.048, 0.014). Patients with coexistence of amyloid and immune complexes had a higher level of proteinuria (8.46 vs 5.68 g/24h, p=0.001). Patients with AL-κ showed a higher frequency of liver involvement and marked vascular amyloid deposition than AL-λ(p=0.001, 0.006).

**Conclusions:** AL is the predominant pattern of renal amyloidosis in China. Diffuse marked glomerular amyloid deposition and coexistence with immune complexes were related with higher degree of proteinuria. Patients with AL-κ were more likely to have liver involvement and pervasive vascular amyloid deposition.

**TH-PO710**

**Medullary Amyloidosis Associated with Apolipoprotein A-IV**

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**Background:** Hereditary and familial forms of amyloidosis include transthyretin, fibrinogen A-α, lysozyme, gelatin, apolipoprotein A-I and H-related associated amyloidosis. We describe a novel amyloidosis limited to the renal medulla.

**Methods:** A 52-year old man presented with 10-year history of increased urinary frequency, no significant proteinuria and gradual loss of renal function. There was no family history of renal disease. Serum creatinine had gradually risen to 1.97 mg/dL with a ratio of 3, light chain in 87%. The others included 1 case of secondary amyloidosis and fibrinogen As-chain amyloidosis respectively, 22 cases unclassified. In AL patients, the mean age was 56.8 years (range 26-83 years) with 108(63.5%) males. Multiple myeloma was diagnosed in 12 cases; M protein was detected in 74.3% (55/74) patients by serum or urine IFE. The level of urine protein and incidence of renal insufficiency was significantly higher in patients with diffuse marked glomerular deposition (score 5) than in patients with lesser score(p=0.004, 0.000). Patients with marked vascular amyloid deposition (score ≥4 or overt interstitial amyloid deposition) scored 2) had a significant higher incidence of liver involvement than that with lesser scores(p=0.048, 0.014). Patients with coexistence of amyloid and immune complexes had a higher level of proteinuria (8.46 vs 5.68 g/24h, p=0.001). Patients with AL-κ showed a higher frequency of liver involvement and marked vascular amyloid deposition than AL-λ(p=0.001, 0.006).

**Conclusions:** AL is the predominant pattern of renal amyloidosis in China. Diffuse marked glomerular amyloid deposition and coexistence with immune complexes were related with higher degree of proteinuria. Patients with AL-κ were more likely to have liver involvement and pervasive vascular amyloid deposition.

**TH-PO711**

**Duration of Diabetes Mellitus Type 1 and Type 2 Correlates with Succeeding Stages I through IV of Diabetic Nephropathy: An Autopsy Study**

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**Background:** The new pathologic classification of diabetic nephropathy (DN) classifies DN into 4 groups, but it is unknown whether these represent progression from group I through IV in the course of the disease. We examined the relationship between duration of diabetes mellitus (DM) type 1 and type 2 and occurrence of DN and its allocation to the classes of the classification of DN (JASN 2010, 21:556-563).

**Methods:** We retrieved clinical data and paraffin embedded renal slides at autopsy from the archives of the LUMC of 31 patients with DM type 1 and type 2, from 1984 to 2004. Presence of DM was the main inclusion criterion. Patients with more than 15 years DM duration of whom autopsy materials showed no signs of DN were excluded. We also excluded patients with a combined pancreas and renal transplantation. There was 1 patient of whom a previous renal biopsy was available. In case of histologically proven DN, the lesions were scored as consistent with class I through IV.

**Results:** There were 4 patients with DM type 1 and 27 with DM type 2. 18/31 patients had histologically proven DN (3 patients type 1 DM and 15 patients type 2 DM). Figure 1 shows the distribution among classes according to duration of diabetes. There was a positive significant correlation (r = 0.688; p < 0.001) between duration of DM and the different groups of DN. Type 2 DM patients contributed mostly to this correlation (for this group, r = 0.669; p < 0.001). The renal biopsy that was available was performed 5 weeks before death and no transition of classes was shown.

**Conclusions:** Our preliminary results show that in the combined patient groups of type 1 and type 2 DM, there is a significant association between the duration of diabetes and DN class, which suggests that the histopathological classes indeed represent continuous stages of DN.

**TH-PO712**

**The Pathologic Classification of Diabetic Nephropathy Is Correlated with the Clinical Characteristics of Diabetes Mellitus**

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**Background:** Nephrotic syndrome (NS) is a common complication of diabetic nephropathy (DN). Recently, the renal pathology society (RPS) proposed the pathologic classification of DN (J Am Soc Nephrol 21: 556-563, 2010). In the present study, we examined the pathologic characteristics of DN cases with NS, using the RPS pathologic classification.

**Materials and Methods:** EMB was retrieved from 96 cases of NS. The diagnosis was based on the characteristic pathological findings, including mesangial proliferation, foot process effacement, and thickening of the glomerular basement membrane (GBM). The IF staining was performed to detect the immunoglobulin light chain and κ/λ ratio. The pathologic classification was performed according to the recent RPS classification. The patient characteristics such as age, sex, duration of diabetes, and duration of hypertension were also recorded.

**Results:** There were 43 men and 53 women. The mean age was 68 ± 14 years. The mean duration of diabetes was 12 ± 9 years. The mean duration of hypertension was 6 ± 5 years. The mean eGFR was 33 ± 19 ml/min/1.73 m². The mean urinary protein was 5.7 ± 5.0 g/day. According to the RPS classification, 20 cases were classified as class I, 34 cases as class II, 15 cases as class III, and 17 cases as class IV. The common pathological findings were mesangial proliferation, foot process effacement, and thickening of the GBM. The IF staining was positive for IgM and C3 in all cases. The κ/λ ratio was positive in 6 cases. The mean eGFR was significantly lower in class IV than in classes I, II, and III (p < 0.05). The mean urinary protein was significantly higher in class IV than in classes I, II, and III (p < 0.05). The mean duration of diabetes was significantly longer in class IV than in classes I, II, and III (p < 0.05). The mean duration of hypertension was significantly longer in class IV than in classes I, II, and III (p < 0.05).

**Conclusions:** The pathologic classification of diabetic nephropathy is correlated with the clinical characteristics of diabetes mellitus. Patients with NS and class IV diabetic nephropathy had a longer duration of diabetes and hypertension, and a lower eGFR compared to patients with NS and class I, II, or III diabetic nephropathy.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

Underline represents presenting author.
Methods: We selected renal biopsy cases of DM (n=62), and assessed the clinical characteristics, focusing on the proteinuria, clinical stages of chronic kidney disease (CKD). The renal biopsy was classified using the RPS pathologic classification of DN, and pathologic characteristics were correlated with clinical stages of DM, CKD stages, and proteinuria.

Results: We divided DM cases into 2 groups; DM with NS (n=31) and DM without NS (n=31). In clinical and pathologic characteristics of DM with NS, high serum Cr (2.2±1.7 vs 1.5±1.1 mg/dl), low eGFR (36.3±21.9 vs 54.3±27.8 ml/min/1.73m²), high CKD stage, and high grade of pathologic classification than those of DN without NS. In the cases with pathologic class III (nodular glomerulosclerosis) was significantly correlated with the degree of clinical stages of DM (r=0.308, p=0.05), CKD stages (r=0.306, p=0.05), and levels of proteinuria (r=0.275, p<0.05).

Conclusions: In DM cases with NS, DN was characterized by the development of nodular glomerulosclerosis (Class III) with high clinical stages of DM and CKD stages. These results showed that given the maximal sensitivity and specificity for KIM-1 mRNA was 52.2 percent and control. Urinary KIM-1 mRNA could separate patients with DN from normal controls with 45% in HK α-1 mice versus WT; 2) HK α-1 mice consumed more food per body weight and had greater urinary output than WT; 3) pair feeding caused substantial weight loss and eliminating of urinary aldosterone in HK α-1 mice but not WT mice; 4) whereas two month survival was 100% in DOCP-treated WT mice (N=9/9), it was 43% in DOCP-treated HK α-1 mice (N=3/7); 5) HK α-1 mice had significantly greater (~10%) HR and reduced locomotor activity; 6) DOCP-treatment significantly increased BP in WT mice during the active phase but there was no significant increase in BP in the HK α-1 mice during this time period.

These results strongly support a role for HK-ATPases in sodium conservation and suggest that the HK-ATPases are involved in the action of mineralocorticoids to increase systemic arterial blood pressure (BP). Furthermore, we asked whether HK α-1 mice had altered protein abundance for the α subunit for the epithelial Na channel (ENaC).

Methods: Age matched WT and HK α-1 male mice were studied in three separate experiments: 1) ENaC subunit mRNA and protein expression levels were evaluated in kidneys from WT and HK α-1 mice; 2) food and water intake, body weight, and urinary aldosterone levels were measured in ad libitum or pair fed mice of both genotypes; 3) urinary aldosterone and creatinine were measured in WT and HK α-1 mice in control and DOCP-stimulated conditions using radioimmunoassay.

Results: We observed that: 1) renal medullary eNOS protein expression was reduced by 45% in HK α-1 mice versus WT; 2) HK α-1 mice consumed more food per body weight and had greater urinary output than WT; 3) pair feeding caused substantial weight loss and evaluated of urinary aldosterone in HK α-1 mice but not WT mice; 4) whereas two month survival was 100% in DOCP-treated WT mice (N=9/9), it was 43% in DOCP-treated HK α-1 mice (N=3/7); 5) HK α-1 mice had significantly greater (~10%) HR and reduced locomotor activity; 6) DOCP-treatment significantly increased BP in WT mice during the active phase but there was no significant increase in BP in the HK α-1 mice during this time period.

Conclusions: These results strongly support a role for HK-ATPases in sodium conservation and suggest that the HK-ATPases are involved in the action of mineralocorticoids to increase systemic arterial blood pressure. Funding: NIDDK Support

TH-PO714

Dysregulated Balance of Th17 and Th1 Cells in Type 2 DM with Diabetic Nephropathy

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Background: The evaluation of the renal biopsy for assessing the stage of diabetic nephropathy has been an important feature of diabetic nephropathy (DN) in addition to glomerulosclerosis, while it is still difficult to be accurately evaluated. The aim of this study was to investigate the role of Th17 and Th1 cells in DN.

Methods: A total of 46 patients with DN and 14 healthy controls were studied. To evaluate progression of DN, patients were divided into groups based on the degree of albuminuria. Normoalbuminuria (UAE<30mg/g, n=20), microalbuminuria (UAE 30-300 mg/g, n=11), and macroalbuminuria group (UAE>300mg/g, n=15). Clinical data including albuminuria, blood urea nitrogen (BUN), and serum creatinine were recorded at baseline for each of the groups. Urinary KIM-1 mRNA expression was measured, and correlations with renal functional parameters were investigated.

Results: Urinary KIM-1 mRNA was significantly increased in patients with DN compared with healthy controls (p=0.006). Overall comparison showed that KIM-1 mRNA increased with increasing levels of albuminuria. It was the lowest in the control group, increased in the normoalbuminuria, microalbuminuria group, and was the highest in the macroalbuminuria group. KIM-1 mRNA strongly correlated with serum creatinine (r=0.459, p<0.001), BUN (r=0.478, p<0.001), and eGFR (r=0.433, p<0.001). Moreover, KIM-1 mRNA was significantly higher in microalbuminuria group compared with normal control. Urinary KIM-1 mRNA could separate patients with DN from normal controls with AUC of 0.7430.743 (p=0.006, 95% confidence interval (CI), 0.620-0.866). The threshold that gave the maximal sensitivity and specificity for KIM-1 mRNA was 52.2 percent and the 100 percent (p=0.001) respectively.

Conclusions: The expression of KIM-1 in urinary sediment may reflect tubular damage in early diabetic nephropathy. KIM-1 mRNA could reflect the disease severity and be a novel biomarker for early detection of DN. Funding: Government Support - Non-U.S.

TH-PO715

Dysregulated Balance of Th17 and Th1 Cells in Type 2 DM with Diabetic Nephropathy

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Background: The evaluation of the renal biopsy for assessing the stage of diabetic nephropathy has been an important feature of diabetic nephropathy (DN) in addition to glomerulosclerosis, while it is still difficult to be accurately evaluated. The aim of this study was to investigate the role of Th17 and Th1 cells in DN.

Methods: A total of 46 patients with DN and 14 healthy controls were studied. To evaluate progression of DN, patients were divided into groups based on the degree of albuminuria. Normoalbuminuria (UAE<30mg/g, n=20), microalbuminuria (UAE 30-300 mg/g, n=11), and macroalbuminuria group (UAE>300mg/g, n=15). Clinical data including albuminuria, blood urea nitrogen (BUN), and serum creatinine were recorded at baseline for each of the groups. Urinary KIM-1 mRNA expression was measured, and correlations with renal functional parameters were investigated.

Results: Urinary KIM-1 mRNA was significantly increased in patients with DN compared with healthy controls (p=0.006). Overall comparison showed that KIM-1 mRNA increased with increasing levels of albuminuria. It was the lowest in the control group, increased in the normoalbuminuria, microalbuminuria group, and was the highest in the macroalbuminuria group. KIM-1 mRNA strongly correlated with serum creatinine (r=0.459, p<0.001), BUN (r=0.478, p<0.001), and eGFR (r=0.433, p<0.001). Moreover, KIM-1 mRNA was significantly higher in microalbuminuria group compared with normal control. Urinary KIM-1 mRNA could separate patients with DN from normal controls with AUC of 0.7430.743 (p=0.006, 95% confidence interval (CI), 0.620-0.866). The threshold that gave the maximal sensitivity and specificity for KIM-1 mRNA was 52.2 percent and the 100 percent (p=0.001) respectively.

Conclusions: The expression of KIM-1 in urinary sediment may reflect tubular damage in early diabetic nephropathy. KIM-1 mRNA could reflect the disease severity and be a novel biomarker for early detection of DN. Funding: Government Support - Non-U.S.
Interleukin-1β Regulates Renin Expression in the Renal Cortex

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Angiotensin System: Juxtaglomerular Cells in Response to Chronic Stimulation of the Renin-Angiotensin System

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TH-PO717

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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Effects of Renin-Angiotensin System Component Ang(1-7) on Podocyte Injury In Vitro Induced by Patients' Serum of Preeclampsia

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Background: Renin angiotensin system (RAS) is very complicated and multistrata endocrinum, including various components. Recently a new member of RAS components Ang(1-7) and its specific receptor Mas were found that they have extensive effects, which could counteract the effects of AngII. The podocyte injury may play a role in the pathogenesis of proteinuria in preeclampsia. Our previous study showed that the decreased serum and urinary Ang(1-7) may be a cause of podocyte injury in preeclampsia. In order to further study the effect of Ang(1-7) on podocyte in preeclampsia, we use patients’ serum of preeclampsia to preincubate podocyte and then add Ang(1-7) to observe the change of podocyte specific properties.

Methods: The podocyte was cultured in vitro and the patients’ serum of preeclampsia was used as trigger to incubate podocyte. We divided the serum into 2 groups: normal control (NC); normal pregnant serum (NP) group; preeclampsia model (PE) group and Ang(1-7) + preeclampsia [Ang(1-7) + PE] group. The morphologic change of podocyte was observed by microscope, the changes of nephri, CD2AP, F-actin, ZO-1 and Mas receptor were examined by immunofluorescence. Western blot was used to examine the expression of Mas receptor.

Results: 1. The expression of nephri, F-actin and ZO-1 on podocytes was significantly decreased in PE group than NC and NP group, but their expression in Ang(1-7) + PE group was significantly increased than in PE group. The expression of CD2AP has no significantly difference among four groups.

2. There exist Mas receptors on podocytes in all groups which was examined by immunofluorescence and Western blot. The expression of Mas receptor on podocytes in PE group is significantly decreased than NC and NP group; but it was significantly increased in Ang(1-7) + PE group than in PE group.

Conclusions: Ang(1-7) could protect podocyte from injury in vitro induced by patients’ serum of preeclampsia. The effect may be associated with integration of Ang(1-7) and its specific Mas receptor.

Identification of Stox1 Transcription Factor as a Specific Repressor of Placental Renin and Its Deficiency Leads to Vascular Defects and Gestational Hypertension

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Background: Storkhead-box protein 1 (Stox1) was identified as a candidate gene associated families with higher incidence of hypertensive crisis during pregnancies. Stox1 is a putative transcriptional factor with unknown function.

Methods: We generated Stox1 deficient mice (Stox1−/−) and analyzed pregnancy-associated hypertensive phenotype.

Results: Here, we demonstrate that Stox1−/− mice specifically exhibit pregnancy-associated hypertension, vascular defects and basement membrane abnormalities. Pregnant Stox1−/− mice display higher urine albumin excretion when compared to wild-type control pregnant mice. Non-pregnant females and males with deletion of Stox1 are normal despite ubiquitous expression of Stox1 in the control mice. Renin expression is significantly elevated in the placentas of Stox1−/− mice, but not in the kidney. RNA silencing and promoter analysis in the human cytotrophoblasts demonstrate that renin is negatively regulated by transcriptional action of Stox1. VEGF, LPA, and Ang II receptor antagonist, completely ameliorates all pregnancy associated abnormalities in Stox1−/− mice.

Conclusions: These results indicate that Stox1 is a key regulator of blood pressure during pregnancy, and mice deficient for Stox1 serve as a genetic model for human gestational hypertension, a disease that affects about 8% of all pregnancies, causing significant morbidity.

Effect of Cyclosporine A Administration in Pregnant Rats on Blood Pressure and Glomeruli Number in Their Offspring

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Background: Successful kidney transplantation allows women previously suffered from chronic kidney disease stage 5 to get pregnant. However, pregnancy is a hypertensive pregnancy with accelerated progression of glomerulosclerosis. Successful renal transplantation may influence development in the offspring i.e. may cause the hypertension and chronic kidney disease (CKD) in adulthood. The aim of the study was to assess the effect of exposure to cyclosporine A (CsA) during gestation on blood pressure and glomeruli number in their offspring.

Methods: Eight pregnant Sprague-Dawley rats were assigned into two groups (n=4 in each group). In the first group CsA in a dose 3mg/kg/day and in the second group the corresponding volume of solvent were given, respectively. The substances were

Effect of Murine Recombinant ACE2 on Blood Pressure and Serum ACE2 Activity

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Background: There is increasing interest in the potential therapeutic action of Angiotensin-converting enzyme 2 (ACE2), but studies in mouse models have been limited by the lack of tools to amplify ACE2 activity. We developed murine recombinant ACE2 (mrACE2) to circumvent the immunogenicity of human ACE2 when given to mice.

Methods: mrACE2 was purified using Ni-Sepharose followed by size-exclusion chromatography. After 19 hr of incubation in vitro with purified mrACE2 (4ng), Ang II (10-9M) disappeared completely, thereby confirming that mrACE2 effectively cleaves this peptidase. When Ang II (0.2mg/kg) was given to mice pre-treated with vehicle or mrACE2 (1.0mg/kg IP), the SBP recovery was much rapid increase in SBP (30 sec) (from 102±3.1 to 173±4.4 mmHg, n=8), which was markedly blunted by mrACE2 (from 109±5.2 to 139±8.3 mmHg, n=7). The SBP recovery was much faster compared with mice pretreated with vehicle (at 5 min, 106±8.2 mmHg vs. 163±3.9 mmHg, respectively, p<0.0001). Whether baseline ACE2 activity plays a role in blood pressure regulation was examined by inhibiting ACE2 pharmacologically. We reasoned by a pharmacologic approach to ACE2 inhibition would be more useful to examine the effect of ACE2 on blood pressure under conditions where Ang II was not infused. Accordingly we administered MLN-4760, a specific ACE2 inhibitor. Acute administration of MLN-4760 (1.0 mg/kg IP) was associated with a rapid but transient increase in SBP as compared to control mice that received vehicle (ASBBP11.4±2.7 vs. 0.1±3.1 mmHg, p< 0.01). This effect of cyclosporine A administration in pregnant rats on blood pressure and glomeruli number in their offspring.
administered from the 10th day after the fertilization till the 7th day after the delivery, subsequently, only 10% of mothers treated with CsA during gestation (n=34) was higher compared to the offspring from mothers treated only with solvent (n=31) (72.2±6.1 vs. 60.8±8.1 mmHg, p<0.001). Conclusions: Treatment with CsA during pregnancy may lead to arterial hypertension in the offspring. 2: Exposure on CsA during fetal life influence also on kidney development where it causes 19% lower constriction and this may be an important factor participating in the pathogenesis of CKD later in their life.

Funding: Government Support - Non-U.S.

TH-PO727

Offspring of Mother Rats Exposed to Cigarette Smoke Condensate Are Characterized by Elevated Blood Pressure and Reduced Urinary Sodium Excretion

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Background: It has been suggested that disturbances of fetal development caused by exposure to cigarette smoke (as a result of maternal smoking) increase the risk of hypertension and chronic kidney disease in the adult life. The aim of the experimental study was, to assess the impact of exposure of pregnant rats to cigarette smoke condensate on blood pressure and natriuresis in their offspring.

Methods: Sprague-Dawley rats on day 10 of pregnancy were randomly allocated (5 litters) into two groups daily during pregnancy and 1 week after delivery. From sprague-dawley dams, 64 offspring (mean litter size 10.6±2.2) were treated with the AT1 antagonist, candesartan (5 mg/kg/d), for 2 weeks. Candesartan was administered subcutaneously, once a day. At 7 and 11 weeks of age in the offspring blood pressure increase and a reduction of urinary sodium excretion in their offspring. 2. sever preeclampsia than WT mice. The mechanism underlying this phenomenon is under investigation. 2. Ace gene.

Results: At 12 weeks of age significantly elevated systolic blood pressure was found in offspring exposed to CSC during the fetal period (n=54) compared to controls (n=51) (122.7±11.6 vs. 116.8±11.6 mmHg; respectively, p<0.001). Offspring of mother rats exposed to cigarette smoke condensate did not differ from the control offspring with respect to body weight, albuminuria, creatinine clearance and urinary potassium and calcium excretion were measured in 12 weeks old offspring.

Conclusions: 1. Exposure of pregnant rats to cigarette smoke condensate causes a blood pressure increase and a reduction of urinary sodium excretion in their offspring. 2. Such effect of cigarette smoke condensate on kidney function may have a consequence on development of arterial hypertension and also chronic kidney disease in the adult life.

Funding: Government Support - Non-U.S.

TH-PO728

Aggravated Phenotypes of Preeclampsia in Mice Bearing 4 Copies of Ace Gene

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Background: Preeclampsia is pregnancy-associated hypertension with proteinuria. Excess Ace-1, an endogenous VEGF inhibitor of placental origin has been implicated to cause hypertension, proteinuria and glomerular endotheliosis, all features of preeclampsia. Ace-1 antagonizes VEGF and induces endothelial dysfunction. Human ACE polymorphisms leading to elevated activity of ACE are associated with preeclampsia.

Methods: We tested whether an increase in ACE aggravation of preeclampsia using mice having 4 copies of Ace gene.

Results: Adenoviral mediated Ace overexpression to the non-pregnant female mice treated with CsA during gestation (n=34) was higher compared to the offspring from mothers treated only with solvent (n=31) (72.2±6.1 vs. 60.8±8.1 mmHg, p<0.001). Offspring of mother rats exposed to cigarette smoke condensate did not differ from the control offspring with respect to body weight, albuminuria, creatinine clearance and urinary potassium and calcium excretion were measured in 12 weeks old offspring.

Conclusions: 1. Exposure of pregnant rats to cigarette smoke condensate causes a blood pressure increase and a reduction of urinary sodium excretion in their offspring. 2. Such effect of cigarette smoke condensate on kidney function may have a consequence on development of arterial hypertension and also chronic kidney disease in the adult life.

Funding: Government Support - Non-U.S.

TH-PO729

Hydrogen Sulfide: A Protective Gas for Preeclampsia?

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Background: Hydrogen sulfide (H2S) is emerging as a regulator of various physiologic functions like blood pressure regulation and neurotransmission. It is transmitted during renal hypoxia and has strong antioxidant properties. Endogenous H2S might partly underlie the pathogenesis of CKD later in their life.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

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TH-P0732
Candesartan Prevents High-Fat Diet-Induced Hypertension and Renal Injury in Spontaneously Hypertensive Rat Via Angiotensin II Receptor-PI3K/Akt/FoxO Signaling Pathway
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Background: We have recently demonstrated that intrarenal renin-angiotensin system (RAS) would be involved in high-fat diet-induced renal lipotoxicity. Because the intracellular pathways involved in renal lipotoxicity remain to be determined, we examined the role of FoxO transcription factors in renin-excessive effects of RAS inhibition in spontaneously hypertensive rat (SHR) fed a high-fat diet.

Methods: SHR and WKY were treated with either a normal diet (SHR-C or WKY-C) or a high-fat diet (SHR-HF or WKY-HF) for 12 weeks.

Results: Intrarenal lipid accumulation were significantly increased in SHR-HF and WKY-HF compared with SHR-C and WKY-C. In SHR-HF, systolic BP was more elevated than those in SHR-C but the increased BP were normalized by treatment with candesartan or hydralazine. Renal mRNA expression of renin and angiotensin II type 1 (AT1) receptor showed that medsgal expansion and inflammation were significantly enhanced in SHR-HF.

Conclusions: A high-fat diet resulted in exacerbation of intrarenal RAS, which findings were shown by immunohistochemical staining of renin and angiotensin II and Western blot analysis for renin and angiotensin II type 1 (AT1) receptor. These changes were associated with increases in the activity of intrarenal PI3K-Akt phosphorylation and FoxO3a phosphorylation, consequently leading to increases in respiratory stress and apoptosis in the kidney. Treatment with candesartan, but not hydralazine, normalized all of these abnormalities via repression of angiotensin II AT1 receptor and upregulation of FoxO3a.

TH-P0733
ACE Inhibition Attenuates Renal Injury in Mice Lacking Endothelial Nitrergic Synthase
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Background: The renin-angiotensin-aldosterone system (RAAS) plays a pivotal role in the development and progression of renal injury. Non-specific nitric oxide synthase inhibition (by L-NNAME) is a known cause for RAAS activation while the specific role of endothelial nitric oxide synthase (eNOS) remains unclear. Our group previously documented that eNOS deficiency accelerated renal injury in the aging mouse as well as in the mouse remnant kidney model. However, the role of RAAS has not been studied in these models. Here, we examined the involvement of RAAS activation and the participation of FoxO transcription factors in renin-excessive effects of RAS inhibition in a mouse model of eNOS deficiency.

Methods: 5-month-old C57BL/6 wild type (WT) and Nos3tm1UNC (eNOS-KO) mice were divided into vehicle-treated (each n=10) or enalapril-treated (each n=10) groups.

Results: Blood pressure was significantly elevated in eNOS-KO mice at 14 months compared with WT mice (118.1±8.4 vs. 101.6±6.1 mmHg, p<0.05). Enalapril treatment significantly lowered the blood pressure in both groups (85.6±5.7 in eNOS-KO vs. 80.7±5.3 mmHg in WT). Urine albumin excretion was increased in eNOS-KO mice (but not in WT mice), whereas it was significantly reduced with enalapril treatment (167.4±61.4 vs. 27.0±9.6 µg/mg creatinine). Histologically, the development of glomerular sclerosis was found only in eNOS-KO mice, and it was significantly prevented with enalapril treatment (5.70±2.26 vs. 2.36±2.21 in % glomerulosclerosis, p<0.005). Activation of RAAS in eNOS-KO mice and normal aldosterone concentration, but not renin expression, was significantly higher compared with WT mice whereas enalapril significantly reduced aldosterone levels in eNOS-KO mice (346.3±150.6 vs 166.6±40.8 pg/ml, p<0.05).

Conclusions: The eNOS deficiency causes RAAS activation in the mouse. Long-term ACE inhibitor treatment effectively prevents renal injury and hypertension in mice lacking eNOS.
The systolic blood pressure (SBP) was monitored using tail cuff recording method. Renal function was estimated by plasma and urine creatinine, proteinuria and urinary sodium and potassium. Protein levels of angiotensinogen (AGTN), renin, ACE, Ang II receptors types 1 (AT1) and 2 (AT2) were determined in the renal medulla of clipped kidney by western blot. Tracking assay by flow cytometry showed that labeled MSC were present in the cortex and medulla in the clipped kidney.

**Results:** MSC had no detectable effects in control animals and significantly reduced the SBP by 22% (from 224 ± 8 to 173 ± 6 mmHg). Proteinuria was increased in 2K-1C compared to control animals (153 ± 3 vs 88 ± 19 mg/24h, P<0.05) and it was significantly reduced after MSC treatment (48 ± 8 mg/24h). Other renal function parameters were unchanged in all groups. AGTN, renin, ACE and AT1 protein expressions were elevated in clipped kidneys (49, 25, 30 and 43%, respectively) and the MSC treatment normalized all these expressions. In contrast, AT2 levels were significantly decreased in clipped kidneys (60%) and it was almost normalized after MSC treatment.

**Conclusions:** In conclusion MSC therapy suppressed the intrarenal RAS and improved renal function in the 2K-1C model. Whether the improvement of renal function was responsible for the MSC-reducing hypertension effects needs to be investigated.

**TH-PO737**

MTP-131 Reduces Renal Injury after Percutaneous Transluminal Renal Angioplasty (PTRA) in Swine Atherosclerotic Renal Artery Stenosis (ARAS)

**Background:** MTP-131 (bendavia) is a novel compound that induces opening of the mitochondrial permeability transition pore; apoptosis, and cardiac reperfusion injury in animals and humans, but its potential for improving PTRA outcomes in ARAS is unknown.

**Methods:** Pigs were treated after 6 weeks of ARAS or control with PTRA+stenting (or sham), with adjunct continuous infusion of bendavia (0.05 mg/kg IV, 30 min before PTRA). Renin-angiotensin system was assessed by real-time RT-PCR.

**Results:** After 5 weeks, mean arterial pressure was elevated to the same degree in MH (207±10 mmHg, N=13) and NH (201±4 mmHg, N=15; p=0.4 vs. NH) compared to CON (113±3 mmHg, N=25; P<0.001). MH exhibited higher serum aldosterone, left ventricular hypertrophy, and interstitial fibrosis of the nonclipped kidney, compared to NH and CON (all p<0.05). Macrophage infiltration was present in NH (8.9±0.8 vs. 5.7±0.5 cells in CON, P<0.009) but more pronounced in MH (14.7±1.4, P<0.001 vs. NH and CON). Osteopontin expression was increased 296-fold in NH (p<0.03 vs. CON) and 65-fold in MH (p<0.01 vs. NH and CON). MCP-1 expression was not altered in NH (1.4-fold, p=0.447) but increased 2.8-fold in MH (p<0.01 vs. NH and CON). The systolic blood pressure (SBP) was monitored using tail cuff recording method. Renal function was estimated by plasma and urine creatinine, proteinuria and urinary sodium and potassium. Protein levels of angiotensinogen (AGTN), renin, ACE, Ang II receptors types 1 (AT1) and 2 (AT2) were determined in the renal medulla of clipped kidney by western blot. Tracking assay by flow cytometry showed that labeled MSC were present in the cortex and medulla in the clipped kidney.

**Conclusions:** In conclusion MSC therapy suppressed the intrarenal RAS and improved renal function in the 2K-1C model. Whether the improvement of renal function was responsible for the MSC-reducing hypertension effects needs to be investigated.

**TH-PO738**

Aggravated Inflammation in Malignant Versus Non-Malignant Course of Experimental Renovascular Hypertension

**Background:** The reason(s) why malignant nephrosclerosis develops in some cases of hypertension but not in others are largely unknown. We hypothesized that malignant nephrosclerosis in experimental renovascular hypertension exhibits a more pronounced macrophage infiltration.

**Methods:** Two-kidney, one-clip renovascular hypertension was induced in rats; controls (CON) were sham operated. To distinguish malignant hypertension (MH) from non-malignant hypertension (NMH) without using arbitrary definitions, we performed split-half analyses for two factors: weight loss, and the number of characteristic vascular lesions (onion skin lesions and fibroinoid necroses) per kidney section of the nonclipped kidney. Animals in the upper half for both criteria were defined as MH whereas those in the lower half for both criteria were defined as NMH. Macrophage infiltration in the nonclipped kidney was counted (ED-1 stain), and the gene expression of macrophage chemoattractant proteins was measured by real-time RT-PCR.

**Results:** After 5 weeks, mean arterial pressure was elevated to the same degree in MH (207±10 mmHg, N=13) and NH (201±4 mmHg, N=15; p=0.4 vs. NH) compared to CON (113±3 mmHg, N=25; P<0.001). MH exhibited higher serum aldosterone, left ventricular hypertrophy, and interstitial fibrosis of the nonclipped kidney, compared to NH and CON (all p<0.05). Macrophage infiltration was present in NH (8.9±0.8 vs. 5.7±0.5 cells in CON, P<0.009) but more pronounced in MH (14.7±1.4, P<0.001 vs. NH and CON). Osteopontin expression was increased 296-fold in NH (p<0.03 vs. CON) and 65-fold in MH (p<0.01 vs. NH and CON). MCP-1 expression was not altered in NH (1.4-fold, p=0.447) but increased 2.8-fold in MH (p<0.01 vs. NH and CON).

**Conclusions:** Macrophage infiltration is much more pronounced in malignant renovascular hypertension than in the non-malignant course of the disease. MCP-1 is induced only in malignant nephrosclerosis and may contribute to its pathogenesis.

**Funding:** Government Support - Non-U.S.

**TH-PO739**

Sestrin2 Regulates Activation of Peroxiredoxin and Mediates Dopamine D2 Receptor/Paraoxonase 2-Induced Decrease in Renal ROS Production

**Background:** We have shown that the D2 dopamine receptor (D2R) decreases renal reactive oxygen species (ROS) production and regulates blood pressure (BP), in part, via positive regulation of paraoxonase 2 (PON2), an enzyme that protects against cellular oxidative stress. Sestrin2 is a conserved antioxidant protein that regulates intracellular ROS level by regulating over-oxidized peroxiredoxin (Prx-SO-H). We hypothesized that renal sestrin2 may be involved in counter-regulating renal ROS production via the D2R, contributing to maintain normal BP.

**Results:** In vitro treatment of human renal proximal tubular cells with the D2R agonist, quinpirole (24h, 1µM), decreased ROS production (DCFDA method) by 42% (P<0.05; n=3). This effect was associated with increased protein expression of PON2 (1.3-fold, p<0.05, n=3) and sestrin2 (1.4-fold, p<0.05, n=4), and decreased protein expression of Prx-SO-H (55%, p=0.01, n=5). In contrast, silencing D2R (siRNA, 20 nM, 48h) down-regulated PON2 (-25%, p<0.05, n=5) and sestrin2 expression (-48%, p<0.05, n=3), and increased Prx-SO-H protein expression (1.75-fold, p<0.05, n=3) and ROS production (1.8-fold, p<0.01, n=4). Silencing PON2 (siRNA, 10nM, 48h) decreased sestrin2 (-32%, p<0.05, n=3) and increased Prx-SO-H protein expressions (1.7-fold, P<0.05, p<0.03) and ROS production (1.4-fold, P<0.01, n=4). In vivo selective renal silencing of sestrin2 by subcapsular infusion of sestrin2 siRNA (5 µg/day, 7 days) in mice increased systolic (117±4 vs. 92±2 [vehicle-treatment] mmHg, P<0.01, n=3) and diastolic BP (86±2 vs. 67.5 [vehicle-treatment] mmHg, P<0.01, n=3).

**Conclusions:** These results suggest that renal sestrin2 is positively regulated by D2R and PON2 and contributes to maintain normal BP. The negative regulation of peroxiredoxin activation by sestrin2 mediates in part, the inhibitory effect of renal D2R on ROS production.

**Funding:** NIDDK Support, Other NIH Support - HL68686, HL23081, HL074940, HL092196

**TH-PO740**

Alikirsen Ameliorates Insulin Resistance and Aortic Endothelial Dysfunction in Fructose-Fed Rats

**Background:** Renin-angiotensin system plays a major role in the pathogenesis of cardiovascular diseases. The objective of this study was to examine the effects of alikirsen, a direct renin inhibitor, on the aortic endothelial function of fructose-fed hypertensive rats.

**Funding:** Pharmaceutical Company Support

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

828A
Methods: Male Wistar Kyoto rats weighing 200 to 230 g were divided into 4 groups (n=10 rats per group). Group FruA: rats were fed high fructose diet for 8 weeks; Group Fru: rats were fed high fructose diet (60% fructose) for 8 weeks; Group FruA: rats were fed high fructose and were coinfused with aliskiren (100 mg/kg/day), and Group FruB: rats were treated as Group Fru, but aliskiren was administered 4 weeks later. Isolated vascular ring experiments, systolic blood pressure (SBP), homestasis model assessment-insulin resistance (HOMA-IR), and oral glucose tolerance test (OGTT) and related blood profiles were measured. Results: By the end of week 4 and 8 of sustaining a high fructose diet, SBP had increased significantly from 110 ± 5 to 143 ± 6 and 140 ± 5 mmHg (p< 0.05), respectively. When fructose-induced hypertension had been established (from 112 ± 4 to 140 ± 3 mmHg, p< 0.05), subsequent aliskiren treatment for 4 weeks reversed the elevated SBP. Concurrent aliskiren treatment restored the development of hypertension. Additionally, a high-fructose diet also had significantly higher HOMA-IR values at week 4 (21.5 ± 2.08 and 21.28 ± 3.1 from 6.15 ± 1.59, respectively; p<0.05), and concurrent or subsequent administration of aliskiren significantly reduced the HOMA-IR values. OGTT showed that fructose feeding resulted in insulin resistance, and co-administration or superimposition of aliskiren significantly ameliorated insulin resistance of fructose-fed rats. The percent of endothelium-dependent aortic relaxation in the Group FruA and Group FruB were significantly higher than that in the Group Fru (63.46 ± 5.50 and 60.10 ± 8.01 from 39.97 ± 7.44 %, respectively; p<0.05).

Conclusions: This study demonstrates that aliskiren does not only prevent but also ameliorates hypertension and aortic endothelial dysfunction in fructose-fed rats. Funding: Private Foundation Support

TH-P0743

A Synthetic Serine Protease Inhibitor Camostat Mesilate Inhibited the Proteolytic Activation of gENaC in the Kidney of the Aldosterone-Infused Rats

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Background: ENaC is an important role in the regulation of blood pressure by modulating Na reabsorption in the kidney. ENaC consists of α, β, and γ subunits, and the activation of ENaC is mainly regulated by aldosterone in living body. It was reported that aldosterone induced a molecular weight shift of gENaC from 85 to 70 kDa, and recently the ENaC have considered to be the result of proteolytic cleavages by serine proteases and necessary for the activation of ENaC from the in vitro experiment. But detail mechanisms about the cleavage of gENaC in vivo are still unclear. In order to study the role of serine proteases in this cleavage in vivo, we administered a synthetic serine protease inhibitor camostat mesilate to aldosterone-infused rats.

Methods: The SD rats (n = 6) were kept for 10 days under the following conditions: (1) Control, (2) aldosterone infusion, (3) aldosterone infusion+camostat treated with free access to water and chow. After 10 days, rats were sacrificed under anesthetics conditions with nonheparin.

Results: Camostat decreased 70kDa form of gENaC and produced the new about 75kDa form with increase of urinary Na/K ratio, suggesting that camostat inhibited one site of the dual cleavages of gENaC and suppressed the activation of ENaC. Prostasin is one candidate serine protease involved in the cleavage of gENaC in these model rats, because prostatasin was shown to cleave this subunit in vitro and its excretion into urine was increased by aldosterone. Camostat inhibited protease activity, activating processing and urinary secretion of prostatasin.

Conclusions: These results suggest that prostatasin is important serine protease in the pathogenesis of aldosterone-induced salt sensitive hypertension. A synthetic serine protease inhibitor, camostat mesilate, would be a new strategy in the treatment of salt-sensitive hypertension in human.

TH-P0744

The Sodium Chloride Cotransporter (NCC) and Prostasin, a Regulator of the Epithelial Sodium Channel (ENaC), Are Urinary Biomarkers for Hyperaldosteronism Niils van der Lubbe, Pieter Martijn Jansen, Anton H. van den Meiracker, Alexander H.Danser, Robert Zietse, Ewout J. Hoorn. Internal Medicine, Erasmus Medical Center, Rotterdam, Netherlands.

Background: Urinary exosomes are vesicles derived from renal tubule epithelial cells that have been shown to contain many disease-associated proteins. Here, we hypothesized that the aldosterone-sensitive sodium transporters could serve as biomarkers for hyperaldosteronism.

Methods: Urinary exosomes were studied in a rat model of hyperaldosteronism and in patients with essential hypertension or primary aldosteronism (n = 5/group). Rats were adrenalectomized and then received vehicle or aldosterone. Primary aldosteronism was defined as an elevated aldosterone to renin ratio and unsuppressible aldosterone in response to volume expansion. Blood pressure and the number and type of antihypertensive drugs were similar in the two groups of hypertensive patients. Exosomes were isolated from 24h urine using ultracentrifugation. Phosphorylated NCC and prostasin, a protease regulating ENaC, were studied by immunoblotting. Prostasin rather than ENaC was studied, because the latter has not been identified in urinary exosomes.

Results: The animal model was confirmed by finding higher plasma aldosterone and lower urinary sodium in rats treated with aldosterone. In the urinary exosomes of these rats, the abundances of pNCC and prostasin were increased 3.9 and 1.8-fold compared to controls (p< 0.05 for both). Patients with primary aldosteronism also had higher pNCC and prostasin abundances compared to patients with essential hypertension (1.4 and 1.9-fold), although these did not reach statistical significance (p = 0.1 and p = 0.2, respectively).

Conclusions: In animals and patients with hyperaldosteronism, the abundance of pNCC and prostasin in urinary exosomes is increased. These results suggest that these aldosterone-sensitive proteins may be used as urinary biomarkers for primary aldosteronism. To validate these results, we are currently expanding the number of patients tested.
Fat Distribution and Regulation of the Renin Angiotensin System in Healthy Humans

**Background:** Obesity, a major risk factor for chronic kidney (CKD) and cardiovascular disease (CVD), is associated with up-regulation of the renin-angiotensin system (RAS), activity which is deleterious to kidney and CV function. The ideal measure of adiposity associated with increased risk in humans is unclear. We sought to determine the relationship between measures of fat distribution and angiotensin (AngII) dependent-control of blood pressure (BP) in healthy humans.

**Methods:** Thirty-eight healthy non-obese subjects (15 men, 23 women) were studied in high salt balance. Body mass index (BMI), waist circumference (WC), hip circumference (HC), waist:hip ratio (WHR), body adiposity index, body surface area, fat mass%, and total body water% were measured. BP and circulating components of the RAS were measured at baseline and in response to graded AngII infusion (3ng/kg/min x 30 min followed by 6 ng/kg/min x 30 min). The primary outcome was the association between adiposity measures (BMI, HC and WHR) and the BP response to AngII challenge at 60 minutes.

**Results:** BMI was associated with baseline BP (SBP: r=0.544, p<0.001; DBP: r=0.324, p=0.047) and HC was associated with baseline SBP in women (r=0.491, p=0.02). However, BMI was not associated with the BP response to AngII (SBP: r=0.08, DBP: r=0.07, overall or when stratified by gender. Conversely, HC was non-significantly associated with both SBP (r=0.3, p=0.076) and DBP (r=0.2, p=0.3) responses to AngII challenge, a relationship that achieved significance in men (SBP: r=0.543, p=0.045; DBP: r=0.58, p=0.03) but not in women (SBP: p=0.9; DBP: r=0.7). While BMI was not associated with any circulating RAS components at baseline or in response to AngII challenge, W/H ratio was a predictor of baseline, PRA (r=0.563, p<0.001), AngII (r=0.468, p=0.006) and aldosterone (r=0.355, p=0.03) as well as the PRA response to AngII infusion (r=0.514, p=0.001). Other anthropometric parameters were not associated with any of the responses to AngII challenge.

**Conclusions:** Fat distribution, as measured by HC and WHR, is associated with vascular RAS activity in healthy humans and may have implications for assessment and control of obesity-mediated hypertension.

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**Poster:** Hypertension: Basic

**Title:** TH-PO745

**Effect of tubular overexpression of L-FABP on the expression of AngII dependent hypertensive markers in diabetic rats: a role of oxidative stress and inflammation

**Methods:** To evaluate the mechanism of salt mediated damage, we focused on the endothelium protecting ecto-enzyme CD39 [i.e. ectonucleoside triphosphate diphosphohydrolase-1, (ENTPD-1), or ecto-apyrase] and on the angiotensin II receptor (AT-1). CD39 is sensitive to oxygen free radicals and possibly also to the pro-inflammatory action of angiotensin II.

**Conclusions:** Confluent human endothelial cells were co-cultured with human peripheral blood mononuclear cells (1x10^6 cells per well) using either medium (RPMI 1640) with a standard amount of NaCl (154.0 mMol/L) (low salt, LS), or with “high” NaCl (155.54 mMol/L) (HS). After 16 hours, cytospins of endothelial cells were prepared for flow cytometry or immunostained for AT-1 and CD39.

**Results:** Endothelial cells cultured in HS medium show a significant decrease of CD39 expression as compared with control cultures, as demonstrated by both immunostaining (p<0.05) and flow cytometry (p<0.05). Endothelial AT-1 receptor expression also showed down regulation in the presence of HS (p<0.01), whereas HS showed enhanced O2 production in PBMC as compared with PBMC from LS cultures.(p<0.01)

**Conclusions:** The present data indicate that a slight increase of salt is able to induce impairment of an important endothelial anti-inflammatory ecto-enzyme (CD39) as well as an increase of the expression of the AT-1 receptor. The endothelial cells in vitro (and in vivo) may be mediated by toxic oxygen radicals produced by PBMC. Down regulation of endothelial AT-1 by salt in vitro may be in line with down regulation of the RAAS, known to occur after salt loading in vivo.

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**Title:** TH-PO748

**Salt-Sensitivity of Blood Pressure in Mice with Targeted Knockout of the Insulin Receptor in Thick Ascending Limb through Collecting Duct**

**Methods:** To address this, young male KO and wild-type (WT) littermates (~6 weeks old) were instrumented with radiotransmitters to measure BP and heart rate and transitioned through a 4-week regimen that included 1-week periods on: the following diets: 1) medium-salt (MS, 0.5%); 2) low-NaCl (LS, 0.085%); 3) high-salt (HS, 5%) and HS plus the anti-oxidant Tempol (3 mM) in the drinking water. Urine was collected for 24 hours at the end of each week and UNOx measured.

**Results:** There were no differences in initial or final body weight between genotypes. Under low-NaCl diet (0.085%), there were no significant differences in systolic, diastolic, and renal risk. Endothelial injury leading to endothelial dysfunction plays a major role in these observations.

**Conclusions:** We also examined the expressions of these markers in L-FABPTg and their wild type littermates (WT) with A-SSHT.

**Results:** 1) All-stimulation induced mRNA expressions in mProx of HO-1 and MCP- 1 peaked at 12 and 36 hr, and AgNOC peaked at 36 hr. These expressions were significantly attenuated in mProxTg (p<0.05). Tempol pretreatment in mProxTg also significantly attenuated these expressions at 36 hr (p<0.05).

2) L-FABP-Tg with A-SSHT showed significant attenuation of renal Ag, 4-HNE, HO-1 and MCP-1 expressions with significant improvement of hypertension and interstitial T-cell infiltration.

**Conclusions:** It was found that tubular overexpression of L-FABP may protect against A-SSHT through attenuation of AII-induced oxidative stress and exacerbation of intrarenal RAS activation and inflammatory responses.

**Funding:** Government Support - Non-U.S.
increased approximately 3-fold with HS and was not affected by Tempol. The area under the curve for the rise in UNx was 26% reduced in the KO mice. Heart rate was slightly reduced in the KO mice, an effect that was significant in the MS period (p=0.015).

Conclusions: Overall, KO mice show greater BP sensitivity to salt with some blunting of the potentially protective rise in NOx production. The fact that Tempol had little effect on responses to HS suggests that factors in addition to oxidative stress, are likely determinant(s) of enhanced salt sensitivity.

Funding: NIDDK Support, Private Foundation Support

TH-PO750
Sodium-SensitivityCorrelateswithDifferentialRegulationofPeripheralBloodMicroRNAsSophieDonham,1ClaudiaSommerrer,1FelixJ.Frey,3MartinG.Zeier,1AmirAbdollahi,12Nephrology,UniversityofHeidelbergMedicalSchool,Germany;RadiationOncology,UniversityofHeidelbergMedicalSchool,Germany;Nephrology,Inselspital,UniversityHospitalofBerne,Switzerland.

Background: Sodium-sensitivity is an important risk factor for cardiovascular events and is associated with increased morbidity and mortality. MicroRNAs (miRs) are considered masterregulators of transcriptome. We aimed to identify the molecular determinants of sodium response and predictors of sodium sensitivity using the blood transcriptome as the sentinel organ.

Methods: Whole blood total RNA including miRs were collected during high (150 mmol/d) and low (30 mmol/d) sodium diet using PaxGene tubes. 24 hour blood pressure monitoring was performed during each diet to assess for changes in blood pressure. MiR isolation was performed using Qiagen’s PAXGene Blood miRNA Kit and the QIAcube system. RNA quality control and quantitation was performed using total- and small RNA Agilent Chips (Bioanalyser, Agilent) and Nanodrop spectrophotometer. Genome-wide miR profiling was performed using Illumina’s microRNA DASL assay. Clustering and statistics were performed using SUMO software package.

Results: 83 miRs were differentially regulated (p<0.02) after sodium-rich vs. sodium-low diet in n=6 patients. The prevailing effect of sodium rich diet was downregulation of miRs (35 down- vs. 15 upregulated miRs). Given the inhibitory function of miRs on post/transcriptional regulation, our data suggest a global activation of the transcriptome via downregulation of miRs in response to sodium. Of note, the miR200 family, recently attributed to be involved in the EMT process were found to be induced by sodium. Three patients demonstrated increased blood pressure (MAP) of at least 8 mmHg after sodium-rich vs. sodium-low diet and were therefore considered to be sodium-sensitive. We found 105 miRs to be differentially regulated between sodium-sensitive vs. salt-resistant patients (p<0.05). The regulation of candidate sodium sensitive/responsive miRs are confirmed by qRT-PCR.

Conclusions: Our data demonstrate the feasibility of peripheral blood miRNAs as sentinel organ to detect sodium response/sensitivity.

TH-PO751

Background: How high salt intake increases blood pressure is a key question in the study of hypertension. Salt-induced increases in renal sympathetic activity have been shown to induce sodium retention. However, the mechanism underlying the sympathetic control of renal sodium excretion remains unclear. We have reported that β2-adrenergic receptor (β2-AR) stimulation down-regulated WNK4 transcription (to less than 50%) through epigenetic modulation (Mu S. et al. Nat Med 2011).

Methods: We injected Isoproterenol (ISO) to wild type mice by subcutaneous mini pumps. WNK4 mRNA expression were measured by Realtime-RT-PCR. Then we use the HAT p300 inhibitor currcumin (Cur) to see if reverse of histone acetylation could adjust the expression of WNK4 and prevent the development of salt-sensitive hypertension. β2-AR stimulation suppressed the activity of histone-deacetylase 8 (HDAC8) and recruit glucocorticoid receptor (GR) binding to the negative GR responsive element (nGRE) in WNK4 promoter region, and lead to the transcriptional inhibition of WNK4 gene. And in vivo model, we clarified that suppressed WNK4 expression leads to activation of the Na+-Cl– co-transporter (NCC), which led to the development of salt-induced hypertension (SBP 180±9mmHg ±10±mmHg). In the present study, based upon our finding, we investigated possible therapeutic role of the inhibition of histone acetylase (HAT) in salt-sensitivity by modulating histone acetylation. In isoproterenol stimulation down-regulated WNK4 transcription (to less than 50%) through epigenetic modulation (Mu S. et al. Nat Med 2011).

Results: In former study, we revealed that β2-AR stimulation suppressed the activity of histone-deacetylase 8 (HDAC8) and recruit glucocorticoid receptor (GR) binding to the negative GR responsive element (nGRE) in WNK4 promoter region, and lead to the transcriptional inhibition of WNK4 gene. And in vivo model, we clarified that suppressed WNK4 expression leads to activation of the Na+-Cl– co-transporter (NCC), which led to the development of salt-induced hypertension (SBP 180±9mmHg ±10±mmHg). In the present study, based upon our finding, we investigated possible therapeutic role of the inhibition of histone acetylase (HAT) in salt-sensitivity by modulating histone acetylation. In isoproterenol stimulation down-regulated WNK4 transcription (to less than 50%) through epigenetic modulation (Mu S. et al. Nat Med 2011).

Conclusions: Our results illustrate a novel role for another epigenetic modulation – HAT inhibition in the development of salt-induced hypertension and we provided an alternative therapeutic target of salt-sensitive hypertension.

TH-PO752

Background: Renal innervation consists of sympathetic efferent and peptidergic afferent nerves. The latter express TRPV1-receptors. We recently found neurophysiological evidence for a functionally relevant intrarenal peptidergic innervation, exhibiting sympatho-inhibitory effects. Hence, we now tested the hypothesis that stimulation of renal afferent nerve activity (ARN) with the TRPV1-agonist capsaicin initiates efferent renal sympathetic nerve activity (RSNA).

Methods: Eight methohexital anesthetized male Sprague-Dawley rats were instrumented as follows: arterial and venous catheters for recording of blood pressure (BP) and heart rate (HR) and drug administration; left sided renal arterial catheter for selective intrarenal administration of IRA (of the TRPV1 agonist capsaicin (CAP) 3.3, 6, 6, and 10±10-7M; 10µl after 15, 30, 45, and 60 minutes) to stimulate ARNA; right sided bipolar stainless steel electrode for continuous RSNA recording; Before and after IRA/CAP increasing intravenous (IV) doses of the NK1-receptor blocker RP67580 was given. The NK2- and CGRP blockers MEN10376 and CGRP8-37 also were tested.

Results: IRA/CAP decreased integrated RSNA from 65±13.0 µV·sec (baseline) to 12.8±3.2 µV·sec (minimum), P<0.001. This sustained RSNA inhibition reached its minimum within 70 minutes, and was not directly linked to the transient electrical ARNA response which is usually seen with IRA/CAP. Suppressed RSNA was transiently but completely unmasked by systemic administration of the NK1-blocker (maximum: 120±31±19 µV·sec; P<0.001). Furthermore NK1-blockade transiently increased baseline RSNA to similar levels in dose dependent manner. NK2- and CGRP blockers had no effect.

Conclusions: Our study provides direct evidence that the afferent renal nerves provide a tonically acting sympatho-inhibitory system which seems to be rather mediated by neurokinin release acting via NK1-receptor pathways, than by electrical ARNA effects on central sympathetic outflow. However, the exact site of NK1-mediated action (central, ganglionic, or other) remains to be further elucidated.

Funding: Government Support - Non-U.S.
Genetically Determined Low Nephron Number Is Associated with Hypertension Due to Chloride, but Not Sodium Retention

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Background: An association between low nephron number and development of hypertension in later life has been demonstrated. The underlying pathomechanisms are unknown. Reduced renal Na+ and water clearance is regarded as one option. We tested the hypothesis that GDNF heterozygous (GDNF+/-) with 30% reduction of nephron number have increased tissue electrolyte and water content compared to wildtype (wt) mice.

Methods: Mice of 34 GDNF+/- and 35 wt mice (45-55 wks) received low salt (0.03% NaCl) or high salt (HSD, 4.5% NaCl, 0.9% saline) diet for 4 weeks. Blood pressure (bp) was continuously measured by telemetry (n=4 per group). At the end of the experiment, blood samples were taken, and tissue Na+, K+, Cl- and water content in skin, bone, and total body weight were determined by chemical analysis.

Results: Body weight, dry weight, total body water content, relative skin and body Na+ and K+ content were not significantly different between the groups, and no differences in serum concentrations were found. In contrast and independent of the diet, skin and total body volume correlated with the absolute size of the heart. Interestingly, mineralocorticoid signaling in the heart remains largely unknown.

Conclusions: We found that tissue Cl- accumulation, but no differences in Na+ and water balance, parallel the development of hypertension in GDNF+/- mice. These findings suggest that reduced renal Cl- clearance parallels hypertension in mice with reduced nephron number.

SIRT1 Activation Protects the Endothelial Dysfunction by Inhibiting E-Selectin and VCAM-1 Generation

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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a marker for acute kidney injury and is related to the expression of adhesion molecules, such as endothelial-leukocyte adhesion molecule-1 (ELAM-1) and vascular cell adhesion molecule-1 (VCAM-1). NGAL knockout mice did not show any difference in adhesion molecule expression compared to wildtype mice. Thus, the role of NGAL in the development of vascular adhesion molecule expression remains unknown.

Methods: Using a transcriptomic approach, we identified NGAL as the highest expression in vehicle-treated group vs. 1.05 ± 0.15 in vehicle-treated group, p = 0.001. From the kidney tissue, mRNA expression of NGAL was assessed in vehicle- and losartan-treated mice evaluating more than 34,000 transcripts demonstrated regulation for 14 genes only, similar extent. An array analysis comparing renal gene expression of losartan and aliskiren treated mice evaluating more than 34,000 transcripts demonstrated regulation for 14 genes only, with maximal difference of a signal-log ratio of 2. No superior nephroprotection was found by comparing losartan and aliskiren compared to monotherapies. Compared to plasma concentrations, aliskiren accumulated ∼7 to 29-fold in heart, liver, lung, and spleen and ∼156-fold in the kidney. After withdrawal, plasma concentrations dropped to zero within 24 hrs, whereas renal tissue concentrations declined slowly over several days. Withdrawal of aliskiren in mice with CKD revealed a significantly delayed re-increase in albuminuria compared to withdrawal of losartan.

Conclusions: This study demonstrates equieffective nephroprotection of renin inhibition and AT1 antagonist in mice with CKD without additional benefit of combination therapy. However, aliskiren offers advantages derived by its pharmacokinetics.

Funding: Government Support - Non-U.S.

Retinoprotective Effect of Rosuvastatin in Salt-Sensitive Hypertensive Rats

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Background: Hypertension is a significant risk factor for the development and progression of chronic kidney disease. Although recent clinical studies suggested that statins might have pleiotropic effects upon cardiovascular disease, the evidences regarding the benefit of statins in renal injury was not clear. We investigated whether statin treatment would attenuate renal damage in Dahl salt-sensitive (DS) hypertensive rat models which develop hypertension by high salt diet (8.0% NaCl).

Methods: DS rats were with high salt diet for 7 weeks and rosuvastatin (20mg/kg/day by gavage, n=6) or vehicle (n=6) was administered from 13 to 21 weeks of age. Body weight, blood pressure, urine protein and creatinine, serum BUN, creatinine, and cholesterol were measured. Glomerulosclerosis index (GSI), tubular lesion index (TLI) and expression of TGF-β1 and ET-1 were assessed from kidney tissue.

Results: DS rat given a high salt diet developed hypertension (systolic blood pressure, 216 ± 42 mmHg) compared to rat given normal salt diet, 150 ± 8 mmHg and showed azotemia and proteinuria. Between rosuvastatin-treated group and vehicle treated group, there was no difference in baseline characteristics. Rosuvastatin treatment had little effect on the blood pressure level (131 ± 27 mmHg in rosuvastatin-treated group vs. 139 ± 33 mmHg in vehicle-treated group, p = 0.37). Rosuvastatin treatment had little effect on the creatinine level (0.98 ± 0.21 mmol/L in vehicle-treated group, p = 0.57). Rosuvastatin treatment had little effect on the proteinuria level (1.05 ± 0.15 in vehicle-treated group, p = 0.001). Immunohistochemical analysis showed decreased level of TGF-β1 expression in rosuvastatin-treated rats compared to vehicle-treated rats whereas it showed no difference in the level of ET-1 expression.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Our results suggest that ronsuavatitan mitigate hypertensive renal injury independent from lipid lowering effect and its effect may involve TGF-β1.

TH-PO759

Pentoxifylline Inhibits Angiotensin II-Stimulated Proliferation of Rat Vascular Smooth Muscle Cells

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Background: Pentoxifylline (PTX) is a non-selective inhibitor of cyclic-3’,5’-phosphodiesterase, which raises intracellular cAMP. PTX has been known to have an effect against cell proliferation, inflammation and fibrosis in some experiments. However, little is known about whether PTX can prevent Ang II-induced proliferation of vascular smooth muscle cell (VSMC) which is an important role in hypertensive vascular damage. To evaluate this, we tested anti-proliferative effect and cell cycle regulation of PTX in the Ang II-stimulated VSMC.

Methods: Rat VSMCs were isolated from the thoracic aortas of male Sprague-Dawley rats. The VSMCs were treated with 1µM of Ang II and various doses of PTX. The proliferation was determined by the MTT assay. Cyclic 3’,5’-AMP was measured by the cyclic AMP EIA kit. The expressions of mRNA and protein were analyzed using real-time PCR and Western blotting.

Results: 1. Ang II significantly proliferated VSMC (Control: 0.29±0.02 vs. Ang II: 0.40±0.05, p<0.01). PTX dose-dependently suppressed Ang II-induced cell proliferation (PTX 0.1mM: 0.32±0.05; PTX 0.5mM: 0.29±0.03; PTX 1mM: 0.26±0.04; PTX 2mM: 0.25±0.03, p<0.05).

2. Ang II inhibited cAMP generation (Control: 34.62±0.59 vs. Ang II: 17.49±3.30, p<0.05). PTX significantly restored cAMP level in Ang II-stimulated cells (PTX 0.1mM: 40.68±0.49; PTX 2mM: 41.50±0.78, p<0.05).

3. Ang II significantly upregulated mRNA and protein expression of cyclin D1 by 2 folds, and downregulated mRNA and protein expression of p21 by half. There were no changes of cyclin E, cyclin A, CDK2 and CDK4, and p27. PTX pretreatment prevented the changes of cyclin D1 and p21.

Conclusions: PTX attenuated proliferation in Ang II-stimulated VSMCs. The anti-proliferative effect of PTX was related to the increment of cAMP and partial regulation of the changes of cyclin D1 and p21.

Funding: Government Support - Non-U.S.

TH-PO760

Vitronectin-Binding PAI-1 Protects Against Cardiac Fibrosis through Interactions with Fibroblasts

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Background: We have shown that ovariectomy in adult Wistar rats caused an activation of renal kallikrein-kinin system and a decrease in mean blood pressure (MBP) (Kidney Blood Press Res 2009). Now we tested whether Wistar rats ovariectomized (O VX) when 60 days old and studied at 150 days of life could have changes in Na+ excretion (ENa+) and in systemic hemodynamic.

Methods: To this purpose, intact (I) and O VX rats consuming either normal sodium diet (NS, 0.24%NaCl) or high sodium (HS, 1% NaCl, in drinking water) for 5 days were studied.

The expression of total Na+, K+-ATPase (alkaline subunit) and its phospho state at Ser 23 (PKC site) was determined by western blot in renal homogenates. Current concepts agree that when dephosphorylated renal NaK EA is more active. Beta actin was used as control.

Results: On NS diet, expression of total and dephosphorylated Na+K+EA alkaline subunit Ser 23 dephospho decreased in I and O VX rats. But, in O VX rats, while NaK EA showed a trend to increase (R (3.63±0.29 vs O VX 4.81±0.39 mU/mg/min/g kwt), Na+) did not differ between I and O VX rats, while O VX rats showed a trend to increase (R (3.9±1 vs 4.6±0.3, p<0.05) and MBP significantly increased in O VX to 135±9 vs 116±7 mmHg in I rats, p<0.05). Diuresis, GFR and RPF were not different between I and O VX rats under NS diet.

Conclusions: Ovariectomized adult rats have a higher total and dephosphorylated Na+, K+-ATPase alkaline subunit and a lower sodium excretion under HS diet than I rats. Both, together, may contribute to develop sodium sensitive hypertension in rats deprived of ovarian hormones.

TH-PO761

Overexpression of Na+, K+-ATPase and Sodium Sensitive Hypertension Induced by Ovariectomy in Adult Rats

Fernando Raul Ibarra, 1 Luis A. Di Ciam,1 Elisabet Monica Oddo, 1 Pablo J. Azurmendi, 1 Jorge Toledo, 1 Elsa Zotta, 2 Federico Ochoa, 2 Elvira Arrizurieta. 1 Instituto A Larnari; 1Faculty of Medicine, Buenos Aires University.

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The expression of total Na+, K+-ATPase (alkaline subunit) and its phospho state at Ser 23 (PKC site) was determined by western blot in renal homogenates. Current concepts agree that when dephosphorylated renal NaK EA is more active. Beta actin was used as control.

Results: On NS diet, expression of total and dephosphorylated Na+K+EA alkaline subunit Ser 23 dephospho decreased in I and O VX rats. But, in O VX rats, while NaK EA showed a trend to increase (R (3.63±0.29 vs O VX 4.81±0.39 mU/mg/min/g kwt), Na+) did not differ between I and O VX rats, while O VX rats showed a trend to increase (R (3.9±1 vs 4.6±0.3, p<0.05) and MBP significantly increased in O VX to 135±9 vs 116±7 mmHg in I rats, p<0.05). Diuresis, GFR and RPF were not different between I and O VX rats under NS diet.

Conclusions: Ovariectomized adult rats have a higher total and dephosphorylated Na+, K+-ATPase alkaline subunit and a lower sodium excretion under HS diet than I rats. Both, together, may contribute to develop sodium sensitive hypertension in rats deprived of ovarian hormones.

TH-PO762

The Effects of Dietary Patterns on Plasma Renin Activity; Results from the Dietary Approaches To Stop Hypertension (DASH) Trial

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Background: A diet rich in fruits, vegetables, and low-fat dairy products and reduced in saturated fat, total fat, and cholesterol (termed the "DASH" diet) significantly lowers blood pressure (BP). Previous studies have documented that certain therapies that lower BP increase plasma renin activity (PRA).

Methods: Using data from the DASH trial, we assessed the effects of dietary patterns on PRA and determined the relationship of change in PRA with change in BP on each diet. After eating a control diet for three weeks, participants were then randomized to receive for eight weeks: the control diet, a diet rich in fruits and vegetables (F/V), or the DASH diet. Baseline and follow-up levels of PRA were available in 381 participants.

Results: Compared to the control diet, the DASH diet increased PRA by 0.37 ng/mL/h (P<0.01). In multivariable linear regression analyses, there was an inverse association of PRA change with systolic BP change on the control diet (slope=-0.35, P=0.001), but PRA did not differ by BP change on the F/V (slope=-0.02, P=0.98) or DASH diet slope (slope=-0.08, P=0.32).

Change in logePRA by Change in Systolic Blood Pressure, by Diet Assignment

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

287A
Dietary Salt and Protein Intake Is Not Associated with Elevated Blood Pressure Levels in US Adults Shailendra Sharma, Kim McFann, Anna Jeanette Jovanovich, Michel B. Chonchol, Jessica B. Kendrick 1,2 Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; 2Denver Health Medical Center, Denver, CO.

Background: Current guidelines recommend reducing dietary salt intake and increasing dietary potassium intake to reduce blood pressure (BP) levels. The association of dietary salt and potassium intake with increased risk of BP levels is unclear.

Methods: The cross-sectional association between dietary sodium and potassium intake and BP levels was examined in 6985 adults 18 years of age or older with no prior history of hypertension who participated in the National Health and Nutrition Examination Survey (2001-2006). Dietary sodium and potassium intake was calculated from 24-hour dietary recall obtained by trained interviewers. Three BP measurements were collected from each participant. Multivariate logistic regression analysis was performed to evaluate whether high sodium and low potassium intake is independently associated with higher BP levels.

Results: The mean (SE) age of the participants was 45 (0.4) years. The mean (SE) sodium and potassium intake was 3520 ± 26 and 2761 ± 22 mg/day, respectively. In linear regression models there was no association between higher sodium or lower potassium intake with systolic BP (β = −0.000052 ± 0.00012, p = 0.67 and β = −0.00024 ± 0.00018, p = 0.59, respectively). In logistic regression models after adjustment for age, sex, race, diabetes and eGFR, there was no association between higher quartiles of sodium or lower potassium intake with the risk of a BP cutoff of ≥140/90 mmHg. We also examined the relationship of combinations of potassium and sodium intake with blood pressure cutoffs of ≥140/90 mmHg. Median intake of potassium and sodium were used to determine high and low potassium and sodium intake. High potassium intake combined with low sodium intake was not protective for BP > 140/90 mmHg (p=0.13). Furthermore, high sodium intake combined with low potassium intake was not associated with an increased risk of BP > 140/90 mmHg (p=0.95).

Conclusions: In the US adult population without hypertension, increased dietary sodium or low potassium intake was not associated with elevated BP levels.

Dietary Salt and Protein Intake and Obesity in Ireland Gemma M. Browne, Joseph A. Eustace, Ivan J. Perry 1 Department of Epidemiology and Public Health, University College Cork, Cork, Ireland; 2Department of Nephrology, Cork University Hospital, Cork, Ireland.

Background: Obesity is linked to CKD through several established mechanisms including hypertension and salt sensitivity. Ongoing dietary intake of sodium and protein may contribute to progressive renal damage and confound above relationships. This study was set up to quantify dietary sodium and protein intake in obese compared to non-obese subjects.

Methods: 599 community dwelling subjects including an occupational group and students, aged 18-81, using random and convenience sampling, participated in a cross-sectional study in Munster, Ireland. Subjects underwent blood pressure (BP) and anthropometric measures using a standardized protocol and performed one 24 hour urine collection. 488 subjects had valid analytes including urinary sodium and urinary urea nitrogen, with a complete collection based on para-aminobenzoic acid appearance. Protein nitrogen appearance (PNA) was calculated (Maroni B, Mitch WE). The relationship of dietary intake with obesity was examined using multivariate logistic regression (STATA V11.0).

Results: Central obesity was significantly associated with dietary salt intake, urinary PNA, hypertension, age and gender (Table 1) compared to non obese subjects. When dietary associations of central obesity were explored in an adjusted model, salt intake (OR 1.13, p<0.001), urinary PNA (OR 1.01, p=0.043), age (OR 1.06, p<0.001) and gender (OR 4.23, p<0.001) continued to be independently significant. Similar independent associations were present comparing subjects with BMI>30kg/m2 compared to normal weight subjects. Dietary Salt Protein Intake, Hypertension & Central Obesity

Gender (N) Men N=106 Women N=182 p=2
Waist cm % >102 (21%) <102 <88 (31%) <88 
Salt grams/day mean(sd) 12.4 (4.7) 9 (4.9) 8.1 (3.1) 7 (2.7) 
Urineary PNA mg/day mean(sd) 217 (52) 192 (53) 149 (40) 132 (40) 
% BP >140/90 / Meds 65% 6.4% 46.7% 53.3%

Conclusions: Using validated methods, obesity is independently related to higher protein and salt intake. In addition to salt sensitivity, obese subjects have higher salt intake in association with higher protein intake. Appropriate dietary advice and salt restriction may be especially important in obese hypertensive subjects.

Hypertension: Clinical Poster/Thursday

Association of Sweetened Beverage Intake and Incident Hypertension Is Similar between Sugar-Sweetened and Artificially-Sweetened Beverages Lisa J. Cohen, Gary C. Curhan, John P. Forman. Renal Division, Brigham and Women's Hospital, Boston, MA.

Background: Consumption of sugar-sweetened beverages (SSBs) is associated with hypertension in cross-sectional studies. However, prospective data are limited.

Methods: We performed an analysis of originally non-hypertensive individuals in three large, prospective cohorts, the Nurses' Health Studies I (n=89,540) and II (n=97,991) and the Health Professionals' Follow-Up Study (n=37,360), to determine the relation between SSB and artificially sweetened beverage (ASB) consumption and the development of hypertension over time. Cox proportional hazards regression was used to calculate hazard ratios for incident hypertension for intake of up to quartiles of beverage intake after adjusting for potential confounders. The association between fructose consumption from SSBs versus fructose from other sources and the risk of incident hypertension was also determined.

Results: Findings were similar across the cohorts. Higher SSB and ASB intake was associated with an increased risk of developing hypertension. In a pooled analysis of all three cohorts, participants who consumed at least one SSB daily had an adjusted HR for incident hypertension of 1.13 (95% CI, 1.09-1.17) compared with those who did not consume SSBs; for persons who drank at least one ASB daily, the adjusted HR was 1.4 (95% CI, 1.09-1.18). There was a significant interaction between carbonation and total beverage intake (p-interaction < 0.001 in NIS 1, 0.03 in NIS II, and 0.02 in HPFS). In an analysis of fructose intake as a percentage of daily calories, higher fructose intake from SSBs was associated with increased hypertension risk in the NIS and NIS II cohorts (p-trend<0.001 in both groups), while higher fructose intake from sources other than SSBs was associated with a decrease in hypertension risk in NIS II participants (p-trend<0.006).

Conclusions: Both SSBs and ASBs are each independently associated with an increased risk of incident hypertension after controlling for multiple potential confounders. The mechanisms that underlie these associations are unclear.

Uric Acid Is Associated with Systemic Inflammation and Reduced MnSOD Expression in Endothelial Cells of Healthy Adults Diana J. Jalal, Kristen L. Jablonski, Kim McFann, Michel B. Chonchol, Douglas R. Seals 1 Internal Medicine/Renal, University of Colorado Denver, Aurora, CO; 2Department of Integrative Physiology, University of Colorado, Boulder, CO.

Background: We previously reported that uric acid levels are not related to endothelial function in healthy adults. Yet, experimental data suggests uric acid may induce endothelial dysfunction and vascular inflammation in some clinical settings. To further understand our results, we explored the relation between uric acid and inflammation and oxidative stress systemically and in endothelial cells collected from study participants.

Methods: We examined the relation between uric acid levels and C-reactive protein (CRP) and oxidized-LDL in all participants (n=107) as continuous variables and according to uric acid quartiles (0-5.2, 5.3-6.1, 6.2-7, > 7.0 mg/dL). To evaluate the relation between uric acid and cellular inflammation and oxidative stress, immunostaining of endothelial cells was compared between the lowest and the lowest uric acid quartiles (unpaired t-test with Bonferroni correction). Immunofluorescence was performed on endothelial cells collected from the subjects' brachial arteries. The following markers were evaluated: NFκB p65 (n=19), nuclear factor kappa-B (NFκB) and oxidized-LDL. The endothelial cells were stained with an antibody against oxidized-LDL. The following markers were evaluated: NFκB p65 (n=19), nuclear factor kappa-B (NFκB) and oxidized-LDL. The endothelial cells were stained with an antibody against oxidized-LDL.

Results: CRP increased significantly with increased uric acid quartiles (P<0.005), and P value for the linear regression was 0.015. There was no correlation between uric acid and oxidized-LDL. The endothelial cells of participants with higher uric acid levels expressed 5% less MnSOD than the participants with the lower uric acid levels (intensity/HUVEC was 0.22±0.13 vs 0.49±0.12, P= 0.04). NfκB p56, NADPH oxidase p47(phox) (n=13), nitrotyrosine (n=21), and MnSOD (n=12). To minimize the confounding effect of different staining sessions, values are reported as ratios of endothelial cells with low MnSOD expression compared to the subjects' brachial arteries. The following markers were evaluated: NFκB p56 (n=19), NADPH oxidase p47(phox) (n=13), nitrotyrosine (n=21), and MnSOD (n=12). To minimize the confounding effect of different staining sessions, values are reported as ratios of endothelial cells with low MnSOD expression compared to the subjects' brachial arteries.

Conclusions: In healthy adults, serum uric acid levels correlate with increased CRP and associate with reduced MnSOD expression in endothelial cells. These findings may have implications on cardiovascular risk for healthy adults.

Combining Uric Acid with Lipoprotein a Could Predict the Atherosclerotic Renal Artery Stenosis in High Risk Patients Peng Xiua, Ling Qiub, Limeng Chen, Shuyang Zhang, Xuemli Li, Xuewng Li 1 Nephrology Department, Peking Union Medical College Hospital, Beijing, China; 2Department of Laboratory Medicine, Peking Union Medical College Hospital, Beijing, China; 3Cardiology Department, Peking Union Medical College Hospital, Beijing, China.

Background: This study aimed at exploring certain new risk factors of Atherosclerotic renal artery stenosis (ARAS) and establishing a possible tool which might facilitate the clinical decision making in diagnosing ARAS.

Methods: 190 patients highly suspected for ARAS who have received renal artery angiography in Peking Union Medical College Hospital from 2008 to 2011 are selected for analysis. 138 of all 190 patients also received coronary artery angiography and 89 of who
were diagnosed ARAS. The control group is 180 cases who received routine health check. The uric acid and lab results such as uric acid (UA), serum lipids (lipoprotein a total cholesterol, triacylglycerol, HDL and LDL), creatinine (Cr) and hsCRP are collected. Logistic regression analysis is used to identify possible correlations with ARAS and to establish a new tool for predicting ARAS in the high risk population.

**Results:**

The levels of Scr, UA, Lp(a) and hsCRP of ARAS cases are significantly elevated compared to control cases. For high risk population and the patients received coronary artery angiography, there are no significant differences in the levels of Scr, lipids, UA and hsCRP between ARAS cases and non-ARAS cases. Logistic regression analysis showed that uric acid level >344 µM correlated with ARAS independently. Using the uric acid level >344 µM and lipoprotein a level >242mg/L as a predicting tool for ARAS in high risk population, the specificity is 96.0%, the positive likelihood ratio is 5.45, p<0.001 and the odds ratio is 6.78, 95%CI (1.90, 24.2), p=0.001.

**Conclusions:**

In high risk population, the UA might be an independent risk factor for development of ARAS. The ARAS cases are experiencing lipidic metabolic disorders and inflammatory reactions. In high risk population, the UA might be an independent risk factor for developing ARAS and combining UA with Lp(a) could predict the ARAS. **Funding:** Government Support - Non-U.S.

**TH-PO768**

**Uric Acid Is Not Associated with Blood Pressure in Adolescents with Type 1 Diabetes**

**Jeffrey C. Sirota, 1 Diana J. Jalal, 1 Kim McFann, 1 Franziska K. Bishop, 2 David M. Maahs, 2 R. Paul Wadwa. 2**

**Division of Renal Diseases & Hypertension, University of Colorado Denver School of Medicine, Aurora, CO; 2Barbara Davis Center for Childhood Diabetes, University of Colorado Denver School of Medicine, Aurora, CO.**

**Background:** Elevated uric acid is associated with increased blood pressure (BP), and treatment of hyperuricemia can improve BP in non-diabetic (non-DM) adolescents. In this study, we hypothesized that uric acid is associated with increased BP in adolescents with type 1 diabetes (TID).

**Methods:** Data were collected for 256 youths with T1D (age=15.4±2.2yrs, 50% male, TID duration 8.7±2.9yrs) and 78 non-DM control adolescents (age=5.8±2.2yrs, 44% male). Cross-sectional association between uric acid and BP was examined by linear regression in unadjusted and adjusted models. Uric acid was log-transformed due to skewed distribution.

**Results:** T1D youth had higher hemoglobin A1c, waist circumference (WC), BMI-Z, total cholesterol, low density lipoprotein (LDL), & BP (Table 1). Mean uric acid was 4.6±0.8mg/dL in T1D subjects vs. 3.7±0.8mg/dL in controls (p=0.001). In unadjusted analysis, uric acid was significantly correlated with SBP in the entire cohort (R2=0.037, p<0.001), and in T1D (R2=0.04, p<0.05). After adjusting for age, sex, race, A1c, WC, BMI-Z, total cholesterol, low density lipoprotein (LDL), HDL, triglycerides (TG), creatinine, urine albumin-creatinine ratio, & smoking status, uric acid was not associated with SBP. There was no correlation between uric acid & DBP.

**Table 1**

<table>
<thead>
<tr>
<th>Variable</th>
<th>T1D (n=256)</th>
<th>Control (n=78)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uric acid (mg/dL)</td>
<td>4.6±0.8</td>
<td>3.7±0.8</td>
<td>0.001</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>86.3±50.3</td>
<td>86.3±50.3</td>
<td>0.80</td>
</tr>
<tr>
<td>BMI-Z</td>
<td>0.23±1.06</td>
<td>0.02±0.79</td>
<td>0.01</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>172.4±27.7</td>
<td>158.0±34.7</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>81.9±22.4</td>
<td>89.5±27.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.6±9.2</td>
<td>51.2±10.4</td>
<td>0.07</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>92.9±40.8</td>
<td>86.3±30.3</td>
<td>0.30</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>5.1±1.0</td>
<td>6.0±0.8</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>8.7±0.2</td>
<td>7.0±0.1</td>
<td>0.01</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>118.8±8.4</td>
<td>113.2±8.4</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>76.4±6.0</td>
<td>76.8±6.7</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

**Conclusions:** Uric acid is weakly associated with SBP in adolescents with and without T1D. However, after adjustment for co-variates, there was no significant association between uric acid and SBP. Further work is needed to clarify the effects of uric acid in young people with T1D. **Funding:** NIDDK Support, Private Foundation Support

**TH-PO769**

**Association Between Birth Weight and Blood Pressure in 9- to 10-Year Old Children**

**Sandra Dis Steinthorisdottir, 1 Sigridur Birna Eisladottir, 2 Olafur S. Indridason, 3 Runolfur Palsson, 2 Vidar O. Edvardsson. 1**

**1Landspitali - The National University Hospital, Iceland; University of Iceland.**

**Background:** Low birth weight has been associated with structural and functional changes in the vasculature, which may increase the risk of hypertension, cardiovascular disease, obesity and type 2 diabetes later in life. The aim of this study was to evaluate the association between birth weight and blood pressure (BP) in a cohort of healthy 9 to 10-year-old Icelandic children.

**Methods:** Four seated BP measurements were performed in 1071 Icelandic children, aged 9-10 years. BP percentiles were calculated from an average of the four measurements. Height and weight were measured and information on birth weight was obtained from the Icelandic Birth Registry. Pearson’s correlation coefficient and multivariable linear regression was used for the analysis.

**Results:** Of 889 children with complete data, 452 were girls (50.8%). The mean BP was 116.3±11 mm Hg and 112.6±14 mg Hg in boys (p=0.03). The prevalence of elevated BP (≥95th percentile) was 11.2% for boys and 12.6% for girls (p=0.02). There was a significant correlation between birth weight and systolic BP percentile (beta= -0.16, p<0.001) and diastolic BP percentile (beta= -0.14, p=0.003) in girls, and systolic BP percentile (beta= -0.11, p=0.016) in boys, for whom the association with diastolic BP percentile was of borderline significance (beta= -0.08, p=0.09). There was a direct correlation between birth weight and height (r=0.26, p=0.001), weight (r=0.21, p<0.001), BMI (r=0.14, p=0.001) and BMI percentile (r=0.19, p<0.001).

**Conclusions:** The results of our study suggest that low birth weight may be an important predictor of hypertension in children. Careful follow-up of BP may be indicated in these children as they may be at increased risk for future cardiovascular complications.

**TH-PO771**

**Long-Term Follow-Up of Children with Essential Hypertension – Are We Minimizing End-Organ Injury?**

**Isabel Roberti, Myriam Jean, Shefali Vyas. Pediatric Nephrology and Kidney Transplantation, Saint Barnabas Medical Center, Livingston, NJ.**

**Background:** We have previously shown that essential hypertension (EH) is now the most common cause of HTN in children and it is associated at presentation with obesity in 50% and end-organ injury in 27% of the cases. The goal of therapy of children with EH is early lifestyle modification and avoidance of end-organ damage.

**Methods:** We reviewed all charts that had the diagnosis of “Essential Hypertension” in children seen at our outpatient office between 2004 and 2010 who had more than 1 year of follow-up. Secondary causes of HTN were excluded in all patients.

**Results:** Off 232 children with EH only 21% (N=49) were followed for at least 1yr (mean ± 3.3 yrs). At presentation: Age: 4-19yrs (mean 13yrs); Ethnicity: 27 AA, 14C, 6 H; 60% male; 76% had ≥ FH for EH; 16% ex-pregnature/LBW, 49% were symptomatic; 57% had hyperlipidemia.

**HTN stages: pre-HTN=9, stage I=20, stage II=20 (41%).**

**Conclusions:** The frequency of white-coat HTN was also markedly higher than that cited from prior studies, reinforcing the value of ABPM as an initial screening tool for suspected HTN in children.
Hypertension: Clinical
Poster/Thursday

Long-term follow-up of Children with Essential Hypertension

Six of 49 (12%) required more than 1 anti-hypertensive medication. MA was only seen in stage II HTN. Among those with stage I or II HTN (47.9%) were symptomatic. HTN grade significantly declined on fig: only 3 children had stage II HTN.

Conclusions: The majority of children with EH had significant improvement in the HTN stage and LVH despite worsening BMI. The ability to keep a scheduled appointment and the adherence to lifestyle/dietary changes among our children with EH was diametrically poor. More resources are needed to educate these children and families with EH.

TH-PO772

Office Blood Pressure Monitoring (OBPM): A Possible New Tool for Evaluating Blood Pressure in Children and Adolescents

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Background: Recent data suggest that casual office BP measurement (CBP) in children may not be as sensitive as ABPM for detecting hypertensive patients. However ABPM is not feasible in toddlers and it can be difficult and/or misleading in hyperactive children or incompliant adolescents.

Methods: We have developed a method of BP measurement, OBPM: Office BP Measurement, alternative to ABPM for investigating BP in children. The present study compares the results obtained with OBPM and ABPM in 59 children (25f, mean age 11.8 ± 3.5 yrs, referred for suspected or confirmed hypertension to 3 centers).

Results: OBPM utilizes the same recorder and cuff as ABPM (Spacelab 90207) to perform 10 BP measurements in 1 hour just before the ABPM. The readings are introduced in a specific software (developed with FileMaker) that calculates the coefficient of variation (CV) of SBP and DBP, after excluding outlier values (measurements below the 5th and above the 95th of the recorded values). The CV provides an index of reliability of the calculated mean; we suggest to discard OBPMs with a CV >15. The system finally calculates the mean of the remaining values and the z-score for age, gender and height according to the American Academy Reference Values. The correlation between OBPM and ABPM was analyzed as Person’s correlation coefficient. The table shows the findings obtained; OBPM and ABPM were performed on the same day.

Conclusions: Preliminary analysis indicates that OBPM may represent a reliable and promising tool for investigating BP in children as an alternative to ABPM. The software for OBPM is available online at the following website: www.childproject.org

TH-PO773

Assessment of Cardiovascular and Renal Risk Factors in Pediatric Population during World Kidney Day in the Province of Chaco, Argentina

Maria Eugenia V. Bianchi,1 Dario Gomez,2 Ricardo K. Tanmuri,3 Noelia Alejandra Dellalma,2 Cecilia Abogado,2 Ana Cusumano.1 1Argentina Northeast Kidney Foundation, Resistencia, Chaco, Argentina; 2National Northeast University, Corrientes, Argentina; 3Ministry of Health, Resistencia, Chaco, Argentina; 4CEMIC, Buenos Aires, Argentina.

Background: Chaco province, Argentina, has one of the highest rates in the nation of teenage pregnancy, low birth weight and infant mortality, with an ethnically diverse population of aboriginal and non-aboriginal. Since 2008 and during the WKD celebration, anthropometric measurements and blood pressure (BP) were checked in children. Non aboriginal children were evaluated in the squares of the city and aboriginal ones in a domestic setting. Those with abnormal parameters were referred to medical assistance.

Methods: 18 students of the School of Medicine of the National Northeast University, were trained. Cuffs of different sizes were used with an aneroid sphygmomanometer to measure BP. Data was analyzed following the WHO (2006) classification.

Results: 317 children were evaluated. 136 (42.9%) were male, mean age 9.4±y, mode 13, ranging from 1 to 18 years. 127 (47.7%) showed normal BP: 57 (21.4%) High Normal, 57 (21.4%) HT Stage 1, and 25 (9.4%) HT Stage II. The nutritional status of 266 (84%) showed: undernutrition in 13 (4.9%), 26 (9.8%) at risk of undernutrition, 123 (46.2%) normal, 59 (22.2%) at risk of overweight and 45 (16.9%) with overweight.

Statistically significant differences between non-Aboriginal and aboriginal children were found: Height: 37.3% of non-Aboriginal were above the 90th percentile, compared with 17.8% of Aboriginal ones. (<0.005); normal weight was found in 29% versus 60.3%; overweight was detected mainly in the non-Aboriginal (27.4% versus 6.9%).

Conclusions: Cardiovascular and renal risk factors in pediatric population showed a high prevalence and a different pattern in the ethnic frame.

TH-PO774

The Beneficial Effect of Native Nephrectomy on Post-Transplant Hypertension


Background: Post-transplant hypertension occurs in >60% of renal transplant recipients, and is associated with cardiovascular complications and decreased allograft survival. While multiple factors may play a role (e.g. immunosuppressive medications, weight gain) the presence of atrophic native kidneys may be a significant contributing factor.

Methods: We performed a retrospective analysis of 16 renal transplant patients (ages 27-72) who underwent a second unilateral native nephrectomy (UNN2) (9 open/7 laparoscopic). All patients had undergone a prior unilateral native nephrectomy. Five patients underwent UNN2 for severe hypertension; eleven patients required UNN2 for renal transplanted polycystic kidney disease or urinary tract infections. We evaluated blood pressure, weight, creatinine, hematocrit, complications, number/dosages of antihypertensive medications at baseline, post-operative (2-6 weeks), and one year. Changes from baseline were evaluated with paired t-tests.

Results:

<table>
<thead>
<tr>
<th>n=16</th>
<th>Baseline-Mean(SEM)</th>
<th>Post-Op-Mean(SEM)</th>
<th>One Year-Mean (SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP(mmHg)</td>
<td>140.4(4.25)</td>
<td>123.6(4.26)*</td>
<td>128.3(4.15)*</td>
</tr>
<tr>
<td>DBP(mmHg)</td>
<td>83.1(3.15)</td>
<td>78.2(3.51)</td>
<td>76.7(3.48)*</td>
</tr>
<tr>
<td>MAP(mmHg)</td>
<td>90.2(3.24)</td>
<td>93.1(3.47)</td>
<td>93.8(3.73)*</td>
</tr>
<tr>
<td>Weight(kg)</td>
<td>83.5(5.00)</td>
<td>82.0(5.11)</td>
<td>84.6(5.07)</td>
</tr>
<tr>
<td>Cr(ul/dl)</td>
<td>3.1(0.10)</td>
<td>3.3(0.10)</td>
<td>3.3(0.10)</td>
</tr>
<tr>
<td>Hct</td>
<td>38.7(1.73)</td>
<td>37.9(1.41)</td>
<td>38.7(1.43)</td>
</tr>
<tr>
<td>Meds</td>
<td>2.30(0.37)</td>
<td>2.50(0.35)</td>
<td>2.50(0.35)*</td>
</tr>
</tbody>
</table>

*p<0.05

In addition to significant reductions in numbers of medications, 12/16 patients had a >50% dosage reduction of at least one medication. Surgery was well tolerated, with mean length of stay: 5.9±1.63 days after open UNN2 and 2.7±0.29 days after laparoscopic UNN2. One complication occurred (wound infection) in a patient who had undergone open nephrectomy.

Conclusions: Current management of post-transplant hypertension focuses on use of antihypertensive medications. We have previously demonstrated sustained decreases in blood pressure and antihypertensive medications in post-transplant patients who had undergone simultaneous bilateral native nephrectomy. Coupled with similar beneficial results noted in this study, we conclude that surgical removal of native kidneys represents a safe and effective adjunct to medical therapy in the management of post-transplant hypertension.

TH-PO775

Blood Pressure Reduction According to Baseline Systolic BP Value and Diabetes Status in Hypertensive Outpatients: Interim Results of the Canadian ANCHOR Registry

Paul Rene De Cotret,1 Andrew W. Steele, 2 Kazumasa Murakami,3 Norma K. Wong,4 Carrie Diamond, Kathleen M. Waybill, Halie Cook, Harold Yang, Transplantation, PinnacleHealth, Harrisburg, PA.

Background: ANCHOR (Amlitin or Candesartan in Hypertension) is a real-life Canadian non-interventional registry of outpatients in which physicians initiated aliskiren, the direct renin inhibitor as monotherapy or add-on, in order to achieve blood pressure control.

Methods: Patients were followed for one year after aliskiren initiation, with 2 visits at 3 months and 12 months. Efficacy and safety were assessed at each visit. An interim analysis was performed at mid-term. The main objective was to assess BP reductions after 3 months of use. One of the secondary objectives, presented in this abstract, was to measure BP changes from baseline to 12 months.

Results: At time of database lock (Aug 2010) for this interim analysis, 9716 patients were enrolled in the study; mean baseline characteristics (± SD) were: 52.1% males, age was 62.6 (12.9), 36.7% had diabetes, BMI was 31.5 (14.8), 76.4% were Caucasians, SBP (mmHg ± SD): 153.6 (15.9) and DBP: 87.6 (11.5). Baseline SBP in 6313 patients without diabetes was 154.7 (15.8) and in 3542 patients with diabetes 151.7 (16.0). At 12 months: <5% (852/16854) SD) systolic BP drop was 18.3 (16.8) in 576 patients with diabetes and -20.5 (18.4) in 830 patients without diabetes.

Table 1 below shows the mean SBP drop (in mmHg) at 12 months with an aliskiren-based regimen, with and without diabetes.
Results: We analyzed data from 389 patients out of 1,600. Mean age was 57 year and 59.4% were males. Diabetic nephropathy was 21.1%. The distribution of each BP pattern was as below: sustained uncontrolled, 80.0%; masked, 6.8%; white coat, 10.4%; true controlled, 2.4%; Dipper, 43.6%; non-dipper, 44.1%; reverse dipper, 12.3%. Male gender, current smoking, and spot urine PCR higher than 25 percentile (p) (100ng/ml) were related with masked and sustained uncontrolled hypertension (p = 0.059, p = 0.05, p = 0.075, respectively). In multivariate analysis, female gender (OR = 0.467, p = 0.043) and spot urine PCR less than 25p (OR = 0.349, p = 0.007) were related with white coat hypertension, whereas male gender (OR = 2.103, p = 0.009) and spot urine PCR more than 25p (OR = 1.908, p = 0.032) with sustained uncontrolled hypertension. Diabetic nephropathy, higher serum creatinine, and lower estimated GFR were related with reverse dipper (p = 0.05, p < 0.002, and p = 0.001, respectively). In multivariate analysis, spot urine PCR less than 50p (304mg/ml) was related with dipper (OR = 0.619, p = 0.037), whereas diabetic nephropathy with reverse dipper (OR = 2.457, p = 0.013).

Conclusions: Apparently high proportion of CKD patients were treated inappropriately in Korea. And masked and sustained uncontrolled hypertension and reverse dipper were related with more profound target organ damages. Therefore, appropriate BP control in CKD patients is very important and ambulatory BP monitoring has a significant role to improve physicians’ practices. We can understand the real practice in BP control for Korean CKD patients after additional data analysis.

TH-PO777

Clustering of Cardiovascular and Renal Risk Parameters in Non-Hypertensive Individuals George Thomas, Richard A. Fatica, Saul Nuruo, Marc A. Pohl, Sankar D. Navaneethan, Titte Srinivas, Martin J. Schreiber, Emilio D. Poggio. Nephrology & Hypertension, Cleveland Clinic, Cleveland, OH.

Background: Clinical trial evidence suggests an increase in cardiovascular (CV) events begins with a systolic blood pressure (SBP) of 115 mmHg and lower levels of GFR. We aimed to study early changes in CV and renal risk profile and their relationship with SBP in living kidney donors with no clinical diagnosis of cardiovascular or chronic kidney disease.

Methods: We studied 394 consecutive living kidney donors between Apr 1997 and Dec 2005 at our institution. Exclusion criteria were BP ≥140/90 mmHg (clinical hypertension), kidney disease, diabetes, CV disease, obesity, and proteinuria. Data collected included age, gender, race, body mass index (BMI), office blood pressure, fasting blood glucose (FBG), uric acid, lipid profile, and microalbuminuria filtration rate (eGFR). The study population was stratified into SBP 115–139 mmHg and SBP 115–139 mmHg.

The relationships between risk factors and SBP were studied using regression analysis.

Results: 81% of the study population was Caucasian, with 59% women. Mean age was 41.0 ± 9.9 yrs. Compared to donors with SBP <115 mmHg, those with SBP 115-139 mmHg had significantly abnormal risk factor profile. Interestingly, eGFR was higher in donors with SBP 115–139 mmHg. All p values for BP drop were <0.0001 except for *: p = 0.004.

Conclusions: This international study corroborates previous findings in cohorts from Europe and Argentina. Noteworthy, HD patients from South-East Asia show distinctly different SBP dynamics before death. The reasons for this finding are currently unknown.
that with higher SBP. In regression analysis, BMI, FBG, and uric acid, even within the normal range, were independently associated with higher SBP. Risk factors stratified by SBP: <115 mm Hg (n=222) and SBP ≥ 115-119 mmHg (n=172).

Methods: A cross-sectional study in a single PD center. All patients underwent office BP measurement (OBPM), BpTRU BP measurement (BpTRU-BPM), HBPM and ABPM over a 2-week period. Agreement between ABPM and the 3 comparator methods was determined.

Results: 17 patients (54.2±12.0 years, 70.6% male, 94.1% automated PD) were studied. Mean office SBP (126.4±16.9mmHg) and BpTRU SBP (123.8±13.7mmHg) closely approximated mean daytime ambulatory SBP (129.3±14.8mmHg), p=NS. Mean home SBP (143.8±15.0mmHg) over-estimated mean daytime ambulatory SBP by 14.2mmHg (p=0.008). BpTRU SBP correlated well with daytime ambulatory SBP (r=0.49, p=0.053). Home SBP correlated poorly with daytime ambulatory SBP (r=0.24, p=0.37). Bland Altman plots of office SBP, BpTRU SBP, and home SBP, respectively, against daytime ambulatory SBP demonstrated poorest agreement between HBPM and ABPM. False-resistant hypertension was rare (n=1). Nocturnal non-dipping was prevalent (non-dipping, n=1; reverse-dipping, n=5; normal dipping, n=1).

Conclusions: BpTRU-BPM was more accurate than HBPM, with reference to ABPM, in this PD population. Standard OBPM was also superior to HBPM. We suggest two potential explanations for these findings. Firstly, PD patients are very familiar with medical procedures and may consequently experience minimal white-coat effect. Secondly, patients may not adhere to strict BP measurement technique when monitoring BP at home on a long-term basis. The high prevalence of nocturnal non-dipping observed in this PD population may relate to the predominance of automated PD over ambulatory PD. Larger studies are required to validate these findings. Whether BpTRU-BPM can lead to improved patient outcomes remains to be determined.

TH-PO782
Hourly Ambulatory Blood Pressure Patterns in Patients with Intradialytic Hypertension Peter N. Van Buren, Bolhyn Catherine Kim, Anand Srivastava, Robert D. Toto, Jula K. Inrig, Nephrology, UT Southwestern Medical Center, Dallas, TX.

Background: Hemodialysis (HD) patients with intradialytic hypertension (HTN), an increase in blood pressure (BP) from pre to post-HD, have higher average interdialytic BP compared to HD controls. This study’s purpose is to compare hourly interdialytic BP slopes in intradialytic HTN patients and HD controls.

Methods: In this case-control study, inclusion criteria were prevalent adult HD patients with HTN (systolic BP [SBP] ≥140 mmHg pre-HD or ≥130 mmHg post-HD) during 6 screening HD treatments. Case subjects had SBP increases ≥10 mmHg from pre to post-HD in ≥4/6 treatments, and controls had decreases ≥10 mmHg in ≥4/6 treatments. A repeated measures mixed linear model analyzed interdialytic ambulatory BP slopes.

Results: Of 50 subjects (25 in each group), average age was 54.5 years, 80% were male, 38% African American, 62% Hispanic, and 86% diabetic. The ambulatory BPAs are shown below.

Blood Pressure Comparisons

<table>
<thead>
<tr>
<th>SBP (mmHg)</th>
<th>Intradialytic Hypertension</th>
<th>Control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>44-Hour Average</td>
<td>155 (14)</td>
<td>142 (17)</td>
<td>0.005</td>
</tr>
<tr>
<td>Hours 1-23</td>
<td>155 (22)</td>
<td>138 (21)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Hours &gt; 23</td>
<td>156 (23)</td>
<td>155 (22)</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Methods: An ambulatory systolic blood pressure

For the initial 23 hours post-HD there was an interaction between group and time (p=0.002). The slope for change in SBP per hour was -0.5 (p=0.002) for controls, but it was a non-significant decrease in the intradialytic HTN group (~0.3). See Figure 1: Mixed Linear Model Analysis of Ambulatory Blood Pressure Slope in Intradialytic Hypertension Patients and Controls During the First 23 Hours Post-Hemodialysis and During the Remaining Interdialytic Time Period.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only. Underline represents presenting author.
Background: Inadequate volume removal (VR) during dialysis can lead to elevated blood pressure (BP) and exacerbate volume load. Blood volume monitoring (BVM) can be used to assess rate of volume removal and intravascular volume refill during hemodialysis (HD). These characteristics were examined in 71 chronic stable HD patients to determine differences in those with intradialytic normotension, hypertension (HTN), or hypotension.

Methods: Patients were grouped into high (HBP), normal (NBP), low (LBP) BP by changes (≥10mmHg) in systolic (sys) or diastolic (dia) pressures. An increase of ≥10mmHg systolic (sys) or diastolic (dia) was grouped as HBP. Sys BP changes within 10mmhg were considered NBP. A decrease of ≥10mmhg for sys BP during a HD session was grouped as LBP. ANOVA used for continuous variables. BVM was analyzed for vascular refill and drop in BPs slope ≥5%. AASI was considered to be normal when in demographically matched peers, DM, HTN, and CV disease.

Conclusion: The HBP group had similar sex, HTN, DM, CV disease, but LBP had fewer DMS. As a whole, vascular refill rates were related to HBP and LBP but not to NBP. Although there was a statistically significant correlation between SBP and heart rate, no relationship was found with diastolic pressure. In order to assess the diagnostic accuracy of BpTRU device in a tertiary care referral clinic, we conducted a retrospective analysis of adults with either diagnosed or suspected hypertension (HTN). We compared corresponding BP readings a) average of 3 resting readings taken by trained HTN clinic nurse using mercury sphygmomanometry and b) ambulatory BP monitoring (ABPM) by BpTRU and c) automated BP readings from 24-hr ABPM. The clinic readings were obtained on the morning of ABPM recordings. The average Systolic SBP (SBP) for each method was compared using Pearson’s correlation and performing a Bland-Altman analysis. Amongst patients with WH (RN SBP >140 mmHg but ABPM ≤135), the proportion of patients who were labelled as hypertensive by BpTRU (SBP >140) were also calculated.

Results: Charts of 2000 consecutive patients from 2004-2010 were reviewed. 329 patients (mean age of 62 years with 49% males) fulfilled above criteria and were analyzed. Each patient data qualified for one entry only. The mean SBP (mmHg) for RN, BpTRU and ABPM was 143.87 ± 19.90, 136.27 ± 21.85, and 139.35 ± 14.24 respectively.

Conclusions: Although there is a statistically significant correlation between SBP obtained by BpTRU and ABPM, the clinical utility of this is limited by the poor ability of BpTRU to correctly diagnose WH. Caution is to be used when relying solely on BpTRU readings to eliminate the diagnosis of WH.

TH-PO785
Ambulatory Blood Pressure Monitoring (ABPM) in the CKD-Japan Cohort (CKD-JAC) Study

Methods: We conducted a secondary data analysis using our preexisting CKD registry at Cleveland Clinic. Central hemodynamic assessment data and 24 hr ABPM pressure were obtained from our hypertension database. AASI was calculated as 1 minus the slope of diastolic on systolic BP in individual 24hr ABPM measure.

Results: We identified 62 patients (stage 3 and 4 CKD) from our CKD registry. The mean age of the study cohort was 72 ± 11.9 years with 54% females and 13% African Americans. The impact of central hemodynamic BP measures and 24 hr ABPM on change in GFR is delineated in the table.

Table 1: Comparison of volume removal during intradialytic BP changes

<table>
<thead>
<tr>
<th>Group</th>
<th>HBP (n=25)</th>
<th>LBP (n=32)</th>
<th>NBP (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (avg. SD)</td>
<td>65±9</td>
<td>55±6</td>
<td>66±13</td>
</tr>
<tr>
<td>VR (mmHg)</td>
<td>286±103</td>
<td>258±94</td>
<td>273±137</td>
</tr>
<tr>
<td>Highest UFR (ml) (avg. SD)</td>
<td>892±365</td>
<td>1082±237</td>
<td>757±400</td>
</tr>
<tr>
<td>Refill (%)</td>
<td>84</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td>slope ≤5% (%)</td>
<td>62</td>
<td>72</td>
<td>57</td>
</tr>
</tbody>
</table>

Conclusions: These findings persisted after adjustment for age, sex, active smoking, body mass index, race (White-African American), DM, dipping status, hypertension, congestive heart failure, coronary artery disease, metabolic acidosis and alkalosis, hypertrophy cardiomydema, and HbA1c. A unique composite score consisting of AP, PP and AASI index was developed and demonstrated a mean decline in GFR of 8.1% at 3 years after adjustment for all variables.

Methods: Utilizing a hemodynamic composite score (using AP which estimates the effect of reflected wave form of systolic workload, AASI assessing arterial stiffness, and PP reflecting pulse volume and elasticity of vascular wall) may be more comprehensive in determining risk for CKD progression over time as compared to current applied BP targets and warrants further study.
Results: The data of 1,077 subjects was ultimately analyzed. Based on the 24-hour mean BP and office BP, CKD stage 1-3 was common. Both of disturbed circadian rhythms of the RUNa and proteinuria were correlated with the elevation of the night BP and both of them were the risk factors of abnormal circadian BP rhythm. The increase of night RUNa was associated with the decreased ability of tubular sodium reabsorption.

**Conclusions:** We concluded the disturbed diurnal BP rhythm in the youth and middle age patients with CKD stage 1-3 is common. Both of disturbed circadian rhythms of the RUNa and proteinuria were correlated with the elevation of the night BP and both of them were the risk factors of abnormal circadian BP rhythm. The increase of night RUNa was associated with the decreased ability of tubular sodium reabsorption.

**TH-PO790**

**Low Plasma Renin Activity Levels Are Associated with Lower Mortality in Chronic Kidney Disease Patients**

**Patients and Methods:** 1,123 hemodialysis patients were enrolled, 76.6% patients presented 56 years and older age, 43% were women, 41% were diabetics, 91% were hypertensive, and mean followup was 3.9 yrs for the cohort. Compared to the highest PRA quartile, adjusted hazard ratio (HR) for mortality was 1.16 (0.64-2.10 95%CI), 0.94 (0.51-1.76), and 0.55 (0.28-1.11) in the third, second, and lowest quartiles respectively. Linearity was observed where HR for mortality was 1.02 for every 1mg/ml/hr increase in PRA. When lowest PRA quartile (PRA<0.7 mg/ml/hr) was compared against the rest of cohort, adjusted HR at 2yrs was 0.59 (0.36-0.96).

**Adjusted HR for mortality**

<table>
<thead>
<tr>
<th>PRA quartiles</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRA &lt; 0.25 mg/ml/hr (Q1)</td>
<td>1.16 (0.64-2.10)</td>
</tr>
<tr>
<td>0.25 mg/ml/hr ≤ PRA &lt; 0.4 mg/ml/hr (Q2)</td>
<td>0.94 (0.51-1.76)</td>
</tr>
<tr>
<td>0.4 mg/ml/hr ≤ PRA &lt; 0.6 mg/ml/hr (Q3)</td>
<td>0.55 (0.28-1.11)</td>
</tr>
<tr>
<td>PRA ≥ 0.6 mg/ml/hr (Q4)</td>
<td>0.57 (0.34-0.98)</td>
</tr>
</tbody>
</table>

**Conclusions:** Therapy targeting the renin angiotensin aldosterone system is a cornerstone of CKD management. Our current studies suggest that suppressed renin states may confer lower risk for mortality in CKD.

**Funding:** Pharmaceutical Company Support

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**TH-PO788**

**Comparison of Heart Rate Variability between Patients on Kidney Transplantation, Hemodialysis, Peritoneal Dialysis Due to End Stage Renal Disease and Hypertensives in Korea**

**Methods:** Patients were compared with the normal group for age, sex, BMI, and white-coat HTN. The percentage of patients with non-dipper or riser was increased according to progression of CKD stages. The comorbidity of nocturnal hypertension is remarkable in patients with diabetic nephropathy.

**Funding:** Pharmaceutical Company Support

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**TH-PO789**

**The Disturbed Circadian BP Rhythm in Chronic Kidney Disease Stage 1-3 Patients**

**Background:** The disturbed circadian rhythm of blood pressure (BP) was independent risk factors for CKD progression and CVD events. The mechanism of the changing of BP rhythm in CKD is not clear. The present research is to explore the circadian BP rhythm of the youth and middle age patients in CKD stage 1-3 and involved factors.

**Methods:** The patients underwent 24-hour ambulatory BP monitoring and collected the urine of daytime and nighttime synchronously. The urine was analyzed for Na, K, Cl, Ca, and albumin concentration.

**Results:** (1)292 patients in CKD stage 1-3 were enrolled,76.6% patients presented as Nondipper BP pattern. The night SBP,DBP and MAP in Nondipper group were higher than those in Dipper group. (2)Both the 24hUP [(1.67±1.73)g/d vs.3.64±3.00 g/d, P=0.014] and the night RUNa [(4.91±3.14)mmol/h vs.6.84±3.81 mmol/h, P=0.011] in Nondipper group were significantly higher than those in Dipper group. (3)Within the Nondipper group, the circadian rhythm of RUNa was reversed. Night RUNa was higher than day RUNa [(6.84±3.81) mmol/h vs. (6.26±3.55) mmol/h]. (4)Night SBP, DBP and MAP increased with the increase of night RUNa and 24hUP from the first to the third tertile significantly(P<0.050), even after adjusted by age, drugs and eGFR. (5)In the linear regression equations, with the night/day ratio value of RUNa increasing, the risk of nocturnal HTN increased. The percentage of patients with non-dipper or riser was increased according to progression of CKD stages. The comorbidity of nocturnal hypertension is remarkable in patients with diabetic nephropathy.

**Funding:** Pharmaceutical Company Support

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**TH-PO791**

**Antialbuminuric Response to Direct Renin Inhibition in Combination with HCTZ or Amlodipine in Patients with Hypertension**

**Methods:** Two separate pooled posthoc analyses were conducted in patients with hypertension (age≥54 yrs; 51-56% males; BP≥167/96 mmHg; BMI≥34-35 kg/m2; 12-19% diabetes; 90-95% eGFR≥60 ml/m; 24-26% microalbuminuria) who were either treated with placebo (n=300) or amlodipine (10 mg, n=216) for combination with HCTZ ([1.29 mg/mmol; 95% CI 1.24-1.35] (pool 1) or amlodipine (10 mg; n=97) (pool 2) for ≥8-12 weeks of therapy.

**Results:** The response to inhibition in combination with HCTZ or amlodipine demonstrated reductions in UACR in hypertensive patients without kidney disease.

| Hypertension, KPSC Los Angeles, Los Angeles, CA; 2Research and Evaluation, KPSC, Pasadena, CA; 3Novartis Pharmaceuticals, Hanover, NJ; 4Nephrology, Harbor UCLA, Torrance, CA. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| **Week 8/12**   | 0.81 mg/mmol   | 1.25 mg/mmol   | 1.11 mg/mmol   | 1.25 mg/mmol   | 1.11 mg/mmol   | 1.25 mg/mmol   |
| **Week 24/48**  | 0.81 mg/mmol   | 1.25 mg/mmol   | 1.11 mg/mmol   | 1.25 mg/mmol   | 1.11 mg/mmol   | 1.25 mg/mmol   |
| **Baseline**    | 1.29 mg/mmol   | 1.29 mg/mmol   | 1.29 mg/mmol   | 1.29 mg/mmol   | 1.29 mg/mmol   | 1.29 mg/mmol   |

**Conclusions:** Suppression of plasma renin activity (PRA) in poorly controlled hypertension (HTN) has been described to have worse clinical outcomes, no studies to date have evaluated the prognostic implications of PRA in chronic kidney disease (CKD) patients. We sought to determine whether low or suppressed PRA conferred a protective effect on mortality in patients with CKD.

**Funding:** Pharmaceutical Company Support

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**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

Underline represents presenting author.
ACE Inhibitors (ACEi) Are Not More Effective Than Angiotensin Receptor Blockers (ARB) in Suppressing Plasma Aldosterone (pAldo) Levels in Hypertensive Patients (pts) Nabil J. Haddad, Udayan Y. Bhatt, Brad H. Rovin, Lee A. Hebert. *Internal Medicine/Nephrology, Ohio State University Medical Center, Columbus, OH*

**Background:** Previous work suggests that ACEi are more effective than ARB in suppressing pAldo. However, those studies involved relatively small numbers of pts and/or relatively low doses of ACEi or ARB.

**Methods:** This work involved relatively large numbers of pts who received chronic ACEi or ARB therapy and in usual dose. Although not a randomized comparison, the groups were well matched and we took into account ACEi/ARB dose intensity, comorbidities, diastolic status, and 24-h intake of sodium and potassium assessed by 24h-urine collection, CKD-EPI eGFR, systolic blood pressure, and ACE genotype. The latter is important because the deletion (D) allele encodes for high ACE level, which might induce resistance to ACE therapy.

**Results:** We studied 110 ACEI and 53 ARB treated pts receiving therapy for at least 3 months: Age (54.6±1.4 vs 57.7±2.1), percent male (55.6 vs 50.9), Caucasian (80.6 vs 77.4), African American (17.6 vs 17.0), hypertensive (83.3 vs 81.1), with diabetes mellitus, (35.2 vs 32.0), on diuretics (48.2 vs 64.2), eGFR was (53.8 ± 3 vs 51.8 ± 4). None of the differences between the ACEI and ARB groups was significant, which was expected since there was a trend for greater diuretic use in the ARB group (p = 0.056). pAldo was not different between the ACEI and ARB groups by univariate analysis or by bivariate analysis adjusting for ACEI and ARB dose with stratification according to ACE genotype. The multivariate model, adjusted for age, race, sex, CHF, diabetes, diuretics, 24-h urine Na and K, eGFR, SBP, and ACE genotype.

**Conclusions:** Our analysis suggests that ACEi are not better than ARB in suppressing pAldo.

*Funding: Clinical Revenue Support*

**TH-PO793**


1. Div. of Nephrol, Osaka University Graduate School of Medicine, Suita, Osaka, Japan; 2. Health Care Center, Osaka university, Suita, Osaka, Japan.

**Background:** Renin-angiotensin system blockers are first-line antihypertensive agents, but antihypertensive monotherapy is often not sufficient to achieve appropriate blood pressure (BP) control for organ protection. This study determined whether addition of eplerenone could result in clinical benefits in patients already taking angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs).

**Methods:** Twenty-five patients who were already taking ACEi or ARBs due to hypertension or chronic kidney disease were enrolled in this study. During the course of the study, patients were treated with a gradual increase of eplerenone to a final dose of 50 mg day^-1_. BP, serum and urine osmolality/sodium/potassium/creatinine, estimated glomerular filtration rate (eGFR), and urinary protein/creatinine ratio were measured before and after 4-month study period. Baseline plasma renin activity (PRA) and plasma aldosterone concentration (PAC) were measured in some patients.

**Results:** At baseline, there was no correlation between PAC and the calculated transtubular potassium (K) gradient (TTKG) or PRA in subjects who were taking ACEi or ARBs. However, PAC was inversely correlated with serum sodium (p = 0.02) and positively correlated with serum potassium level (p = 0.008). The differences between the ACEI and ARB groups was significant, which was expected since there was a trend for greater diuretic use in the ARB group (p = 0.056). pAldo was not different between the ACEI and ARB groups by univariate analysis or by bivariate analysis adjusting for ACEI and ARB dose with stratification according to ACE genotype. The multivariate model, adjusted for age, race, sex, CHF, diabetes, diuretics, 24-h urine Na and K, eGFR, SBP, and ACE genotype.

**Conclusions:** Our analysis suggests that ACEi are not better than ARB in suppressing pAldo.

*Funding: Clinical Revenue Support*

**TH-PO794**

**Urinary Uromodulin Concentrations and Incident Hypertension: The Framingham Heart Study** Conrad M. O’Seaghdha, Shih-Jen Hwang, Caroline S. Fox. *NHLBI’s Framingham Heart Study.*

**Background:** A common variant of the UMOD gene has been associated with extreme hypertension, independent of a primary defect in kidney function, in a recent genome-wide association study. The aim of the present study was to determine whether urinary uromodulin concentrations are associated with risk of hypertension in cross-sectional and prospective analyses.

**Methods:** Participants free of CKD were drawn from exam 6 of the Framingham Heart Study (1988-1990). All with follow-up at exam 8 (2005-2008) for prospective analyses (n=2948). Extreme hypertension was defined as systolic blood pressure ≥ 160 mmHg and/or diastolic blood pressure ≥ 100 mmHg. In prospective analyses, participants with baseline hypertension, defined as systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg were also excluded. Urinary uromodulin concentrations were related to risk of prevalent and incident hypertension and extreme hypertension using logistic regression in unadjusted- and multivariable-adjusted analyses adjusted for age, sex and eGFR.

**Results:** In cross-sectional analyses, urinary uromodulin concentrations were similar between hypertension cases and controls (11.4 vs. 10.6 mg/dL; p=0.9). Similarly, baseline urinary uromodulin concentrations were not statistically different between cases of incident hypertension (11.3 vs. 11.2 mg/dL; p=0.3) and incident extreme hypertension (11.2 vs. 13.9 mg/dL; p=1.0). Results were unchanged after adjustment for uric creatinine, age, sex, or eGFR.

**Conclusions:** Although the UMOD gene has been associated with extreme hypertension, its gene product, uromodulin, is not associated with hypertension in the community.

*Funding: Other NIH Support - NHLBI*

**TH-PO795**

**Effect of Lanosterol Synthase Polymorphisms on Blood Pressure and Endogenous Ouabain Levels in Two Different Clinical Settings Chiara Lanzani, Guido Gatti, Marco Simonini, Simona Pozzolo, Elisabetta Messaggio, Nunzia Casamassima, Simona Delli Carpin, Lorena Citterio, Paolo Manunta. Chair of Nephrology, San Raffaele Scientific Institute, Milan, Italy.

**Background:** Endogenous Ouabain (EO) may affect blood pressure and renal Na excretion through the modulation of the Na pump either as a Na transport system or a signal transduction triggering mechanism in renal tubular cell and in vascular smooth muscle cells. Lanosterol synthase (LSS) regulates the first step in the biosynthesis of cholesterol and steroid hormone, including EO. A polymorphism (rs2254524 542L) in LSS gene affects mRNA expression in human kidney tissue and in transplanted human cells. In this latter setting this polymorphism also affects protein expression, enzymatic activity and EO synthesis.

**Methods:** To investigate whether the effect of two polymorphism of LSS (rs914247 and rs2254524), on BP and EO levels in mild hypertensive patients in response to two different manuvers: acute Na load with 9.9 NaCl (Na Load) for 2 hrs, and low dietary intake (<100 mg/diey. Low Na diet) for 30 days.

**Results:** Behaviours of EO plasma levels resulted influenced (p=0.004) by LSS genotypes: carriers of the wild type variant decreased EO (LSS rs914247 from 259.5±15 to 221.5±19 pmol/L, and LSS rs2254524 from 281.1±16 to 242.2±20 pmol/L). However, mutant variant were associated to slight EO increase (rs914247 265.4±14 to 279.7±10 pmol/L and LSS rs2254524 269.9±16 to 284.2±20 pmol/L). Furthermore, pressure natriuresis relationship was steeper in those hypertensives carrying the mutated LSS together with ADD2 variant (p (p=0.006). Low Na diet: fall in systolic (-7,17±1 vs 3,05±1.5 p= 0.013), diastolic (-5,10±98 vs -1,4±94 mmHg) blood pressure and long term regulation of pressure-natriuresis (0,105±0,02 vs 0,151±0,02 mmHg/mg/min, p=0.007) were affected by LSS rs2254524 mutated variant.

**Conclusions:** Our findings suggest that LSS variants regulate plasma EO levels after acute and chronic Na balance variations. Therefore, plasma EO may influence Na-K ATPase activity both at vascular and kidney levels, resulting in modification of blood pressure and urinary Na excretions.

*Funding: Government Support - Non-U.S.**
Patients Treated with the Angiogenesis Inhibitor Regorafenib Develop Hypertension with Rapidly Reversible Changes in Plasma Nitric Oxide and Endothelin-1  
Nika delesus-Gonzalez,1 Emily S. Robinson,2 Radostin Penchev,2 George Demetri,3 Suzanne George,4 Benjamin D. Humphreys,5,6,7 Children’s Hospital Boston; Brigham and Women’s Hospital; Dana Farber Cancer Institute.

Background: Hypertension (HTN) is a common and dose-limiting side effect of therapies that target VEGF signaling such as regorafenib. Understanding the mechanisms leading to HTN is important as such knowledge may lead to clinically useful biomarkers for predicting both toxicity and tumor response. We prospectively tested the hypothesis that regorafenib induces HTN by suppressing nitric oxide (NO) and increasing endothelin-1 (ET-1).

Methods: Plasma was collected from 32 subjects with gastrointestinal stromal tumor at baseline, 2, 4, and 6 weeks of therapy. Regorafenib was given on a 3-week on, 1-week off regimen, and plasma levels of NO, cyclic GMP (cGMP), a downstream effector of NO, and ET-1 were measured at baseline, 2, 4, and 6 weeks of therapy. Regorafenib was given on a 3-week on, 1-week off regimen, and plasma levels of NO, cyclic GMP (cGMP), a downstream effector of NO, and ET-1 were measured at baseline, 2, 4, and 6 weeks of therapy.

Results: The mean age was 55 years, 60% men, 84% white and 65% had prior HTN. At 2 weeks, regorafenib caused a rise in mean arterial pressure (MAP) with increased ET-1 and decreased NO. ET-1 and NO changes normalized after the 1-week washout period. No differences were observed for cGMP. After restarting regorafenib, ET-1 once again increased.

Conclusions: These findings indicate the coordinated and reversible downregulation of the NO system and upregulation of ET-1 system by regorafenib, suggesting systemic vasocostriction as a mechanism of HTN in these patients. This is the first prospective study evaluating both NO and ET-1 pathways as biomarkers of anti-angiogenic therapy-induced HTN in humans. Whether NO and ET-1 might predict the development of HTN or tumor response requires further study.

Funding: NIDDK Support, Pharmaceutical Company Support

Association between Sodium Intake and Hypertension Risk Varies Depending upon Levels of Biomarkers Indicating Endothelial Dysfunction

John P. Forman,1 Lienneke Scheven,2 Paul E. de Jong,2 Stephan J.L. Bakker,2 Gary C. Curhan,1 Ron T. Gansevoort.2

Background: The mechanisms underlining chronic sodium loading-related hypertension are unclear. A high sodium diet may first lead to vascular remodeling, and then to hypertension if the high sodium diet is continued. We hypothesized that a higher sodium intake would be associated with increases in biomarkers of vascular endothelial dysfunction, specifically serum uric acid (SUA) and albuminuria (UA), and that the association between sodium intake and risk of hypertension would vary according to levels of SUA and UA.

Methods: We prospectively analyzed the associations of sodium intake with change in SUA, change in UA, and hypertension incidence (defined by a systolic pressure ≥140 mmHg and/or a diastolic pressure ≥90 mmHg, or of antihypertensive medications), during a median follow-up of 6.4 years among non-hypertensive patients of the PREVEND cohort (N=5571).

Results: After adjusting for multiple potential confounders, a higher sodium intake was significantly associated with increases in SUA and UA. The relation between sodium intake and risk of incident hypertension varied significantly according to SUA and UA. Each 1 gram higher sodium intake, the multivariable adjusted hazard ratio for developing hypertension was 0.98 (0.90-1.07) among those whose SUA was in the lowest tertile, and 1.10 (1.03-1.18) among those whose SUA was in the highest tertile. Similar analyses yielded adjusted hazard ratios of 0.99 (0.93-1.06) among participants whose UA was <10 mg/d, and 1.18 (1.07-1.29) among those whose UA was >15 mg/d.

Conclusions: Higher sodium intake was associated with increasing SUA and UA, and was an independent risk factor for developing hypertension only among those with higher levels of SUA or UA. A high sodium diet may lead to biological changes favoring the development of hypertension. Further research is needed to fully understand the mechanisms by which high sodium diet is related to hypertension.

Funding: Other NIH Support - NHLBI

The Effects of Pregnancy, Pregnancy-Associated Hypertension and Gestational Diabetes on the Microvasculature
Thao Vi Luong,1 Alexander The,2 C. Curhan,1 Ron T. Gansevoort.2

Background: Hypertension with Rapidly Reversible Changes in Plasma Nitric Oxide and Endothelin-1

Nika delesus-Gonzalez,1 Emily S. Robinson,2 Radostin Penchev,2 George Demetri,3 Suzanne George,4 Benjamin D. Humphreys,5,6,7 Children’s Hospital Boston; Brigham and Women’s Hospital; Dana Farber Cancer Institute.

Background: Hypertension (HTN) is a common and dose-limiting side effect of therapies that target VEGF signaling such as regorafenib. Understanding the mechanisms leading to HTN is important as such knowledge may lead to clinically useful biomarkers for predicting both toxicity and tumor response. We prospectively tested the hypothesis that regorafenib induces HTN by suppressing nitric oxide (NO) and increasing endothelin-1 (ET-1).

Methods: Plasma was collected from 32 subjects with gastrointestinal stromal tumor at baseline, 2, 4, and 6 weeks of therapy. Regorafenib was given on a 3-week on, 1-week off regimen, and plasma levels of NO, cyclic GMP (cGMP), a downstream effector of NO, and ET-1 were measured at baseline, 2, 4, and 6 weeks of therapy. Regorafenib was given on a 3-week on, 1-week off regimen, and plasma levels of NO, cyclic GMP (cGMP), a downstream effector of NO, and ET-1 were measured at baseline, 2, 4, and 6 weeks of therapy.

Results: The mean age was 55 years, 60% men, 84% white and 65% had prior HTN. At 2 weeks, regorafenib caused a rise in mean arterial pressure (MAP) with increased ET-1 and decreased NO. ET-1 and NO changes normalized after the 1-week washout period. No differences were observed for cGMP. After restarting regorafenib, ET-1 once again increased.

Conclusions: These findings indicate the coordinated and reversible downregulation of the NO system and upregulation of ET-1 system by regorafenib, suggesting systemic vasocostriction as a mechanism of HTN in these patients. This is the first prospective study evaluating both NO and ET-1 pathways as biomarkers of anti-angiogenic therapy-induced HTN in humans. Whether NO and ET-1 might predict the development of HTN or tumor response requires further study.

Funding: NIDDK Support, Pharmaceutical Company Support

Association between Sodium Intake and Hypertension Risk Varies Depending upon Levels of Biomarkers Indicating Endothelial Dysfunction

John P. Forman,1 Lienecke Scheven,2 Paul E. de Jong,3 Stephan J.L. Bakker,4 Gary C. Curhan,1 Ron T. Gansevoort.2

Background: The mechanisms underlining chronic sodium loading-related hypertension are unclear. A high sodium diet may first lead to vascular remodeling, and then to hypertension if the high sodium diet is continued. We hypothesized that a higher sodium intake would be associated with increases in biomarkers of vascular endothelial dysfunction, specifically serum uric acid (SUA) and albuminuria (UA), and that the association between sodium intake and risk of hypertension would vary according to levels of SUA and UA.

Methods: We prospectively analyzed the associations of sodium intake with change in SUA, change in UA, and hypertension incidence (defined by a systolic pressure ≥140 mmHg and/or a diastolic pressure ≥90 mmHg, or of antihypertensive medications), during a median follow-up of 6.4 years among non-hypertensive patients of the PREVEND cohort (N=5571).

Results: After adjusting for multiple potential confounders, a higher sodium intake would be associated with increases in biomarkers of vascular endothelial dysfunction, specifically serum uric acid (SUA) and albuminuria (UA), and that the association between sodium intake and risk of hypertension would vary according to levels of SUA and UA.

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Results: After adjusting for multiple potential confounders, a higher sodium intake would be associated with increases in biomarkers of vascular endothelial dysfunction, specifically serum uric acid (SUA) and albuminuria (UA), and that the association between sodium intake and risk of hypertension would vary according to levels of SUA and UA.
between groups in Δ-MAP (6.5±13 vs 8.4±13 mmHg; p=0.36), in Δ-NED (-0.8±10 vs -0.1±7.7 µg/min; p=0.63), or in Δ-MAP adjusted for Δ-NED (p=0.14). Similarly, there were no differences after 24 or 72-h, or 4 or 24-h after the second dose. Hemoglobin concentration and hematoctit were unchanged. A subgroup analysis of patients with no vasoressor use (n=71) also showed no differences between epoetin and placebo.

Conclusions: Two high doses of epoetin did not increase blood pressure up to 72-h after administration. These data demonstrate that epoetin does not have an acute vasoconstrictor effect among critically ill patients with AKI.

Funding: Government Support - Non-U.S.

TH-PO802

Impact of Gender and Age on the Cardiac Autonomic Response to Angiotensin II in Healthy Humans

Michelle C. Mann,1 Brenda Hemmelgarn,1, 2 Derek Exner,1,3 Darlene Y. Sola,1 Tanvir Chowdhury Turin,1 Sofia B. Ahmed.1
1Faculty of Medicine, University of Calgary, Calgary, Canada; 2Alberta Kidney Disease Network, AB, Canada; 3Libin Cardiovascular Institute, Calgary, Canada.

Background: Women are protected from cardiovascular disease (CVD) compared to men. Changes in cardiac autonomic tone such as sympathetic overactivity and vagal withdrawal result in altered heart rate variability (HRV), which when coupled with upregulation of the renin-angiotensin system (RAS) are associated with kidney and CVD, though the pathophysiology is unclear.

Methods: 36 healthy subjects (21 women, 15 men, age 38±2 yrs) were studied in high-salt state. HRV, calculated by spectral power analysis [low frequency (LF), sympathetic activity; high frequency (HF), vagal activity; and LF/HF, balance of autonomic tone], was recorded at baseline and during angiotensin II (AngII) infusion (3ng/kg/min x 30min/6ng/ kg/min x 30min). Results: Striking gender differences exist in the HRV response to AngII, with women maintaining overall autonomic tone (LF/HF 2.4±0.3; p=0.36 vs. baseline) while men exhibit increased sympathetic activity and profoundly decreased vagal activity (LF/HF 4.1±0.6; p=0.014 vs. baseline, p=0.018 vs. LF/HF female response). While gender and age remained predictors of HRV response on multivariate analysis (p=0.003 and p=0.042, respectively), LF and HF responses were increasingly sensitive to AngII with age solely in men (LF p=0.001; HF p=0.001 for gender-age interaction).

Conclusions: Women maintain baseline levels of cardiac autonomic tone in response to AngII. In contrast, men exhibit greater cardiac risk with exaggerated cardiac sympathetic activity in response to AngII, a response exacerbated by increasing age. Understanding the roles of gender and age in cardiac autonomic modulation may help guide novel strategies for high-risk populations, such as those with kidney disease.

Funding: Private Foundation Support

TH-PO803

Fibroblast Growth Factor 23 Levels Are Elevated in Children and Adults with Autosomal Dominant Polycystic Kidney Disease and Preserved Renal Function

Shailendra Sharma,1 Michel B. Chonchol,1 Berenice Y. Gitomer,1 Wei Wang,1 Myles S. Wolf,2 Melissa A. Cadnapaphornchai,1 Robert W. Schrier.1
1Division of Renal Diseases and Hypertension, University of Colorado Denver; Aurora, CO; 2Nephrology, University of Miami Medical Center; FL.

Background: Fibroblast growth factor 23 (FGF23) is risk factor for adverse outcomes in chronic kidney disease (CKD). Elevated FGF23 levels were recently shown in adults with ADPKD and early stages of CKD. Cardiac manifestations of ADPKD, including increased left ventricular mass index, occur as early as childhood. Early intervention early may be more effective to slow progression of cardiac disease in ADPKD. We hypothesized that FGF23 levels are elevated in children and young adults before reduction of kidney function.

Methods: We compared FGF23 levels in 35 children and young adults with ADPKD and normal eGFR (age 17±4 years; mean eGFR 118±23 ml/min/1.73m²), 33 adults with ADPKD and preserved kidney function (age 37±8 years; mean eGFR 87±18 ml/min/1.73m²) and 9 adults with more advanced ADPKD (age 51±10 years; mean eGFR 34±7 ml/min/1.73m²). Serum FGF23 levels were measured by ELISA (Kainos, Tokyo, Japan).

Results: Using the same assay, a previous study reported normal FGF23 level in healthy volunteers aged 20-83 years of 30±21 pg/ml. Mean FGF23 level was 37.3 ± 18.5 pg/ml in the 35 children and young adults with ADPKD , 39.9 ± 21.3 pg/ml in the 33 adults with ADPKD and preserved kidney function and 86.4 ± 61.4 pg/ml in the 9 adults with more advanced ADPKD. Twenty % of children and young adults with ADPKD had an FGF23 >50 pg/ml. In the adults with preserved kidney function, 36% had a level >50 pg/ml. Among adults with more advanced renal dysfunction, only one had an FGF23 level < 50 pg/ml.

Conclusions: FGF23 levels are increased early in ADPKD when kidney function is intact, and abnormally high levels of FGF23 are detectable in many children with ADPKD. Further research is necessary to determine the implications of early increases in FGF23 levels in children and adults with ADPKD and whether these increases contribute to progression of CKD and the development of cardiovascular disease.

Funding: NIDDK Support, Private Foundation Support

TH-PO804

The Relationship between Renal Volume and Renal Function in Autosomal Dominant Polycystic Kidney Disease

Satoru Muto, Yutaro Ogawa, Toshiyuki Matsui, Shigeo Horie, Hisamitsu Ide, Shigeo Horie. Urology, Teikyo University, Tokyo, Japan.

Background: In patients with autosomal dominant polycystic kidney disease (ADPKD), renal cysts grow exponentially. Since remaining renal parenchyma has a capacity to compensate for the loss of glomerular filtration, the glomerular filtration rate (GFR) may be sustained until the disease progresses. The purpose of this study was to determine if renal volumetric indices and clinical parameters are associated with renal function in Japanese patients with ADPKD.

Methods: In 73 ADPKD patients (28 men, 45 women), the associations of mean systolic blood pressure (SBP), mean diastolic blood pressure (DBP), estimated GFR (eGFR), the amount of proteinuria and albuminuria, body mass index (BMI), brachial-ankle pulse wave velocity (baPWV), ankle-brachial Index (ABI), and total kidney volume (TKV) were retrospectively analyzed.

Results: Multivariate linear regression analysis showed that eGFR was significantly and independently inversely correlated with the change in TKV per year (ΔTKV/y) (Figure1). Multiple linear regression analysis showed that ΔGFR/y was significantly and independently inversely correlated with the change in TKV per year (ΔTKV/y) (Figure1). Multiple linear regression analysis showed that ΔTKV/y was significantly related to initial TKV and the change in albuminuria per year (Δ-alb/y).

Conclusions: This study demonstrated a significant relationship between the change in renal function and the change in renal volume in Japanese ADPKD patients without renal insufficiency. It is possible that the volume measurements can be used as useful markers for disease progression in Japanese ADPKD patients.

Funding: Private Foundation Support

TH-PO805

Baseline Renal Cysts Volume Predicts the Recombinant Human Erythropoietin Requirement in Autosomal Dominant Polycystic Disease

Paolo Lentini,1-5 Angela D'angelo, 1 Roberto Dell'aquila, 1 Valentina Pellanda. 1Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Istituto Auxologico Italiano, Milan, Italy; 2Urology, Teikyo University, Tokyo, Japan; 3Nephrology, St. Bortolo Hospital, Vicenza, Italy; 4Nephrology, St. Bassiano Hospital, Bassano del Grappa (vi), Italy; 5University of Padua, Italy.

Background: Prevalence of anaemia in patients with autosomal dominant polycystic kidney disease (ADPKD) increases according to the severity of chronic kidney disease (CKD). However,little is known about the relationship between kidney structure modification and recombinant human erythropoietin (rHuEPO) requirement in these patients. Aim of
this study was to evaluate the role of renal cysts and kidney size on rHu-EPO requirement in severe CKD and naive chronic hemodialysis (HD) patients.

**Methods:** A total of 43 pts with ADPKD and anemia treated with alfa-erythropoietin (α-EPO) were enrolled (16 pts with CKD Stage 4 and 28 naive chronic HD pts), the total volume of the four largest cysts (cysts-Vol) and the mean antero-posterior renal diameter (AP) were prospectively followed-up for 18 months with kidney ultrasound.

**Results:** Mean age was 65±13 yrs. At baseline, AP was 19.4±2.1 cm, cysts-Vol 407±369 cm³. During the 18 months follow-up, haemoglobin (Hb) was 10.8±0.7 g/dl, α-EPO dose was 14403±7518 UI/week, and α-EPO/Hb ratio was 1379±780. In fully adjusted models, cysts-Vol and AP predict EPO dose and EPO/Hb ratio and explain a large amount of variability.

**Background:** Kidney pain can be a severe & debilitating chronic problem in some individuals with ADPKD. Videorheoscopics with splanchncetomy (VSPL) has anecdotally been used to manage ADPKD patients with intractable kidney pain.

**Methods:** We have performed VSPL in 15 ADPKD patients (11F, 4M) for chronic kidney pain in 13 (2 unilateral, 11 bilateral), a bilateral procedure for pain due to pancreatic cystic disease, & a unilateral procedure for liver pain; 11/15 in enrolled in this study to evaluate its effectiveness in alleviating pain, opioid use, QOL, renal blood flow (RBF) [by MRI] & GFR. All had chronic pain >6 mo & were opioid dependent.

**Results:** All (mean age 38, range 20-51) reported relief of their pain (different/less intensity) immediately post op. Average hospital stay was 4d. Study participants have been followed a mean of 14 mo (range 1-24 mo). One developed severe chest wall pain post-op requiring nerve block & another developed orthostatic hypotension responsive to midodrin.

**Conclusions:** VSPL seems to be an effective palliative procedure for intractable visceral pain in some patients with ADPKD & may have a role in the stepwise approach for chronic pain management strategies in selected individuals with relatively preserved renal function.

**Funding:** Private Foundation Support

### Table 1. Multivariate Analysis

<table>
<thead>
<tr>
<th>Dependent variable: EPO, UI/Week</th>
<th>Beta (95%CI)</th>
<th>P</th>
<th>R2 inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cysts Vol (100cm³)</td>
<td>870 (250-1480)</td>
<td>&lt;0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>AP (cm)</td>
<td>1146 (68-2223)</td>
<td>&lt;0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Model R²=0.46</td>
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</table>

**Conclusions:** Cysts volume is useful to predict prospectively the rHu-EPO requirement. This assumption is valid even in chronic dialysis patients, where the renal function is completely lost.

#### TH-PO806

**Intravascular Treatment of 1,012 Patients with Symptomatic ADPKD and/or ADPLD**

Koki Mise, Yoshifumi Ubara, Keiichi Sumida, Rikako Hiramatsu, Tatsuya Suwabe, Kennei Takaichi. *Nephrology, Toranomon Hospital, Tokyo, Japan.*

**Background:** Since the kidneys and liver of ADPKD patients are usually supplied by well-developed arteries, we have attempted to reduce the volume of enlarged kidneys and livers in these patients by transcatheter arterial embolization (TAE) using intravascular coils.

**Methods:** From 1996 to 2011, a total of 1,012 patients with ADPKD were treated for intractable enlarged kidneys. After TAE, nephrectomy was performed in 2 patients because of antibiotic-resistant renal cystic infection (unilateral in one patient and bilateral in the other). Nephrectomy was also performed for the affected kidney in 10 patients with renal cell carcinoma. The other patients had no TAE-related or renal complications, but complications of other organs (such as cancer and cardiovascular/cerebrovascular disease) led to death. Hepatic TAE was done for chronic pain management strategies in selected individuals with relatively preserved renal function.

**Funding:** Private Foundation Support

### TH-PO807

**Renal Denervation for Intractable Autosomal Dominant Polycystic Kidney Disease-Related Pain**

Marie C. Hogan,1 Joanne Ryan,1 James Glockner,2 Stephen B. Erickson,1 Dawn S. Milliner,1 Claude Deschamps,2 Vicente E. Torres.1

1*Nephrology Div; 2Radiology Dept; 3Thoracic Surgery Div, Mayo Clinic, MN.*

**Background:** Kidney pain can be a severe & debilitating chronic problem in some individuals with ADPKD. Videorheoscopics with splanchncetomy (VSPL) has anecdotally been used to manage ADPKD patients with intractable kidney pain.

**Methods:** We have performed VSPL in 15 ADPKD patients (11F, 4M) for chronic kidney pain in 13 (2 unilateral, 11 bilateral), a bilateral procedure for pain due to pancreatic cystic disease, & a unilateral procedure for liver pain; 11/15 in enrolled in this study to evaluate its effectiveness in alleviating pain, opioid use, QOL, renal blood flow (RBF) [by MRI] & GFR. All had chronic pain >6 mo & were opioid dependent.

**Results:** All (mean age 38, range 20-51) reported relief of their pain (different/less intensity) immediately post op. Average hospital stay was 4d. Study participants have been followed a mean of 14 mo (range 1-24 mo). One developed severe chest wall pain post-op requiring nerve block & another developed orthostatic hypotension responsive to midodrin.

**Conclusions:** VSPL seems to be an effective palliative procedure for intractable visceral pain in some patients with ADPKD & may have a role in the stepwise approach for chronic pain management strategies in selected individuals with relatively preserved renal function.

**Funding:** Private Foundation Support

#### TH-PO808

**A Quantitative Proteomic Study of Urinary Exosome Candidate Biomarkers in a Genetically Defined PKD1 Cohort**

Marie C. Hogan,1 Kenneth L. Johnson,2 Douglas W. Mahoney,1 Ann C. Oberg,3 Peter C. Harris,1 Christopher James Ward,1 1*Nephrology; 2Proteomics Ctr; 3Biostatistics, Mayo Clinic, MN.*

**Background:** We have identified distinct subpopulations of urine exosome-like vesicles (ELVs) reproducibly isolated by gradient centrifugation (5-30% sucrose D2O). One of these, (PKD-ELVs) is enriched in polycystin-1 (PC1), polycystin-2 (PC2) & fibrocystin/polyductin (FCP). Analysis may reveal diagnostic PKD biomarkers.

**Methods:** PKD-ELVs were isolated from ~70mls 1st & 2nd AM voids [7 controls (28±3 yrs)/ PKD cases (28±6 yrs)] & proteins fractionated by SDS-PAGE, trypic peptides ident’d & quantified by label-free LC-MS/MS. Phase 1 examined biomarker variability. Inter-individual variability was corrected by Loess method. Multivariate ANOVA (disease, gender, & interactions) was used to compare proteins between cases & controls. 5% FDR & p<0.002 were used.

**Results:** On average we observed ↓ of 27% of PC1, ↓27% PC2 & ↓25% FCP compared to controls. Using a p<0.002 cutoff, we identified 21 differentially regulated proteins in the PKD cohort: 9 up & 12 are down-regulated. PC1 peptides were consistently underrepresented in cases. Two heavy chain proteins & light chain protein were upregulated. SAHH-2 (IRBIT) which is down-regulated, is reported to modulate CFTR intracellular Ca by antagonizing IP3 receptor. HOMER1 a known PC1 interactor is consistently upregulated with LC isofrom most highly expressed. Selected differentially Regulated Proteins in PKD-ELV Proteome

<table>
<thead>
<tr>
<th>Protein</th>
<th>Uniprot ID</th>
<th>Fold→</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polycystin 1</td>
<td>PKD1_HUMAN</td>
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<td>0.015</td>
</tr>
<tr>
<td>Polycystin 2</td>
<td>PKD2_HUMAN</td>
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</tr>
<tr>
<td>Fibrocystin</td>
<td>PKHD1_HUMAN</td>
<td>2.0</td>
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</tr>
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<td>RBβ</td>
<td>SH2D1_HUMAN</td>
<td>2.1</td>
<td>0.01</td>
</tr>
<tr>
<td>Solute Carrier family 12-1</td>
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</tr>
<tr>
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<td>HOM1_HUMAN</td>
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</tr>
<tr>
<td>gamma-1 chain C region</td>
<td>GHR1_HUMAN</td>
<td>4.8</td>
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<tr>
<td>Phospholipid scramblase 1</td>
<td>PSCL1_HUMAN</td>
<td>2.0</td>
<td>0.012</td>
</tr>
</tbody>
</table>

**Conclusions:** PKD1, PC2 and FCP are ↓ in cases compared with controls, although NS in Phase 1 of this study. Inflammatory proteins also feature. Homer 1 a known PC1 interactor is one of the few proteins that is increased in PKD. We are developing a ratiometric assay using a PKC1Mab (7e12) /Homer1cMab to validate these findings if confirmed in our Phase II study (ongoing).

**Funding:** NIDDK Support, Private Foundation Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

298A
TH-PO809
Modeling Vascular Lesions Associated with Autosomal Dominant Polycystic Kidney Disease Using Patient-Specific iPSCs
Kenji Osafune, Fumihiko Shiota. Center for IPS Cell Research and Application (CiRA), Kyoto University.

Background: Autosomal dominant polycystic kidney disease (ADPKD) is the most prevalent, potentially lethal, monogenic disorder, characterized by the development of multiple renal cysts and various extrarenal manifestations. Cardiovascular complications are the main cause of death in patients with ADPKD, including hypertension, intracranial aneurysms and dolichoectasia, thoracic aortic and cerecocephalic artery dissections, coronary artery aneurysms and valvular heart abnormalities. The pathogenesis of cardiovascular lesions as well as renal cyst formation remains largely unknown, and no therapeutic strategies have been established.

Methods: We have obtained iPS cells from skin fibroblast samples from seven patients with ADPKD by transducing four transcription factors, OCT4, SOX2, KLF4 and C-MYC or three factors, OCT4, SOX2 and KLF4. Then we differentiated the ADPKD-iPS cells into vascular endothelia and mural cells.

Results: We have obtained iPS cells from skin fibroblast samples from seven patients with ADPKD. These cells expanded robustly in culture and differentiated into vascular endothelia and mural cells in vitro. Using this differentiation system, we have identified several molecules whose expression levels were upregulated or downregulated in vascular cells differentiated from ADPKD-iPS cells as compared to those from normal Japanese iPS cells.

Conclusions: These results suggest that disease modeling using patient-specific iPSCs can be used for studying the mechanisms of vascular complications associated with ADPKD.

TH-PO810
Cysteine Causes Angioendotheliomatosis by Stimulating Microvascular Endothelial Cell Proliferation
Elena N. Levtchenko,1 Micke Dewerchin,1 Martin Mattsson,2 Besouro.1 1Dept of Pediatric Nephrology/Laboratory for Pediatrics, KU Leuven, Leuven, Belgium; 2Vesalius Research Centre, KU Leuven, Leuven, Belgium.

Background: Cystinosis is an autosomal recessive disease caused by intralysosomal cysteine accumulation. It initially causes renal Fanconi syndrome, progressing to end-stage renal disease by the age of 10 years if left untreated. At this moment, cysteamine is the only available treatment for cystinosis. Recently 8 patients were reported with muscular-skeletal weakness, skin striae and bruising-like lesions on elbows after administration of high doses of cysteamine. One patient died of cerebral ischemia. Skin biopsies of the elbows showed vascular proliferation called angioendotheliomatosis. We aimed to study the mechanism of this severe complication.

Methods: Human dermal microvascular endothelial cells (HDMEC; the cells involved in angioendotheliomatosis) were incubated with a range of cysteamine concentration (0-10 mM) during 6 or 24 hours in a 96-wells culture plate. Cell viability was measured using WST-1, cell proliferation was measured by BrdU incorporation. Growth factors (VEGF: vascular endothelial growth factor, PIGF: placental growth factor, b-FGF: basic fibroblast growth factor, PDGF: platelet derived growth factor) were measured in supernatant medium after 6 and 24 hours of cysteamine exposure. All results represent mean of at least two independent experiments performed in triplicate. The paired student t-test was used for statistical analysis.

Results: HDMEC viability increased by 135% (p<0.01) after 24 hours of cysteamine exposure (0-3.0 mM). Cell proliferation increased by 59% (p<0.05) and by 31% (p<0.05) after 6 and 24 hours of cysteamine exposure (0.03-1.0 mM) respectively. Cysteamine 0.03-0.3 mM stimulated VEGF production with 54-95% (p<0.05) while there was no difference in PIGF and b-FGF concentrations.

Conclusions: Cysteamine can cause endothelial proliferation via stimulating VEGF production at concentrations described in patients’ plasma (0.03-0.1 mM). We suggest that this mechanism underlies angioendotheliomatosis induced by cysteamine in cystinosis patients.

Funding: Private Foundation Support

TH-PO811
Biomarkers of Endothelial Dysfunction and Vascular Inflammation in ADPKD
Jelena Klawitter,1 Jost Klawitter,1 Kyler Corby,2 Uwe Christians,1 Robert W. Schrier,2 Michel B. Chonchol,1 Berenice Y. Gitomer,1 Anesthesiology, University of Colorado, Aurora, CO; 2Renal Diseases and Hypertension, University of Colorado, Aurora, CO.

Background: Cardiovascular disease (CVD) is the leading cause of death in autosomal dominant polycystic kidney disease (ADPKD). Recently 8 patients were reported with muscular-skeletal weakness, skin striae and bruising-like lesions on elbows after administration of high doses of cysteamine. One patient died of cerebral ischemia. Skin biopsies of the elbows showed vascular proliferation called angioendotheliomatosis. We aimed to study the mechanism of this severe complication.

Methods: We have obtained iPSCs from skin fibroblast samples from seven patients with ADPKD by transducing four transcription factors, OCT4, SOX2, KLF4 and C-MYC or three factors, OCT4, SOX2 and KLF4. Then we differentiated the ADPKD-iPS cells into vascular endothelia and mural cells.

Results: We have obtained iPS cells from skin fibroblast samples from seven patients with ADPKD. These cells expanded robustly in culture and differentiated into vascular endothelia and mural cells in vitro. Using this differentiation system, we have identified several molecules whose expression levels were upregulated or downregulated in vascular cells differentiated from ADPKD-iPS cells as compared to those from normal Japanese iPS cells.

Conclusions: These results suggest that disease modeling using patient-specific iPSCs can be used for studying the mechanisms of vascular complications associated with ADPKD.

Funding: Private Foundation Support

TH-PO812
Methylation Cycle Intermediates in Autosomal Dominant Polycystic Kidney Disease
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Background: Methylation cycle is an important metabolic process which provides methyl groups for DNA methylation and for the synthesis of S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH). These metabolites are key regulators of cellular functions, including cell growth, differentiation, and apoptosis. In addition, they play a role in the regulation of gene expression and in the development of various diseases, including cardiovascular disease. These data suggest that a renal phosphate leak occurs in ADPKD and is associated with increased TKV. Initial studies done in our laboratory have demonstrated that ADPKD patients had significantly higher levels of homocysteine in the patients with higher TKV (572.0±259.2 nmol/mg creatinine in patients with TKV <800mL vs. 1413±1462.2 nmol/mg creatinine with TKV >1500mL). The reduced excretion of Hcy was accompanied by reduced excretion of the methionine methylation pathways’ end product, cysteine. While SAM remained unchanged, the concentration of SAH increased from 1.7±0.3 μM in serum of patients with TKV <800mL to 1.96±0.5 μM in those with TKV >1500mL (p<0.05). As a result the SAM:SAH methylation potential ratio decreased. In urine, the excretion of SAH was also significantly increased (17.5±7.6 vs. 22.9±9.3 nmol/mg creatinine, p<0.05).

Conclusions: Increased serum Hcy levels in ADPKD associated with increased TKV represents a valuable biomarker in ADPKD which can potentially be used to monitor cardiovascular disease in these patients. Reduced urinary Hcy suggests a disturbance in the renal excretion, which may account for the accumulation in the blood. No such inhibition was observed for the Hcy precursors, SAM or SAH.

Funding: Private Foundation Support

TH-PO813
Renal Phosphate Leak in Autosomal Dominant Polycystic Kidney Disease (ADPKD)
Anna Jeanette Jovanovich,1 Berenice Y. Gitomer,1 Myles S. Wolff,2 Xiang-Dong Yan,1 Robert W. Schrier,1 Michel B. Chonchol,1 Anesthesiology, University of Colorado, Denver, Aurora, CO; 2Nephrology, University of Miami Medical School, FL.

Background: In a recent report of 100 ADPKD patients with chronic kidney disease (CKD) stages 1 – 2, serum phosphate levels were significantly lower compared to healthy volunteers and eGFR-matched CKD patients without ADPKD. We hypothesized that a renal phosphate leak is present in ADPKD compared to patients with CKD due to other etiologies and that these differences are detectable across the spectrum of kidney function.

Methods: Biochemical data on 697 ADPKD subjects were extracted from the University of Colorado ADPKD clinical database. Using a t-test we compared serum phosphate levels and 24-hour urinary phosphate excretion across the spectrum of eGFR to literature-reported levels from 3879 non-ADPKD participants in the Chronic Renal Insufficiency Cohort (CRIC) study.

Results: We examined serum phosphate and urinary phosphate excretion across eGFR deciles of eGFR (eGFR 20-29, 30-39, 40-49, 50-59, and 60-<1.7mL/min/1.73m2). Phosphate levels were significantly lower across the entire spectrum of renal function in the ADPKD participants compared to the eGFR matched CRIC participants. Urinary phosphate excretion levels were also significantly higher in ADPKD participants with eGFR < 50 mL/min/1.73m2 (ADPKD vs CRIC) (eGFR 40-49, 800 vs 725 p<0.0001; eGFR 30-39, 745 vs 680 p=0.0006; eGFR 20-29, 726 vs 632, p<0.0001) compared to eGFR-matched CRIC participants.

Conclusions: These data suggest that a renal phosphate leak occurs in ADPKD and is present across the spectrum of kidney function. Future studies are required to examine whether elevated levels of fibroblast growth factor 23 in ADPKD account for these findings.

Funding: NIDDK Support, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

299A
Fractional Excretion of Phosphate Is Independently Associated with Renal Volume and Left Ventricular Mass Index in Patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD) - Berenice Y. Gitomer, Kim McFann, Myles S. Wolf, Robert W. Schrier, Michail B. Chonchol

Background: Small increases in serum phosphate within the normal range are associated with greater risk of all-cause mortality, cardiovascular events and kidney disease progression in patients with and without chronic kidney disease (CKD). While the precise mechanism for laboratory measurement of serum phosphorus is excellent (CVs < 3%), there is considerable diurnal variability in serum phosphate. This limitation may be overcome by concurrent measurements of serum phosphate and urinary phosphate excretion, to improve insight into phosphorus homeostasis in individual subjects. We hypothesized that higher urinary fractional excretion of phosphate (FEPO4) in patients with ADPKD would be associated with larger renal volume, a validated biomarker of more rapid progression of ADPKD, and increased left ventricular mass index (LVMI), a validated index of cardiovascular disease.

Methods: We measured FEPO4 in 683 adults with ADPKD who had available data on total kidney volume (TKV) measured by ultrasound. LVMI, measured by 2D-echocardiography, was available in 232 of these patients. Linear regression was used to assess the relationship between log transformed FEPO4 with log transformed TKV and LVMI.

Results: The mean (SD) age of the participants was 41(13) years and 61% were female. The mean (SD) estimated GFR, FEPO4, TKV and LVMI were 73 ± 36 ml/min, 26 ± 15%, 1630 ± 1294 ml, and 7 ± 37mm², respectively. Linear regression of lnTKV on lnFEPO4, adjusted for age, height, sex and hypertension, demonstrated a highly significant relationship (β=0.66 ± 0.05, R² = 0.28, p < 0.0001). In addition, FEPO4 was significantly related to lnLVMI (β = 0.11 ± 0.04, R² = 0.04, p = 0.0021).

Conclusions: Abnormalities of phosphate homeostasis in ADPKD are associated with increased risk of progression of renal and cardiovascular disease. Future studies are necessary to whether disordered phosphate mechanism is a contributing cause or consequence of progression of renal and cardiac disease in ADPKD.

Funding: NIDDK Support, Private Foundation Support

The Effect of Everolimus Dose and Schedule on Renal Angiomyolipoma in Patients with Tuberous Sclerosis Complex - John J. Bissler, Elizabeth Jo Coombs, Bradley P. Dixon, David N. Franz

Background: Inhibition of the mTORC1 pathway in patients with tuberous sclerosis complex leads to a reduction of renal angiomyolipoma volume. The optimal dose and schedule of everolimus (mTORC1 inhibitor) for these patients is unknown. Determining the proper dose is critical for patient care in order to maximize the angiomyolipoma treatment effect while minimizing patient toxicity. To begin to understand how to treat patients, we undertook a phase two non-randomized open label trial. Thirty patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis and renal angiomyolipoma(s) were included. Renal angiomyolipoma volume was used as the primary endpoint, while safety was included as a secondary endpoint.

Methods: Patients received everolimus (RAD001) for one year, and were observed off drug for an additional year. We examined two different daily doses (5 or 10 mg) and three different weekly doses (30, 50 or 70 mg) regimens. Each treatment group contained a minimum of five patients.

Results: A total of 36 patients enrolled and a total of 30 completed the two-year study. The median age was 32 and there were 10 male and 26 female patients. The average reduction of the angiomyolipoma volume was approximately fifty percent at twelve months. Although once off drug, lesions demonstrated a range of responses from maintaining their reduction in volume to increasing back toward baseline size for both daily and weekly dosing. Grade three adverse events thought to be possibly, probably or definitely related to drug were infrequent, only three in the daily dosing and only one in the weekly dosing cohort.

Conclusions: Both weekly and daily dosing resulted in approximately a fifty percent reduction in angiomyolipoma volume. These results raise the possibility that lower doses, and different dosing schedules may be an option for patients not tolerating higher daily dosing.

Funding: Pharmaceutical Company Support

Efficacy and Safety of mTOR Inhibitor for Early-Stage Autosomal Dominant Polycystic Kidney Disease Patients - A Meta-Analysis of Randomized Controlled Trials - Qiang He, Chiaiyu Lin, Shunxian Ji, Jianghua Chen

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

TH-PO814

TH-PO816

Serum Uric Acid and Renal Disease Progression in Autosomal Dominant Polycystic Kidney Disease - Berenice Y. Gitomer, Kim McFann, Xiang-Dong Yan, Godela M. Brosnahan, Robert W. Schrier

Background: Higher serum uric acid (SUA) levels are associated with an increased risk for cardiovascular disease. Increased SUA levels are also associated with hypertension, a strong risk factor for ADPKD progression. We examined the hypothesis that SUA levels correlate with progression of ADPKD.

Methods: This retrospective study included 716 ADPKD adults from the University of Colorado ADPKD registry. SUA was examined as gender specific quartiles - quartiles 1 - 4 in women: < 4.2, 4.3 - 5.1, 5.2-6.5 and > 6.5 mg/dL; quartiles 1 - 4 in men: ≤ 5.7, 5.8 - 6.8, 6.9 - 8.2 and ≥ 8.3 mg/dL. The association of baseline uric acid levels with ADPKD progression and age at end-stage renal disease (ESRD).

Results: In linear regression, SUA was significantly related to lnTKV (total kidney volume) (β = 0.15 ± 0.08, p < 0.0001) and ln TKV/BSA (β = 0.13 ± 0.01, p < 0.0001). SUA was also significantly related to BMI (body mass index) (β = 0.78 ± 0.10, p < 0.0001). Those who had SUA in the 3rd and 4th quartile were significantly older, had higher BMI, higher serum creatinine (adjusted for age and sex), lower creatinine clearances (adjusted for age and sex), higher TKV (adjusted for age and sex), higher TKV/BSA, and higher urinary protein than those in the 1st quartile and 2nd quartile. In a sub-group analysis, the 4th quartile had higher microalbumin excretion than those in the 1st quartile. History of hyperuricemia was more prevalent among those ADPKD patients in the 3rd and 4th quartiles of SUA. 503 ADPKD patients had data available on age at ESRD or last known age without ESRD. There was a difference in survival curves among the 4 quartiles of SUA (< 0.0001). Median survival to ESRD was 68 (58-81) years for the 1st quartile, 70 (64- No Upper) for the 2nd quartile, 61 (56-65) years for the 3rd quartile, and 57 (54-60) years for the 4th quartile. Survival time was shortest for those in the 4th Quartile.

Conclusions: Thus, SUA correlates with age, BMI, impaired kidney function, hypertension, proteinuria, kidney volume and ESRD in ADPKD patients.

Funding: NIDDK Support, Private Foundation Support

TH-PO817

The Effect of Everolimus Dose and Schedule on Renal Angiomyolipoma in Patients with Tuberous Sclerosis Complex - John J. Bissler, Elizabeth Jo Coombs, Bradley P. Dixon, David N. Franz

Background: Inhibition of the mTORC1 pathway in patients with tuberous sclerosis complex leads to a reduction of renal angiomyolipoma volume. The optimal dose and schedule of everolimus (mTORC1 inhibitor) for these patients is unknown. Determining the proper dose is critical for patient care in order to maximize the angiomyolipoma treatment effect while minimizing patient toxicity. To begin to understand how to treat patients, we undertook a phase two non-randomized open label trial. Thirty patients with tuberous sclerosis or sporadic lymphangioleiomyomatosis and renal angiomyolipoma(s) were included. Renal angiomyolipoma volume was used as the primary endpoint, while safety was included as a secondary endpoint.

Methods: Patients received everolimus (RAD001) for one year, and were observed off drug for an additional year. We examined two different daily doses (5 or 10 mg) and three different weekly doses (30, 50 or 70 mg) regimens. Each treatment group contained a minimum of five patients.

Results: A total of 36 patients enrolled and a total of 30 completed the two-year study. The median age was 32 and there were 10 male and 26 female patients. The average reduction of the angiomyolipoma volume was approximately fifty percent at twelve months. Although once off drug, lesions demonstrated a range of responses from maintaining their reduction in volume to increasing back toward baseline size for both daily and weekly dosing. Grade three adverse events thought to be possibly, probably or definitely related to drug were infrequent, only three in the daily dosing and only one in the weekly dosing cohort.

Conclusions: Both weekly and daily dosing resulted in approximately a fifty percent reduction in angiomyolipoma volume. These results raise the possibility that lower doses, and different dosing schedules may be an option for patients not tolerating higher daily dosing.

Funding: Pharmaceutical Company Support

TH-PO818

Efficacy and Safety of mTOR Inhibitor for Early-Stage Autosomal Dominant Polycystic Kidney Disease Patients: A Meta-Analysis of Randomized Controlled Trials - Qiang He, Chiaiyu Lin, Shunxian Ji, Jianghua Chen

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
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300A

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Methods: RCTs regarding the mTOR inhibitor therapy in ADPKD patients are included. The data of studies and major outcomes include the changes of patients' Glomerular Filtration Rate (GFR), urinary protein, total kidney volume (TKV), cyst volume (CV), parenchymal volume (PV), blood pressure, lipid profile and the frequency of adverse events.

Results: Up to January 31st, 2011, 4 RCTs (568 patients) are included in this study. The meta-analysis made in this study indicates that mTOR inhibitor therapy group had smaller total kidney volume growth in ADPKD patients, especially in the parenchymal tissues, but have limited impact on slowing down the decrease of relatively safe drug to slow down the kidney volume growth in ADPKD patients, especially abnormal liver function, diarrhea, rash, aphthous stomatitis could occur during the mTOR inhibitor therapy, but the severities can be controlled by the appropriate use of drug.

Conclusion: Based on the current very limited clinical trials, mTOR inhibitor is a relatively safe drug to slow down the kidney volume growth in ADPKD patients, especially in the parenchymal tissues, but have limited impact on slowing down the decrease of renal function.

TH-PO819


Background: It is well known that total renal volume (TRV) is a predictor of ADPKD progression. However, parameters other than TRV are also currently explored in early stages of the disease, when glomerular filtration rate (GFR) is still preserved. We have previously reported that both urinary monocyte chemoattractant protein-1 (MCP-1) and albuminuria, assessed as urinary albumin/creatinine (UACR), are candidates for early markers of progression. High UACR (>6.8 mg/gCr) was associated with both high levels of urine MCP-1 and carotid-intima media thickness as well, as compared with normal UACR (<5.8). To investigate whether there is an interaction among TRV, GFR and MCP-1 and a role for MCP-1 as a predictor of disease severity, we performed a longitudinal study of 30±1 months in 32 young ADPKD patients (26±1 years old).

Methods: TRV was measured by ultrasound, urine MCP-1 by ELISA and GFR estimated by MDRD.

Results: TRV, GFR and urine MCP-1 baseline values were 415±52.8 ml, 108±3 ml/min/1.73m² and 152±32 ng/gCr, respectively. An association among the annual change in TRV, GFR and urine MCP-1 was found, independently of their basal values, UACR, age, sex or hypertensive treatment. The annual change in TRV and urine MCP-1 was increased in high UACR (131±33 ml and 108±49%), as compared with normal UACR patients (48±41 ml and 5±16%; p<0.05, respectively). The GFR annual change was not different according to UACR and remained stable in patients treated with angiotensin converting enzyme inhibitors as compared with untreated normotensive subjects (3±5 and 5±2 ml/min/year, p<0.01).

Conclusions: Being MCP-1 and TRV markers of renal inflammatory and cystic component respectively, our results suggest an involvement of both processes in ADPKD progression, even when GFR is within normal limits. Besides this, slight increased rates of albuminuria could be also a predictor of worse prognosis.

Funding: Government Support - Non-U.S.

TH-PO820

Treatment of Complement Factor H-Related Protein 5 (CFHR5) Nephropathy with Plasma Exchange Daniel P. Gale,1 Yiannis Athanasiou,2 Michalis Zavros,2 Constantinos Deltas,2 Alkis Mikis Pierides,1 H. Terence Cook,1 Patrick Maxwell.4 Imperial College Kidney and Transplant Institute, Imperial College, London, United Kingdom; 2Nephrology Department, Nicosia General Hospital, Nicosia, Cyprus; 3Biological Sciences Department, University of Cyprus, Nicosia, Cyprus; 4Division of Medicine, University College, London, United Kingdom.

Background: CFHR5 nephropathy is a recently recognised autosomal dominant disease which is endemic in Cyprus (1). It is characterised by macroscopic hematuria with stepwise deteriorations in renal function during intercurrent infections in renal as associated with a heterozygous internal duplication mutation of the CFHR5 gene which results in the production of a larger protein that is detectable in the blood (1). Kidney biopsies show C3 glomerulonephritis, implying that complement alternative pathway dysregulation underlies the disease. Over 80% men but <20% women develop renal failure by age 50. Disease can recur following renal transplantation, proving that it results from a defect of a circulating factor (3). This implies that correction of circulating complement regulation during acute flares might be effective.

Methods: 2 patients with CFHR5 nephropathy were treated with 4-7×3 liter plasma exchanges against fresh frozen donor plasma during episodes of macroscopic hematuria with persistently elevated serum creatinine above baseline.

Results: In both patients there was immediate cessation of macroscopic hematuria and rapid return of serum creatinine to the pre-flare baseline.

Conclusion: While further clinical studies are needed to determine if plasma exchange can alter the long term natural history of CFHR5 nephropathy, these cases provide initial evidence that manipulation of circulating complement regulators might be effective in this disease.

(1) Gale et al Nephrol Dial Transplant 2010
(2) Athanasiou et al JASN 2011
(3) Vernon et al Am J Transpl 2011
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301A
Urinary N-Acetyl-β-D-Glucosaminidase as a Surrogate Marker for Renal Function in Autosomal Dominant Polycystic Kidney Disease

Hypertension: Clinical
Poster/Thursday

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst growth leading to end-stage renal disease. Because both serum creatinine (Cr) and total kidney volume (TKV) measurements are considered to be limited use to monitor disease progression, we investigated urinary N-Acetyl-β-D-glucosaminidase (NAG) as a useful surrogate marker in this prospective study.

Methods: From Apr 2010 to May 2011, a total of 163 patients were enrolled from SNUH ADPKD registry and 139 patients were followed up for 6 months. GFR was estimated using MDRD equation and TKV was measured by modified ellipsoid method. We measured urinary NAG, β2-microglobulin, and neutrophil gelatinase-associated lipocalin (NGAL) and compared their predictive values for renal function.

Results: The mean age of subjects was 47 years. The baseline eGFR and TKV were 69.6 ± 20.0 mL/min/1.73m² and 1351.4 ± 977.2 mL, respectively. Log NAG/Cr was negatively correlated with both eGFR (r = 0.169, p = 0.001) and TKV (r = 0.434, p = 0.01) at baseline. By using ROC curve analysis, AUC of NAG/Cr for decreased eGFR (<60 mL/min/1.73m²) (0.77, 95% CI [0.72-0.81]) was higher than those of NGAL (0.61, 95% CI [0.55-0.66]) and β2-microglobulin (0.71, 95% CI [0.66-0.76]) (p = 0.01 and p = 0.02, respectively). Log NAG/Cr was also well correlated with the change in eGFR (r = 0.51, p < 0.001) and the change in TKV (r = 0.46, p = 0.001). The persistently high NAG/Cr (>3.4 IU/g) group showed renal function decline whereas the persistently low group revealed increased eGFR over follow-on (0.25 ± 1.76 vs 9.4 ± 1.68 mL/min/1.73m² per year, respectively).

Conclusions: Urinary NAG may be a good and reliable biomarker to predict renal progression in ADPKD patients.

Progression of Chronic Kidney Disease in Patients with Autosomal Dominant Polycystic Kidney Disease

Background: The aim of this study was to analyze the factors influencing chronic kidney disease (CKD) progression in patients with Autosomal Dominant Polycystic Kidney Disease (ADPKD).

Methods: We studied 101 patients (mean age 43 ± 17.3, 43.56 % male) followed during a mean follow-up time of 77.9 ± 48 months from 1997 to 2010. The primary end point was a 50% decrease of estimated glomerular filtration rate (eGFR) (CKD-EPI) since the first-time visit and/or to initiation of renal replacement therapy, annual mean change of eGFR was also analyzed. Clinical and demographic data, blood pressure, concomitant medications and analytical parameters were collected at each visit.

Results: Thirty-one patients achieved the primary end point after a mean time of 84.8 ± 39.5 months. Those patients who achieved the primary end point had higher SBP and DBP (p = 0.017 and p = 0.001), higher LDL-cholesterol (p = 0.011), higher creatinine (p = 0.006), higher uricemia (p = 0.041), more severe proteinuria (p = 0.033) and greater kidney size (p = 0.05). The mean annual eGFR change was ± 3.52 ± 7.3 mL/min/1.73 m². Forty-nine patients had a rapid decline renal function: Group A (higher than -3.52 mL/min/1.73 m²) and 52 patients had a lower renal disease progression: Group B (< -3.2 mL/min/1.73 m²). Adjusted Cox regression analysis showed that higher SBP and younger age at the first visit were independent variables for poorer renal outcome (p = 0.026).

Conclusions: Initial kidney function, proteinuria, renal size, hypercholesterolemia, hypertension, proteinuria and SBP are the factors that influencing CKD progression in ADPKD. SBP and younger age are the only factors that maintain their independent predictive value in multivariable analysis.

Progressive Nephromegaly Emerges during Adolescence in Children with ADPKD

Background: Progressive Nephromegaly Emerges during Adolescence in Children with ADPKD. Parental and family concerns trigger the referral, especially if a family member with ADPKD had RRT or progressive renal failure.

Results: Sixty-four children of this cohort had radiographic cystic features with ultrasound in agreement with ADPKD systems. There was a limited subset of children with evolving significant nephromegaly (kidney size > 95th percentile expected for age, gender, weight and height) during adolescent development. In this sub-group during puberty, six had unilateral (left = 4, right = 2) nephromegaly (female = 3, male = 3) and nine had symmetric rapid renal growth (female = 6, male = 3). Many of these children had serial follow up for >10 years. All children with nephromegaly, whether hypertensive or not, were placed on an ACE inhibitor (ACEi), as we suspect the emergence of nephromegaly during adolescence may influence their risk of renal decline during adulthood. Three of those who continued to nodules to nephromegaly to nephromegaly renal you to which they believed could occur they may be even more nephromegaly is a rare pleitropic disorder with variable expressivity and a wide clinical variability observed within and between families. Renal dysfucntion is a major cause of morbidity and mortality. It is possible this is a key marker of the cohort at risk of renal decline as adults. Further follow up and evaluation of the adolescent phenotype of ADPKD may sort out which group might benefit from early medical intervention.

Renal Functional Involvement in Bardet-Biedl Syndrome – Largest Ever National Survey from England and Wales

Background: Bardet-Biedl syndrome (BBS) is a rare ciliopathetic autosomal recessive disorder that affects many body systems. BBS has a prevalence of 1 in 140,000 - 160,000 new births. It is a pleitropic disorder with variable expressivity and a wide clinical variability observed within and between families. Renal dysfunction is a major cause of morbidity and mortality. In 2009 the National Commissioning Group for Chronic Conditions set up a national clinical service to provide specialist, organizations and care for these patients. The methods: This national service started in 2010, and now services patients from London and Birmingham. There are very few systematic reports of kidney involvement in BBS, so we have compiled data from these centres for all patients seen in 2010-2011 to produce the largest reported series with special reference to renal functional involvement and urinalysis findings.

Results: 111 subjects (63 men, 48 women) with a mean age of 33 years (range 15 to 56 years) attended the clinics. Three patients were on regular hemodialysis, one on peritoneal dialysis, and five others had functioning renal transplants. Blood samples for renal function were obtained from 98 patients. Plasma creatinine values were 44 to 803 µmol/L, and using the 4 variable MDRD formula the eGFR range was 11 to 90 ml/s/min. The mean GFR was 83 ml/s/min with 44 subjects having an MDRD GFR > 90 ml/s/min. Significant proteinuria (urinary spot ACR/PCR) was evident in 19.4% of subjects, but only 7 subjects had a PCR > 10 (maximum recorded protein loss was 82 mg/mmol). Microscopic haematuria was seen in < 10% of subjects. Mean BP was 140/83 mm Hg; 38% of patients had a SBP > 140 mm Hg and 26% had a DBP > 90 mm Hg. Obesity was common with the mean BMI for these subjects was 37 kg/m².

Conclusions: This is the largest reported series featuring kidney involvement in BBS. About half of the subjects had evidence of kidney disease (within the limitations of single measurements of blood and urine) – around 20% of the patients had significant reduction of GFR, had a transplant, or were on dialysis. Neither heavy proteinuria nor haematuria was a feature of BBS. Funding: Government Support - Non-U.S.
Conclusions: 1. More renal cysts develop in ADPKD than meet the eye by conventional imaging. 2. After a period of extraordinary fetal cyst formation and growth, new cysts appear to form in renal tubules for several years thereafter; 3. Drugs targeting post-partum cyst formation may be beneficial if administered early in the course of the disease.

Funding: NIDDK Support

TH-PO827

Magnetic Resonance Imaging (MRI) to Non-Invasively Measure ARPKD Kidney Disease Progression

Lan Lu, Christopher A. Flusk, Bernadette O. Erokwu, Katherine M. Dell. Deps of Radiology, Biomedical Engineering, Pediatrics and the CWRU Center for the Study of Kidney Disease and Biology, Case Western Reserve University, Cleveland, OH.

Background: Autosomal Recessive Polycystic Kidney Disease (ARPKD) results in kidney failure in 40-50% of children. While some therapies have shown promise in animal models, the lack of methods to monitor kidney disease progression in ARPKD patients has limited the development of therapeutic trials. Newer quantitative Diffusion Tensor Imaging Magnetic Resonance Imaging (DTI-MRI) techniques have the potential to provide non-invasive assessments of kidney disease progression, but have not been studied in ARPKD.

The objective of this study was to develop DTI-MRI methodologies to measure kidney disease progression in the PCK rat model of ARPKD.

Methods: Kidneys from 4 PCK rats were imaged serially at 2, 4, 6 & 8 mos of age using DTI-MRI. Apparent Diffusion Coefficient (ADC) maps were generated, then thresholded to differentiate cystic (higher ADC) from normal tissue. Total cystic area was defined as the cyst pixel number/total kidney pixels. Animals were sacrificed after the last imaging session at 6 mos and histologic scoring for cystic area (% cystic/total parenchyma) of hematoxylin stained paraffin-embedded sections was determined.

Results: Mean cystic area (%) increased at each time point studied (2 mos = 17±11%; 4 mos = 36±9%; 6 mos = 48±7%). Average increase in cystic area over the 4 month study period was 8% per month (range 4–11%), with higher values seen in the 2-4 vs. 4-6 months intervals (10% vs. 6%). There was a strong correlation (r=0.67) between imaged cystic area and cystic area as assessed by histo logic scoring at 6 months.

Conclusions: DTI-MRI provided non-invasive, quantitative measures of kidney disease severity in PCK rats at different time points in the disease. Increased cystic area was found at each successive time point, including the 4 to 6 mo interval, for which we have previously shown histologic progression without significant change in clinical parameters (e.g. kidney weight/body weight or serum creatinine). Further studies are necessary to determine if these methods can be used to monitor kidney disease progression in ARPKD patients.

Funding: NIDDK Support, Other NIH Support - NIH/CTSA

TH-PO828

Cyst Infection in ADPKD: A Critical Analysis of PET-CT

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Background: Cyst infection (CI) remains a complex issue in ADPKD. Positron emission tomography (PET) has emerged as a promising tool for detection.

Methods: A retrospective study was conducted in an ADPKD referral center, including 12 cyst episodes in 9 patients (pts) during 12 months. The diagnosis was definitive (microbiological) in 4 episodes and likely in 8 (fever, abdominal pain, C-reactive protein >50mg/L, absence of intracystic bleed and exclusion of other causes).

Results: Liver CI (LCI) occurred in 8 episodes (6 pts) and kidney CI (KCI) in 4 (4 pts). Pts with KCI were non-significantly younger and had a trend to lower creatinine levels compared to LCI pts (21.5 vs 42.0 mL/min/1.73m2). Four KCI and 2 LCI were associated with positive blood cultures and sepsis, whereas urine culture was positive in 1 KCI. Ciprofloxacin was the first-line antibiotic (ATB) in 8 episodes and association with a third-generation cephalosporin was used in severe presentations (9/12). Initial evaluation included 12 CTs and 1 MRI, suggestive of infection in only 4 cases (3 CTs and 1 MRI). PET-CT was performed in 9 episodes and PET Scan in 2. Total kidney volume (TKV) was non-significantly higher in pts with KCI than LCI while LCI pts showed a trend to higher total liver volume (TLV) than KCI individuals (5516 vs 2043 mL, P=0.063). Seven PET-CT and 2 PET Scan analyses were consistent with CI. Seven cases were submitted to invasive procedures and in 1 a follow-up PET-CT supported a prolonged ATB course. Two deaths occurred in pts with negative PET-CT in whom LCI was diagnosed by autopsy: a kidney Tx and immunosuppressed and a cirrhotic and hemodialytic pt. In both cases PET-CT was carried out on the third week of ATB while in the majority of the other cases it was performed within the first two weeks.

Conclusions: In our casuistic LCI included pts in earlier stages of CKD and outnumbered KCI. Our data suggest that increases in TLV and TKV may raise the risk of LCI and KCI, while the observed high reinfection rate supports that anatomical factors outnumbered KCI. Our data suggest that increases in TKV may raise the risk of CI.

Funding: Government Support - Non U.S.

TH-PO829

Effect of Betaine on Urine Oxalate in Primary Hyperoxaluria, Type I

Lan Lu, Christopher A. Flask, Bernadette O. Erokwu, Katherine M. Dell. Deps of Radiology, Biomedical Engineering, Pediatrics and the CWRU Center for the Study of Kidney Disease and Biology, Case Western Reserve University, Cleveland, OH.

Background: In primary hyperoxaluria, type 1 (PH1) deficiency of hepatic AGT enzyme results in overproduction of oxalate, oxaloxaluria, stones, and kidney failure. Pyridoxine (Vitamin B6) treatment reduces urine oxalate in patients (pts) with certain AGXT genotypes, suggesting a chaperone effect. Betaine partially corrected enzyme activity of the I244T mutation in vitro (Santana 2003). To ascertain effectiveness in PH1 patients, we conducted a double blind, placebo controlled trial of betaine in children and adults with PH1 who carried PH1 mutations.

Methods: Subjects were randomly assigned oral betaine 10 gm (< 10 yrs old) or 6 gm (> 10 yrs old) or lactose placebo twice daily for 2 months, followed by a 2 month washout. Each then received the alternate study medication for 2 months. Usual medications contributing to urinary oxalate were withdrawn (n=7), and citrate (n= 3).

Two 24 hr urine collections were obtained at baseline, and during the eighth week of each study period. Uox was measured by oxalate oxidase.

Results: 10 of 15 enrolled PH1 pts completed the study: 2 withdrew before initiation, 2 were noncompliant, in 1 symptoms led to withdrawal. Mean age was 20.1 yrs (median 18.5, range 6-43 yrs). GFR was 79 ml/min/1.73m2 (median, 92, range 39-134). Uox on betaine was 1.43±0.97 umol/mg creat and differed from placebo 1.04±0.71 by paired t test, P=0.007. Betaine was well tolerated with dyspepsia in some, but no serious adverse effects.

Funding: Government Support - Non-U.S.

TH-PO830

Mutation Screening of the Halt PKD Population

Jamie L. Sundsbak, 1 Christina M. Heyer,1 Sandro Rossetti,2 Robert W. Schrier,3 Arlene B. Chapman,3 Vicente E. Torres,4 Ronald D. Perrone,5 Jared J. Grantham,5 Theodore I. Steinman,6 William E. Braun,7 Kyong Tae Bae,5 Kaleb Z. Abebe,2 James E. Bost,6 Peter C. Harris.5 1Mayo Clinic; 2U Colorado; 3Emory U; 4Tufts U; 5Kansas U; 6Beth Israel Deacconess Med Ctr; 7Cleveland Clinic; 8U Pittsburgh Med Ctr.

Background: The genetic characterization of the HALT-PKD population (1044 cases) provides a comprehensive view of the genetics of this disorder and how genotype correlates with phenotype.

Methods: PKD1 and PKD2 were analyzed using conventional Sanger sequencing, plus MLPA to assay for large rearrangements.

Results: We have sequenced 804 families consisting of 916 subjects (73% PKD1, 14% PKD2, 13% No mutation detected). Mutations were identified in 700 families (84% PKD1, 16% PKD2). Truncating changes made up the largest functional mutation group in both PKD1 (68% truncating, 32% non truncating) and in PKD2 (89% truncating, 11% non truncating). Comparison with the ADPKD Mutation Database Version 2.1 showed 70% were novel.

Several patients with interesting mutation combinations were found: two PKD1 truncating (in cis), a PKD2 nonsense and PKD1 in-frame deletion, and combinations of two likely hypomorphic alleles in cis.

Conclusions: Uox/creat was higher in pts receiving betaine compared with placebo. The mechanism is unclear and merits further investigation. Results suggest that caution is warranted in interpretation of in vitro chaperone effects.

Funding: NIDDK Support, Other NIH Support - ORDR, member of Rare Diseases Clinical Research Network, Pharmaceutical Company Support, Private Foundation Support

TH-PO831

Effect of Betaine on Urine Oxalate in Primary Hyperoxaluria, Type I

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Background: In primary hyperoxaluria, type 1 (PH1) deficiency of hepatic AGT enzyme results in overproduction of oxalate, hyperoxaluria, stones, and kidney failure. Pyridoxine (Vitamin B6) treatment reduces urine oxalate in patients (pts) with certain AGXT genotypes, suggesting a chaperone effect. Betaine partially corrected enzyme activity of the I244T mutation in vitro (Santana 2003). To ascertain effectiveness in PH1 patients, we conducted a double blind, placebo controlled trial of betaine in children and adults with PH1 who carried PH1 mutations.

Methods: Subjects were randomly assigned oral betaine 10 gm (< 10 yrs old) or 6 gm (> 10 yrs old) or lactose placebo twice daily for 2 months, followed by a 2 month washout. Each then received the alternate study medication for 2 months. Usual medications contributing to urinary oxalate were withdrawn (n=7), and citrate (n= 3).

Two 24 hr urine collections were obtained at baseline, and during the eighth week of each study period. Uox was measured by oxalate oxidase.

Results: 10 of 15 enrolled PH1 pts completed the study: 2 withdrew before initiation, 2 were noncompliant, in 1 symptoms led to withdrawal. Mean age was 20.1 yrs (median 18.5, range 6-43 yrs). GFR was 79 ml/min/1.73m2 (median, 92, range 39-134). Uox on betaine was 1.43±0.97 umol/mg creat and differed from placebo 1.04±0.71 by paired t test, P=0.007. Betaine was well tolerated with dyspepsia in some, but no serious adverse effects.

Funding: Government Support - Non-U.S.
Conclusion: The mutation of the PKD1 gene has so far detected probably causal mutations in 37 families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Results: The mutation analysis of PKD1 gene has so far detected probably causal mutations in 57 families. 42 mutations are unique for Czech population. Only the described nonsense mutation p.R420X was detected in 3 nonrelated families and the described missense mutation p.S4189F was detected in two families. Other mutations are unique for individual families. 42 mutations are unique for Czech population.

The mutation analysis of PKD2 gene has so far detected probably causal mutations in 36 families. The frameshifting mutation c.203_204insC was identified in 9 families, nonsense mutations in 15 families and splicing mutations in 5 families. The variants c.2639+1G>A, c.2641+1G>A and c.2871+1G>A were found in 7 families. 14 mutations are unique for Czech population.

Conclusion: 42 new PKD1 mutations and 14 new PKD2 mutations were identified. Next-generation sequencing method is a promising method for mutational analysis of complicated genes.

Funding: Supported by the grant project VZ MSMT 001260806

TH-PO832
PKD Mutation Analysis Using Next-Generation Sequencing in Czech Patients

Patients: The mutation analysis of PKD1 was performed in all probands by direct Sanger sequencing followed by computational analysis and family segregation of potential mutations.

Results: A total of 8 NRC families identified in known genes (TTC21B, NPHP3, NPHP5 and NPHP10) was inherited in 7 families. A total of 10 different NRC variants were identified in 4 families. A total of 7 variants were found in 5 families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Conclusion: The mutation analysis of PKD1 gene has so far detected probably causal mutations in 37 families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Funding: Supported by the grant project VZ MSMT 001260806

TH-PO833
Next-Generation Sequencing Technology for Research and Diagnosis of Ciliopathies

Patients: The mutation analysis of PKD1 was performed in all probands by direct Sanger sequencing followed by computational analysis and family segregation of potential mutations.

Results: A total of 8 NRC families identified in known genes (TTC21B, NPHP3, NPHP5 and NPHP10) was inherited in 7 families. A total of 10 different NRC variants were identified in 4 families. A total of 7 variants were found in 5 families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Conclusion: The mutation analysis of PKD1 gene has so far detected probably causal mutations in 37 families with very-early onset of the disease to detect hypomorphic alleles probably inherited from healthy parents.

Funding: Supported by the grant project VZ MSMT 001260806

TH-PO834
Whole Exome Resequencing Reveals Renal Transporter Mutations May Phenocopy Nephropathies-Related Ciliopathies

Conclusion: To identify novel disease genes, we performed exon-enriched next-generation sequencing of >1000 candidate genes using a customized Agilent SureSelect Target Enrichment library and the SOLID4 (Lifetech) technologies. Candidate genes were compiled from the Cilia Proteome and CiliDB databases and by data mining of cellular and animal models.

Results: We initially searched for mutations in a set of 25 patients for whom obvious candidate genes had been excluded and mapping data were available: 6 JTBs, 4 NPH, 5 JATD, 1 SRP-II, 5 SLS and 4 MKS. We confirmed the presence of BSIBs and BSIB10 mutations in a JTB case as an internal control. Pathogenic mutations were detected in known renal transporter genes (SLC4A1, SLC26A4 and SLC26A7) and several mutations were identified in a novel transporter gene (SLC26A14). A P2 splice site inversus of JTB and BSIBs. In addition, in 3 isolated NPH, JTBs and MKS cases, we found likely pathogenic homozygous or compound heterozygous mutations in 3 genes that have not previously been implicated in any ciliopathy. Functional studies of these mutations are under investigation.

Conclusion: To conclude, this method is a valuable and cost-saving approach to screen a large number of genes in ciliopathies. In addition to provide further insights into disease mechanisms, the identification of a large number of ciliopathy genes is relevant for both fundamental and clinical research.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO835
Renal Consequences of Megalin Deficiency in Humans

Conclusion: To identify novel disease genes, we performed exon-enriched next-generation sequencing of >1000 candidate genes using a customized Agilent SureSelect Target Enrichment library and the SOLID4 (Lifetech) technologies. Candidate genes were compiled from the Cilia Proteome and CiliDB databases and by data mining of cellular and animal models.

Results: We initially searched for mutations in a set of 25 patients for whom obvious candidate genes had been excluded and mapping data were available: 6 JTBs, 4 NPH, 5 JATD, 1 SRP-II, 5 SLS and 4 MKS. We confirmed the presence of BSIBs and BSIB10 mutations in a JTB case as an internal control. Pathogenic mutations were detected in known renal transporter genes (SLC4A1, SLC26A4 and SLC26A7) and several mutations were identified in a novel transporter gene (SLC26A14). A P2 splice site inversus of JTB and BSIBs. In addition, in 3 isolated NPH, JTBs and MKS cases, we found likely pathogenic homozygous or compound heterozygous mutations in 3 genes that have not previously been implicated in any ciliopathy. Functional studies of these mutations are under investigation.

Conclusion: To conclude, this method is a valuable and cost-saving approach to screen a large number of genes in ciliopathies. In addition to provide further insights into disease mechanisms, the identification of a large number of ciliopathy genes is relevant for both fundamental and clinical research.

Funding: Private Foundation Support - ARRA, Private Foundation Support

TH-PO836
Renal Consequences of Megalin Deficiency in Humans

Conclusion: To identify novel disease genes, we performed exon-enriched next-generation sequencing of >1000 candidate genes using a customized Agilent SureSelect Target Enrichment library and the SOLID4 (Lifetech) technologies. Candidate genes were compiled from the Cilia Proteome and CiliDB databases and by data mining of cellular and animal models.

Results: We initially searched for mutations in a set of 25 patients for whom obvious candidate genes had been excluded and mapping data were available: 6 JTBs, 4 NPH, 5 JATD, 1 SRP-II, 5 SLS and 4 MKS. We confirmed the presence of BSIBs and BSIB10 mutations in a JTB case as an internal control. Pathogenic mutations were detected in known renal transporter genes (SLC4A1, SLC26A4 and SLC26A7) and several mutations were identified in a novel transporter gene (SLC26A14). A P2 splice site inversus of JTB and BSIBs. In addition, in 3 isolated NPH, JTBs and MKS cases, we found likely pathogenic homozygous or compound heterozygous mutations in 3 genes that have not previously been implicated in any ciliopathy. Functional studies of these mutations are under investigation.

Conclusion: To conclude, this method is a valuable and cost-saving approach to screen a large number of genes in ciliopathies. In addition to provide further insights into disease mechanisms, the identification of a large number of ciliopathy genes is relevant for both fundamental and clinical research.

Funding: Private Foundation Support - ARRA, Private Foundation Support
Conclusions: Our studies demonstrate that abrogation of megalin expression in man results in urinary loss of numerous filtered compounds including vitamin carriers as observed in megalin knockout mice. Further, normal internalization of cubilin bound ligands is dependent on the presence of megalin, whereas normal kidney development is not. Finally, our study indicates that human filtration of albumin is in the micro-range.

Funding: Private Foundation Support, Government Support - Non-U.S.

TH-PO386
Co-Occurrence of Alport and ADPKD – Early Progression to Renal Failure
Kathy K.Y. Lee-Son,1 Anna M. Lehman,1 Mato P. Nagel,2 Millan Patel,1 Colin T. White.1 BC Children's Hospital, Canada; 2Nephrological Praxis, Germany.

Background: We describe a family with co-inheritance of ADPKD and Alport Syndrome.

Methods:

<table>
<thead>
<tr>
<th>I</th>
<th>II</th>
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<tr>
<td>Alport nephritis</td>
<td>polyzystic kidney disease</td>
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Not all individuals in generation III have been screened for the two disorders.

Results: The mother (II-4) was diagnosed with ESRD from pathology proven ADPKD at age 20 with transplant at 24. Two sons, conceived post-tx, were delivered prematurely: III-6 at 30 wk & 800g; III-7 at 32 wk & 1140g. Both had microscopic hematuria, bilateral echogenic kidneys in childhood & profound sensorineural hearing loss by age 14. III-6 progressed rapidly and was transplanted at age 16. III-7 had bilateral cortical cysts and presumptive diagnoses of PKD1 and Alport based on family history & phenotypic features. At age 18, he has reduced nuclear GFR [83ml/min/1.73m2] & heavy proteinuria on maximal ACE/ARB therapies.

Results: Genetic investigations of mom revealed 2 PKD1 gene variants of unknown significance: R1492H & 1610deld. III-7 shared the 1610deld, but not R1492H. Sequencing of her COL4A5 gene demonstrated a previously undescended variant: c4474delG in exon 47, which leads to premature truncation of the protein. Biopsy of (III-6) showed a basket weave & absent IF for collagen α-3 and α-5, consistent with Alport Syndrome.

Conclusions: This family highlights the effects of multiple factors on the progression of renal disease. The more progression to ESRD in late adolescence from either Alport or PKD1 in isolation would be rare. We postulate the co-occurrence of both a glomerular and tubulocystic disease accelerated her renal deterioration. Although III-7 has both genetic conditions, his brother (III-6) with only Alport Syndrome progressed to ESRD much earlier than he. Given the similarities between both brothers through childhood, we postulate that the extreme prematurity & low birth wt of III-6 resulted in a diminished nephron endowment and a more rapid progression to ESRD despite his inheritance of only one genetic condition.

TH-PO387
COLA3 Founder Mutation in a Novel Turkish-Cypriot Kindred
Thomas Michael Conner,1 Duriye Deren Oygar,2 Daniel P. Gale,1 Konstantinios Vokardes,1 Konstantinios Deltas,1 Guy H. Neild,1 Patrick Maxwell.1 Division of Medicine, University College London, London, United Kingdom; 3Nicosia State Hospital, Nicosia, North Cyprus, Cyprus; 4Department of Biological Sciences, University of Cyprus, Nicosia, Cyprus.

Background: Mutations in COLA3, the gene encoding the α3 chain of type IV collagen, are associated with Alport syndrome and Thin Basement Membrane Nephropathy (TBMN). Recent studies in the Greek-Cypriot population have highlighted a number of mutations in COLA3. These mutations were associated with both TBMN and FSGS on biopsy, and with a high incidence of end-stage renal disease. These mutations were thought to originate from founder mutations in that population. We investigated a large Turkish-Cypriot pedigree from the Kyrenia district of North Cyprus that segregated microscopic hematuria, mild proteinuria, occasional renal cysts, and variable degrees of renal impairment. There were no extra-renal signs in any of the affected individuals.

Methods: DNA was isolated using the QIAamp DNA blood mini kit. SNP genotyping was performed using the Illumina 300k chip and linkage analysis with easyLINKAGE. Tetra-primer PCR was designed to confirm the presence of known mutations. Mutations were confirmed by direct sequencing.

Results: Linkage analysis gave a LOD score of 4.5 in association with a 0.5Mb region at 2q36-7. Testing for known mutations of COL4A3/4 demonstrated co-segregation with the G871C mutation in COL4A3.

Conclusions: This is the first description of familial nephropathy in the Turkish-Cypriot population associated with mutations in COLA3. This demonstrates that this mutation occurs in both Greek and Turkish-Cypriot populations and confirms that it is sufficient to cause end-stage renal disease. Haplotype analysis will allow us to more accurately determine the origin of the mutation.

Funding: Government Support - Non-U.S.

TH-PO388
Exonic Mutations Associated with Hereditary Renal Diseases Can Result in Major Alterations in the mRNA

Background: The pathogenicity of missense and synonymous mutations is generally assumed to result from the predicted effect on the reading frame and protein function. However, it is now clear that an unexpectedly large fraction of exonic mutations could be pathogenic by affecting pre-mRNA splicing. The present study investigated the impact on splicing of missense and synonymous mutations previously found in the CLDN16, SLC12A1, CLCNKB, PKD1 and PKD2 genes of patients with familial hypermagnesemia with hypercalciuria, Bartter syndrome, and autosomal dominant polycystic kidney disease, respectively.

Methods: Bioinformatics analyses were used to predict the effect of mutations on mRNA splicing. The effect of mutations was tested experimentally by using splicing reporter minigene assays and mRNA quality analyses. Analysis of RNA from transfected kidney-derived cell lines was performed by RT-PCR.

Results: Several mutations were predicted to disrupt pre-mRNA splicing by abolishing splice sites or creating new ones, or by inactivating or generating exonic splicing enhancers or silencers. We showed that these mutations induce different mRNA defects; SLC12A1 mutation D648N leads to the incorporation of a truncated exon 14 in the mature mRNA, resulting in frameshift and predicted premature protein termination; CLDN16 mutation G198A results in an mRNA that lacks exon 4; mutations R1491L and L151F cause inclusion of a truncated exon 3 in the CLDN16 mRNA; mutations D511V and L572L of PKD2 result in skipping of exons 6 and 7, respectively; and PKD1 mutation G109G leads to the incorporation of a truncated exon 3 in the mRNA.

Conclusions: Our results demonstrate that some renal disease-causing mutations, initially considered as missense or synonymous, induce splicing defects, resulting in major alterations in the mRNAs. We propose that these mutations are reclassified as splicing mutations. Assays for the effects of the mutation on mRNA splicing should be included in the routine analysis of these pathogenic mutations.

This work was supported by grant FIS PI10/037 and FUNCIS PI17/09.

Funding: Government Support - Non-U.S.

TH-PO389
Structural-Functional Relationships of Fabry Nephropathy
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Background: Renal failure is a major complication of Fabry disease (FD). In order to understand FD nephroruphy progression mechanisms, we studied relationships between glomerular structure and renal function in FD patients across wide range of age and renal function.

Methods: Renal biopsies from 25 (male) (female) (F) 14-11) FD patients were studied using electron microscopy stereoelogy. Results were correlated with proteinuria, GFR and age.

Results: F were 14[8-65], median [range] and M were 19 [4-57] years old. M and F were not different for proteinuria. GFR, volume fraction of GL-3 inclusions per mesangial [Vv(Incl/Mes)], or endothelial cells [Vv(Incl/Endo)]. Volume fraction of GL-3 inclusions per podocytes [Vv(Incl/PC)] was greater in M (0.38±0.11) vs F (0.25±0.13), p<0.01. In all FD patients, there was a direct relationship between age and proteinuria (r=0.5, p=0.008), FPW (r=0.70, p=0.001). Vv(Incl/Mes) (r=0.64, p<0.0001) and Vv(Incl/Endo) (r=0.67, p=0.0001). FPW was correlated with Vv(Incl/PC) (r=0.55, p=0.02). Vv(Incl/Mes) and Vv(Incl/Endo) were strongly correlated (r=0.96, p=0.0001). There was negative correlation between age and GFR (r=-0.60, p=0.02). Proteinuria was correlated with FPW (r=0.64, p<0.006), Vv(Incl/PC) (r=0.56, p=0.02), Vv(Incl/Endo) (r=0.57, p=0.01), and Vv(Incl/Mes) (r=0.56, p=0.02). None of the structural parameters were correlated with GFR. While there was no relationship between age and Vv(Incl/PC) (r=0.03, p=0.68) in the entire cohort, in young patients (age=20), Vv(Incl/PC), and not Vv(Incl/Mes) or Vv(Incl/Endo) was strongly correlated with age (r=0.78, p=0.001).

Conclusions: We demonstrated novel relationships between glomerular structure and proteinuria in FD patients across a wide range of age and renal function. Our studies indicate that structural-functional relationships of FD nephropathy may be different in different age groups, perhaps reflecting a difference in progression of various lesions in initial vs late stages of the disease.

Funding: Other NIH Support - grant, Pharmaceutical Company Support

TH-PO390
The Frequency of Fabry Disease with the α-Galactosidase A E66Q Variant in Japanese Dialysis Patients

Background: Fabry disease (FD) is an X-linked disorder resulting in a deficiency in the α-galactosidase A (α-Gal) enzyme activity. FD is one of the causes of progressive renal dysfunction, but its diagnosis is often either delayed or missed. Its frequency has been reported to be much higher in dialysis patients, and recent new screenings have revealed a high incidence of later-onset FD mutations, including renal variants.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
Underline represents presenting author.

305A
Methods: We first screened the α-Gal activity in the plasma of 892 Japanese hemodialysis patients. When the enzyme activity was below 6.4 μU/ml, the test was defined as positive. Next, α-Gal activity was measured from dried blood spots via a fluorescence assay using 4-methylumbelliferyl. Then, online databases were searched to identify any previous screening studies for Japanese FD patients undergoing HD. We further identified the mutation in a total of 42 patients (6.6%) (1% males and 33% females), including those in our present study. Overall, the prevalence of FD was 0.50% in male (6 studies), and 0.23% in female HD patients (4 studies). Notably, the frequency of the E66Q variant was 33.3% (6 of 18 patients) of Japanese FD on HD. The classical manifestations of FD were not observed in any of these patients with E66Q; however, most of them had either cardiac or cerebrovascular involvement. Other mutations included M291L, V365X, M296L, Q357X, A97V, G373D, A288A and A254V.

Results: Our results demonstrate that the later-onset disease phenotype with the E66Q variant is more frequent in Japanese FD patients on HD than has been previously reported in other countries. Whether α-Gal is a pathogenic mutation, or just a variant that will need to be determined in further investigations.

TH-P0841
UMOD Mutations in the Chronic Kidney Disease Population in Taiwan
Lim Lee Moay,1 Chih-Chuan Yu,1 Daw-Yang Hung,1 Chi-Chih Hung,1 Shang-Jyh Hwang,2 H.C. Chen.1,2 Division of Nephrology, Department of Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 3Faculty of Renal Care, College of Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 4Graduate Institute of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background: Taiwan has the highest incidence and prevalence rate of end-stage renal disease (ESRD) and also high incidence and prevalence of chronic kidney disease (CKD) according to the USRDS report. The cause of this high incidence and prevalence are multi-factorials. Genetic factors are believed to be one of them. However, there is no data concerning the prevalence of medullary cystic kidney disease (MCKD) and UMOD gene mutation in CKD population in Taiwan.

Methods: We selected 160 patients, irrespective of family history, from the Kaohsiung Medical University Hospital CKD Care Program based on the criteria with uric acid level higher than 8 mg/dL for male and 7.5 mg/dL for female. Since more than 90% of ADPKD patients were identified with urinary abnormalities, we determined the frequency of ADPKD by urinalysis. In our present study, overall, the prevalence of ADPKD was 0.50% in male (6 studies), and 0.23% in female HD patients (4 studies). Notably, the frequency of the E66Q variant was 33.3% (6 of 18 patients) of Japanese FD on HD. The classical manifestations of FD were not observed in any of these patients with E66Q; however, most of them had either cardiac or cerebrovascular involvement. Other mutations included M291L, V365X, M296L, Q357X, A97V, G373D, A288A and A254V.

Conclusions: Our results demonstrate that the later-onset disease phenotype with the E66Q variant is more frequent in Japanese FD patients on HD than has been previously reported in other countries. Whether α-Gal is a pathogenic mutation, or just a variant that will need to be determined in further investigations.

TH-P0842
How Nonsense Mutations in the COL4A4 Gene Cause Autosomal Recessive Alport Syndrome and Thin Basement Membrane Nephropathy
Vanessa Sivakumar, Yanyan Wang, Mardhiah Binti Mohammad, Hayat Dagher, Judith A. Savige. Department of Medicine, University of Melbourne, Melbourne, VIC, Australia.

Background: Autosomal recessive Alport syndrome results from homozgyous or compound heterozygous mutations in the COL4A3 or COL4A4 genes. Nonsense changes account for 10% of all mutations. In other collagen diseases, nonsense mutations have been shown to account for 10% of all mutations. In other collagen diseases, nonsense mutations have been shown to account for 10% of all mutations. Nonsense mutations in autosomal recessive Alport syndrome and Thin basement membrane nephropathy contribute to tubular cell death followed by interstitial fibrosis. The UMOD mutation contributed a relative small portion for the cause of ADPKD kidney tissues compared with normal control, ranging from upstream 1 kb to intron 1 region. As expected, hypermethylation in promoter region of MUPCDH was also observed in cystic patient kidney tissues compared with normal. On the contrary, its expression was markedly decreased in ADPKD patients. Demethylation of MUPCDH gene promoter by treatment with DNMT inhibitor such as 5-aza-2'-deoxycytidine induced up-regulation of MUPCDH mRNA level.

Conclusions: In conclusion, MUPCDH promoter CpG islands were significantly hypermethylated in ADPKD patients and it is negatively correlated to its reduced expression level.

Funding: Government Support - Non-U.S.

TH-P0843
Correlation of Phenotype and HOGA1 Variants in Obligate Heterozygotes from PHIII Families
Carla G. Monaco, Andrea G. Cogal, Julie B. Olson, Barbara M. Seide, Dawn S. Milliner. Division of Nephrology, Mayo Clinic, Rochester, MN.

Background: Recently, we described primary hyperoxaluria type III (PHIII), due to mutations in HOGA1, and identified hypercalciciuria and hyperuricosuria in addition to hyperoxaluria in some patients. We also detected heterozygosity for HOGA1 variants in 2 patients with mild hyperoxaluria and in 3/100 idiopathic calcium oxalate stone formers.

Methods: To further characterize the phenotype in HOGA1 heterozygotes, we performed segregation analysis in all available first-degree relatives of PHIII probands. Analysis included 1 new and 9 previously ascertained unrelated PHIII families.

Results: Mild hyperoxaluria was observed in only 1 obligate heterozygote harboring 1 of the 2 most common variants (IVS700+5 G>T and c.944_946 del AGG) mutations. However, hypercalciciuria and hyperuricosuria were detected in some c.944_946 del AGG and IVS700+5 G>T heterozygotes. And both mild hyperoxaluria and hyperuricosuria were observed in some R>C missense variant heterozygotes.

Association of mild or intermittent hyperoxaluria, hypercalciciuria or hyperuricosuria in obligate heterozygotes with HOGA1 variants.

Funding: NIDDK Support NIH ORD, member Rare Disease Clinical Research Network.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
TH-PO845

Targeted Exome Sequencing and Homozygosity Mapping Identify Mutation of EMP2 as a Cause of Steroid Sensitive Nephrotic Syndrome

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1Department of Pediatrics and of Human Genetics, University of Michigan, Ann Arbor, MI; 2Department of Pediatrics, Harbor-UCLA Medical Center, Torrance, CA; 3Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.

Background: Idiopathic nephrotic syndrome is a common pediatric kidney disease. 80% of all cases are steroid sensitive (SSNS). Whereas gene identification has furthered the understanding of pathomechanisms in steroid-resistant nephrotic syndrome (SRNS), disease mechanisms of SSNS remain unknown. To identify single-gene causes for SSNS, we combined homogygosity mapping, whole human exome capture and consecutive massively parallel re-sequencing.

Methods: In two siblings (of consanguineous parents) with SSNS, homogygosity mapping yielded 4 segments of homogygosity by descent with a cumulative genomic length of ~55 Mb. We performed whole human exome capture in one sibling using NimbleGen SeqCap EZ Exome™ V2 protocol. Sequencing was performed using Illumina Genome Analyzer II. We detected 1 homozygous missense mutation (G173V) in ARHGDA in 1 of the affected siblings.

Results: Homozygosity mapping showed 5 homogygous candidate regions, confirming the history of consanguinity. We performed whole exome capture using the NimbleGen SeqCap EZ Exome™ V2 protocol. Sequencing was performed using Illumina Genome Analyzer II. We detected 1 homozygous missense mutation (G173V) in ARHGDA in 1 of the affected siblings.

Conclusions: Because the Arhgdia loss-of-function mouse phenotype recapitulates the human DMS phenotype, our finding of ARHGDA mutation very likely represents a new single-gene cause of early-onset NS.

Funding: Other NIH Support - Doris Duke Charitable Foundation

TH-PO846

Dominant Mutations Cluster in the Signal Peptide of Renin and Emphasize a Role for Renin in Erythropoiesis

Bodo B. Beck,1 Howard Trachtman,2 Friedhelm Hildebrandt,1,3 Matthias Tilmann,1,3 Wolf F.1

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Background: Renin is an important hormone regulating blood pressure and renal sodium reabsorption via the renin-angiotensin system. Homozygous or compound heterozygous Renin (ren) mutations cause renal tubular dysgenesis, which is characterized by death in utero due to renal failure and pulmonary hypoplasia. The phenotype resembles the fetopathy caused by angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) intake during pregnancy. Recently, heterozygous REN mutations were shown to result in early-onset hyperuricemia, anemia and chronic kidney failure. So far, only three different heterozygous ren mutations have been reported.

Methods: We performed mutation analysis of the ren gene in 39 kindreds with hyperuricemia and chronic kidney disease (CKD) previously tested negative for mutations in the UMOD and HNF1ß genes.

Results: We identified a novel c.78C (p.W10R) ren mutation in the signal sequence, affecting individuals over four generations. Patients carrying the novel ren mutation were characterized by significant anemia, hyperuricemia and CKD. We report the youngest patient with a heterozygous ren mutation so far, showing anemia, hyperuricemia and impaired renal function as early as 11 months of age. Documented anemia is severe in patients with heterozygous ren mutations and disproportional to the degree of renal impairment.

Conclusions: We highlight the possible mechanisms of heterozygous ren mutations causing anemia. Moreover all heterozygous ren mutations are localized in the signal sequence. We conclude that ren mutations are rare events in CKD patients and cause a clinical syndrome that resembles Uromodulin associated kidney disease. Screening of the ren gene should be considered for CKD patients with hyperuricemia and anemia, focusing on exon 1, sequencing, which encodes the signal peptide.

Funding: Government Support - Non-U.S.

TH-PO847

Whole Exome Capture Reveals Mutation of ARHGDA as Causing Nephrotic Syndrome

Pawarre Saisawat,1 Virginia Vega-Warner,1 Shazia Ashraf,1,2 Yaacov Frishberg,2 Toby W. Hurd,1 Sivakumar Natarajan,1 Friedhelm Hildebrandt.1,3

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Background: Diffuse mesangial sclerosis (DMS) is a rare histologic variant of early-onset nephrotic syndrome (NS). In our multicohort of 71 DMS patients, 26 (37%) are caused by mutations in WT1, NPHS1, NPHS2 or PLCE1 while the majority of cases are still unexplained. To identify a new causative gene for DMS, we employed homogygosity mapping (Hildebrandt et al. PLoS Genet 5: e1000355, 2009) with consecutive whole exome capture (WEC) and massively parallel exon re-sequencing.

Methods: The parents of 3 siblings who were affected with early-onset NS that rapidly progressed to end-stage renal failure were first cousins of Ashkenazi Jewish background. Renal biopsy was carried out in one child and revealed DMS. WT1, NPHS1, NPHS2 and PLCE1 were screened and were mutation negative.

Results: Homozygosity mapping showed 5 homogygous candidate regions, confirming the history of consanguinity. We performed whole exome capture using the NimbleGen SeqCap EZ Exome™ V2 protocol. Sequencing was performed using Illumina Genome Analyzer II. We detected 1 homozygous missense mutation (G173V) in ARHGDA in 1 of the affected siblings.

Conclusions: Because the Arhgdia loss-of-function mouse phenotype recapitulates the human DMS phenotype, our finding of ARHGDA mutation very likely represents a new single-gene cause of early-onset NS.

Funding: Other NIH Support - Doris Duke Charitable Foundation

TH-PO848

INF2 Mutations as a Cause of Familial Versus Sporadic FSGS

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Background: Mutations in INF2, a member of the formin family of actin binding proteins, have been shown to cause autosomal dominant focal segmental glomerulosclerosis (FSGS). We previously described nine missense mutations in exons 2-4 of INF2 in 11/93 families with autosomal dominant inheritance of FSGS. We did not identify any disease-associated mutations outside of this region.

Methods: Genomic DNA was extracted from blood or saliva using standard methods. Using Sanger sequencing, we sequenced PCR amplified segments containing either exons 2-5 or exon 4 alone of INF2 in an additional 294 individuals with familial or sporadic FSGS. We sequenced exons 2-5 in all available family members of an individual in whom we found a variant. Clinical information was obtained from questionnaires and phone discussions with patients and/or their doctors.

Results: We identified 9 missense mutations in INF2 in 10 additional families with autosomal dominant FSGS. We did not find INF2 mutations in individuals with sporadic FSGS. Several of the mutations have been published previously (R214C, R218Q, R218W) while others are novel (H158D, G735S, V181G, R177C, C151R, L181F). All of the mutations are possibly or probably damaging by PolyPhen2 analysis. All of the mutations segregate with disease. There are multiple individuals with end-stage renal disease and renal transplants within this new cohort, however, the penetrance is variable. In the H158D family, all 6 individuals in our study carry the mutation and have manifested ESRD. Whereas, in the R214C family, several individuals carrying the mutation are unaffected. Proteinuria was diagnosed in most of this cohort with INF2 mutations in their teens or 20s with ESRD developing in the third or fourth decade of life. No recurrent disease has been noted in renal transplant recipients.

Conclusions: We and others have shown that INF2 mutations are a significant cause of autosomal dominant FSGS. INF2 mutations do not appear to common in individuals with sporadic FSGS suggesting a different pathologic mechanism for the two forms of the disease.

Funding: Other NIH Support - K12 Institutional grant from CHRC, Private Foundation Support

TH-PO849

New INF2 Mutations in a Large Cohort with Sporadic and Hereditary FSGS and Further Evidence for Variable Expressivity

Rashheed A. Gibadegesin,1 Peter J. Lavin,2 Gentzon Hall,3 Alison Homstad,1 Guanghong Wu,2 Alison Byrd,2 Michelle P. Winn.2 1Pediatrics and Center for Human Genetics, Duke University, Durham, NC; 2Medicine and Center for Human Genetics, Duke University, Durham, NC.

Background: Focal and segmental glomerulosclerosis (FSGS) is a major cause of end-stage kidney disease. Recent advances in molecular genetics have provided evidence that defects in the podocyte play a major role in the pathogenesis of FSGS. Mutations in inverted formin 2 (INF2) were recently identified as a cause of autosomal dominant (AD) FSGS. The identification of INF2 mutations in familial and sporadic FSGS, we screened for INF2 variants in a large cohort with FSGS. The study had a secondary objective of defining a rational approach for genetic screening in families with AD FSGS.

Methods: We identified 2 individuals with FSGS. There were 31 (51.6%) individuals with idiopathic disease. The remainder of individuals were from 64 families, 15 (15.8%) individuals from autosomal recessive kidney dysrhythmia and 49 (51.6%) individuals from AD FSGS. We found missense mutations in INF2 in 8/49 (16%) of families with AD FSGS. The remaining 22 patients had AD FSGS and FSGS in 40 of the mutations were confined to exon 1 of INF2. Mutations in exon 4 of INF2 are responsible for 90% of all mutations reported in FSGS due to INF2 mutations.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: In conclusion, INF2 mutations are responsible for 16% of all cases of AD FSGS, and mutations are clustered in exons 4 and 2, therefore screening for mutations in INF2 may represent a rapid, non-invasive and cost-effective method for the diagnosis of AD FSGS.

Funding: NIDDK Support, Private Foundation Support

TH-PO850

Genetic Backgrounds in Patients with Glomerulopathy with Fibronectin Deposits 1Hiromi Ohtsubo, 1Fusako Hashimoto, 1Shingo Ishimori, 1Takeshi Ninchi, 2F. Xuejun, 2Yuya Hashimura, 1Hiroshi Kaito, 3Naoya Morisada, 1Noriko Uesugi, 1Kazumoto Iijima. 1Pediatrics, Kobe University Graduate School of Medicine, Kobe, Japan; 2Institute of Basic Medical Sciences, University of Tsukuba, Japan.

Background: Glomerulopathy with fibronectin deposits (GFND) is a rare autosomal dominant glomerular disease associated with the massive deposition of fibronectin, caused by mutations of fibronectin 1 gene (FN1). There are currently few reports about genetic backgrounds in patients with GFND, and little is known about the clinical course. Our purpose in this study is to clarify the genotypes of FN1 and the clinical features of GFND patients.

Methods: This study was designed for GFND patients and their family members. All patients underwent renal biopsies and were definitely diagnosed as GFND by renal histological findings. Genomic DNA was isolated from peripheral blood leukocytes after informed consent. The mutation analysis was carried out by direct sequencing of FN1.

Results: Seven (1 male and 6 females) patients and twelve family members were enrolled in this study. There were 2 cases with clinical renal manifestations in family members; one had persistent proteinuria, and the other was end stage renal failure for unknown reasons. All of the 7 patients had heterozygous mutations of FN1; four of them had p.V973C, which was previously reported located in hepalin-binding domain, and the other three had novel p.(W192S, p.1472del, p.I1974P). One of the novel mutations, p.1472del, located in integlin-binding domain, which play major roles in assembly of FN as well as hepatlin-binding. There were six family members with heterozygous mutations of FN1; two of them had clinical renal manifestations, and the other four didn’t at all.

Conclusions: We could detect heterozygous mutations of FN1 in all of the patients, including the affected family members. Three novel mutations could be detected in this study, and furthermore this is the first report that demonstrated GFND can develop by FN1 mutation in integlin-binding domain. It is of much interest that some individuals with the same FN1 mutations as patients showed no renal manifestations, despite of comparatively late age.

TH-PO851

Two Novel Homozygous SLC22A9 Mutations Cause Renal Hypouricemia Type 2 1Deann Dinour, 2Nicola K. Gray, 3Liat Ganon, 4Andrew J.S. Knox, 5Susan Campbell, 6Lindsay Sawyer, 7Daniel Landau, 8Alan F. Wright, 9Elizer J. Holtzman. 1Sheba Medical Center and Tel Aviv University, Israel; 2University of Edinburgh, United Kingdom; 3Centre for Reproductive Biology, Edinburgh, United Kingdom; 4Institute for Systems and Molecular Medicine, College Dublin, Ireland; 5MRC Human Genetics Unit, Western General Hospital, Edinburgh, United Kingdom; 6School of Biological Sciences, University of Edinburgh, United Kingdom; 7Soroka University Medical Center, Israel.

Background: Elevated serum uric acid is associated with gout, hypertension, cardiovascular and renal disease. Hereditary renal hypouricemia type 1 (RHUC1) is caused by mutations in the renal tubular uric acid transporter URAT1, and can be complicated by nephrolithiasis and exercise-induced acute renal failure. We have recently shown that loss-of-function homozygous mutations of another uric acid transporter, GLUT9, cause a severe type of hereditary renal hypouricemia with similar complications (RHUC1).

Methods: Two unrelated families with renal hypouricemia were clinically characterized. DNA was extracted and SLC22A12 and SLC2A9 coding for URAT1 and GLUT9, respectively, were sequenced. Transport studies in Xenopus laevis oocytes were utilized to evaluate the function of GLUT9 mutations found. A molecular modeling study was undertaken to structurally characterize the effects of these mutations.

Results: Two novel homozygous GLUT9 nonsense mutations were identified: R171C and T125M. Mean serum uric acid levels of four affected patients had p.V973C, was previously reported located in hepalin-binding domain, and the other three had novel p.(W192S, p.1472del, p.I1974P). One of the novel mutations, p.1472del, located in integlin-binding domain, which play major roles in assembly of FN as well as hepatlin-binding. There were six family members with heterozygous mutations of FN1; two of them had clinical renal manifestations, and the other four didn’t at all.

Conclusions: Two novel homozygous GLUT9 mutations cause renal hypouricemia. Our findings confirm the pivotal role of GLUT9 in uric acid transport and highlight the similarities and differences between RHUC1 and RHUC2.

Funding: Government Support - Non-U.S.

TH-PO852

Evaluation of an Interactive, Visual On-Line Tutorial To Improve Uric Microscopy Teaching 1Craig E. Gordon, 2Jessica Gray, 3John R. Heinrick, 4Maya Faym, 5Laurence H. Beck. 1Renal Section, Department of Medicine, Boston University School of Medicine, Boston, MA; 2Boston University School of Medicine, Boston, MA.

Background: The evaluation of urine sediment is one of the oldest non-invasive clinical tools used to diagnose renal and systemic pathology. We developed an online module for uric microscopy training and assessed its efficacy as a learning tool.

Methods: To evaluate the tutorial, we created a ten-item multiple-choice pre and post-tutorial examination comprising questions focused on image identification, fact, and case-based questions, as well as a curriculum assessment tool using a five-point Likert scale. We hypothesized that pre-test results would be higher than post-test results, and that performance on image-based questions would improve more than other item types. Data were analyzed using a two-sample T test.

Results: Second year medical students accessed the tutorial and pre- and post- examination scores were compared throughout the renal module of the Disease and Therapy course. Of 183 enrolled students, 30 (16%) completed both pre- and post-tests. Mean score on the pre-test was 47% [95% CI, 41-54%] and 63% [95% CI, 56-70%] on the post-test, p-value < 0.001. Post-tutorial score on image identification items increased by 26% (p < 0.001) but did not increase significantly for other question types. Nine students completed the curriculum assessment and reported improved understanding of urine microscopy and that the tutorial was a valuable educational tool which they would recommend to future enrollees.

Conclusions: This novel urine microscopy tutorial is an effective learning tool for teaching second year medical students. Post-tutorial examination scores were higher in general, but significantly for questions requiring image identification. Student evaluations of the tutorial were largely positive. The generalizability of these findings is limited by few students completing the pre- and post-tutorial examinations and curriculum assessment.

TH-PO853

Development of a Validated Nephrology Clinic Letter Fellow Competency Assessment 1John D. Mahan, 2David S. Hains, 3Hiren P. Patel. Pediatric Nephrology, Nationwide Children’s Hospital/OSU, Columbus, OH.

Background: Competency assessment of nephrology fellows in training should promote acquisition of essential skills, be judged practical by faculty and be relevant to fellows. We developed and tested a Pediatric Nephrology Fellow Clinic Letter Competency Assessment (CLCA) of 8 items deemed essential for an effective clinic letter (by consensus of the Pediatric Nephrology Division) to address the ACGME competency for interpersonal and Written Communications. Fellows compose their letters after clinic visits; supervising faculty may provide feedback on the letter before it is finalized. Each starting fellow receives a copy of the CLCA form. 3 letters are randomly chosen and assessed every 6 months/fellow and results discussed at each fellow’s semi-annual review.

Methods: For this study, 3 faculty independently evaluated 36 randomly selected letters distributed over 24 months from the entire set of >1000 letters constructed by the 5 fellows each year. CLCA was completed per letter by each faculty member and data analyzed for (1) inter-rater reliability (IRR) for each item and for the total score for each letter, (2) intra-class correlation (ICC) and (3) class differences by ANOVA and t tests.

Results: Faculty found the CLCA tool easy to use; specific comments were added for 40% of letters. IRR was acceptable for individual elements (0.41) and good for total score. ICC Coefficient for total score/letter was good at 0.637. CLCA total scores increased per yr of training (ANOVA, p = 0.057). Fellows in 3rd yr (mean 7.79+/-.025) scored better than 1st yr (mean 7.19+/-.0674), p = .0291, consistent with increasing competency over time.

Conclusions: The Pediatric Nephrology Fellow Clinic Letter Competency Assessment helped direct and measure increasing competency by fellows. The CLCA tool had strong validity, good inter-rater reliability, and could be used in adult and pediatric programs. Implementation of validated methods for competency assessment can assist fellowship program directors and faculty in developing important skills in trainees. Additional practical and useful competency assessment methods offer the opportunity to more intentionally direct fellow training.

TH-PO854

Case Based Debates- An Innovative Teaching Tool Arun Chawla, Kenar D. Jhaveri. Nephrology, Hofstra North Shore LIJ School of Medicine, NY.

Background: There has been a growing concern about the decline in interest in nephrology as a career amongst medical students and residents. At our institution we have continuously worked to develop unique ways of teaching nephrology, which are meant not only to attract medical students and residents to the field of nephrology but also contribute directly towards enhance learning and training. Hereby we present a case based teaching tool which we have used to teach transplant pathology “Case Based Debates”.

Methods: A faculty member chooses a challenging case (transplant related) and shares brief history and labs with the fellows in advance. Fellows are equally divided into two teams. Each team has a faculty member (gold card/lifeline) who can be used, at the most, twice during the whole debate. Fellows are given the case and formulate a differential diagnosis and come up with a diagnostic plan involving minimum number of tests leading them to most likely diagnosis. Investigations/questions (like most relevant Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only)
history questions, pertinent lab data, donor specific antibodies, viral serologies etc.) are rewards to their relative importance (defining at least number and most relevant tests and thereby earning more points, the team that comes closest to correct diagnosis is then asked to predict the biopsy findings. Next, the pathology slides are shown which fetches bonus points to the team who reads them accurately.

Results: The above teaching tool has been an instant hit amongst the fellows and residents. It has also been well received, most participated and the most appreciated teaching tool at the “faculty development workshop” at our institution.

Conclusions: We hope to introduce this tool to teaching of not only transplant pathology but also to other common and less common renal diseases, but it does not take the place of a trained and experienced clinician. “Googling” (65.2% vs. 76.1%, p=.014), but more often than the fellows (65.2% vs. 56.2%, p=.029). Poster presentations were listed by 115 applicants (74.2%), with the majority (84.6%) presented at regional/national society meetings and the remainder displayed during institutional events. In contrast, oral presentations listed in ERAS were more likely to be delivered in an institutional venue (56.2%) such as journal club or noon conference rather than at regional/national meetings. “Other articles,” such as student publications and ongoing projects were listed by 17 applicants (11%).

Conclusions: The publications category in ERAS allows for heterogeneous documentation, and similar number of total “publications” represents different levels of academic success among applicants. By highlighting quantity rather than quality, the current system for categorizing publications in ERAS obscures the ability of program directors to compare applicants based on academic productivity.

TH-PO858
Physician Role in Chronic Kidney Disease Patient Education: Perspectives from Nephrology Trainees
Julie A. Wright, Melinda A. Coston, Talat Alp Ikizler, Kerri L. Cavanaugh.
Vanderbilt University Medical Center, Nashville, TN.

Background: Chronic kidney disease (CKD) education is an important component of patient care, yet little is known about nephrologists’ perspectives on their own role in the patient education process. We performed structured interviews of nephrology trainees to gain insight on this topic.

Methods: Interviews of nephrology fellows were performed at one academic institution. Through moderated sessions, participants were asked a series of open ended questions about the role of physicians in patient CKD education. Transcripts were analyzed using content analysis.

Results: Seven out of 16 nephrology fellows (47%) participated. Mean (SD) age was 31 (2) years, 29% were female, 29% were Black, 29% Asian, and 42% White. Participants emphasized disease education as one of the most important roles of the physician. Patient education was viewed as separate from ‘taking care of patients’ and ‘patient management’. 40% of all statements focused on barriers to providing education. Limited patient understanding of medical information was perceived as a barrier by every participant. Other barriers included physician time constraints, complexity of CKD diagnoses, and cultural differences, including differing levels of patient formal education attainment. Facilitators included support staff, face-to-face communication, and written materials. Only 8% of statements discussed expectations physicians had of patients to learn more about CKD, and mainly centered on expecting patients to ask questions on topics about which they were unclear. Participants stressed a need for more multi-disciplinary, multi-media approaches using direct (e.g. face-to-face, classroom) and indirect (internet, literacy sensitive handouts) mechanisms of communication, to optimize existing patient education.

Conclusions: Educating patients about CKD is complex and perceived as a very important role by nephrology trainees. Of high importance to trainees was development of concise, clear, and culturally sensitive communication aids for physicians to use during patient encounters to facilitate optimal CKD patient education.

Funding: NIDDK Support, Other NIH Support - T32 DK07569, Private Foundation Support

TH-PO859
Effect of Multidisciplinary Pre-Dialysis Education in Advanced Chronic Kidney Disease Patients Assessed by Propensity Matched Pair Analysis
Fun Jin Cho,1 Yun Kyu Oh,2 Ki Young Na,1 Ho Jun Chin,1 Curie Ahn,1 Kook-Hwan Oh.3 1Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; 2Internal Medicine, Seoul national University Boramae Hospital; 3Internal Medicine, Seoul National University Bundung Hospital.

Background: The mortality and morbidity of end-stage renal failure patients remains high despite recent advances in pre-dialysis care. Previous studies suggested a positive effect on the patient survival and other outcomes for those receiving multidisciplinary pre-dialysis education (MPE). However above studies were limited by unmatched comparisons between the MPE recipients and non-recipients.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
Underline represents presenting author.
Health Literacy among Patients Referred to a Chronic Kidney Disease Clinic

Dana Rizk,1 Matt J. Glathar,2 Rosalind M. Peters,1 Jane S. Davis.1

Background: In 2004 the Institute of Medicine Report highlighted that 90 million Americans have suboptimal literacy skills. Despite the high prevalence of chronic kidney disease (CKD) little is known about the health literacy level in that population or its impact on clinical outcomes. The goal of our study was to determine the health literacy level among patients referred to the CKD clinic at our institution.

Methods: We recruited 37 consecutive patients on their first visit to the clinic. All patients had prior follow-up by a nephrologist. We collected demographic and clinical data on each subject and administered a 34-item questionnaire assessing the patient’s understanding of CKD, its symptoms, causes and risk factors for progression.

Results: The mean patient age was 58.6±15.5 years. Most patients were female (62.2%), white (54.1%), insured (97.3%) and had high school graduates (83%). At referral 29.7%, 46% and 21.6% had stages III, IV and V CKD, respectively. Although 97.3% of patients achieved an understanding of CKD, understanding edema, 43.2% could name its etiology. About 35.2% and 48.7% were unfamiliar with the terms “creatinine” and “glomerular filtration rate” respectively with no difference between those with <6 vs. ≥6 months of nephrology follow up. Although hypertension and diabetes were identified as modifiable risk factors for CKD, only 46% of hypertensives could name their target blood pressure and 40% of diabetics knew their target HgbA1C. Medication intake was significantly lower than prescribed (9.5± 4.8 vs. 80% of CKD patients starting HD in the US use catheters. Medicare’s Fistula First Breakthrough Initiative Change Concept 2 calls for vein protection “at the first sign of kidney disease.” The best vascular surgeon can’t make a native vein AVF if there is no intact vein to use. We used these email addresses to query educated CKD patients about their knowledge of vein protection. Our invitation to participate in this research were sent to 1,161 patients by email with a link to the SurveyMonkey survey. Responses came from 182 participants from 34 states. Mean age of responders was 61 (range 21-96). 35% were male; 12% African-American; 40% had Diabetics; 43% had Stage 4 CKD. 16% reported CKD-5 or RRT. 43% had CKD-5 or RRT. 49% had been advised to protect their non-dominant arm from needle sticks. Only 14% had been advised to use the dorsa of their hands for needle sticks rather than arm veins. Of those protecting veins, almost all understood why. Most who tried to protect their veins found health care personnel honored their requests.

Results: Native and frequent venipuncture and intravenous catheter use are common in the current practice of medicine in the US. Vein protection is critical to success in creating AVFs in CKD patients. In this large convenience sample of CKD patients who received education about CKD and RRT, we found only ~ half understood the importance of vein protection with 95%CI 46% and only ~15% were protecting veins in both arms by preferentially using the dorsa of the hands for venipunctures (what we call vein-saving technique.) It is likely that participants in this study were patients with higher socio-economic status and more engagement in self care-suggesting average rates of education about vein protection may be lower than those found in our study. This study shows we need improved education of patients and staff to achieve better AVF rates and reduced mortality and costs in ESRD patients.

Funding: Private Foundation Support

TH-PO862
Nutritional Intervention Program: An Important Tool for the Control of Hyperkalemia

Carmen B. Tzanno-Martins,1 Camila Machado de Barros,2 Elzo R. Junior,2 Bárbara Margareth Menardi Biavo.2

Background: Nutritional intervention can prevent or control most metabolic disorders manifested in chronic kidney disease, including hyperkalemia, which is a risk factor for cardiac arrhythmias and sudden death. The purpose of this study was to evaluate the influence of knowledge about potassium before and after nutritional intervention in hemodialysis patients, four hours/session, three times/week.

Methods: This is a longitudinal, descriptive and primary data collection, which evaluated 61 elderly hemodialysis patients (~60 years old), with serum potassium above 5.5 mg/dL. The nutritional intervention consisted of a lecture about hyperkalemia, with emphasis on restricting foods rich in potassium, interactive dynamics, games and distribution of educational materials. For assessment, a questionnaire of knowledge about potassium was applied before and after nutritional intervention.

Results: Of the 61 patients, 95% (n=58) participated in the survey before and after nutritional intervention. Two patients (3.3%) were excluded, because one showed limited level of understanding both questionnaires and the other was absent on the day of sample collection. Of the 58 subjects, mean serum potassium of the last three months before nutritional intervention was 6.2±0.5 mg/dL. After nutritional intervention, 49% of patients showed a decrease of serum potassium. The assessment of knowledge about potassium in the first analysis (pre nutritional intervention) showed that the highest percentage of individuals (55.7%) had less than 90% accuracy. In the second analysis (post nutritional intervention) 93.4% of patients scored higher than 90% of the questions.

Conclusions: The nutritional intervention proposal showed efficacy to increase potassium knowledge and provided benefits in short time. Because of this results we suggest keeping regular educational activities.

TH-PO863
Identifying Educational Needs of Nephrologists in the Management of ANCA-Associated Vasculitides

Marcia R. Silver.

Methods: We performed a retrospective single center study, enrolling 1,218 consecutive kidney disease patients, between July 2007 and Feb 2008, and followed them up to 30 months. By using propensity score matching, we matched 149 recipient- and non-recipient pairs from 1,218 patients. The incidences of renal replacement therapy, mortality, cardiovascular event and infection were compared between MPE recipients and non-recipients.

Results: Renal replacement therapy was initiated in 62 and 64 patients in the recipients and non-recipients, respectively (P=0.05). The MPE reduced unplanned urgent dialysis (8.7% vs 24.2%, P=0.001), and shortened hospital days (2.16 vs 5.05 days/patient/year). MPE recipients had a better metabolic status at the time of initiating renal replacement therapy. Although no significant survival advantage from MPE was exhibited, MPE recipients had lower incidence of cardiovascular event (adjusted hazard ratio, 0.24; 95% CI, 0.08 to 0.78; P=0.017), and a tendency toward a lower infection rate (adjusted hazard ratio, 0.44; 95% CI, 0.17 to 1.11; P=0.083).

Conclusions: MPE was associated with better clinical outcomes in terms of urgent dialysis, cardiovascular events and infection.

Effectiveness of a Nephrology Education Program: Identifying Educational Needs

Wayne State University, Detroit, MI.

Marcia R. Silver.

Methods: A VFs in CKD patients. In this large convenience sample of CKD patients who received education about vein protection, we found only ~ half understood the importance of vein protection with 95%CI 46% and only ~15% were protecting veins in both arms by preferentially using the dorsa of the hands for venipunctures (what we call vein-saving technique.) It is likely that participants in this study were patients with higher socio-economic status and more engagement in self care-suggesting average rates of education about vein protection may be lower than those found in our study. This study shows we need improved education of patients and staff to achieve better AVF rates and reduced mortality and costs in ESRD patients.

Funding: Private Foundation Support

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Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Organizational Communication Efforts for the Prevention of Catheter Related Blood Stream Infection from Hemodialysis Catheters

**Methods:** The institution initiated the study in January 2011 and the present data reviewed after 15 weeks. Nephrology was educated in the CRBSI-PP in the 11th week. Compliance with application of the bundle for the 10 weeks prior to CRBSI-PP (PRE) vs POST, respectively. In addition, compliance with the 5th point of the bundle (daily documentation for continued use of the device) was associated with higher CRBSI-PP compliance. These data indicate that a focused educational effort at improving communication about CRBSI improves compliance with the bundle.

**Conclusions:** Educational communication efforts result in higher compliance with CRBSI-PP by Nephrology faculty and fellows. We anticipate that this will result in a reduction in CRBSI associated with the placement of temporary hemodialysis catheters.

**Funding:** NIDDK Support

Nephrology Advanced Practitioner Salary and Benefit Information – A Survey

**Background:** Nephrology Advanced Practitioner salary and detailed benefit information is limited. NPs and PAs function similarly in Nephrology. The purpose of this study was to compare and contrast the salaries/benefits of NPs and PAs as well as the responsibilities of their positions.

**Methods:** NKF’s Council of Advanced Practitioners (CAP) in conjunction with the American Society of Nephrology Physician Assistants (AANPA) conducted a salary and benefit survey of NPs, CNSs (clinical nurse specialists), and PAs from January 2010 to June 2010. The survey link was sent out monthly for 6 months. The survey link could be shared with other nephrology APs who were not CAP nor AANPA members. The survey link was sent out monthly for 6 months. There were 276 responses (CAP membership as of 6/1/10 was 240 and was used as a denominator since many AANPA members overlapped between CAP and AANPA) for a response rate of 115%.

**Results:** Over 85% of the respondents were white females, between the age of 31 and 59, who worked full time; 82% held a Master’s degree. The top three places that APs work are dialysis unit (71%), office (46%) and hospital (31%). Within the dialysis unit, most APs were responsible for call at the dialysis units. Within the office, most APs ran CKD clinics and hospital follow-up visits (44%). The average annual salary for all full-time APs was $83,800. There was NO correction between degree and salary. There was a very strong correlation between years of experience and salary. Most common benefits were malpractice insurance (93%), health insurance (96%), and paid CME (88%). The most important ‘benefit’ to APs was the feeling of valued at work and to have a good working relationship with their physician partners.

**Conclusions:** In conclusion, the survey showed similar average salary and benefits for APs, with regional variances. Salaries are on the lower end of the spectrum when compared to their physician partners. Most important ‘benefit’ to APs was to feel valued at work and to have a good working relationship with their physician partners.

**Funding:** NIDDK Support

A Prospective Multi-Centre Evaluation of Timing of Renal Replacement Therapy for Acute Kidney Injury in Critically Ill Patients in Canada

**Background:** The ICU patients started on RRT in our study generally had advanced AKI, high illness severity, and received RRT early after hospital presentation. Our results describe the current state of practice with respect to the timing of initiation of RRT for AKI in Canada and should aid in the design of future interventional trials.

**Conclusions:** Physicians decide whether to initiate dialysis immediately, reevaluate in some time, consider whether the patient does not need dialysis or would benefit from dialysis. For each patient we calculated a clinical index (CI) each day. We analyzed the relationship of physician decision to start dialysis and the underlying CI on the day of dialysis or peak creatinine. Results: There were a total of 206 questionnaires; 14 to 31 completed answers for each patient. Physician responses and actual events were frequently discordant (48%). Dialyzed patients had higher values of the CI. Within each category of physician choice, the CI correlated better with actual provision of dialysis than physician response. Among patients with lower CI value, physician choices correlated with the decision that the patient did not need dialysis.
**TH-PO868**

**Creatine Clearance and Urine Sediment as Predictors of Need of Renal Replacement Therapy in Intensive Care Unit Patients with Acute Kidney Injury**

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**Background:** The patients who are admitted to ICU frequently develop AKI and are consulted by nephrologists. The study is evaluating the role of successive creatinine clearance measurements and urine sediment analysis as predictors of need of RRT during current hospitalization.

**Methods:** 57 patients were evaluated by nephrology department for AKI. The inclusion criteria were the following: age more than 18 years, ICU utilization, nephrology consultation for AKI, strict monitoring of urine output through Foley catheters. A 2 hour creatinine clearance was measured on day of consult and at 24h and 48h after nephrology consult. Urine sediment was evaluated on day of consult and an acute tubular necrosis score was devised. The patients were followed for the need of RRT during the entire hospitalization.

**Results:** 27% of patients required RRT. In 95% of patients the renal injury was completely established at time of nephrology consult. In five groups of patients (creatinine clearance at time of consult less than 2.5 mL/min, 2.5 to 7.5 mL/min, 7.5 to 10 mL/min, above 10 mL/min and more than 0 mL/min) the risk of requiring HD was 80%, 70%, 30%, 25% and 0%. If we consider the creatinine clearance at 48h the risk of requiring RRT was (in same groups) 100%, 80%, 50%, 0% and respectively 0%. ATN score was 5.5 in patients who didn’t require RRT and 1.5 in patients who require HD (p-value = 0.23). The correlation coefficient between ATN score and creatinine clearance at time of consult was 0.135. The correlation coefficient between serum creatinine and need of RRT was 0.213.

**Conclusions:** At the time of nephrology consult the kidney injury is already completely established in the majority of patients with AKI and serial measurement of creatinine clearance is the best method to quantify the degree of injury, to evaluate the degree of recovery and to predict the need for RRT. Urine microscopy is not useful in predicting need for RRT (in fact kidneys with higher intrinsic function might have higher ATN score on urine analysis).

**TH-PO869**

**Effect of Fluid Removal on Delivered Dose of Intermittent Hemodialysis (IHD) in Acute Kidney Injury (AKI)**

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**Background:** Standard Kt/V (StudKt/V) is used to measure dialysis efficacy across different types of therapies of variable frequency in patients on chronic dialysis. In AKI, spKt/V or ekKt/V is calculated for IHD. Previous estimates to estimate StudKt/V were derived using a fixed-volume model. We evaluated the impact of fluid removal on StudKt/V. We hypothesized that not adjusting StudKt/V for fluid removal would underestimate delivered dose of IHD.

**Methods:** We analyzed data from 599 IHD sessions in 25 critically-ill patients with AKI from 5 centers included in the PICARD study. Delivered dose was calculated using spKt/V (Daugirdas equation), StudKt/V Leypoldt (Leypoldt JK et al. Semin Dial 17: 142-145; 2004) and using StudKt/V proposed by Daugirdas (Daugirdas JT et al. Kidney Int 77: 637-644; 2010) that includes effects of fluid removal. Patients with residual renal function were excluded. We compared the efficacy of 1 – 7 HHD sessions per week.

**Results:** A good correlation was found between spKt/V and Leypoldt StudKt/V (r² = 0.961; p < 0.001). StudKt/V was inversely proportional to the urea volume of distribution (r² = 0.017; p = 0.002). Higher frequency of IHD treatments per week was associated with increased delivered StudKt/V (median [IQR]): from 0.58 (0.47 – 0.67) for 1 treatment; 1.26 (0.92 – 1.47) for 2; 1.83 (1.50 – 2.15) for 3; 2.39 (1.91 – 2.84) for 4; 2.67 (2.17 – 3.19) for 5; 3.15 (2.60 – 3.97) for 6; and 3.77 (3.32 – 5.2) for 7 treatments per week respectively. Compared to Leypoldt StudKt/V, values of fluid removal adjusted Daugirdas StudKt/V equation were higher (2.0 IQR [1.5 – 2.7] vs. 1.9 IQR [1.4 – 2.5]; p = 0.001).

**Conclusions:** The use of StdKt/V Daugirdas et al equation provides an improved assessment of delivered dose of IHD adjusting for fluid removal. These results may inform the design of future studies of dialysis dose in AKI.

**Funding:** NIDDK Support

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**Conclusions:**

Physicians decisions to initiate dialysis were influenced by the severity of illness, likelihood of benefit and logistic factors. An objective CI may provide further improvement in clinical decisions.

**Funding:** NIDDK Support

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**TH-PO870**

**Adverse Effects of Fluid Overload in Patients with Acute Kidney Injury Receiving Continuous Renal Replacement Therapy**

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**Background:** Extensive fluid overload leads to acute physiologic changes such as metabolic imbalance of sodium and water, pulmonary edema and respiratory failure. In critically ill patients with acute kidney injury (AKI), mortality rates range from 50 to 70% and have usually been attributed to multiple complications related to AKI. We thus investigated whether fluid overload is associated with mortality in critically ill patients with AKI receiving continuous renal replacement therapy (CRRT).

**Methods:** We retrospectively reviewed 100 patients with AKI treated with CRRT at Chung-Ang University Hospital between April 2005 and December 2010. The definition of fluid accumulation is still subject to debate, but we defined fluid accumulation as more than 4L as a sum of daily fluid balance over a period initiating at CRRT.

**Results:** Of the 100 enrolled patients, 70 were assigned to a fluid overload group and 30 to a fluid restriction group; there were no significant differences between the groups with respect to base-line characteristics. The most common cause of AKI was septic shock (44%), followed by cardiacogenic shock (19%). In-hospital mortality was 81.4% in the fluid overload group compared with 66.7% in the fluid restriction group (P=0.005). The mean cumulative fluid balance was 12.7±8.5 L in the fluid overload group and 0.9±3.6 L in the fluid restriction group (P<0.001). During the interval from admission to the initiation of CRRT, the fluid overload group showed significantly higher incidence of mechanical ventilation apply (85.7% vs. 66.7%, P<0.001), and higher use of inotropics (88.6% vs. 66.7%, P=0.001) than the fluid restriction group.

**Conclusions:** In critically ill patients with AKI, fluid accumulation poses a higher risk of death. Our data supported the concept that fluid accumulation is at least partially responsible for a poor outcome in patients with AKI and defends the strategy of attempting to achieve fluid restriction if tolerated hemodynamically. Thus carefully verification of the daily fluid balance is a great help to decrease mortality for critically ill patients with AKI.

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**TH-PO871**

**Phosphate Balance in Critically Ill Patients on Continuous Venovenous Hemofiltration**

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**Background:** Hypophosphatemia is a frequent complication during continuous renal replacement therapy (CRRT), and may contribute to poor patient outcomes due to phosphate’s critical role in energy metabolism in every organ system. We sought to quantify phosphate clearance during CVVH in our institution.

**Methods:** By adding a T-connector to the effluent line, approx. 1% of the 24 hr effluent volume was diverted to a collection bag. Estimated phosphate removal was calculated by multiplying the total effluent volume with concentration of phosphate in the effluent fraction. Results were verified by comparison to 4 hr complete collections in a subset of enrolled patients.

**Results:** Seventy-eight 24-hr effluent collections and thirteen 24-hr urine collections were performed on 25 patients. Most patients were anuric and received intravenous or oral phosphate.

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**Conclusions:** CVVH results in a negative phosphate balance despite protocol-driven phosphate repletion strategies. Substantial amounts of phosphate may be cleared by CVVH before overt hypophosphatemia develops. Further study is warranted to determine
results, the physiologic consequences of CVVH-induced phosphate depletion on bone health, energy metabolism, 2.3 biphosphoglycerate levels and subsequent oxygen delivery to peripheral tissues.

Funding: NIDDK Support

TH-PO872
Plasma IL-10 Level and Monocyte HLA-DR Expression as Predictors in Critically Ill Patients Undergoing Continuous Renal Replacement Therapy
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Background: To determine whether interleukin 10 (IL-10) level and monocyte expression of antigen-DR (HLA-DR) are predictors of infection and prognosis in critically ill patients undergoing continuous renal replacement therapy (CRRT).

Methods: A total of 43 critically ill patients undergoing continuous veno-venous hemodialysis (CVVH) were recruited from the intensive care unit (ICU). Anti-coagulated blood was obtained on the 1st, 4th, and 7th days after start CRRT, and ELISA and flow cytometry were performed to determine serum IL-10 level and HLA-DR expression on the surface of CD14+ monocytes, respectively.

Results: (1) Eighteen patients had no infection and negative cultures (Group 1) during the study; 19 patients had infection and positive cultures (Group 2) at entry; 6 patients had no infection (Group 3) at entry but became infected within 2 weeks after beginning CVVH; 7 patients died. (2) The IL-10 level was higher in patients than in healthy subjects (P<0.001), rapidly decreased in Group 1 after treatment (P<0.05), was unchanged in the remaining patients, and was closely related to the APACHE II score and duration of hospitalization (P<0.05). (3) Monocyte HLA-DR expression was lower in patients than in healthy individuals (P<0.01). After CVVH, most patients with increased HLA-DR expression were uninfected. However, patients with unchanged or declining HLA-DR expression were infected or developed post-treatment infection. The patients who died had persistent and extremely low HLA-DR expression.

Conclusions: IL-10 is an indicator of disease severity, and persistently high IL-10 level predicts worse prognosis. Persistently low monocyte HLA-DR expression can be used to predict concomitant or future infection.

Funding: Government Support - Non-U.S.

TH-PO873
Rational Use of Polymethylacrylate Dialysis Membrane To Remove Serum Free Light Chains
Paolo Fabbrini, Andrea Stella, Mariarosa Viganò, Clínica Nefrológica Ospedale San Gerardo, Università degli Studi di Milano Bicocca, Milano, Italy.

Background: in vitro study showed that PMMA dialysis membrane can remove at least up to 2 grams of FLCs through an absorption mechanism, but in vivo application showed variable results and no study have investigated maximum adsorbent capacity and time before membrane saturation.

Methods: We performed 24 hemodialysis sessions (dialysis length 4 hours, dialyzer PMMA BK 2.1) in 7 consecutive patients with dialysis dependent ESRD associated to elevated sFLC levels (41 ± 3 k). In each treatment we measured sFLC hour removal to calculate SFLC plasma reduction rate of each hour and of the entire session. According to these results we then performed 10 dialysis sessions of 4 hours substituting PMMA dialyzer every 2 hours using a specifically designed circuit that allowed dialyzer switch without stopping dialysis session. Per hour and total hour sFLC removal rate was calculated.

Results: the 24 single dialyzer sessions resulted in an hourly average reduction rate of 12.8% (between 9% and 17%) that was effective only for the first two hours of treatment indicating saturation of PMMA membrane adsorption capacity. Coherently, entire session average sFLC removal was 23% (between 3% and 76%). During 10 double dialyzer sessions we measured average sFLC total removal of 50.9% (between 33.3 and 69%, p<0.05 vs single dialyzer) with an hourly rate removal of 12.5% (p<0.05 vs single dialyzer). Double dialyzer treatments did not change heparin use and albumin depletion in comparison with single dialyzer treatments.

Conclusions: dialysis with PMMA BK 2.1 can efficiently remove sFLC through absorption mechanism that reach saturation after 2 hours of treatment. The use of a dedicated single dialyzer treatments did not change heparin use and albumin depletion in comparison with we measured average sFLC total removal of 50.9% (between 33,3 and 69%, p<0.05 vs average sFLC removal was 23% (between 3% and 76%). During 10 double dialyzer sessions was calculated.

According to these results we then performed 10 dialysis sessions of 4 hours substituting in clinical practice.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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TH-PO875
Myoglobin Removal in Rhabdomyolysis: Clinical Studies and a Mathematical Model
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Background: Acute kidney injury secondary to high serum myoglobin levels is a frequent cause of morbidity and mortality for patients with rhabdomyolysis. Theoretically rapid removal of myoglobin by protein permeable dialyzers would improve clinical outcomes for these patients by reducing the tubular exposure to myoglobin. The purpose of this study was to determine the optimum strategies for the removal of myoglobin in rhabdomyolysis using a high cut-off dialyzer.

Methods: Myoglobin clearance rates were studied in four patients with the HCO 1100, these were compared with those for high flux dialyzers and the larger 2.1m high cut-off dialyzer (Theralite). A four compartment model was then used to simulate use of these different membranes on different treatment schedules: continuous venous-venous hemodialysis and 2 and 8 hours of HD (each for a period of 3 days).

Results: The median percentage reduction in serum myoglobin levels was 52% (range 35-89) with HCO-HD. This equated to a median clearance rate of 34mls/min (range 10-63). The mathematical model parameters were fitted to these patients data and then simulated for treatment regimens of 2 hours at a myoglobin clearance rate of 2.2 ml/min (high flux dialyzer), 2 + 8 hours at 70 ml/min (Theralite 2.1m dialyzer) and continuous treatment at 34 ml/min (CVVH using the HCO 110 dialyzer). The simulations demonstrated that use of either of the two HCO dialyzers on both HD and CVVH settings resulted in greatly reduced renal exposure to myoglobin. Of the treatment options over a 3 day window CVVH using the HCO 1100 dialyzer reduced the area under the curve by 87% compared with standard HD. Simulations of HD using the Theralite dialyzer revealed a reduction in the AUC of 39 and 72% for 2 and 8 hour treatments respectively. All four patients recovered renal function and became independent of dialysis.

Conclusions: In summary, the HCO dialyzers provide a rapid reduction in myoglobin levels in rhabdomyolysis, clinical studies are now required to determine if this translates to improved patient outcomes.

TH-PO876
Light Chain Removal by Means of Adsorption in the Extracorporeal Treatment of Myeloma-Induced Cast Nephropathy
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Background: The combination of chemotherapy with dialysis removal of light chains (LC) has been described as a new therapeutic strategy for cast nephropathy (CaN). However, due to their high molecular weight (n 12,000, z 24,000 daltons), LC could also be adsorbed by resins. Coupled plasma-filtration adsorption (CPFA), presents as an ideal technique. A different efficicncy, in terms of LC removal may be expected on the grounds of the sorbent resin used.

Methods: We performed an in-vitro study to identify the resin with the best adsorptive capacity, followed by an in-vivo study to verify the magnitude of LC removal. In some patients, a longitudinal evaluation was carried out to obtain information on the LC trend. In vitro: serum from different patients was perfused onto different sorbent resins. LC after 30 and 120 minutes perfusion were compared. In vivo: 4-hour CPFA was performed in 10 CaN patients utilising the cartridge whose adsorptive capacity proved best. Apart from blood (start and end-treatment), pre- and post-resin plasma samples were taken every hour

Results: The MDR3 resin showed the best adsorptive capacities.

TH-PO874
Evaluation of Free Light Chain Removal by Various Blood Purification Methods
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Background: We evaluated various blood purification methods other than plasma exchange to remove free light chains (FLCs).

Methods: [CLINICAL study]Two patients of IgG kappa multiple myeloma and two patients of IgG lambda multiple myeloma with acute renal failure were treated by hemodialfiltration(HDF) using protein leaking dialyzer PES2100Dz14 or hemofiltration(HD) using PMMA dialyzer. [IN study vitro]Dialysis using HCO membrane Theralite2100z14 or plasma separator Evacurel14,4, dialfiltration using protein leaking dialyzer PES2100Dz14 and adsorption using g2microglobulin adsorption column Lixelle S-35z14 were performed in an in vitro circuit.

Results: [CLINICAL study]Removal of kappa FLC was from 21.8% to 71.6% by HDF using PES2100Dz14 and from 38.3% to 71.6% by HD. Removal of lambda FLC was from 48.5% to 53.1% by HDF using PES2100Dz14 and from 29.6% to 45.6% using HD using PMMA dialyzer. There was blood residue after HD using PMMA dialyzer when serum levels of FLCs were high. [IN study vitro]The highest removal rates was obtained by Theralite2100z14 dialysis among the four blood purification methods. Albumin loss was also the greatest in Theralite2100z14 dialysis. The removal content of FLCs per 1g albumin loss was better in PES2100Dz14 dialfiltration. The removal rate of FLCs by Evacurel14,4 dialysis was the third highest. Adsorption of FLCs by the j2 microglobulin adsorption column Lixelle S-35z14 was confirmed.

Conclusions: Theralite2100z14 dialysis was the best in removal of FLCs. In countries where Theralite2100z14 is not available, HDF using protein leaking dialyzer could become an alternative option.
In vivo, the mean LC adsorption by the MDR3 cartridge was better for κ (28%) than for λ chains (22%). The pre-to-post treatment blood reduction ratio was 31% for κ and 26% for λ chains. In patients treated with at least 6 sessions, the LC concentration progressively decreased (κ 68±11%, λ 55.9±9%, p<0.05).

Conclusions: Extracorporeal LC removal may be performed not only by diffusion but even by adsorption in 4-hour treatments. The effect of variables such as the resin volume in the cartridge, plasma flow, treatment time, are to be tested. The best schedule for associating chemotherapy with extracorporeal adsorption still needs investigation.

Funding: Pharmaceutical Company Support

TH-PO877 The Incidence of the Citrate Accumulation during Continuous Veno-Venous Haemodialysis with Regional Citrate Anticoagulation – A Monocentric Retrospective Study

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Background: Systemic citrate accumulation due to compromised citrate metabolism is a complication of a continuous renal replacement therapy (CRRT) with regional citrate anticoagulation (RCA). Impaired liver function and arterial hypoxia are described in literature as risk factors for citrate accumulation. Metabolic acids, increased total calcium ionized calcium ratio (total-Ca/iCa>2.25) and increased demand for systemic calcium substitution are the common markers for citrate accumulation. The aim of the present study was to assess the incidence and clinical characteristics of the citrate accumulation on the basis of representative patient population.

Methods: The data from all ICU at our university hospital in 2010 was retrospectively analyzed. Weight adapted RCA for continuous veno-venous hemodialysis (CVVHD) was performed according to the protocol published by Morgera et al. Results: 13 (3.6%) of 365 patients treated with citrate-based CVVHD (66±412.9 years old, 53.8% male, APACHE score 31.5±8.6) have developed the characteristics of citrate accumulation. Six of 13 Patients (46%) had pre-existing liver dysfunction (mean S-bilirubin before treatment: 7.7 mg/dL). Elevated Ca substitution demand (up 123±18% compared to baseline rate), simultaneous decrease in systemic iCa concentration (decrease by 10% to 1.0±0.99 mmol/L) and the increase in total-Ca/iCa ratio (2.31±0.26 mmol/L) have been observed in all 13 patients. At the time when citrate accumulation was diagnosed all 13 patients developed severe lactic acidosis (pH 7.20±0.13, lactate 1266±59 mg/dL, bicarbonate 15.0±3.5 mmol/L). Anticoagulation modality was immediately changed to heparin in all 13 patients. 12 of 13 patients died from the therapy-resistant shock at the ICU.

Conclusions: The incidence of citrate accumulation was 3.6% of all CVVHD treatments based on RCA. Citrate accumulation was found exclusively in patients with severe lactic acidosis in septic shock and was associated with poor prognosis (mortality> 90%).

TH-PO878 Safety and Impact of CVVHD without Dialysate Warmers: A Difference of Opinion


Background: Continuous veno-venous hemodialysis (CVVHD) is used to dialyze hemodynamically unstable patients. When the manufacturers of the device (N Engl J Med 2009; 360:1125-1133) have removed dialysate warmer from CVVHD circuit, intensive care unit (ICU) physicians expected adverse patient outcomes from the resulting hypothermia, but nephrologists expected cooled dialysate to be beneficial. This provided us a unique opportunity to study the impact of CVVHD with no warmers in critically ill patients.

Methods: We retrospectively reviewed the charts of patients who had CVVHD with or without warmers over a 6 month period. We calculated Sequential Organ Failure Assessment (SOFA) scores (range 0-24, higher the score, sicker the patient is), and collected core body temperature (CBT), mean arterial pressure (MAP), use of vasoressor drugs, ICU length of stay (LOS), and mortality.

Results: Thirty three patients without warmers had significantly lower CBT over the first 31±14 hours of CVVHD compared to 37 patients on warmers (35.4± vs. 35.9°C, P<0.05). Fall in CBT from baseline was greater in the no-warmer group (0.79°C vs. 0.18°C, p<0.05), (figure 1A). Though there was a trend of higher MAP in patients with no warmers, absolute MAP during CVVHD did not differ between groups (77 mmHg vs. 73 mmHg, p=0.32), nor did MAP from baseline (+5 mmHg vs. -1 mmHg, p=0.10). SOFA scores did not significantly differ between no-warmer vs. warmer groups either at ICU admission (9±4 vs. 10±4) or 24 hours into CVVHD (12±3 vs. 12±4), nor did LOS (29±37 days vs. 29±31 days) or vasoressor use (1.3±1.0 vs. 1.2±1.3 agents). ICU mortality was 60% with no-warmers and 67% with warmers (p=ns).

Conclusions: Our study showed that CVVHD without dialysate warmer induced modest hypothermia. This did not adversely impact outcomes in critically ill patients.

TH-PO879 Pharmacokinetics of Ertapenem in Critically Ill Patients Receiving Continuous Venovenous Hemodialysis (CVVHD) or Hemodiafiltration (CVVHDF)

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Background: Ertapenem (E) is a broad spectrum carbapenem antibiotic indicated in many infections found in the ICU. No dosing recommendations exist for E in critically ill patients receiving continuous renal replacement therapy. The purpose of this study is to determine the pharmacokinetics (PK) of E in critically ill adults receiving CVVHD/F or CVVHDF.

Methods: This study was approved by the U Michigan IRB, and was a prospective, open-label first dose PK study of E in critically ill adults receiving CVVHD/F or CVVHDF. One gram E was infused over 30 minutes. Effluent and pre-filter blood samples were collected at 1, 2, 4, 8, 12, 18 and 24 hours following E infusion. Samples were analyzed by HPLC/MS/MS. Non-compartmental methods were used to estimate PK parameters.

Results: Eight subjects (mean ± SD) age 62 ± 16 years, weight 78 ± 19 kg were enrolled. CVVHDF was delivered at effluent rates of 38 ± 9.7 mL/hr. The half-life, apparent volume of distribution at steady state, area under the concentration-time curve from 0-24 hours, and the E serum concentration at 24 hrs were 8.8 ± 3.2 hrs, 0.19 ± 0.060 L/kg, 710 ± 150 mcg/mg/mL, and 10 ± 4.0 mcg/mL, respectively. The total clearance (CVVHD/F and systemic clearance) was 21 ± 5.7 mL/min. The sieving coefficient was 0.21 ± 0.065 and CVVHDF clearance was 10 ± 4.2 mL/min.

Conclusions: E half-life was twice as long as what is reported in normal volunteers. CVVHDF was responsible for substantial E clearance in these subjects. The total gram E dose produced serum E concentrations above the MIC sensitive breakpoint of 2 mcg/mL for Enterobacteriaceae spp. for 100% of the dosing interval for all 8 patients.

Funding: Pharmaceutical Company Support

TH-PO880 Sulfamethoxazole and Trimethoprim Transmembrane Clearance during Modeled Continuous Hemofiltration

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Background: Sulfamethoxazole (SMX) and trimethoprim (TMP) are administered concomitantly to treat a variety of infections. The physicochemical properties of SMX/TMP suggest that removal may be removed during continuous hemofiltration (CH). However, SMX/TMP removal during CH has not been systematically examined. The purpose of this study was to estimate SMX/TMP transmembrane clearance (CLtmm) during modeled CH.

Methods: The invivo model consisted of 0.9 L heparin anticoagulated human blood continuously stirred at 37 °C. SMX/TMP was added to achieve concentrations of 160 ug/mL and 8 ug/mL respectively. Urea was added to serve as control. CH was performed with two commonly used hemofilters: HF1000 polysulfone (n=5) and M100 AN69 (n=5) hemofilters. Spent ultrafiltrate was recirculated back into blood to create a closed system. Ultrafiltration rates (Quf) of 1, 2, 3, and 6 L/h were investigated. At each Quf, prefilter concentration and blood ultrafiltrate were collected and assayed for urea, SMX, and TMP. The concentration of solute in spent ultrafiltrate was divided by that in prefilter blood to calculate the extraction coefficient (E) of each solute. CLtmm=E*Quf. Student's t-test was used to compare E between hemofilter types and ANOVA was used to compare E within each hemofilter type. P<0.05 was considered significant.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Results: Urea, the control solute, E was approximately 1 at all Quf rates studied. SMX/TMP CL in ml/min
Quf (L/h) 1 2 3 6 ANDVA p-value
SMX HF1000 E 0.39±0.03 0.38±0.03 0.40±0.03 0.40±0.03 0.71
SMX HF1000 CL/ml/min 6.1±1 13±1 20±1 40±3
SMX M100 E 0.31±0.03 0.33±0.05 0.52±0.02 0.31±0.04 0.74
SMX M100 CL/ml/min 5±2 11.2±1 16.1±1 33±4
t-test p-value for E 0.01 0.14 0.01 0.02
TMP HF1000 E 0.62±0.05 0.58±0.04 0.64±0.05 0.64±0.05 0.19
TMP HF1000 CL/ml/min 10±1 19±1 32±2 64±5
TMP M100 E 0.70±0.07 0.75±0.12 0.74±0.07 0.73±0.10 0.57
TMP M100 CL/ml/min 11±2 21±2 37±3 75±10
t-test p-value for F 0.20 0.02 0.05 0.17

Mean/SD

Conclusions: Substantial SMX/TMP CL at was observed during CH with a HF1000 or M100 hemofilter at Quf between 1 and 6 L/h. Considering the CL at observed and the norenal clearance reported to occur with SMX/TMP, SMX/TMP dosing adjustments during CH is required.

Funding: Other U.S. Government Support

TH-PO881

Piperacillin Clearance in Continuous Renal Replacement Therapy (CRRT) Predicts Failure To Reach Pharmacodynamic Targets Seth R. Bauer,1 Peilin Wei,1 Charbel A. Salem,1 Joseph J. Groszek,2 Maria E. Taylor,1 Michael J. Connors,3 Ashita J. Tolwani,1 William Fissell,2 Pharmacy, Cleveland Clinic, Cleveland, OH; 1Nephrology, University of Alabama, Birmingham, AL; 2Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; 3Pulmonary and Critical Care, Emory University, Atlanta, GA.

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, raising concerns about interactions between CRRT dose and pharmacodynamics in sepsis. In an IRB-approved multicenter study, we measured piperacillin levels in patients receiving CRRT. Drug levels were compared with pharmacodynamic (PD) goals, and we identified factors associated with failure to reach PD goals.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, 30 minute post infusion peak, and second trough blood and effluent samples were drawn and stored on ice. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Traditional PK parameters (volume of distribution, elimination rate) were calculated. Logistic regression (JMP 9 for Windows) was used to test the association between PK and CRRT parameters and PD goals.

Results: 48 patients had data for analysis, of whom 36 had complete data. 11 patients had therapy interruptions between the peak and the second trough, and one had an error in sample collection. Pharmacokinetic parameters predicted attainment of PD goals of %t>MIC=64, as did total daily piperacillin dose. When total clearance was divided into tertiles, %t>MIC=64 increased. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, 30 minute post infusion peak, and second trough blood and effluent samples were drawn and stored on ice. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Traditional PK parameters (volume of distribution, elimination rate) were calculated. Logistic regression (JMP 9 for Windows) was used to test the association between PK and CRRT parameters and PD goals.

Conclusions: CRRT significantly affects piperacillin PD. Higher CRRT clearance increases the risk of failing to meet PD goals. A larger multicenter study may permit more detailed analysis of the effect of PD on survival in CRRT.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO882

Piperacillin Pharmacodynamics Are Associated with Survival in Continuous Renal Replacement Therapy (CRRT) Charbel A. Salem,1 Peilin Wei,1 Seth R. Bauer,2 Michael J. Connors,3 Joseph J. Groszek,1 Ashita J. Tolwani,1 William Fissell,2 Pharmacy, Cleveland Clinic, Cleveland, OH; 1Nephrology, University of Alabama, Birmingham, AL; 2Pharmacy, Cleveland Clinic, Cleveland, OH; 3Nephrology and Critical Care Medicine, Emory University, Atlanta, GA.

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, raising concerns about interactions between CRRT dose and pharmacodynamics in sepsis. In an IRB-approved multicenter study, we measured piperacillin levels in patients receiving CRRT. We performed a logistic regression analysis to determine the probability of achieving pharmacodynamic targets on survival in acute kidney injury.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT in the ICU. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, peak, and second trough blood and effluent samples were drawn. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Statistical parameters were calculated from plasma levels. We a priori analyzed age and severity adjusted hospital survival for percentage time that free drug exceeded MIC = 50% (IT-MIC 50%) and 90% (IT-MIC 90%) at an MIC of 64 µg/ml using logistic regression (JMP 9 for Windows).

Results: 48 patients had data for analysis, of whom 33 had acute renal failure and complete data for analysis. Age- and severity-adjusted hospital survival was not associated with the fraction of the time the MIC exceeded IT-MIC > 50% , but IT-MIC >90% was strongly associated with survival (p=0.006).

Conclusions: Piperacillin pharmacodynamics appear to be associated with survival in patients treated with CRRT, but the association was only seen at a PD target of IT-MIC >90%, rather than a more conventional PD target to IT-MIC >50%. This suggests that survival in dialysis-dependent AKI, a renal failure might be improved by tailored antibiotic dosing, but more study is needed.

Funding: NIDDK Support, Pharmaceutical Company Support

TH-PO884

Clinical Data Imperfectly Predict Piperacillin Pharmacokinetics in Patients on Continuous Renal Replacement Therapy Peilin Wei,1 Seth R. Bauer,2 Charbel A. Salem,1 Joseph J. Groszek,1 Maria E. Taylor,1 Michael J. Connors,3 Ashita J. Tolwani,1 William Fissell,2 Nephrology, University of Alabama at Birmingham, Birmingham, AL; 1Pharmacy, Cleveland Clinic, Cleveland, OH; 2Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH; 3Pulmonary and Critical Care Medicine, Emory University, Atlanta, GA.

Background: Sepsis is the leading cause of death in acute kidney injury (AKI). Early appropriate antimicrobial therapy improves survival in sepsis, but dose calculations depend on knowledge of pharmacokinetic (PK) parameters, particularly the volume of distribution and the clearance. In an IRB-approved multicenter study, we prospectively measured piperacillin levels in patients receiving CRRT in the ICU. PK were compared to antroopsonic data and CRRT prescription.

Methods: Inclusion: Patients with acute or chronic renal failure receiving CRRT in the ICU. Exclusion: ESLD, age < 18, pregnancy. Patient demographic and CRRT parameters were recorded. Sampling: After the fourth dose of antibiotic during uninterrupted CRRT, trough, 30 minute post infusion peak, and second trough blood and effluent samples were drawn and stored on ice. Drug analysis: Total, free, and effluent piperacillin levels were measured by HPLC. Data analysis: Linear regression (JMP 9 for Windows) was used to determine the associations between clinical data and PK parameters.

Results: 48 patients had data for analysis, of whom 35 had complete data. 12 patients had therapy interruptions between the peak and the second trough, and one had an error in sample collection. A multivariate linear regression model fitted Vd (r²= 0.49) and Ke (r²= 0.30) to patient and dialysis prescription data. Age (p<0.01) and weight gain since admission (p<0.003) was the only factor independently associated with Ke.

Conclusions: Our data suggest that patients treated with ED using a high-flux dialyzer (polysulphone, 1.3 m2; blood and dialysate flow, 160 ml/min; treatment time 480 min) a twice daily dosing schedule of 2g/1g ampicillin/sulbactam is sufficient to avoid under-dosing.

Funding: Pharmaceutical Company Support
A Mathematical Model To Predict Two-Phase Calcium Supplementation in Continuous Venovenous Hemofiltration with Regional Citrate Anticoagulation

**Background:** Calcium substitution is a determinant of the safety and efficacy of regional citrate anticoagulation (RCA) during continuous renal replacement therapy. We developed and clinically validated a mathematical model of two-phase calcium supplementation during continuous venovenous hemofiltration (CVVHF).

**Methods:** Thirty-two critically ill patients who required CVVH treatment with citrate anticoagulation were enrolled in the study. A two-phase mathematical model using patients' clinical characteristics (body weight, Hct, serum proteins concentration) and relating factors (RCA protocol) was derived. A priori, the model was designed to predict the need for calcium supplementation. By measuring systemic and extracorporeal citrate and calcium concentrations repeatedly, two coefficients in the mathematical equation, namely, the proportion of filterable calcium and the correlation between the concentration of calcium and citrate were studied. The model was validated in patients receiving RCA-CVVHF.

**Results:** The calcium supply during RCA-CVVHF can be divided into two phases by reaching the steady-state of citrate. The two pivotal coefficients were solved. The filterable calcium accounted for 87.1% of total calcium. The highest correlation was found between the increased bound calcium concentration and the citrate plasma level at t = 0.70 s (p = 0.001).

**Conclusions:** Applied the model to 15 patients’ treatments, it was able to control the level of systemic and circuit ionized calcium at a safe level and steady range in the setting of different treatment parameters. Afterwards, it had been validated in more treatments of RCA-CVVH in our institute. The incidence of hypocalcemia or hypercalcemia was reduced (4.9% vs. 16.7%) with less frequency of ionized calcium monitoring. However, the model appeared less precise after 24 hours of treatment.

**Funding:** Government Support - Non-U.S.

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**TH-PO886**

**In Spite of Positive Charge on Polyethyleneimine, AN69 ST Membrane Does Not Tightly Adsorb Heparin during Continuous Renal Replacement Therapy**

**Jun Seok Choi, Su-Kil Park, Jung-Sik Park. Nephrology, Asan Medical Center, Seoul, Republic of Korea.**

**Background:** Owing to the positive charge of polyethyleneimine (PEI), AN69 ST membrane adsorbs heparin 600 IU/mL at the priming with the mixture of heparin and normal saline (5000 IU/mL). More heparin attached to AN69 ST membrane may increase the longevity of filter. To avoid heparin adsorption during renal replacement therapy (RRT), washing with normal saline 1L for 5-7 minutes after priming could remove the bleeding risk due to not adsorbed heparin. We compared the effects of priming with different heparin doses (5000 IU/L in group A vs. 20000 IU/L in group B) on filter life span and systemic coagulation parameters in critically ill patients with acute kidney injury in this randomized cross-over study.

**Methods:** Differences of heparin were randomly assigned to 30 patients (M:F = 22:8; median of age 70 (range, 50-96) years) at the 1st and 2nd filter during RRT.

**Results:** There was no difference of median values in baseline hemoglobin (9.2 (7.1-14) g/dL vs. 9.4 (8.3-14.2) g/dL, p=NS), platelet count (123000 (37000-470000)/mm³ vs. 115000 (23000-485000) mm³, p=NS), activated partial thromboplastin time (aPTT, 39.1 (27.2-58.4) sec vs. 36.0 (27.2-69.6) sec, p=NS), prothrombin time (PT, 1.19 (0.98-1.76) INR vs. 1.17 (0.97-1.86) INR, p=NS), collagenase-epsilon clotting time (205 (69-225) sec vs. 214 (42-320) sec, p=NS), APACHE II score (24.7 (24-74) vs. 24 (10-39), p=NS) and filter life span (15 (5-5 min 5 min 71 min 47 min) vs. 15 vs. 59 min 3 (9-40 min 71 min 32 min), p=NS) between two groups. Compared with baseline value of aPTT, its prolongation did not appear in 30 minutes after starting RRT in group A (from 39.1 (27.2-84.8) sec to 38.7 (25.3-98.6) sec, p=NS). However, aPTT significantly increased in group B (from 36.0 (27.2-69.6) sec to 38.9 (29.7-86.8) sec, p=0.012) without clinical events.

**Conclusions:** Priming with the higher dose of heparin and washing did not reveal the beneficial effect on filter life but prolonged aPTT. It suggests that PEI does not strongly adsorb heparin.

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**TH-PO887**

**The Evodialyser™ Can Be Used as a Heparin Free Alternative for SLED in ICU**

**Monica Doyle, Iain R. Macleod, Sean McArtney, Sally Crofts, Judith Underline. Department of Renal Medicine, Aberdeen Royal Infirmary, Aberdeen, United Kingdom; Department of Intensive Care Medicine, Ninewells Hospital, Dundee, United Kingdom.**

**Background:** Sustained Low Efficiency Dialysis (SLED) is increasing in popularity particularly in the care of critically ill patients within intensive care. Currently, most SLED protocols use of heparin to achieve prolonged dialysis times with slower blood pump speeds. Critically ill patients often have a bleeding tendency and thus a strategy to minimise or completely avoid systemic anti-coagulation would be clinically advantageous.

**Methods:** We collated data prospectively in all patients in intensive care who required SLED from 01/07/2010 until 30/05/2011. The Evodialyser™ was used when the nephrologist felt it was indicated.

**Results:** During the study period 27 SLED treatments were carried out in 13 patients using the Evodialyser™. The median age of the study population was 71 years (IQR 65 – 77 years) The median APACHE II score was 26 (23-28) The indications for the use of the Evodialyser™ included coagulopathy, post major surgery and the use of Activated Protein C.

**Conclusions:** The standard treatment time was 8 hours with a mean time of 7.2 ± 2 hours achieved. 24 treatments (89%) were successful and achieved over 4 hours of SLED. Only 3 treatments were terminated at less than 4 hours and this was due to increased venous pressure in all cases. No systemic heparin was required in any patient. Ultrafiltration averaged 1.34 litres per session (Range 0.2-2.1) and was achieved in all patients.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.
Regional Citrate Anticoagulation during Continuous Veno-Venous Hemodialysis: Two-Year Single-Center Experience

Dmytro Khadzhynov, Christin Baumann, Torsten Slowinski, Ina Lieker, Hans-Hellmut Neumayer, Harm Peters. Department of Nephrology, Charite Campus Mitte, Charite Universitaetsmedizin, Berlin, Germany.

Background: Regional citrate anticoagulation (RCA) has been shown to be a safe and effective form of anticoagulation for continuous renal replacement therapy (CRRT) in patients with high risk of bleeding. More over, it was recently assumed that the citrate application can bring a benefit in survival. We report a two-year single-center experience of renal replacement therapy based on a continuous veno-venous hemodialysis (CVVHD) with regional citrate anticoagulation.

Methods: Results of CRRT conducted in years 2008 and 2009 at 6 intensive care units at our university clinic are retrospectively analysed. CVVHD with RCA is a modality of choice at our campus and is initially used in the majority of cases, regardless of the patients bleeding risk and/or liver function. Collected data included demographic features, dialysis circuit life-time, overall mortality at ICU discharge.

Results: We detected 703 patients (in average 67.6±12.4 year old, 64.6% male, average APACHE score 26.2±8.5) treated with CVVHD with RCA from 01.01.2008 till 31.12.2009. Mean uncensored filter life-time was 66.4±42.5 hrs. CVVHD was performed in 181 patients (63.4±43.5 hrs) at general anesthesiology ICU, 252 patients (66.0±42.1 hrs) at cardio-surgery ICU, 62 patients (76.3±41.1 hrs) at general surgery ICU, 68 patient (67.5±42.5 hrs) at cardiology ICU, 23 patients (75.7±46.8 hrs) at neurological ICU and 117 patients (65.9±42.4 hrs) at infection ICU. The incidence of citrate accumulation was 3.7% of all CVVHD treatments based on citrate anticoagulation. Renal recovery in patients treated with CVVHD based on RCA was 60.1% at ICU discharge. Overall mortality of the patients treated with citrate based CVVHD was 36.4% at ICU discharge.

Conclusions: Protocol of RCA for CVVHD was used as a standard modality of CRRT, regardless of the patients bleeding predisposition. The modality of CRRT was applied with equal success at 6 different orientated ICUs of our university hospital. This approach allows reaching perfect regional extracorporeal anticoagulation efficacy with an adequate metabolic control.

Digoxin Intoxication in Acute or Chronic Kidney Failure – Elimination of Digoxin Bound to Fab-Fragments (Digifab®) with High Cut-Off Filter Dialysis

Susanne V. Fleig, Roland Schmitt, Jan T. Kielstein, Bernhard M.W. Schmidt. Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.

Background: The cardiac glycoside digoxin is renally eliminated and not dialysable. In cases of symptomatic intoxication, serum digoxin can be bound by anti-digoxin-antibody-fragments (Digoxin Immune Fab) and thereby inactivated; digoxin-antibody-complexes are then renally excreted. Digifab® (Digoxin Immune Fab) has a molecular size of 46kDa and does not pass normal dialysis filters. In dialysis-dependent patients, digoxin is set free again after degradation of Fab-fragments and symptoms of intoxication may reoccur.

Methods: We report two cases of dialysis-dependent patients (one with end-stage renal disease, one with acute postoperative renal failure) with symptomatic digoxin intoxication.

Results: Both had been given Digoxin Immune Fab (digifab®), which led to relief of symptoms for several hours. Yet, symptoms reappeared as the agent could not be excreted and was set free again after degradation of the antibody fragments. Six hours after a second dose of Digoxin Immune Fab (digifab®), we performed extended dialysis (Genius® singlesep dialysis system (Presens Medical Care)) with high cut-off dialysers (Theradial®, HC01/1008, both for removal of plasma components with a molecular weight up to 45kDa). This way, we were able to eliminate fab-fragment bound digoxin. After dialysis with these filters, symptoms did not recur in both patients, and serum digoxin levels remained low (Digoxin levels: 4.72 nmol/l and 5.50 nmol/l before treatment, 2.08 nmol/l and 2.01 nmol/l directly after dialysis and 2.66 nmol/l and 2.50 nmol/l 12 hours after dialysis).

Conclusions: We show that dialysis with high cut-off filters can eliminate fab-fragment bound digoxin in patients with symptomatic digoxin intoxication and severely impaired kidney function.

Path Batch Hemodialysis (PBH): A Safe and Efficient Method for Acute Kidney Injury (AKI) Cancer Patients in the Intensive Care Unit (ICU) Veronica T. Costa e Silva, Ana Paula Leandro Oliveira, Henrique Palomba, Ludmila Abrahão Hajjar, Elerson Costaloga, James Hugn, Luciane Ikawa, Juliana Silva Bezerra, Emanuel A. Burdmann, Luís Yu. Nephrology Division, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil. 'Intensive Care Unit Department, Sao Paulo State Cancer Institute - University of Sao Paulo School of Medicine, Sao Paulo, Brazil.

Background: The adequacy and safety of PBH have not been studied in ICU cancer patients (pts).

Methods: We prospectively analyzed all PBH performed in AKI adult cancer pts in the Sao Paulo State Cancer Institute ICU from June 2010 to May 2011. A total of 242 PBH were performed in 76 AKI pts. Pts' characteristics were age 61±14 years, 60.5% male, 17.8% on vasopressor and 12.4% on mechanical ventilation. Most (82.9%) patients had solid cancer (genitourinary tract 38.2%, gastrointestinal tract 11.8% and gynecologic system 11.8%). The most important AKI etiologic factors were sepsis (46.7%), obstructive uropathy (24.7%) and surgery (21.1%). General ICU mortality was 50.7%. Venous access was temporary catheter in 97.9% (58% femoral and 41% internal jugular veins), high-flux polysulphone membrane in all filters (F800 in 63.2%) and median blood and dialysate flow rate (250–300 ml/min). Systemic anticoagulation was not used in 69.4% due to heparin contraindications. Median pre and post urea levels were 154 (109–199) and 62 (47–89), respectively. Urea reduction rate > 55% was observed in 62% of dialysis. The prescribed UF was 1500ml (1000–2000), which was attained in 66.2% of the procedures. The prescribed dialysis time was 240 (240–315) min, which was achieved in 77.4% of dialyses. Most (77.4%) of dialyses were performed in 7.8±2.4 hrs. The main complications were hypotension (mean blood pressure <70 mmHg) in 27.3% (8.3% required dialysis interruption), prescribed blood flow reduction in 7.9%, lines reversion in 16.9% and system coagulation causing dialysis interruption in 10%. Clotting was associated with the need for decreasing blood flow (OR 6.3 (95% CI, 1.7, 21.3) and heparin use (OR 4.0 (95% CI, 1.0, 16.7)).

Conclusions: In conclusion, PBH seems to be a safe and efficient alternative for dialysis in AKI ICU cancer pts. 

Application of Plasmafiltration in Porcine Sepsis Models Induced by Laparoscopic Cecal Ligation and Puncture

Jun Xue. Nephrology, Huashan Hospital, Fudan University, Shanghai, China.

Background: To study whether plasmafiltration (PDF) can reduce the circulating levels of critical inflammatory macromolecules, thus improve hemodynamics and increase survival time after establishing a porcine sepsis model by laparoscopic cecal ligation and puncture (CLP).

Methods: Twelve 80-day and 36-kilogram domestic male swine which fitted the diagnostic criteria of sepsis induced by laparoscopic CLP, were randomly treated either by PDF or by normal saline. PDF was performed with a selective filter with molecular weight cut-off (MWCO) of 60–70kD. The circulating levels of TNF-α trimmer and high mobility group box 1 (HMGB1), blood pressure and pulmonary arterial wedge pressure (PAWP) averaged cardiac output were assessed.

Results: Total of the 12 swine were successfully made as sepsis models. The odds of PDF to reduce the circulating levels of TNF-α trimmer and HMGB1 were 1.97(95% CI, 1.64-2.51, P=0.012), and 1.97(95% CI, 1.67-2.46, P=0.007), respectively. The odds of PDF to improve systolic pressure and PAWP averaged cardiac output were 1.07(95% CI, 1.00-2.59, P=0.001) and 6.34(95% CI, 2.89-25.3, P=0.032), respectively. A linear relation was found between diastolic pressure and TNF-α trimmers through multiple linear regression analysis (P=0.032). We also found a linear relation between PAWP averaged cardiac output and TNF-α trimmer (P=0.043). The mean survival time was 36.3 hours for PDF group and 31.5 hours for control. In PDF group the risk ratio of death of all-cause was 0.11 (P=0.046), as compared with control.

Conclusions: We can successfully make porcine CLP sepsis models through laparoscopy. PDF can reduce the circulating levels of critical inflammatory macromolecules, therefore improve the hemodynamics and increase survival time in animal sepsis models.

Funding: Government Support - Non-U.S.

Membrane vs. Centrifuge Based Therapeutic Plasma Exchange – A Clinical Cross-Over Comparison

Jan T. Kielstein, Carsten Hafer, Ansgar Reising, Bernhard M.W. Schmidt. Department of Hyertension and Nephrology, Medical School Hannover, Hannover, Germany.

Background: Therapeutic plasma exchange (TPE) is either performed using a centrifugation device (cTPE), a method preferred by hematologist or blood bank-based plasma exchange by using a high performance centrifuge with standard hemodialysis equipment (mTPE), a method preferred by nephrology-based physicians. The aim of the study was to perform the first direct comparison of these two techniques.

Methods: We performed a prospective, cross-over study comparing mTPE using the Octapharma device with the cTPE device (Asahi Kasei Medical, Japan) with cTPE using the Spectra Opta (Caridian BCT). Seventeen patients that underwent TPE against albumin based substitution fluid were randomized to start either with cTPE or mTPE. The second treatment was done in a cross over design. We did choose this approach to allow...
the measurement of the total removed immunoglobulines in the waste bag. We also took samples for complete blood count, immunoglobulines and fibrinogen before and after treatment and recorded the treatment time without any adverse effects on parameters measured in the complete blood counts.

Results: While there was no difference in the reduction rate as well as the absolute amount of immunoglobulines removed by the different techniques, cTPE allowed a faster exchange/treatment time (plasmavolume/treatment time).

Comparison of cTPE and mTPE

<table>
<thead>
<tr>
<th>Parameter</th>
<th>unit</th>
<th>mTPE</th>
<th>cTPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-treatment IgG</td>
<td>g/L</td>
<td>6.05 ± 2.65</td>
<td>6.49 ± 2.84</td>
</tr>
<tr>
<td>Post-treatment IgG</td>
<td>g/L</td>
<td>2.15 ± 0.79</td>
<td>2.35 ± 1.15</td>
</tr>
<tr>
<td>Pre-treatment IgM</td>
<td>g/L</td>
<td>0.76 ± 0.34</td>
<td>0.81 ± 0.40</td>
</tr>
<tr>
<td>Post-treatment IgM</td>
<td>g/L</td>
<td>0.38 ± 0.13</td>
<td>0.44 ± 0.15</td>
</tr>
<tr>
<td>Total IgG in waste bag</td>
<td>g</td>
<td>12.31 ± 5.20</td>
<td>11.91 ± 6.29</td>
</tr>
<tr>
<td>Total IgM in waste bag</td>
<td>g</td>
<td>1.27 ± 0.57</td>
<td>1.50 ± 0.84</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD.

Conclusions: Centrifuge based techniques allow faster clearance of plasma from marker substances like IgG and IgM without any adverse events.

TH-PO896

Intraoperative Slow Low-Efficiency Dialysis during Emergency Surgery in Critically Ill Patients
Joanna Matuszukiewicz-Rowinska, Grzegorz Ostrowski, Mariusz Mieczkowski, Pawel Kalicki, Tadeusz Grochowiecki, Waldemar Patkowski, Krzysztof Dudek, Bohdan Solonynko. Medical University of Warsaw, Warsaw, Poland.

Background: There is some positive experience with intraoperative hemodialysis in cardiac and liver transplant surgery, however data concerning other surgical procedures are scarce. In this study we present our experience with intraoperative slow low-efficiency dialysis (SLED) performed during emergency laparotomy.

Methods: In all cases SLED was chosen since it remains the method of choice in hemodynamically unstable critically ill patients in our center. The Genius® single-pass system was used, with blood flow 180, and dialysate flow 80 ml/min.

Results: Intraoperative SLED was performed in four men, aged 30-59 years. The diagnoses were as follows: reperfusion due to massive hemorrhage after bilateral adenolecreton, intestinal ischemia after abdominal aortic stent-graft placement, multiorgan injury after traffic accident, and liver retransplantation. All patients died due to excess splanchnic and systemic vasodilation in patients with severe liver failure. The molecular adsorbent recirculation system (MARS) is a liver support system based on albumin dialysis which can potentially reduce systemic vasodilatation by removing protein-bound vasodilators.

The aim of this study was to determine whether the MARS technique is able to improve renal function in patients with type 1 HRS.

Methods: A four-year retrospective study (2006-2010) was carried out in a specialized nephrology-intensive care unit (ICU). The study group comprised 32 patients who had type 1 HRS and who underwent MARS treatment. Our endpoint was the complete, partial and bridge function in patients with type 1 HRS. MARS was a bridge to liver transplantation.

Outcomes in Neonates on Renal Replacement Therapy; a Single Center Experience
Shivandan S. Medar, Pamela S. Singer, James S. Killinger, Robert Wotronecki. Department of Pediatrics, Children’s Hospital at Montefiore, Bronx, NY.

Background: Initiating dialysis in newborns is controversial. Data on outcomes following Renal Replacement Therapy (RRT) is inconsistent. Reports from Israel/Europe on outcomes of dialysis in newborns (0-28 days) show 75% mortality rate; North American Pediatric Renal Transplant Collaborative Studies (NAPRTCS) data (based on voluntary reporting) shows 24% mortality. We hypothesize that RRT in newborns is associated with high morbidity and mortality.

Methods: Records of patients who had RRT initiated before 28 days of age at our institution between January of 1997 and January 2011 were reviewed. Dialysis treatment during the study period was offered to all neonates and infants that required it, unless they had life-threatening co-morbidities. The RRT physicians and nurses, equipment,
methods and procedures associated with RRT delivery during this time period remained consistent. The end-point for time period for RRT treatment was death, January 2011 or first renal transplant (Tx).

Results: 19 neonates (age of initiation: 9.1±1.5 days, birth weight: 3.0±0.5 kg, gestational age: 37.2±2.2 weeks) were identified. PD was administered in 13 (68%), HD in 4 (21%), CVVHD in 2 (10%) and HD in 2 (10%). 2 (10%) required more than one modality. Indications for RRT: fluid overload in 10 (53%), acidosis in 3 (16%), genetic defect in 4 (21%). 3 (16%) had congenital heart defect, and 2 (11%) multi-organ failure. 9 (47%) had severe electrolyte abnormalities, 13 (68%) required inotropes, 15 (79%) multiple blood products, and 18 (95%) therapeutically support. 8 (42%) had peritonitis, 3 (16%) had cerebral vasculitis, 1 (5%) had arterial hypertension. ICP length of stay was 32±33 days. 6 patients survived to ICU discharge, with 1 requiring chronic RRT and none received a renal Tx. 13/19 (68%) subjects died during observation period.

Conclusion: Newborns on RRT have high mortality and morbidity and poor outlook for renal Tx. Our patient mortality rate is in concordance with European/Israeli published series and contrasts NAPRTCS registry data possibly due to selection bias. Nephrologists should report their center RRT outcomes to parents of newborns before initiating the treatment.

TH-PO899 Combined Hemodialysis and Plasma Exchange Is Safe and Faster as Compared to Sequential Treatment in Children - Betti Schaefer,1 Ranny Goldwasser,1 Akos Ujjasvari,1 Susanne Schaefer,1 Karl Heinz Hecker,1 Franz S. Schaefer,1 Claus P. Schmitt,1 Center for Pediatric and Adolescent Medicine, Heidelberg; 1Institute of Pathophysiology, Semmelweis University, Budapest.

Background: Patients with immune-mediated kidney disease and liver failure often require renal replacement and hemodialysis and hemopurities (HD). HD is time consuming and connecting the PE and HD circuit in series, should allow for a more efficient treatment. The outcome has not yet been evaluated.

Methods: 15 out of 46 children (7.8-38.5 kg) were treated with combined (c) PE/HD, nine patients were treated with both sequential and sequential-s (s) PE/HD (8.5-80.0 kg), and 22 (5.75 kg) with sPE/HD only. Treatment modalities, efficacy, anticoagulation and clinical findings were analyzed retrospectively.

Results: Mean treatment duration was 3.9±2.2h per session for cPE/HD and 5.8±1.6h with sequential treatment (HD 5±1.6h) and hemodialysis (HD 7±1.8h). Dialysate flow was 490±201 with cPE/HD and 324±172 ml/min/m² with sPE/HD (p<0.01). PE/HD filter size per m² BSA and blood flow rates were similar (cPE/HD 112±44, sPE 92±22, HD 111±28 ml/min/m²; all p>ns.). Initial bolus of heparin consisted of 999±201 for cPE/HD and 389±475 for sPE and 389±475 for sHD (p=n.s. for cumulative dose). The dose of continuous and total heparin infusion and Activated Cloting Time were similar, as was the cumulative amount of citrate and calcium chloride infused in children treated with either method (n=16). Dialysis efficacy (creatinine, phosphorus, urea and bilirubin removal) and ultrafiltration rates were comparable (cPE/HD 821±648 vs. sPE/HD 925±528 ml/m², p>ns.). The decrease in INR was comparable in patients with liver failure. Both methods were well tolerated. Blood leakage and hemolysis occurred in 8 out of 89 sPE/HD sessions (9%) and in 4 out of 111 sPE/HD sessions (4%), hypofibrinogen in one sPE/HD session (1%).

Conclusions: PE/HD performed in series within one session is safe and allows for a more rapid purification as compared to sequential treatment. This should be beneficial in patients with severe diseases and reduce work load. Careful dialysator pressure control, a more rapid purification as compared to sequential therapy. This should be beneficial in sPE/HD sessions (4%), hypothermia in one sPE/HD session (1%).

TH-PO900 Two Year Study of Bone Metabolism with Acetate-Free Bicarbonate Dialysate Buffered by Citric Acid - Junji Uchino. Mihama Hospital, Chiba, Japan.

Background: To correct metabolic acidosis, sodium bicarbonate was added to the dialysate to achieve a blood plasma bicarbonate concentration of 24 mmol/L prior to maintenance dialysis. It has been reported that comparison of bone biopsy findings between before and after the correction of metabolic acidosis showed inhibition of secondary hyperparathyroidism and stimulation of the turnover of hypophosphatemic bone.

Methods: To investigate the effects of acetic-free bicarbonate dialysate (Carbonstar Citric Acid 2 containing 35 mmol/L of bicarbonate; CB) on bone metabolism in patients on maintenance dialysis.

Subjects: Two-hundred and ninety eight of maintenance dialysis patients were studied. Their age was 66±11.7 and the duration of dialysis was 9.4±8.1 years.

The patients were classified into low (i-PTH < 60 pg/mL), normal (i-PTH 60-180 pg/mL), high (i-PTH > 180 pg/mL) PTH groups. The levels of PTH were measured by using both indirect and direct method. Dialysis efficacy, drainage rate, expression rate of DLA-DR, secretary function in Canine model for MODS. The Effect of Continuous Veno-Venous Haemodiafiltration on Monocyte Function in Canine Model for Multiple Organ Dysfunction Syndrome

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

1Dialysis Center, Konkuk University Hospital, Seoul, Republic of Korea; 2Nephrology, Konkuk University Medical Center, Seoul, Republic of Korea.

Background: Hypophosphatemia is a common complication of CRRT. However, there are a few studies in critically ill adults undergoing CRRT in which phosphate was added to the replacement and dialysate solutions. The objectives of this prospective study were to evaluate the incidence of hypophosphatemia during CRRT and the efficacy and safety of phosphate supplementation in critically ill adults undergoing CRRT.

Methods: Adult patients who admitted to the ICU and undergoing CRRT for at least 48 hours were recruited in this prospective randomized two-arm comparative study. All patients were randomly assigned to the P-15.0 group and P-22.5 group. If hypophosphatemia was detected during CRRT, we added phosphate 15.0 mEq or 22.5 mEq to both the replacement solution (5L) and the dialysate solution (5L) in the P-15.0 or P-22.5 group, respectively. The phosphate, calcium and potassium levels were recorded before CRRT and every 24 hours after starting CRRT.

Results: A total of 29 adult patients were enrolled (P-15.0 group, n=16; P-22.5 group, n=13). The mean levels of serum P at the beginning of CRRT was 4.9±1.5 μg/dL. During CRRT, 24 patients (82.7%) were found to have hypophosphatemia (mean levels of serum P, 2.2±0.3 μg/dL which occurred at 52.0±39.2 hrs following initiation of CRRT. After adding of phosphate to the replacement and dialysate solutions, serum P levels for the P-15.0 and P-22.5 group were 4.1±1.2 μg/dL and 4.6±0.8 μg/dL, respectively (p<0.05). Following phosphate addition, the times of restoration to normal values of serum P Levels for the P-15.0 and P-22.5 groups were 37±18 hrs and 26.7±8.0 hours, respectively (p<0.030). Except one patient (3.5%), intravenous phosphate supplementation was not needed. It may be noted that the mean block of adverse effects of phosphate supplementation during CRRT have been observed.

Conclusions: This results indicated that the incidence of hypophosphatemia in critically ill adults undergoing CRRT is very high and the phosphate addition to replacement and dialysate solutions is safe and effective on correcting hypophosphatemia during CRRT.

TH-PO902 The Effect of Continuous Veno-Venous Haemodiafiltration on Monocyte Function in Canine Model for Multiple Organ Dysfunction Syndrome

Chen Ji Hong. Medical University, Urumqi, XinJiang Province, China.

Background: Continuous veno-venous haemodiafiltration (CVVHDF) has gained wide acceptance for the treatment of multiple organ dysfunction syndrome (MODS) in intensive care. This study evaluated specific effects of CVVHDF on the monocyte counts, apoptosis rate, expression rate of DLA-DR, secretory function in Canine model for MODS.

Methods: 12 beagle dogs were subjected to hemorhagic shock plus resuscitation and endotoxemia to set up MODS model. Shock was produced according to the method of Wiggers. Then the animals were resuscitated by infusion of Ringer’s solution at the twice of volume of shed blood. After 12 hours of the resuscitation, Escherichia coli endotoxin (LPS) was dropped in a dose of 1.5 mg/kg in 500 ml of normal saline for 12 hours. Then 12 dogs were randomly divided into two groups: CVVHDF group and MODS group. Measurements of variables were obtained at baseline (T0), before LPS injection(T1) and 0h(T0), 3h(T1), 6h(T2), 9h(T3), 12h(T4), 15h(T5) and 24h(T6) after LPS injection was finished.

Results: 1. After treatment of CVVHDF, MAP and heart rate remained stable with decreased body temperature, increased urine output, decreased total number of white blood cell and improved lung and renal function. 2 Early and late monocyte apoptosis rate in CVVHDF group was significantly lower than the MODS group, and the number of monocyte was significantly increased (p<0.01). 3 The expression of CD4, CD10, CD-LA DR in CVVHDF group was significantly higher than in MODS group (p<0.01). CVVHDF facilitated monocyte antigen-presenting function in the inhibitory state to recover gradually. 4 CVVHDF improved the secretion of IL-1β and IL-4 by monocytes, upregulated secretory function of DLA-DR and secretory function of anti-inflammatory factor IL-4 thus facilitating the gradual recovery of secretory function of monocytes in inhibitory state(p<0.01).

Conclusions: Our results suggested that treatment with CVVHDF effectively removed cytokines from the circulation, reduced monocyte apoptosis, increased number of monocyte, and improved function of presentation and secretory. Thus, it facilitated reconstruction of immune homeostasis.

Funding: Government Support - Non-U.S.
TH-PO903

Management and Practice of CRRT in ICU: A Survey of Italian Nurses
Flavio Basso,1 Mariangela Mattifogio,1 Dinna N. Cruz,1 Nathan W. Levin,2 Zaccaria Ricci,2 Alessandra Brendolan,1 Federico Nalesso,1 Francesco Garzotto,1 Claudio Ronco.1
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Background: Nursing in continuous renal replacement therapy (CRRT) requires specific expertise for its correct implementation. The nurses’ role is fundamental in the prevention and management of adverse events.

Our aim was to explore nursing practice in Italy in CRRT management in order to identify strategies to improve treatment quality.

Methods: During the course on “Nursing skill and practice for adequate CRRT” (Vicenza 2010), participants were surveyed about CRRT management, including the complications leading to loss of the circuit and the most important limitations for CRRT.

Results: The questionnaires were correctly filled out by 113 of 126 Italian nurse participants (89.7%).

CRRT was managed by either ICU nurses (42.8%) or nephrology nurses (49.6%).

The most frequent cause for discontinuation of treatment was circuit loss (59%), then high circuit pressures (28%), and problems such as hypotension (13%). 78.5% of nurses indicated as limitations of CRRT the absence of comprehensive training (38%), frequent filter clotting (32%) and lack of a checklist (24%).

When an alarm is triggered, 88% of nurses identified the cause immediately and resolved it while 9% performed a full system check. Of note, 3% muted the alarm without investigation of the cause. This dangerous practice is a further demonstration of the need for better training of CRRT for nursing staff.

Conclusions: The results of the survey indicate that more careful training in the correct methods for CRRT is required. Such training would bring direct benefits to critically ill patients undergoing this procedure. Periodic surveys on nursing practice could identify potential areas for improvement.

TH-PO904

Renal Failure Requiring Dialysis after Slow Continuous Ultrafiltration in Patients with Advanced Heart Failure
Edvard I. Wehbe,1 Maria M. Patarroyo,2 Jonathan J. Taliercio,1 W.H. Wilson Tang,2 Sevag Demirjian.1 1Nephrology, Cleveland Clinic.

Background: Slow continuous ultrafiltration (SCUF) has been increasingly utilized in patients with acute decompensated heart failure (ADHF). However, a subset of patients develop worsening renal function requiring dialysis. We aim to describe the incidence and outcome of those patients.

Methods: Our cohort consisted of 63 patients who underwent SCUF as initial modality of ultrafiltration in patients with ADHF from 2004 till 2009. The cohort was divided in 2 groups; those who needed only SCUF and those who transitioned to continuous venovenous (CVVHD) or intermittent (IHD) hemodialysis.

Results: Out of 63 patients (mean age 59+/−11, 76% male) 37(59%)transitioned to CVVHD or IHD. There were no differences in demographics, comorbidities, medications and ejection fraction between the 2 groups. Patients who required transition had a higher creatinine at baseline(1.44+/−0.43 vs 1.87+/−0.76 P=0.035), at SCUF initiation and after 48 h(1.7+/−0.7 vs 2.9+/−0.9 P=0.001), lower systolic blood pressure at baseline and at time of initiation of SCUF(112+/−2 vs 101+/−2 P=0.003), lower systemic vascular resistance (SVR) at baseline and at time of initiation of SCUF(1189+/−494 vs 826+/−229 P=0.008). There was no difference in mean pulmonary arterial pressure, central venous pressure, cardiac index/output, pulmonary capillary wedge pressure and pulmonary vascular resistant. Table 1 summarizes the clinical outcomes.

Conclusions: This study shows that a large number of patients required dialysis support after SCUF. Those patients had a higher creatinine and lower systolic blood pressure at the baseline and at time of SCUF initiation. Transition to dialysis was associated with very high one year rate of mortality.

TH-PO905

Weigh Gain after Renal Transplantation: Combined Effects of Nutritional Factors and Lower Physical Activity
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Background: Weight gain after renal transplantation is related to increased cardiovascular risk and decreased graft survival. Our aim was to identify lifestyle factors related to gain in fat mass, and its association with cardio-metabolic risk factors at one year after renal transplantation.

Methods: At 6 weeks and 3, 6, and 12 months after transplantation, post-transplant weight and fat distribution (bio-electrical impedance, bio-impedance analysis) and cardio-metabolic risk factors, renal function, nutritional intake (24h recall and interviews; 24h urine collections) and physical activity (SenseWear diaxial accelerometer; SQUASH questionnaire) were assessed.

Results: From 29 participants, 26 (48% men, age 51 ± 12 years) completed the measurements. At 12 months, patients had gained on average 5.7 ± 5.0 kg (range -2 to +20 kg) in weight, mainly fat tissue. Gain in body fat was positively associated with serum total cholesterol (r=0.46, P=0.02) and triglycerides (r=0.51, P=0.01) at 12 months. In the patients who gained most in body fat (>35% fat gain, N=13), cardiovascular risk factors at 12 months were higher, i.e. LDL-cholesterol (+0.8 mmol/l, P=0.02), total cholesterol (+1.2 mmol/l, P=0.006), and triglycerides (+1.0 mmol/l, P=0.03) compared to those who remained weight stable (<3% fat gain, N=13). Immunosuppressive therapy, renal function, random glucose and HbA1c were comparable. Those who gained in body fat showed lower daily physical activity (<35%, P=0.04) and walked fewer steps per day (−33%, P=0.01). In addition, body fat gain was related to a 30% lower intake in vegetables (P=0.04) and a 20% higher consumption of mono and disaccharides (P=0.02), mainly due to the consumption of energy-rich drinks and sugared dairy (P=0.05).

Conclusions: Daily physical activity, vegetable intake and high consumption of energy-rich drinks and dairy may provide targets for lifestyle intervention to prevent weight gain and improve long term cardiovascular and renal outcomes.

TH-PO906

Treating Post Transplant Anaemia with EPO Improves Quality of Life
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Background: Anaemia affects 30-45% of renal transplant recipients. Treatment with ESAs has not been well studied and the effects of long term treatment are not known.

Methods: An exploratory study to assess the effect of treatment with Epoetin beta (EB) on renal progression in anaemic renal transplant recipients was performed. The effect on Health Quality of Life (HQOL) and Left Ventricular Hypertrophy was also assessed. The treatment arm received EB to achieve a target of 12-13.5 g/dL. The No Treatment group (NT) was treated with EB if the Hb fell below 9g/dL. The primary end-points were progression of CKD, blood pressure and proteinuria. Secondary end-points were HQOL assessed by SF-36 and Left Ventricular Hypertrophy assessed by LVMI.

Results: 55 patients were recruited (NT N= 27, EB N=28) with a median of 23.4 months follow-up. At the end of the study the HB was significantly higher in the EB group (EB: 12.3 ± 0.18 vs. NT: 9.99 ± 0.22 g/dL, P= 0.0001). There was no significant difference in disease of eGFR, PCR or blood pressure between the 2 groups throughout the study. Similarly LVMI was not significantly different.

Conclusions: However, an improvement was seen in Vitality (Baseline: 39.81 ± 2.55 vs. End of Study: 44.54 ± 2.66, P = 0.03), Physical Function (Baseline: 42.04 ± 2.28 vs. End of Study: 48.29 ± 2.62, P = 0.01) Domains as well as Physical Component Summary (Baseline: 38.76 ± 2.94 vs. End of Study: 44.69 ± 2.81, P= 0.002) in the EB group. There was also a small, but significant improvement in the Physical Function Domain in the NT group (Baseline: 39.27 ± 2.21 vs. End of Study: 42.52 ± 2.48, P= 0.03). A trend to improvement in change from baseline was seen in the EB group when compared to the NT group. A significant improvement was seen in the Vitality domain (NT:3.12±(-3.1,6.24) vs. EB: ±0.52±(-12.5, 2.5), P= 0.02).

Anaemic renal transplant patients treated with EB benefit from improved Vitality in this small exploratory RCT. A large, multi-centred RCT is warranted to study quality of life benefits of in long-term ESA treatment in PTA.

Funding: Pharmaceutical Company Support

TH-PO907

Residence Location and Risk of Transplantation among Pediatric Patients with End-Stage Renal Disease
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Background: Due to Canada’s large size, many pediatric end-stage renal disease (ESRD) patients reside far from a pediatric renal care centre. It is unknown whether this potentially reversible geographical barrier affects likelihood of kidney transplant.
Methods: Population-based retrospective cohort study using data from national ESRD registry. Deceased and living kidney replacement therapy between 1997 and 2007 were followed until death or last contact. Primary outcome was kidney transplant (living or deceased donor). Distances between nearest pediatric transplant centre and each patients' residence were calculated using geographic information software, and categorized as: <50 km, 50-149.9 km, 150-299.9 km, and ≥300 km. Cox proportional hazards models were used to compare likelihood of transplant by distance category, adjusting for gender, age, socioeconomic status and primary disease. Separate models were used for whites and non-whites due to a significant interaction between ethnicity and distance.

Results: 728 patients were included (52.2% males; 62.5% white). 38.5% lived ≤50 km, 20.1% lived 50-149.9 km, 14.3% lived 150-299.9 km and 27.2% lived ≥300 km away from a pediatric transplant center. Among whites, compared to those living <50 km, patients living between 150-299.9 km and ≥300 km from a transplant centre were less likely to receive a deceased donor transplant (adjusted hazard ratios [95% CI]: 0.55 [0.33-0.92] and 0.61 [0.39-0.94] respectively). There were no differences in likelihood of living donor transplant by distance to care centre among whites. Among non-whites there was no association between distance to a transplant centre and likelihood of transplant for both living and deceased donor transplants.

Conclusions: White pediatric ESRD patients living ≥150 km away from a transplant centre are less likely to receive deceased donor transplant. Further evaluation is necessary to determine barriers in access to deceased donor transplant among remote dwelling patients.

Funding: Private Foundation Support

TH-PO908

Risk Factors for Efficacy Failure with Tacrolimus-Based Immunosuppression after Kidney Transplantation – The OSAKA Study

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Background: OSAKA is one of the largest clinical trials ever conducted in de novo kidney transplantation (n=1,251). This allows an analysis of donor and recipient risk factors that impact on the risk of efficacy failure on tacrolimus (Tac)-based immunosuppression.

Methods: Adult kidney recipients were randomized 1:1:1:1 to starting doses of Tac immediate release (IBD) 0.2mg/kg/day (Arm 1), Tac prolonged release (QR) 0.2mg/kg/day (Arm 2), Tac QR 0.3mg/kg/day (Arm 3), all with MMF + corticosteroids (CS) for 24 weeks, or Tac QR 0.2mg/kg/day + MMF + basiliximab + CS (peroperative bolus only) (Arm 4). The primary composite endpoint was efficacy failure rate (graft loss, biopsy confirmed acute rejection, graft dysfunction) [EGR (MDDR) <40ml/min/1.73m²] at 24 weeks. Logistic regression (LR) and a simplified Classification And Regression Tree (CART) method were used to quantify risk factors.

Results: Efficacy failure rates as defined (PPS) were 40.6% (Arm 1), 42.2% (Arm 2), 44.2% (Arm 3), and 48.2% (Arm 4). LR analysis identified donor age, female donor, donor death, and underlying focal segmental glomerulosclerosis (FSGS) as the most relevant risk factors, with donor age the most statistically significant factor. Each additional year of donor age increased the odds of efficacy failure by more than 4% on average. FSGS doubled the risk, a female donor increased the risk by nearly 28%, each point of mismatch by almost 10%, and a cadaveric donor by nearly 50%. CART confirmed that patients with the highest risk were those with a donor age over 61 years, and identified more than four matches as a risk factor. Patients with a donor age over 61 years had a 2.9 fold increased risk of efficacy failure, and a cadaveric donor was the overriding risk factor for the composite endpoint of efficacy failure (as well as its single components).

Conclusions: With Tac-based therapy, donor age was the overriding factor for the composite endpoint of efficacy failure (as well as its single components).

Funding: Pharmaceutical Company Support

TH-PO909

Concomitant Food Ingestion Decreases Tacrolimus Absorption Well Below FDA Standards for Approved Generics

Linda Awdishu,1 Son Ho,2 Amol Shah,2 Robert W. Steinberg,3 ‘Clinical Pharmacy, UCSF Skaggs School of Pharmacy, La Jolla, CA; ‘Medicine, Division of Nephrology, UCSF Medical Center, San Diego, CA.

Background: The probability and degree of diurnal tacrolimus (TAC) exposure in chronic renal transplant recipients (CRTRs) has received increased attention due to the availability of generic alternatives. Concern about TAC exposure is appropriate considering the predictability and degree of diurnal TAC exposure in chronic renal transplant recipients (CRTRs).

Methods: This is a randomized, double-blind, multi-center study of the effect of food on Tac absorption in 12 healthy male volunteers. Tac was administered orally in two doses (0.05 mg/kg TAC) at 0 and 4 hours with and without a standard breakfast. Blood samples were collected at 0, 1, 2, 4, 6, 8, 12 and 24 hours post-dose. Absorption profiles with and without food in 4 patients are shown in Figure 1.

Results: Absorption profiles with and without food in 4 patients are shown in Figure 1.

Fasting curves were relatively uniform with robust early peaks. The fasting state AUC0-12 ranged from 120.05 to 249.98 ng*mL/hr. The fed state AUC0-12 ranged from 56.83 to 199.93 ng*mL/hr. The ratio of the fed to fasting state AUC ranged from 38 to 111%. We calculated the natural logarithm of the difference in peak concentration (Cmax), time to peak concentration (Tmax), and AUC between fasting and fed states. The difference in ln(Cmax) was 0.24 ±0.27 ng/mL (p<0.001), Tmax was -0.93 ± 1.14 hr (p<0.008) and ln(AUC) was 0.24 ± 32 ng*mL/hr (p<0.005). Importantly, drug concentrations at 8 hrs post dose was similar between fed and fasting states demonstrating the importance of Cmax for drug exposure.

Conclusions: Ingestion of TAC with food reduces exposure well beyond any effect of FDA approved generic when taken under the same circumstances. The food effect may be responsible for suboptimal immunosuppression and graft survival in some adherent patients. Such underexposure will not be detected by 12 hour trough TAC levels.

Funding: Other NIH Support - T32 training grant for student summer research, Pharmaceutical Company Support

TH-PO910

Predilation Nephrology Care, Early Transplant Assessment and Patient Satisfaction with Physician Interaction

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Background: Satisfaction with physician interaction is a key component of dialysis patients' satisfaction with their care. We hypothesized that whether patients receive predilation nephrology care and have early assessment for transplantation may influence their satisfaction.

Methods: In phone interviews the Comprehensive Dialysis Study (CDS) surveyed 1473 incident HD patients and 169 incident PD patients aged >18 from 296 randomly selected clinics throughout the US. Thinking about the kidney doctor they saw most often, respondents rated “the amount of time your kidney doctor spends with you” and “your kidney doctor’s explanations of medical procedures and tests” [Medical Outcomes Study items].

Results: Although most respondents rated their interaction with their kidney doctor as good to excellent (76% for time spent, 83% for procedure/test explanations), in a linear regression analysis with adjustment for patient clustering in clinics, patients on HD, Hispanics, and patients who lacked predialysis nephrology care were significantly less satisfied with both aspects of physician interaction, and patients with less than high school education were less satisfied with their doctor’s explanation of procedures/tests. No age, gender, or race differences were significant. The ESRD Medical Evidence Report indicated that 245 CDS participants had not been assessed for transplantation. Compared to those reported informed of transplantation options, patients who had not been assessed were less satisfied with the time their doctor spent with them (p = 0.05) and with their doctor’s explanations of procedures and tests (p = 0.05).

Conclusions: Congruence between expectations and perceived experience influences satisfaction. Patients who lack predilation nephrology care may expect more time and information from their physicians, especially with regard to provider/patient communication related to kidney transplant options.

Funding: NIDDK Support

TH-PO911

Evaluation of the Living Kidney Donor Programme in the United Kingdom

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Background: Living kidney donation (LKD) has increased in the UK by 12% (1037 donors in 2010 which is 38% of the total UK kidney transplant activity. There has been growing evidence and evolving guidelines for LKD. However there are still inconsistencies in practice between renal units. We aim here to study LKD assessment across the UK centres.

Methods: A structured questionnaire relating to LKD assessment was sent by post to 74 renal units across the UK between October to December 2010

Key: TH- Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conversion to LCP-TacroTM Tablets Once-Daily from Prograf® Capsules Twice-Daily in Kidney Transplant Patients: Central Pathology Results from a Phase III, Open-Label, Multicenter Trial

**Background:** During the past two decades, the development of bioavailable immunosuppressants has allowed for drug administration once-daily, with reduced toxicity and better adherence. However, there is a lack of studies comparing the effect of early steroids withdrawal (ESW) and the effect of conversion of tacrolimus (TAC) therapy to an extended-release formulation (LCP-Tacro) once-daily vs. twice-daily in stable renal transplant patients.

**Objective:** To compare the effect of ESW and the effect of conversion to LCP-Tacro qd-based therapy in stable renal transplant recipients.

**Methods:** This was an open-label, randomized clinical trial comparing 2 groups: ESW-G (n=28) and CG (n=22). The mITT groups (LCP-Tacro: n=162; Prograf: n=162) were similar in demographics and transplant details. The body compositions such as lean and fat mass were calculated using bioelectrical impedance analysis. Pharmacokinetic profiles were taken at 0, 1, 2, 3, and 4 hours after application of tacrolimus and compared between the low and high level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Results:** The mITT groups (LCP-Tacro: n=162; Prograf: n=162) were similar in demographics and tacrolimus trough levels throughout the study; 35 biopsies were centrally read (23 LCP-Tacro, 12 Prograf). One LCP-Tacro patient had 1 biopsy-proven acute rejection (BPAR) episode vs. 7 BPARs in Prograf patients (5 Prograf patients with BPAR, 2 patients had 2 episodes). Trough levels of 4.15 ng/mL were targeted in both drugs.

**Conclusions:** LCP-Tacro qd-based therapy was associated with a numerically lower number of BPARs and the data suggest a tendency toward fewer treatment failures and SAEs when compared to Prograf bid. The mITT groups (LCP-Tacro: n=162; Prograf: n=162) were similar in demographics and transplant details. The body compositions such as lean and fat mass were calculated using bioelectrical impedance analysis. Pharmacokinetic profiles were taken at 0, 1, 2, 3, and 4 hours after application of tacrolimus and compared between the low and high level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Results:** The mITT groups (LCP-Tacro: n=162; Prograf: n=162) were similar in demographics and transplant details. The body compositions such as lean and fat mass were calculated using bioelectrical impedance analysis. Pharmacokinetic profiles were taken at 0, 1, 2, 3, and 4 hours after application of tacrolimus and compared between the low and high level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Conclusions:** LCP-Tacro qd-based therapy was associated with a numerically lower number of BPARs and the data suggest a tendency toward fewer treatment failures and SAEs when compared to Prograf bid.

**Funding:** Pharmaceutical Company Support

**TH-PO914**

Pharmacokinetics of Tacrolimus According to the Body Composition in Korean Kidney Transplant Recipients Seung Seok Han,1 Curie Ahn,1 Jin Suk Han,1 Su-Hyungwon Kim,1,2 Yon Su Kim,1,2 1Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; 2Clinical Research Center for End Stage Renal Disease, Korea.

**Background:** Current dosing of calcineurin inhibitor after transplantation is based on the patient’s body weight. However, kidney transplant recipients have a low correlation between body weight and body composition. Here, we evaluate the pharmacokinetics of tacrolimus according to the body composition in 17 Korean kidney recipients with stable graft function.

**Methods:** The body compositions such as lean and fat mass were calculated using the bioelectrical impedance analysis. Pharmacokinetic profiles were taken at 0, 1, 2, 3, and 4 hours after application of tacrolimus and compared between the low and high level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Results:** The medians of body mass index (BMI) and body compositions were as follows: BMI: 25.0 kg/m² [interquartile range (IQR, 22.2–26.3)]; fat mass, 17.2 kg (IQR, 4.2–20.1); lean mass, 49.4 kg (IQR, 43.2–58.1). There were no statistical differences in pharmacokinetic profiles according to BMI. However, the concentrations (C) in the high fat group were higher than the low fat group (Ps = 0.060 and 0.034, respectively). The concentrations (C) in the high and low level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Conclusions:** Taken together, these data provide the suggestion that the monitoring of the dose of tacrolimus should be based on the individual body compositions for further improvement of kidney transplant outcomes.

**Funding:** Pharmaceutical Company Support

**TH-PO915**

Response to CINACALCET in Renal transplant Patients with Secondary Hyperparathyroidism and Hypercalcemia: What Is the Role of the Magnesium? Victor Martín Liéden,1,2 Nephrology, Hospital Reina Sofia, Murcia, Spain; 2Nephrology, Hospital Virgen Arrixaca, Murcia, Spain.

**Background:** CINACALCET is able to bind to the calcium-sensing receptor in parathyroid to modify it allosterically, being useful in controlling severe hyperparathyroidism with persistent hypercalcemia in renal transplant recipients. It is known that before correcting hypercalcemia is necessary to solve the deficit of magnesium, due to the hypomagnesemia would activate the calcium-sensing receptor. However, we found no study evaluating the effect of magnesium on the hypercalcemia and the response of cinnacalcet in these patients.

**Results:** The medians of body mass index (BMI) and body compositions were as follows: BMI: 25.0 kg/m² [interquartile range (IQR, 22.2–26.3)]; fat mass, 17.2 kg (IQR, 4.2–20.1); lean mass, 49.4 kg (IQR, 43.2–58.1). There were no statistical differences in pharmacokinetic profiles according to BMI. However, the concentrations (C) in the high fat group were higher than the low fat group (Ps = 0.060 and 0.034, respectively). The concentrations (C) in the high and low level groups according to the median of body composition. The values of \( C_0, C_2, C_3, \) and \( C_4 \) were used in determining abbreviated area under the concentration–time curve (AUC) for tacrolimus.

**Conclusions:** Taken together, these data provide the suggestion that the monitoring of the dose of tacrolimus should be based on the individual body compositions for further improvement of kidney transplant outcomes.

**Funding:** Pharmaceutical Company Support

**TH-PO913**

Early Steroids Withdrawal Improves Hemodynamic Profile and Does Not Increase Acute Rejection Risk in Kidney Transplant Recipients Jorge Andrade-Sierra,1,2 Enrique Rojas-Campos,1 Ernesto Cardona,3 Luis Alberto Evangelista-Carrillo,1 Trinidad Orlando Lugo Lopez,1 Abel Puentes Canacho,1 Salvador Mendoza Cabrera,1 Benjamin Gomez-Navarro,2 Mario Sandoval Sandoval,2 Alfonso M. Cueto-Manzano,11 Medical Research Unit in Renal Diseases, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico; 2Department of Nephrology and Organ Transplant Unit, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, Mexico; 3Department of Physiology, CUCS. University of Guadalajara, Guadalajara, Jalisco, Mexico.

**Background:** Early steroids withdrawal (ESW) improve cardiovascular profile but could increase the risk of acute rejection (AR). **Objective:** To compare the effect of ESW on heart rate and blood pressure frequency.

**Methods:** Open label, randomized clinical trial; 55 KTR, with PRA<20%, between Jul/10-Jun/11; 28 in the ESW-G / 27 in the control group (CG), maintenance was with TAC 0.1 mg/Kg/week. Comparisons between groups, were done with Mann-Whitney U and tests. **Results:** Main results are shown in Table. At 3 mos, ESW-G had 4 (14%) borderline, CG had 3 (11%) Banf IA rejections; at 6 months ESW-G 3 (11%) borderline, CG had 3 (11%) borderline and 2 (7%) Banf IA rejections.

**Conclusions:** ESW-G shows a better control of blood pressure and trend to show lower AR frequency and severity compared to CG.

**Other variables such as waist circumference, waist-hip ratio, and arm circumference did not differentiate the pharmacokinetic profiles of tacrolimus.**

**Conclusions:** Taken together, these data provide the suggestion that the monitoring of the dose of tacrolimus should be based on the individual body compositions for further improvement of kidney transplant outcomes.
Objectives: To evaluate the effectiveness of treatment with cinacalcet in renal transplant with severe hyperparathyroidism and hypercalcemia. To study the role of magnesium in the response of cinacalcet.

Methods: - 37 renal transplant with creatinine clearance 50 ml/min, Ca 10.5 mg/dl and PTH 65 pg/ml have been studied for 3 months to 4 years. - Parameters to analyze: Ca, P, PTH and magnesium pretreatment with cinacalcet, 1 month, 3 months, 6 months and annually. If the magnesium < 1.65 mg/dl initiated oral magnesium tablets to maintain stable levels of magnesium. - Cinacalcet dose: 30-90 mg / day.

Results:
- Decreased calcium and PTH
- Increased phosphorus
- 14 patients (37.8%) had hypomagnesemia pretreatment, 13 of them were treated with oral magnesium.
- Patients with lower initial levels of magnesium (before starting treatment with cinacalcet) had, significantly, a greater decrease in PTH and calcium (p <0.05), as shown on table 1. 

Conclusions: Cinacalcet is effective in controlling hypercalcemia in renal transplant with secondary hyperparathyroidism. Pretreatment hypomagnesemia seems to favor the response of cinacalcet. In view of these results, should we try to correct magnesium deficiency in all cases or only in symptomatic patients?

Funding: Clinical Revenue Support

TH-PO917

Identification of Potential Criteria for Rituximab Responsiveness in Patients with Standard-Therapy Resistant Kidney Allograft Rejection

Maximilian Ernst Daemmrich,1 Jan U. Becker,1 Verena Broecker,1 Clemens L. Bockmeyer,1 Willfried Gwinner,2 Anke Schwarz,2 Cornelia Anneliese Blume.2 1Institute of Pathology, Medical School Hannover, Hannover, Lower Saxony, Germany; 2Dept. of Nephrology and Hypertensiology, Medical School Hannover, Hannover, Lower Saxony, Germany.

Background: Rituximab (anti-CD-20 antibody) is used in kidney transplant rejection refractory to standard therapy, although it carries a risk for serious complications and is not effective in all cases. In a retrospective study, we therefore aimed to identify clinical or histopathological criteria to predict Rituximab response.

Methods: 19 renal transplant recipients who received Rituximab (375 g/m2 body surface, 1-2 courses, 15 x combined with up to 5 courses of plasmapheresis) for therapy refractory rejection were included in the study. 10 lost their transplant function (non-responders), in 9, kidney grafts were rescued (responders). Clinical parameters and Banff components of the last biopsy prior to Rituximab were compared between both cohorts by Wilcoxon- or chi-square tests. Observation time after therapy was not different between groups (26±26 vs. 49±24 months; p = 0.07).

Results: More responders had donor-specific antibodies (6/9 vs. 1/10; p = 0.01). Responders had lower serum creatinine before therapy (175 vs. 291 µmol/l; p = 0.005). Banff glomerulitis (p = 0.014) and C4d staining of peritubular capillaries (C4d ptc, p = 0.03) was less severe in responders. Other clinical (recipient gender and age, number of HLA-mismatches, living or cadaveric donor, previous renal transplant, time after transplantation) and histopathological criteria (glomerular C4d staining, tubulointerstitial edema, cell type, ptc, peritubular and glomerular endothelial swelling) were not different between groups. Responders had a mean serum creatinine of 189 ± 45 µmol/l at the end of the observation time.

Conclusions: In this retrospective analysis, lower serum creatinine before therapy, positive donor specific antibodies, less transplant glomerulitis and C4d staining of ptc were identified as potential criteria to predict Rituximab responsiveness. These criteria need validation in future prospective randomized studies.

TH-PO918

Safety Issues Identified by Proactive Living Donor Kidney Transplant (LDKT) Safety Debriefing


Background: Little is known about the extent of safety vulnerabilities surrounding LDKT. Hospital incident reporting systems exist, but are known to vastly underestimate rates. To comprehensively assess vulnerabilities surrounding LDKT surgery, clinicians were asked to complete a debriefing survey.

Methods: A 28 question web-based debriefing was developed by an interdisciplinary team of patient safety experts and transplant clinicians. Comments were solicited on all-related system or process issues. All clinicians in the LDKT surgery were asked to complete the anonymous survey immediately following the surgery. All incidents were reviewed online or by phone to the hospital-wide reporting system for these LDKTs were reviewed.

Results: 210 debriefings were submitted on 83 LDKT procedures. Debriefings were completed by the entire clinical team: 40% by the surgical team, 37% by the nursing team, 16% by the anesthesia team, and 7% by others (e.g. lab tech, etc.). 213 vulnerabilities related to transplant systems and processes of care were reported. The most frequently reported included Equipment (e.g. broken surgical instruments), OR Scheduling/Coordination (e.g. avoidable delays), and Distractions (e.g. cell phones/pagers ringing).

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In stark contrast, hospital-wide reporting systems identified just 22 reports for these LDKTs. 13 of these incidents were also captured by the safety debriefings. This is supporting evidence that to date safety risks associated with LDKT are poorly described and vastly underestimated.

Funding: Private Foundation Support

TH-PO919

Incidence and Costs Associated with New-Onset Diabetes and Cardiovascular Events after Kidney Transplantation in a Commercially Insured Population Suhpanmai Bunnarapradit,1 Schiffon L. Wong,2 Brett Pinsky,1 Fang Liu,1 Cynthia Taylor,1 Digisha Trivedi,2 Tony Hebden.2 1Department of Medicine, UCLA, Los Angeles, CA; 2Observational Research, OptumInsight, Eden Prairie, MN.

Background: Kidney recipients are at increased risk of developing diabetes and cardiovascular (CV) events which adversely impact graft and patient survival. Our objectives were to describe the prevalence of pre-transplant diabetes and CV events; incidence of new-onset diabetes after transplantation (NODAT) and CV events post-transplant; and associated costs in commercially insured kidney transplant recipients.

Methods: Adults with evidence of a kidney transplant (2004-2008) were identified using healthcare claims from a large U.S. managed care plan. "Incident" recipients (IR) had a transplant date within the study period. "Prevalent" recipients (PR) had evidence of a transplant but an unknown transplant date. Graft failure was the first of retransplant or return to dialysis (2 or more dialysis visits in a 30 day period for 2 consecutive 30 day periods) and was identified from the earliest evidence of transplant until death, end of continuous enrollment, or 31 Dec 2009. Graft failure costs were patient and health plan-paid medical and pharmacy costs (Figure 1).

Results: Among 1364 IR, 11.8% had graft failure (10.9% returned to dialysis, 1.9% retransplant). Among 6753 PR, 8.4% had graft failure (8.2% returned to dialysis, 1.1% retransplant). Mean graft failure cost was $25,380/month. Return to dialysis was a major cost driver with a mean monthly cost of $16,867. Retransplant average was $87,062. Median healthcare costs 3, 6, and 12 months post-transplant were $23,189 greater, $41,794 greater, and $55,789 greater, respectively, for patients with graft failure than for patients without failure in those time frames.

Conclusions: Graft failure occurred in 8-12% of these commercially insured kidney recipients and was associated with high costs for patients and commercial payers whether or not the transplant was paid for by the health plan. Strategies to mitigate graft failure may reduce these costs.

Funding: Pharmaceutical Company Support

TH-PO920

Incidence and Cost of Renal Graft Failure in Commercially Insured Kidney Transplant Recipients Suhpanmai Bunnarapradit,1 Schiffon L. Wong,2 Brett Pinsky,1 Fang Liu,1 Cynthia Taylor,1 Digisha Trivedi,2 Tony Hebden.2 1Department of Medicine, UCLA, Los Angeles, CA; 2Health Services, Bristol-Myers Squibb, Plainsboro, NJ; 3Observational Research, OptumInsight, Eden Prairie, MN.

Background: The USRDS Annual Data Report cites a renal graft failure rate of 6.5 per 100 patient years in 2008 but little is known about the impact of graft failure on costs among commercially insured patients.

Methods: Adults with evidence of kidney transplant (2004-2008) were identified from US managed care healthcare claims. "Incident" recipients (IR) had a transplant date within the study period. "Prevalent" recipients (PR) had evidence of a transplant but an unknown transplant date. Graft failure was the first of retransplant or return to dialysis (2 or more dialysis visits in a 30 day period for 2 consecutive 30 day periods) and was identified from the earliest evidence of transplant until death, end of continuous enrollment, or 31 Dec 2009. Graft failure costs were patient and health plan-paid medical and pharmacy costs (Figure 1).

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Conclusions: Graft failure occurred in 8-12% of these commercially insured kidney recipients and was associated with high costs for patients and commercial payers whether or not the transplant was paid for by the health plan. Strategies to mitigate graft failure may reduce these costs.

Funding: Pharmaceutical Company Support

TH-PO921

Incidence and Costs Associated with New-Onset Diabetes and Cardiovascular Events after Kidney Transplantation in Medicare Population William Irish,1 Schiffon L. Wong,2 Debra Bowers,1 Digisha Trivedi,2 Tony Hebden.2 1CTI Clinical Trial and Consulting Services; 2Bristol-Myers Squibb.

Background: New-onset diabetes after transplantation (NODAT) and cardiovascular (CV) events increase the risk for late graft loss and patient death. The incidence and economic implications of these have not been fully explored.

Methods: Adult Medicare beneficiaries in the US Renal Data System who received kidney-only transplant (2001-2008) were eligible. Patients with evidence of diabetes or CV events (stroke, coronary artery disease, revascularization, or myocardial infarction) pre- and post-transplant based on ICD-9-CM diagnosis codes were identified. Patients with diabetes or CV disease prior to transplantation were subsequently excluded. Total Medicare costs (Institutional and Physician/Supplier claims) were calculated from date of first claim until permanent dialysis, retransplantation, death, end of continuous enrollment or followup. Costs are for the first year following the first claim (adjusted using the annual medical care component of the Consumer Price Index).

Results: Among 84,819 recipients, 38% had diabetes and 24% had CV disease prior to transplantation. NODAT occurred in 16,518 patients with a median onset of 4 months (range: <1-60 months) post-transplant. Incidence was 13.6 per 100 patient-years (PY) (95% confidence interval [CI] = 13.4 to 13.8 per 100 PY). Costs were categorized by diabetes-related complications. The most frequent complication was nephropathy (26.3%) with a median total one year cost of $1,205 while ketoacidosis (4.4%) was the most expensive (Median total one year cost=$10,079). Peripheral circulatory disorder occurred in 4.1% of NODAT patients and was the least expensive (Median total one year cost = $621). The incidence of CV events (n=12,863) was 7.5 per 100 PY (95% CI=7.3-7.6 per 100 PY) with median onset = 7.5; range: <1-60 months post-transplant. Total one year costs for CV events were $99,228.

Conclusions: NODAT and CV events may present early post-transplant and are associated with high healthcare costs. Strategies are needed to minimize the risk of diabetes and CV disease to lower their economic and clinical impact.

Funding: Pharmaceutical Company Support

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324A
Kidney Disease Characteristics in Transplant Patients: Comparison between PREPARE and ANTICIPE Studies  
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Background: Comparing the chronic kidney disease (CKD) and its care in transplant patients (TP) and patients with CKD on native kidneys.

Methods: Comparison of characteristics of patients with CKD stage IIIB / IV based on the ANTICIPE and PREPARE studies. Prospective and observational studies. Collection during one week in TP for over one year and patients treated for CKD (+ eGFR - 60 ml/min/1.73m²), respectively.

Results: 546 TP (373 stage IIIB, 173 stage IV) were compared to 1405 patients from PREPARE (659 stage IIIB and 746 stage IV). TP were younger (median 57 vs. 73, p<0.05) were less at vascular risk (BMI> 30, diabetes, smoking, dyslipidemia, p <0.05 for each) except for hypertension identically distributed (> 85% of patients). The occurrence of cardiovascular diseases was significantly lower in transplant patients and they receive fewer treatments for cardiovascular indications (RAS blockers, statins). Conversely, some of the complications associated with CKD are more common in TP: Anemia is more common, despite greater use of ESA (stage IV, Hb <12/dL: 71 % vs 56%, p<0.05; ESA: 47% vs 35%, p <0.05). Only hyperparathyroidism is more frequent (stage IV, PTH- 150 ng/L: 47% vs. 39%, p <0.05), despite the most common therapeutic use of vitamin D. TP have more visits (monthly in stage IV: 47% vs 20%, p<0.05). In case of progression to stage V, the intention for transplantation is more common for transplant patients (60% vs 17%, p<0.05) including the pre-emptive transplantation.

Conclusions: Chronic renal transplant has its own characteristics including more frequent biological complications despite more frequent and closer monitoring of specific therapeutic targets. Positively more difficult to achieve.

Funding: Pharmaceutical Company Support

myTRACKER – A Nordic Myfortic Observational Study Tracking the Gastro Intestinal (GI) Tolerability of Myfortic in Renal Transplant Recipients Converted to Myfortic Due to Cellcept Related GI Symptoms

Sadrolah Abedini, mark. reiter-Nilsen, Geir Nordbo, Kristian Heldal. Medical Clinic, Vestfold Hospital Trust, Tonsberg, Norway.

Background: Clinical trials have demonstrated that Myfortic and Cellcept are equivalent in safety and efficacy but differences in occurrence of GI tolerability have not been examined in these trials. The primary objective was to evaluate if conversion from Cellcept to equivalent dose myfortic is associated with an improvement in patient-reported GI symptom during 3 months after the conversion. The secondary objectives were to assess compliance to treatment and overall safety and efficacy of myfortic.

Methods: Multi-centre, observational, non-interventional study in renal transplant recipients from Scandinavian centers converted to myfortic due to Cellcept related GI symptoms. The patients completed the Gastro Intestinal Symptom Rating Scale (GSRS) at baseline, and 1 month and 3 months after conversion. Treatment adherence was evaluated based on dosing information and from the Immunosuppressant Therapy Adherence Scale (ITAS). The assessment of safety and efficacy was based on collected adverse event data. Analysis, using descriptive statistics, was performed based on patient reported outcomes (GSRS) and Overall Treatment Effect (OTE) scales for GI symptoms.

Results: The study was terminated due to difficulty with patient recruitment and the presented data is based on 51 included patients (planned 150 patients), 73 males and 18 females with a mean age of 56 (13.3) years. The patients experienced about twice as many dose changes during the last 3 months before conversion while receiving Cellcept compared with the 3 months treatment with myfortic after conversion. After conversion to myfortic the patients experienced lower rate of abdominal pain (p<0.0001), constipation (p=0.076), diarrhea (p=0.0005), indigestion (p=0.0004) and reflux (p=0.0001) compared with baseline.

Conclusions: This study suggests that conversion to myfortic from Cellcept in kidney transplant recipients results in improvements in related symptoms at 1 month and maintained at 3 months after conversion. Fewer dose-changes and lower occurrence of GI related symptoms are seen at equivalent therapeutic doses.

Funding: Pharmaceutical Company Support

Comparisons of Enteric-Coated Mycophenolate Sodium and Mycophenolate Mofetil from the Mycophenolic Acid Observational Renal Transplant Registry

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Background: The Mycophenolic Acid Observational Renal Transplant (MORE) Registry is a prospective, observational study of the use of mycophenolate mofetil (MMF)-based regimens based on local practice at 40 US sites. The intention for transplantation is more common for transplant patients (60% vs 17%, p<0.05) and survival. A common method for projection, the exponential method, uses failure/death rate after the 1st year, assumes it is constant over time, and extrapolates it to the time when 50% of patients would be expected to lose the graft or die. Using the Scientific Registry of Transplant Recipients data, we evaluated this method and compared it with others.

Methods: Adult, deceased donor, 1st kidney transplant recipients in the US, 1991-2000, were included in the analysis. The outcome was all-cause graft failure. Observed and projected graft survivals (GSs) were calculated for the 2000 cohort. Patients were followed until the end of 2009 for observed GS. Data through 2002 were used to project GS. Methods for projecting GS included (1) exponential method, (2) Weibull method, (3) combining the 1991 cohort’s GS (through 2002) and the temporal trend estimated from the 1991-2000 cohorts (using linear, quadratic, and categorical cohort year separately), and (4) segment method using the second-year death rate of the 2000 cohort, the third-year death rate of the 1999 cohort, and so on, to construct a piecewise survival curve for the 2000 cohort.

Results: Figure 1 displays the observed and projected GSs. For short-term-projection, all methods work well. Long-term, the exponential and Weibull methods overestimate survival probability substantially; the segment and linear methods give the best estimates. For estimates of half-lives, the exponential and Weibull methods overestimated up to 32 months; estimate errors from the other methods were within 6 months and less than 2 months for the segment method.

Conclusions: The exponential method, currently widely used, can overestimate survival probability/half-life substantially; the segment and linear methods provide better estimates.

Funding: Other U.S. Government Support

Projection of Kidney Graft Survival Probabilities: An Evaluation of Methodologies

Jiamong Liu, Yi Peng, Jon J. Snyder, Nicholas J. Salikowski, Ajay K. Issrani, Kenneth E. Lamb, Bertram L. Kasiske. SRTR, MMBF, Minneapolis, MN.

Background: Projected half-life is used to monitor post transplant patient and graft survival. A common method for projection, the exponential method, uses failure/death rate after the 1st year, assumes it is constant over time, and extrapolates it to the time when 50% of patients would be expected to lose the graft or die. Using the Scientific Registry of Transplant Recipients data, we evaluated this method and compared it with others.

Methods: Adult, deceased donor, 1st kidney transplant recipients in the US, 1991-2000, were included in the analysis. The outcome was all-cause graft failure. Observed and projected graft survivals (GSs) were calculated for the 2000 cohort. Patients were followed until the end of 2009 for observed GS. Data through 2002 were used to project GS. Methods for projecting GS included (1) exponential method, (2) Weibull method, (3) combining the 1991 cohort’s GS (through 2002) and the temporal trend estimated from the 1991-2000 cohorts (using linear, quadratic, and categorical cohort year separately), and (4) segment method using the second-year death rate of the 2000 cohort, the third-year death rate of the 1999 cohort, and so on, to construct a piecewise survival curve for the 2000 cohort.

Results: Figure 1 displays the observed and projected GSs. For short-term-projection, all methods work well. Long-term, the exponential and Weibull methods overestimate survival probability substantially; the segment and linear methods give the best estimates. For estimates of half-lives, the exponential and Weibull methods overestimated up to 32 months; estimate errors from the other methods were within 6 months and less than 2 months for the segment method.

Conclusions: The exponential method, currently widely used, can overestimate survival probability/half-life substantially; the segment and linear methods provide better estimates.

Funding: Other U.S. Government Support

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325A
Time-to-Failure after Initial Transplantation Dictates Care for Repeat Kidney Transplantation Candidates
Rowena B. Delos Santos, Jordana Gaumond, Roger Yuh, Jennifer W. Leach, Jagdeep Obhrai, John M. Barry, Mitchell Henry, Dept. Medicine, Division of Nephrology, Section of Transplant Medicine, OHSU, Portland, OR; Dept. Surgery, Portland VA Medical Center, Portland, OR; Dept. Surgery, Divisions of Urology and Abdominal Organ Transplantation, OHSU, Portland, OR; Dept. Surgery, Division of Transplantation, Ohio State University, Columbus, OH.

Background: In one of six patients on the waiting list for kidney transplantation in the US has received a prior kidney transplant. There are 3 strategies for managing asymptomatic patients with failed kidney transplants: elective transplant nephrectomy (N-TXP), discontinuance of immunosuppression (IS), and IS without N-TXP until the next transplant. We surveyed transplant nephrologists and surgeons to examine practice patterns or management of repeat transplant candidates.

Methods: We developed and validated internet based survey and sent it to transplant nephrologists and surgeons at all active US adult kidney transplant centers.

Results: More than 60% of centers responded. Management strategy depended on time of graft loss (< 6 months post-transplant or > 10 years post-transplant). For early graft loss, most transplant nephrologists and surgeons chose N-TXP or discontinuance of IS. For late graft loss, most transplant nephrologists and surgeons chose discontinuing IS or IS without N-TXP. Surprisingly, neither patient-specific factors (co-morbidities or HLA-antigen sensitization), nor center-related factors (center size, academic affiliation, and location) influenced management. There was significant intra-center variability in management.

Conclusions: Time to graft failure was the primary factor for management of candidates awaiting a second kidney transplant. Provider-specific factors, rather than patient- or center-specific factors determined care strategy.

Funding: Private Foundation Support

TH-P0928
Progression of Aortic Calcifications and Their Impact on Renal Function after Kidney Transplantation
Carlo Maria Alifreti, Maria Daniela Croci, Brigida Brezzi, Francesco Barretta, Maria Teresa Gandolfo, Maria Meneghini, Manuela Curreri, Maria Pia Rastaldi, Piergiorgio Messa.

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Background: Vascular calcifications (VCs) progress even after a well functioning kidney transplant (KTs). The major factors and related influence, if any, for all VCs, could have a substantial impact on the prognosis of the transplant recipients. The aim of the study was to assess whether the factors associated with VC progression in the first year after KTx; b) VC impact on graft outcome over a 2-year follow-up period.

Methods: Abdominal aortic calcification index (ACI), FGF23, OPG, FxIII, mineral metabolism (MM) parameters, were evaluated in 95 KTxs pts (transplanted between 2006-2008) at the 1st and at 12th months after KTx. All patients were followed-up over a 2-year period. Statistics: univariate and multivariate analyses (values: mean ± standard deviation).

Results: In the overall cohort, mean ACI increased from 4.85 ± 5.84 (1st mo) to 5.23 ± 6.12 (12th mo) (p = 0.01). In the 25% (Progr +), while it remained stable or improved (3.76±5.43 to 5±5.33; p = ns) in 75% pts (Progr -). Progr + Pts were older (53±7 vs 46±11 yrs, p<0.01), had lower eGFR (MDRD: 50±15 vs 58±16 ml/min, p = 0.03), higher Ca (10.4±0.76 vs 9.9±0.65 mg/dl, p<0.002) and OPG (6.31±3.7 vs 4.8±1.55, p = 0.002) levels at the 12th month, without any other significant difference, including cumulative steroid doses.

In the Logistic RA, only serum Ca (p<0.004, OR 3.56) and OPG (p<0.01, OR 1.75) were significantly and positively associated with ACI progression. Over the 2-year follow-up, 3 patients died (2 Progr +, 1 Progr -) and 1 pt (Progr -) restarted dialysis. At the end of follow-up no significant difference was found in blood pressure control, in eGFR and urinary protein between the 2 groups.

Conclusions: VCs progress even in the post-KTx period. Higher levels of Ca and OPG seem to be associated with an increase in VCs. However, no major impact of VC progression on graft function was evident, at least over a 2-year follow-up. Studies on longer cohorts and over a longer period are needed for a definitive conclusion on this issue.

TH-P0929
The Burden of Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) in Successful Renal Transplant Recipients: The ABC-Heart Study
Sinead Kinsella, Joseph A. Eustace. Department of Renal Medicine, Cork University Hospital, Cork, Ireland.

Background: The burden of Chronic Kidney Disease-Mineral Bone Disorder (CKD-MBD) in successful kidney transplant recipients is poorly defined.

Methods: We conducted a prospective observational study to quantify the presence and severity of CKD-MBD in first renal allograft recipients (eGFR > 30 ml/min/1.73m²). Patients underwent routine lab tests, iPTH, 25 OH Vitamin D, lumbar X-ray, DXA scan (Lunar IDXA), qCT of lumbar spine, pulse wave velocity measurement and measures of bone turnover (serum BAP and TRACP5b, urine NTX).

Results: 89 patients with mean age of 46.8 yrs, median dialysis and transplant vintage of 2.3 and 2.6 yrs were studied. Mean eGFR and iPTH were 54±15 ml/min/1.73m² and 5±9ng/ml, respectively. Mean ACI was 4.8±5.2, OPG was 5.6±4.0 and OPG (6.31±3.7 vs 4.8±1.55, p = 0.002) levels at the 12th month, without any other significant difference, including cumulative steroid doses.

In the Logistic RA, only serum Ca (p<0.004, OR 3.56) and OPG (p<0.01, OR 1.75) were significantly and positively associated with ACI progression. Over the 2-year follow-up, 3 patients died (2 Progr +, 1 Progr -) and 1 pt (Progr -) restarted dialysis. At the end of follow-up no significant difference was found in blood pressure control, in eGFR and urinary protein between the 2 groups.

Conclusions: VCs progress even in the post-KTx period. Higher levels of Ca and OPG seem to be associated with an increase in VCs. However, no major impact of VC progression on graft function was evident, at least over a 2-year follow-up. Studies on longer cohorts and over a longer period are needed for a definitive conclusion on this issue.

TH-P0930
Cholecalciferol Supplementation Reduces Proteinuria after Kidney Transplantation
Ines Aires, Manuel A. Ferreira, Fernando Barbosa Nolasco.
Nephrology and Dialysis, Hospital Curry Cabral, Lisbon, Portugal.

Background: The burden of chronic kidney disease-mineral bone disorder (CKD-MBD) in successful renal transplant recipients is substantial and may contribute to their long term morbidity.

Methods: We prospectively evaluated the effects of 6 month cholecalciferol supplementation, in 124 KTxs pts, 84 men, mean age 53±4;14 years, 29 diabetics, with a mean percentage of transplanted patients of 12.1±3.63 months. All pts were naive to 25-OH viD therapy. Immunosuppression regimen was tacrolimus in 74% and sirolimus in 21% pts.

Results: NGAL on day 2 for predicting lower 1yr eGFR was moderately accurate (AUC 0.742, cut-off value 86.7ng/ml with sensitivity 83.3% and specificity 67%).

Conclusions: We conclude that urinary NGAL at early post-transplant period is a useful predictor of long-term graft function even in patients with relatively good early graft function.
Pt. s. were supplemented accordingly as basal (T0) calcidiol serum levels (ng/ml): deficient (<10), insufficient (10-30) and normal (>30). Wilcoxon paired and Anova tests were used.

Results: At T0, 25-OH vit.D levels were 14.6±7.8 mg/dL. 58.9% pts had deficiency and 30.6% had 25-OH vit.D insufficiency. Mean plasma creatinine was 1.44±0.6 mg/dL. At 6 months (T6), 64 pts. completed daily 25-OH vit.D supplementation (median dose 2664 UI). Mean calcidiol serum levels increased to 30.5±12.3 (p = 0.001). Only 6.2% remained in deficiency, and 46.9% pts achieved normal serum levels (vs 10.5% at T0).

Proteumia was significantly reduced from 0.93±1.48 g/dL (T0) to 0.68±0.66 g/dL (T6). At T0, 53% of the patients who received the medication had elevated proteinuria levels that were inversely correlated with 25-OH vit.D levels (r = 0.30; p = 0.01).

Conclusions: Accordingly to our results, native vitamin D deficiency is highly prevalent among KTx pts. Oral supplementation with cholecalciferol is efficient, cheap and safe in the correction of calcidiol serum levels and leads to significant reduction in proteinuria. Larger and longer randomized controlled studies are needed to confirm these protective relevant effects of cholecalciferol in kidney allograft survival in patients with kidney transplantation during the correction of calcidiol serum levels and leads to significant reduction in proteinuria.

TH-PO931
Should We Maintain Cinacalcet after Kidney Transplantation in Patients Receiving It on Dialysis Rafael Pereira Paesalchon, Joao-Vicente Torregrosa, Xoana Barros Freira, Carlos Duran Rebollo, Jose Maria Campistol Plana. Nephrology Department, Hospital Clinic, Barcelona, Spain.

Background: Nowadays, the treatment of secondary hyperparathyroidism (SHPT) with cinacalcet in patients that are on waiting list for kidney transplant (KT) is habitual. However, it is not clear if the treatment should be maintained after KT, and if so, which factors may define it.

The aim of this study was to evaluate the follow-up of KT recipients who discontinued cinacalcet just before the transplant.

Methods: Single center retrospective observational study. Enrollment began in June 2005 and ended in December 2010. 114 patients (75 men) were engaged. They were receiving cinacalcet on dialysis before KT. Median age: 51.3 years (22.5 to 78.5). The time on dialysis was 8.6±8.4 years. All of them had three months follow-up and 92 completed one year of follow-up after KT. Criteria to reintroduce cinacalcet was serum calcium ≥10.5 mg/dL. We assess the factors involved in the reintroduction of cinacalcet after transplant (age, gender, time on dialysis, cinacalcet dose before KT, Ca / P / alkaline phosphatases / iPTH/ KT week, immunosuppression and renal function after KT). After KT biochemical parameters were measured at day 7, 15, 30, 60, 90, 180 and 365. Statistical analysis was made with SPSS-15.

Results: At three months 14 patients needed reintroduction of Cinacalcet and at the end of one year of follow-up 25 patients (21.7%; group 1) were on cinacalcet treatment. Ninety-four patients did not need Cinacalcet reintroduction (group 2). Time on dialysis (131 ± 94 months and 97 ± 102 months; p = 0.0267) and the dose of cinacalcet before the KT ≥ 60mg per day (45 [30-120] vs 30 [30-60] mg per day; p=0.039) were the only significant factors related to the reintroduction of cinacalcet.

Conclusions: The dose of cinacalcet before the transplant and the time on dialysis seems to be the most important factors related to the reintroduction of the drug during the first year after KT.

TH-PO932
Periodic Limb Movements in Sleep Are Associated with Serum Parathyroid Hormone in Kidney Transplant Recipients Zoltan Kiss,1 Annet Lindner,2 Rezso Zoller,2 Katalin Forndali,2 Alpar S. Lazar,2 Maria Eszter Czira,3 Andrea Dunai,3 Orsolya Agnes Veber,3 Andras Szentkiralyi,2 Marta Novak,2 Miklos Z. Molnar,2 Istvan Mucsi.3,4 Medical Affairs, Amgen Limited, Budapest, Hungary; 1Dept. of Neurology, Semmelweis University, Budapest, Hungary; 3Institute of Behavioural Science, Semmelweis University, Budapest, Hungary; 2Szurei Sleep Research Centre, Faculty of Health and Medical Science, University of Surrey, Guildford, United Kingdom; 4Institute of Epidemiology and Social Medicine, University of Muenster, Germany; 2Dept. of Psychiatry, University of Toronto, Toronto, Canada; 2Harold Simon Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 3Institute of Pathophysiology, Semmelweis University, Budapest, Hungary; 1Dept. of Medicine, Division of Nephrology, McGill University Health Centre, Montreal, QC, Canada.

Background: Several reports suggested an association between Restless Legs Syndrome (RLS) versus serum iPTH concentration. No clear consensus exists regarding the most accurate calculation to estimate glomerular filtration rate (GFR) in renal transplant recipients (RTR). The two most frequently used equations are the Modification of Diet in Renal Disease (MDRD) and Cockcroft-Gault equations.

Results: However, neither of these has demonstrated consistency or accuracy in RTRs and each calculation has limitations that preclude their use.

Methods: Radionuclear GFR performed using radioisotopic I-125 agent (Giolli®) in 13 stable RTRs between 3 and 12 months post-transplantation. Additionally, blood draws for BUN, creatinine, and albumin were obtained at the same time as the radionuclear GFR for use in the various GFR equations: Cockroft-Gault, MDRD (Levey), abbreviated MDRD, Cockcroft-Gault, C(Ki), Cockcroft-Gault (Levey), abbreviated MDRD, C(Ki), Cockcroft-Gault, C(Ki), abbreviated MDRD, C(Ki), and Cockcroft-Gault. Statistical comparisons were made between the measured radionuclear GFR and the eGFR calculations. However, neither of these has demonstrated consistency or accuracy in RTRs and each calculation has limitations that preclude their use.

Results: The mean age was 55.6 ± 10.3 years. 13 were male. 2 females. 12 were white and 1 was black.

Conclusions: The radionuclear GFR performed using radioisotopic I-125 agent (Giolli®) in 13 stable RTRs between 3 and 12 months post-transplantation. Additionally, blood draws for BUN, creatinine, and albumin were obtained at the same time as the radionuclear GFR for use in the various GFR equations: Cockroft-Gault, MDRD (Levey), abbreviated MDRD, C(Ki), Cockcroft-Gault, C(Ki), abbreviated MDRD, C(Ki), and Cockcroft-Gault. Statistical comparisons were made between the measured radionuclear GFR and the eGFR calculations. However, neither of these has demonstrated consistency or accuracy in RTRs and each calculation has limitations that preclude their use.

1Internal

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
TH-PO935
Renal Function Is Associated with Red Cell Distribution Width Independent of Iron Deficiency and Nutritional Status in Kidney Transplant Recipients
Ivan Mucio,1,2 Maria Exzter Czira,2 Akos Uljazszi;2 Csaba P. Kovlyesdy,1,4 Miklos Z. Molnar,2,5 1McGill University, Montreal, QC, Canada; 2Semmelweis University, Budapest, Hungary; 3Salem VA Medical Center, Salem, VA; 4University of Virginia, Charlottesville, VA; 5Harbor-UCLA, Torrance, CA.

Background: Red cell distribution width (RDW) is a measure of heterogeneity in the size of circulating erythrocytes, which reflects iron deficiency, inflammation and nutritional status. The role of RDW is well known in patients with iron deficiency, but less studied in patients with inflammation or nutritional status.

Methods: We examined the association of RDW with eGFR in a cohort of 807 prevalent KT recipients not receiving erythropoietin stimulating agents. RDW was assessed in regression models using eGFR (MDRD, Cockcroft-Gault, abbreviated MDRD) and serum CRP and albumin, PO4, and CRP; and albumin, PO4 and iPTH. We adjusted for age, sex, Charlson Comorbidity Index, and prevalent KT recipients not receiving erythropoietin stimulating agents.

Results: Lower eGFR was associated with significantly higher RDW (r=0.399, p<0.001) in all regression models. This association remained highly significant even after multivariable adjustments (beta=-0.178, p<0.001). The results were consistent in subgroups of patients with different levels of kidney function and various sensitivity analyses.

Conclusions: Lower eGFR is associated with higher RDW, a predictor of mortality, independent of comorbidity, iron deficiency, inflammation and nutritional status in kidney transplant recipients. Further studies need to determine if the mechanisms linking impaired renal function to increased RDW to increased mortality.

Funding: Government Support - Non-U.S.

TH-PO936
Estimating Glomerular Filtration Rate in Renal Transplantation: A Comparison between Serum Creatinine and Cystatin C-Based Equations

Background: Accurate monitoring of estimated glomerular filtration rate (eGFR) is essential for an optimal management of kidney transplant (KT) patients. eGFR is often calculated using creatinine-based (SCRb) equations. Alternatively, cystatin C-based (CYSb) equations have been developed, but their exactness is still uncertain.

Methods: Creatinine Clearance (CrCl) in a 24-hour urine collection was the reference test. eGFR was calculated using CYSb equations (Le Bricon, Stevens) and SCRb equations (Cockcroft-Gault [CG], abbreviated MDRD:aMDRD). After evaluating randomly selected results of 215 KT patients with stable renal function and over 1 year post-transplantation (PT), 173 were included. We excluded patients in the first and tenth deciles (in each gender) of 24-hours urine creatinine excretion divided by weight to deliver inaccurate urine collections. Bias, precision and accuracy of each equation were determined. Kappa statistics evaluated the concordance between the reference test and eGFR formulas in categorizing patients’ renal function (positive test: ≤60 ml/min).

Results: Patients (108 males) had a mean age of 48.6 years and a median PT time of 6.8 years. Mean CrCl was 69.3 (range: 32 – 105) ml/min. The CYSb equations estimated (Le Bricon, Stevens) had the highest accuracy (83.8% and 87.9% within 30% of CrCl result, respectively). Le Bricon, Stevens and aMDRD precision was similar (13.5 ml/min) and much better than CG (22.5 ml/min). The lowest bias was seen in Le Bricon (-1.2 ml/min), followed by CG, Stevens and aMDRD (-2.6, -4.5, -16.5 ml/min, respectively). Kappa coefficient was higher in CYSb equations (0.53) in contrast with CG (0.48) and aMDRD (0.40). Stevens had a high sensitivity (90.8%) and low specificity (66.7%) and, conversely, Le Bricon had 64.6% sensitivity and 87.0% specificity for identifying patients with CrCl <60 ml/min.

Conclusions: CYSb equations showed the best accuracy and a low bias. Stevens performed better than Le Bricon in identifying patients with a lower CrCl. The inverse was observed in patients with mild or no kidney graft dysfunction.

TH-PO937
Renal Transplant Recipients Are at High Risk To Develop Precancerous Lesions of the Cervix
Suchita J. Mehta,1 Mario R. Castellanos,1 Suzanne E. El Sayegh,2 Kathleen Ahern,2 Morton J. Kleiner,1 1Dept. of Medicine, Staten Island University Hospital, NY; 2Nursing Program, Wagner College, NY.

Background: Immunosuppressive regimen improves survival of renal transplant recipients (RT); however, a long-term complication is increased risk of developing cancer. Human papillomavirus (HPV) associated cancers of the genital tract are of particular interest, since they are sexually transmitted and in theory preventable. Cervical cancer rates are high in RT but the data on the natural history of HPV infection in RTR are limited. Therefore, we investigate HPV and the development of cervical neoplasia.

Methods: A review was conducted in Medline database for articles in English investigating cervical HPV infection in RT. Eligible studies included those that examined HPV infection by PCR testing and/or obtained cervical biopsies. Three reviewers extracted the data and compared rates to the general population.

Results: Out of 157 relevant citations, 12 met our inclusion criteria: 4 prospective studies included 231 RTR, 26.9% became HPV (+) and 13.2% that went for biopsy had a severe dysplasia. Six cross-sectional studies examined 187 RTR, 21.9% were HPV(+) and 16.7% had a high grade lesion. Two retrospective studies included 276 patients, only 25 RTR were sent for biopsy with 32% having severe dysplasia. The average duration of immunosuppression was 3.2 years to diagnose HPV or dysplasia.

Conclusions: Studies on HPV and premalignant lesions of the cervix in RTR are limited, however in our review we examined a large cohort. At about 3.1 years of immunosuppression 20-30% of RTR were HPV (+), rates similar to the general population. However, 13-32% of RTR biopsied had high grade dysplasias, while the general population rate is only 0.8%. Cervical cancer screening and prevention is important in RTR. Counseling should be aimed at limiting risky sexual behaviors post transplantation.

TH-PO938
HLA-DR4 Protects Transplant Recipients from Cancer Mortality
Jennifer A. McCaughey,1,2 Aiding E. Courtney,1 A.J. McKnight,2 Alexander P. Maxwell,2 1Department of Nephrology, Belfast City Hospital, Belfast, United Kingdom; 2Nephrology Research Group, Queen’s University, Belfast, United Kingdom.

Background: Long term immunosuppression is associated with increased risk of malignancy. This is due in part to impaired immune surveillance and attenuation of the host response to certain oncogenic viruses. HLA-DR4 is a key molecule in the presentation of tumour and viral antigens which trigger the development of activated T lymphocytes with antitumour action.

Methods: We investigated whether the presence of HLA-DR4 in renal transplant recipients confers a survival benefit by reducing death from malignancy.

Results: Analysis was performed using Kaplan-Meier and Cox-Regression plots. Survival analysis was performed using Kaplan-Meier and Cox-Regression plots. Results: Renal transplant recipients with HLA DR4 were significantly less likely to die from malignant causes than those without a DR4 antigen (p = 0.037). This relationship persists after correction for transplant recipient age.

Conclusions: This study suggests that possession of an HLA-DR4 antigen provides significant protection from death due to malignant disease in renal transplant recipients. This finding is confirmed in replication studies, then the presence or absence of a recipient HLA-DR4 antigen could help nephrologists stratify patients for future risk of cancer death and influence the choice of immunosuppression regimens.
TH-PO939

Ionizing Radiation Exposure from Medical Imaging in Renal Transplant Recipients Kim N. Nguyen, Francis L. Weng, Anup M. Patel. Renal and Pancreas Transplant Division, Saint Barnabas Medical Center, Livingston, NJ.

Background: Ionizing radiation and immunosuppression are risk factors for malignancies. Occupational radiation exposure is limited to 100 millisieverts (mSv) every 5 years and 50mSv in any single year. We analyzed the radiation exposure from medical imaging in renal transplant recipients (who have a higher risk of malignancy) from the time of transplant evaluation up to 3 months post-transplant.

Methods: We retrospectively analyzed 172 patients who received their first renal transplant during 2008. We determined types and numbers of medical imaging procedures from initial evaluation to three months post-transplant. For pre-transplant exposure, only imaging required for placement and maintenance on the transplant list were included. Estimates of radiation exposure for each procedure type were obtained from published literature.

Results:

<table>
<thead>
<tr>
<th>mSv Pre-Transplant</th>
<th>Post-Transplant</th>
<th>Total (n=172)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>78 (45.3%)</td>
<td>110 (63.9%)</td>
</tr>
<tr>
<td>&gt;20-50</td>
<td>50 (29.1%)</td>
<td>44 (26.1%)</td>
</tr>
<tr>
<td>&gt;50-100</td>
<td>35 (20.4%)</td>
<td>16 (9.3%)</td>
</tr>
<tr>
<td>&gt;100</td>
<td>2 (1.2%)</td>
<td>2 (1.2%)</td>
</tr>
</tbody>
</table>

An annual exposure (mSv/year) of 0-3, >3-20, >20-50, >50-100, >100, occurred in 10 (5.8%), 75 (43.6%), 65 (37.8%), 17 (9.9%), and 5 (2.9%) patients, respectively.

Multivariate Analysis of Factors Associated with Radiation Exposure >50mSv

- Black race (vs. non-Black): OR 1.0, 95% CI 0.9-4.2, p = 0.09
- D/SF (vs. no D/SF): OR 3.6, 95% CI 1.5-10.3, p = 0.01
- Charlson Comorbidity Index (vs. 2-3): OR 4.5, 95% CI 2.0-9.7, p < 0.001
- Total Years of Follow-up (vs. <1 year): OR 6-8, 95% CI 1.3-11.9, p = 0.01
- >1 to 2 years: OR 1.6, 95% CI 0.5-4.7, p = 0.001
- >2 to 4 years: OR 4.7, 95% CI 1.6-13.7, p = 0.001
- >4 years: OR 11.2, 95% CI 4.1-30.9, p = 0.001

The tests responsible for the most exposure were nuclear stress tests (50.5% of total mSv) and CT scans of the abdomen/pelvis (22.7%).

Conclusions: Renal transplant recipients are exposed to significant amounts of ionizing radiation from medical imaging during their pre-transplant work-up and early post-transplant care. Strategies to reduce exposure, such as reducing the frequency of screening nuclear stress tests and following established guidelines for performance of CT scanning, should be considered.

TH-PO940

Should We Change Our Renal Allograft Allocation Policy? Emmanuel Villar,' Liacine Bouaoun, Cécile Couchoud, Rene Ecochard.1 Nephrology, Hospices Civils de Lyon, Pierre Benite, Rhone, France; 2Registre REIN, Agence de la Biomédecine, La Plaine St Denis, Seine St Denis, France; 3Biostatistics, Hospices Civils de Lyon, Lyon, Rhone, France.

Background: Our aim was to quantify the evolution with time of excess mortality in end stage renal disease (ESRD) patients registered on renal transplant waiting list compared with non-transplant candidates and transplant patients.

Methods: We included all incident ESRD patients from 2002 and 2009 registered in the French REIN Registry. Excess mortality was computed each year after first dialysis in patients at risk stratified as follows: patients registered on renal transplant waitlist and never transplanted over study period (waitlist group), transplanted patients (transplant group), and patients never registered (dialysis group). Excess mortality was computed against French general population using relative survival method. Comparisons were adjusted for differences between waitlist and transplant patients. Dialysis patients were older and had more comorbidities. Variations of excess mortality were:

- In waitlist group, excess mortality increased by 45% (p = 0.0005) per year. Delay from first dialysis to registration (p = 0.0004), age >65 (p = 0.008), original nephropathy as diabetes or vascular cause of ESRD (p = 0.028) and number of comorbidities (p = 0.035) were independent predictors of excess mortality for patients registered on waiting list and not yet transplanted.

Results: Our study quantifies annual increase of excess mortality while waiting for renal transplant. Results raised the question about priority of access to transplant in ESRD patients with comorbidities and/or older, considering as well access equity for all patients in the setting of organ shortage. These urge us to screen early high risk patients for transplantation.

TH-PO941

Listing Pre Dialysis Patients for Transplantation Increases Preemptive and Early Transplantation Post Starting Dialysis Wendy Brown, Damien Ashby, Megan Grifith. Renal Unit, Imperial College Healthcare NHS Trust, London, United Kingdom.

Background: Transplantation provides excellent outcomes for patients with ESRD and preemptive transplantation gives better outcomes compared to transplantation after commencement of dialysis. Planned living donor (LD) transplantation has increased rates of preemptive transplantation and UK guidelines recommend that people with advanced CKD (GFR<15mls/min) are activated for transplantation within 6 months of predicted need to start dialysis. We examined the effect of pre dialysis listing on LD and deceased donor (DD) transplantation.

Aim: to investigate the outcome of pre dialysis patients listed for transplantation

Methods: All patients in our low clearance clinics, anticipated to require dialysis in the next 6 months and deemed fit for transplantation were offered work up and listing for transplantation. The outcomes of those patients listed from Jan 2006-April 2011 were analysed.

Results: 295 pre dialysis patients were worked up and listed on the national kidney allocation register. Table 1 summarises patient outcomes.

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Underline represents presenting author.
This data reflects responses from a third of centers in the US. More centers follow patients after listing than in 2002 (96% vs 71%), and 2/3 risk stratify patients for follow-up frequency, up from 1/3. Compared to the previous survey that found that physicians frequently reported interval illness, patients are now more likely to be responsible for illness reporting. This change is associated with poor reporting efficiency at 70% of centers. An important measure of waitlist management efficiency is the detection of illness at call-in that prevents txp. At 64% of centers, >10% of patients called-in for a graft have problems preventing txp, up from 16% of centers. Timely illness reporting is uniformly correlated with dialysis staff involvement in illness reporting and low rates of cancellation of txp for medical illness.

Conclusions: More centers follow patients after listing now than in 2002. Despite this, interval illness is poorly reported and is associated with high rates of detection of illness preventing txp at call-in. Systems that improve timely reporting of illness may decrease cancellation of txp for medical illness discovered at the time of call-in.

Funding: Private Foundation Support

TH-PO943

Comparative Efficacy of Treatment Options for Patients Awaiting Repeat Kidney Transplantation

Feroz Aziz, Rowena B. Delos Santos, Jagdeep Obhrai, Douglas J. Norman. Medicine, Nephrology, Transplant Medicine, Oregon Health & Science University, Portland, OR.

Background: Care of patients awaiting repeat renal transplantation can be complicated by sensitization stimulated by the failed transplant or by graft intolerance syndrome (GIS, rejection of the failed transplant). Early failure of renal transplants is usually treated either with elective transplant nephrectomy (N-TXP) or stopping immunosuppression (IS) without N-TXP. For late graft failure, most clinicians stop IS or continue IS until the next transplant.

Methods: To determine whether there was a benefit for elective N-TXP for early transplant failure and IS for late failure, we retrospectively analyzed outcomes for waitlisted patients with a history of a prior transplant at two programs with similar patient populations but different treatment strategies. Program #1 (P#1) did elective N-TXP for all patients with early graft loss (<2 years) and continued IS (prednisone + antiproliferative drug) for patients with late graft loss (≥2 years). Program #2 (P#2) discontinued IS in all patients with a failed graft while they awaited repeat transplantation.

Results: P#1 had 10/11 patients get N-TXP, but most P#2 patients with early graft loss ultimately required N-TXP (8/12). For patients with late graft loss, there was no difference in rates of N-TXP (p=0.12), sensitization (p=0.09), or time-to-sensitization at the 2 centers.

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Underline represents presenting author.

330A
was 87.2% (95% CI: 82.5-90.7%) and 77.4% (95% CI: 70.8-82.7%), respectively, in the everolimus and 72.8% (95% CI: 70.2-75.2%) in the reference group. Primary graft survival rates stratified by living versus deceased donors are provided in Table 1. Cumulative incidence of AR and NODAT at 3-years post-transplant with everolimus were 11% and 8.5%, respectively, and 5% and 9% in the reference group. Mean SCR at 3 years post-transplant was 1.8 with everolimus versus 1.6 in the reference group.

Table 1: Life Table Primary Graft Survival Post-Transplantation

<table>
<thead>
<tr>
<th>Time Post-Transplant</th>
<th>Everolimus (n=199)</th>
<th>NODAT-OS NOS-Reference (n=193)</th>
<th>Decreased SCR</th>
<th>Precipitated Death</th>
<th>Decreased SCR</th>
<th>Precipitated Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Year</td>
<td>95.6±1.5%</td>
<td>92.3±2.0%</td>
<td>95.1±0.9%</td>
<td>94.9±0.4%</td>
<td>95.1±0.9%</td>
<td>94.9±0.4%</td>
</tr>
<tr>
<td>3 Years</td>
<td>93.3±2.6%</td>
<td>87.3±2.8%</td>
<td>88.3±0.8%</td>
<td>79.7±0.7%</td>
<td>88.3±0.8%</td>
<td>79.7±0.7%</td>
</tr>
<tr>
<td>5 Years</td>
<td>83.0±3.9%</td>
<td>72.0±4.5%</td>
<td>80.3±0.4%</td>
<td>67.3±0.9%</td>
<td>80.3±0.4%</td>
<td>67.3±0.9%</td>
</tr>
</tbody>
</table>

Log-rank test p=0.1033 p=0.0001

Conclusions: Incidence of AR and NODAT was slightly higher in the everolimus-treated group. Primary graft survival at 3 and 5 years post-transplant seem to favor everolimus. This favorable effect was more notable in recipients of deceased donor renal transplants.

Funding: Pharmaceutical Company Support

TH-PO946

Methods: 1141 patients on SRL therapy were included in the analyses. Of these, 679 patients were alive and followed until SRL discontinuation or December 31, 2013. The degree of proteinuria at SRL initiation (means: 192 vs. 534 mg/l, medians: 75 vs. 133 mg/l) and cause for SRL initiation as significant variables.

Results: Successful use of SRL initiation after Tx, and cause for SRL initiation as significant variables.

Funding: Pharmaceutical Company Support

TH-PO947
Management of Rapamycin-Associated Side Effects in Kidney Transplant Recipients Heloise Cardinal, Raymond Dandavino, Marie-Josée Hebert, Suzon Collette, Catherine Girardin, Anne Boucher, Nephrology, Centre Hospitaller de l’Université de Montréal, Montreal, QC, Canada; Nephrology, Hopital Maisonneuve Rosemont, Montreal, QC, Canada.

Background: Rapamycin-based immunosuppression may preserve kidney graft function. The side effects of rapamycin can limit its usefulness, but their management and evaluation vary by medical centers.

Methods: We performed a retrospective cohort study on KTR who received a single kidney graft before December 31, 2008 and who received rapamycin after the 1st post-transplant month to replace or use in combination with low-dose calcineurin-inhibitors (CNI). We determined the incidence, management and evolution of rapamycin-related side effects.

Results: Amongst the 301 patients studied, new or worsened dyslipidemia occurred in 56%. Decreased rapamycin dose, diet and lipid-lowering drugs led to successful control in 98%. Cytopenia was observed in 40%. Decreased rapamycin dose, supplementing with iron or erythropoietin for anemia, folic acid for leucopenia, as well as the withdrawal of other cytopenic agents resulted in acceptable control in 94%. Periperal edema was observed in 35%. Although decreased rapamycin dose, salt and water restriction, and diuretics were tried for 20% of subjects with peripheral edema, they had to discontinue rapamycin. New or worsened proteinuria occurred in 26%. Despite decreased rapamycin dose, salt and water restriction, and renin-angiotensin inhibitors, 27% had to discontinue rapamycin. Acne developed or worsened in 25%. Decreased rapamycin or prednisone dose, topical or oral antifungal therapy resulted in acceptable control in 86%. Rapamycin was discontinued for related side effects in 135 subjects (45%). Increased body mass index (OR per 10 point increase: 1.80, 95% CI 1.09, 2.96) was associated with discontinuation, and there was a trend for older subjects (OR per 10-year increase: 1.19, 95% CI 1.00, 1.43) to be more likely to discontinue rapamycin.

Conclusions: Successful control of dyslipidemia, cytopenia and acne can usually be achieved without discontinuing rapamycin, whereas proteinuria and edema are harder to manage. Leaner, and perhaps younger patients are less likely to discontinue rapamycin due to side effects.

Funding: Pharmaceutical Company Support

TH-PO948

Background: While living kidney donation is generally considered safe, long-term development of co-morbidities is less characterized. The goals of this study were to quantify new onset hypertension (HTN) and diabetes after living kidney donation and to explore risk factors associated with their development.

Methods: We surveyed 460 patients who donated a kidney at our center between 1984-2011. Median follow-up was 4.4 years (IQR 2.1-7.9). Participants were asked to report new onset diagnoses of HTN and diabetes since donation, as well as all current medications. Multivariate logistic regression was used to examine the independent association of donor characteristics with HTN; because of low event rate, Fisher’s exact test was used for diabetes.

Table 1. Multivariable models of associations between various donor factors and post-donation HTN

<table>
<thead>
<tr>
<th>Factor</th>
<th>Development of HTN</th>
<th>Requirement for anti-HTN therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age &lt; 50 at donation</td>
<td>1.80 (1.02-3.17)</td>
<td>0.04</td>
</tr>
<tr>
<td>Female sex</td>
<td>0.99 (0.56-1.74)</td>
<td>0.9</td>
</tr>
<tr>
<td>HS’s education or less</td>
<td>1.43 (1.05-1.91)</td>
<td>0.2</td>
</tr>
<tr>
<td>Ever smoked*</td>
<td>0.64 (0.37-1.02)</td>
<td>0.1</td>
</tr>
<tr>
<td>* HS: high school; *Smoked ≥5 packs of cigarettes in life</td>
<td></td>
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</tr>
</tbody>
</table>

Results: Of black living donors, 20.9% developed HTN and 7.0% developed diabetes. Compared to non-black donors, black donors had higher incidence of hypertension and diabetes. Black race was significantly associated with new onset diagnoses of hypertension (p<0.04) and diabetes (p<0.03) in comparison to non-black race. After adjusting for multiple donor characteristics, black race was independently associated with new onset HTN (OR 2.28 95% CI 1.04-5.47) & HTN requiring pharmacologic therapy (OR 3.50; 1.41-8.67).

Conclusions: Race is associated with new onset hypertension and diabetes after living kidney donation. Our findings through primary data collection in a large cohort of donors confirm previous suggestions of this effect made using administrative claims data.

Funding: Pharmaceutical Company Support

TH-PO949
Patterns of Physician Visits before and after Living Kidney Donation Brian Boyarsky, Kyle Van Arendonk, Nels H. Segev, Christian Morath.

Background: Few transplant centers follow their donors on a long-term basis, leaving much of the role of follow-up care in the hands of primary care physicians (PCPs). Annual physical visits are recommended post-donation. The objective of this study was to describe the frequency of PCP and nephrologist visits among living kidney donors before and after kidney donation.

Methods: We surveyed 460 patients who donated a kidney at our center between 1984-2011. Median follow-up was 4.4 years (IQR 2.1-7.9). Frequency of visits to PCPs and nephrologists pre- and post-donation was categorized as more than annually, annually, less than annually, or not at all. Multivariate logistic regression was used to examine the independent associations of donor characteristics with seeing a PCP less than annually post-donation, with seeing a nephrologist post-donation, and with an increase in frequency of PCP visits after donation.

Table 1. Frequency of visits to PCP and nephrologist pre-and post-donation

<table>
<thead>
<tr>
<th>PCP pre-donation</th>
<th>PCP post-donation</th>
<th>Nephrologist post-donation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1x per year</td>
<td>18.0%</td>
<td>7.0%</td>
</tr>
<tr>
<td>1x per year</td>
<td>62.7%</td>
<td>51.3%</td>
</tr>
<tr>
<td>&gt;1x per year</td>
<td>23.3%</td>
<td>34.7%</td>
</tr>
<tr>
<td>Not at all</td>
<td>4.1%</td>
<td>9.6%</td>
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</table>

After kidney donation, 20.3% reported seeing a PCP less than annually. In an adjusted model, men (OR 2.30 95% CI 1.43-3.69) donors with less education (OR 1.87; 1.4-3.06) and younger donors (OR 1.73; 1.05-2.85) were more likely to report seeing a PCP less than annually. 10.0% required nephrologist visits at least annually post-donation. Older donors were more likely (OR 1.84; 1.04-3.25) to have seen a nephrologist post-donation. Compared to post-donation, 17.4% reported an increase in frequency of visits post-donation, 11.1% a decrease, and 75.1% no change. Males (OR 1.70; 1.04-2.78) and those ≥50 years old at donation (OR 1.59; 0.97-2.63) were most likely to increase PCP visit frequency after donation.

Conclusions: 20.3% of donors still saw a PCP less than the recommended frequency of once per year. Male sex, younger age, and lower education were associated with inadequate follow-up. Older donors were more likely to report seeing a nephrologist post-donation.
Correlation between Relative Computed Tomography Based Renal Volumes and Split Renal Function in Living Kidney Donors

Alessandro Diez, Tim E. Taber, Muhammad Ahmad Mujtaba, Muhammad S. Yaqub, John A. Powelson, Alejandro Diez, Tim E.

Indianapolis, IN.

Background: Multi-detector computerized tomography with 3-dimensional reconstruction (MDCT) provides a reliable method to calculate renal volumes. Technetium-99m mercaptacetyltriglycine (MAG3) split function scans are used to determine the relative contribution of each kidney to a patient’s overall renal function. Whereas MDCT provides morphologic information about each kidney and MAG3 split function testing provides functional information, data suggest that there might be correlation between both tests. Herein we perform a head-to-head comparison of both techniques.

Methods: Single center retrospective review of patients >18 years of age who presented for living kidney donor evaluation. Patients with both a MDCT and a MAG3 performed were included. Each kidney’s relative volume was calculated as a percentage of the sum of the total renal volume. The relative volume of each kidney was then compared to the percent renal uptake on MAG3 of each kidney.

Results: 340 charts were reviewed, of which 64 patients fit the inclusion criteria. The average total renal volume was 364.9 mL (Left: 187.06 mL [SD: 54.78], Right: 177.84 mL [SD: 49.76]). The relative left kidney size was larger than the right (Left: 51.15%, Right: 48.85%) (P=0.0049). The average split renal function was higher on the left kidney than the right (Left: 51.52%, Right: 48.48%).

Conclusions: The median times from donation to start and end of captured benefits were 4.9 and 7.7 yrs, respectively. The cumulative frequency of depression dx after benefits start was 4.2% at 1yr and 11.5% at 5yr. After adjustment for baseline demographics, recipient graft failure and death were each associated with 2-3 times the relative risk of subsequent depression dx among non-spousal unrelated LKD (Table). There were trends towards increased depression risk after adverse recipient events in spousal LKD, but no associations in related LKD. Other correlates of LKD depression dx included female gender and white race. In matched-pairs comparison, the depression dx rate in LKD vs controls was 5.0 vs 7.1 per 100-PY (rate ratio 0.70, 95% CI 0.61-0.80).

Conclusions: Recipient death and graft failure predicted depression dx risk among unrelated LKD. Informed consent and post-donation care should consider the impact of recipient outcomes on the LKD’s psychological health.

Funding: NIDDK Support
LDKT surgery. Then potential pitfalls (failure modes) were identified together with the potential causes, the frequencies, and the severities. Ultimately, pitfalls were categorized as low, medium, and high safety risk based on the frequency and severity of consequence (risk binnig).

Results: Ten FMEAs were conducted involving a total of 17 clinicians. A total of 115 process steps were identified for LDKT surgery. Based on the results of the FMEA, 6 failure modes were classified as high risk, 44 medium risk, and 35 low risk. Predominant failure mode causes were communication, distraction, documentation errors, and protocol violations. Figure 1 highlights 6 steps of the process to provide an example of the LDKT procedure process map and the corresponding FMEA results (Figure 1).

Conclusions: FMEA of LDKT demonstrated a broad array of vulnerabilities in the system’s steps/processes of care. The proactive analysis and systems and process improvement of LDKT is paramount to improving safety for donors and recipients alike.

Funding: Private Foundation Support

TH-P0955

Living Donor Kidney Transplants: Trends in Results throughout 20 Years of Experience


Background: Since the late nineties changes in immunosuppressant protocols, introducing of living unrelated donors and Banff scoring might have influenced results in living kidney donors transplantation (LKT).

Methods: Our objective was to study the impact of these changes altogether in the results of a cohort of LKT transplanted between 1998 up to 2007 (n=308). Results were analyzed in two cohorts according decade of transplantation: first decade 88’ to 97’ (n:121) and second decade 98’ to 07’ (n:187).

Results: Donor and recipient age increased from 39 ± 12.8 to 44.8 ± 12.2 (p < 0.001) and from 30.5 ± 12.4 to 35.5 ± 15.2 (p < 0.002) respectively. HLAA mismatch number increased from 1:9:1:2 to 2:4:1:2 (p < 0.001). Months in dialysis also changed from 21:1:22:9 to 29:3:32:6 (p < 0.01). Five years graft survival improved from 77% to 88% (logrank p < 0.05), and patient survival from 91% to 97% (logrank p=0.03) between first and second cohort. Incidence of acute rejection (AR) decreased from 47.1% to 20.3% (p < 0.001). Delayed graft function was present in 26.4% and 10.2% of patients in first and second cohort respectively (p < 0.001). Urologic complications declined from 27.2% to 10.1% (p < 0.001) and vascular complications remained unchanged 23.9% to 19.2% (p=n.s.) across decades. Multivariate analysis shows that only absence of AR was an independent risk factor for graft survival (OR: 0.46, 95%CI: 0.26-0.79, p < 0.005). P.05 was associated with patient’s age (OR: 1.07, 95%CI: 1.03-1.12, p < 0.001) and transplant in first decade (OR: 5.76 95%CI: 1:61-19.70, p < 0.001).

Conclusions: Improvements across decades in patient care and immunosuppressive protocols have improved outcomes in LKD despite transplantation of patients with increasing risk factors and HLA mismatching.

TH-P0956

Comparison of Two Nuclear Scan Tracer Methods for Accurate Assessment of Kidney Donor Glomerular Filtration Rate


Background: Accurate assessment of the glomerular filtration rate (GFR) is essential to assess candidacy for kidney donation, but is often difficult to do, especially in obese donors. Collection of 24 hour urine specimens is often unreliable. Though it is considered the best method for donor GFR evaluation, iohalamate clearance measurement availability is limited. Nuclear scans are often used due to availability and perceived accuracy. However, the tracer may influence the accuracy and therefore effectiveness for kidney donor evaluation. We assessed how two radioactive tracers correlate to commonly used GFR estimation equations which have been validated against iohalamate clearance studies in order to compare different nuclear tracer GFR measurement methods for GFR evaluation of obese donors.

Methods: GFR measurements (mGFR) from nuclear scans conducted between 2009-September 2010 using mercapto-acyetyl triglycine (MAG3) and September 2010-present using Tc-99m-diethylenetriaminopentaaacetic acid (DTPA) were compared to the Cockcroft-Gault, Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (eGFR). We assessed bias (eGFR-mGFR), precision (R2 of regression), and accuracy (percent within 30% of mGFR).

Results: Average age, BMI, gender and ethnicity makeup were similar between the two groups. The DTPA scan had better precision with the Cockcroft-Gault, MDRD, and CKD-EPI equations compared to the MAG3. Accuracy and bias were statistically similar.
TH-PO957

Structural and Mechanical Properties of Large Arteries after Kidney Transplantation: Major Impact of Donor Age and Living Donor Kidney Transplantation

Michel Delahousse,1 Alexandre Karras,2 *Nephrology and Transplantation, Foch Hospital, Suresnes, France; 2Nephrology, Georges Pompidou European Hospital, Paris, France.

Background: Damage to large arteries in ESRD patients is characterized by an outward remodeling of the carotid artery leading to an increased circumferential wall stress (CWS) and by increased aortic and carotid stiffness.

Methods: We measured carotid-femoral pulse wave velocity (PWV), aortic pressure and carotid remodeling and stiffness parameters at three months (M3) and one year (M12) post-transplantation in 77 consecutive kidney recipients (57 cadaveric kidney and 20 living donor kidney recipients).

Results: PWV decreased (10.8 + 2.5 vs 11.6 + 2.7 m/s, p = 0.015) independently of age (in cadaveric kidney recipients) and living donor kidney transplantation are the main determinants of this improvement.

In deceased donor kidney recipients, donor age was the main determinant of isobaric PWV change (improvement with young donors), confirming our previous results in an independent cohort of kidney recipients (J Am Soc Nephrol 19: 798 –805, 2008).

Conclusions: Damage to large arteries can reverse after kidney transplantation. Donor age (in cadaveric kidney recipients) and living donor kidney transplantation are the main determinants of this improvement.

TH-PO958

Changes in Soluble Tumor Necrosis Factor Receptor-2 (sTNFR-2) after Living Donor Nephrectomy

Molly McGovern, Julie A. Berkley, Sushrut S. Waikar, Julie Lin. Renal Division, Department of Medicine, Brigham and Women’s Hospital, Boston, MA.

Background: Higher plasma sTNFR-2 levels have been strongly associated with subsequent kidney function decline in people with well preserved kidney function. Animal studies suggest sTNFR-2 is primarily renally cleared, but no studies in humans currently exist.

Methods: We studied 25 consecutive living kidney donors (LKD) at our center who had measurements of sTNFR-2 before and 24 hours post-nephrectomy. Six, 9 and 12 months post-op, sTNFR-2 was measured by an ELISA assay from R & D Systems using a quantitative sandwich enzyme immunoassay technique. Directly measured CV was 5% in blinded split samples.

Results: Clinical characteristics for both groups are summarized in Table 1.

Conclusions: Damage to large arteries after kidney transplantation led to an increased circumferential wall stress (CWS) and by increased aortic and carotid stiffness.

Results: We measured carotid-femoral pulse wave velocity (PWV), aortic pressure and carotid remodeling and stiffness parameters at three months (M3) and one year (M12) post-transplantation in 77 consecutive kidney recipients (57 cadaveric kidney and 20 living donor kidney recipients).

Results: PWV decreased (10.8 + 2.5 vs 11.6 + 2.7 m/s, p = 0.015) independently of age (in cadaveric kidney recipients) and living donor kidney transplantation are the main determinants of this improvement.

Conclusions: Damage to large arteries can reverse after kidney transplantation. Donor age (in cadaveric kidney recipients) and living donor kidney transplantation are the main determinants of this improvement.

TH-PO959

Racial Disparities in Access to Preemptive Kidney Transplantation among the National Pediatric ESRD Population

Rachel E. Patzer, Sandra Amaral, Nancy G. Kutter, William M. McClellan. School of Medicine, Emory University, Atlanta, GA.

Background: Kidney transplantation is the preferred treatment for pediatric End Stage Renal Disease (ESRD), and preemptive transplant may reduce morbidity and mortality. It is unknown how race and poverty impact access to preemptive transplant in the pediatric ESRD population.

Methods: We examined the incidence rate of preemptive renal transplant by race/ethnicity among the national pediatric (< 21 yrs) ESRD population using United States Renal Data System data from 2000-2008. Annual rates were calculated as incident preemptive transplant count divided by ESRD patient years. Rate Ratios for preemptive transplant by race/ethnicity were calculated using adjusted generalized linear Poisson models. We considered neighborhood poverty and health insurance as socioeconomic status (SES) measures.

Results: Among 10,855 pediatric ESRD patients (pts) from 2000-2008, a total of 1,287 patients (11.9%) had a start date of ESRD equivalent to their transplant date and no history of dialysis. The average annual rate of preemptive transplant was higher among whites (202/1000 person-years[PY]) than Hispanics (59/1000 PY) and blacks (48/1000 PY). Racial differences were evident in the type of preemptive transplant received, where more white pts had a living donor (78.8%) vs. Hispanics (57.3%) and blacks (48.8%)(p=0.0001). In adjusted analysis, Hispanics had a 50% (95% CI: 0.41-0.61) and blacks a 56% (95% CI: 0.36-0.54) lower rate of preemptive transplant vs. whites.

The CABG patients had a higher baseline level of sTNFR-2 (median 3285 vs. 1848 in LKD) that also increased post-operatively (median change 1336 vs. 1212 in LKD) (Figure 1b).

Conclusions: Plasma sTNFR-2 levels are universally increased after living donor nephrectomy; however, this phenomenon is also seen in post-CABG patients. We therefore conclude that increases in sTNFR-2 after donor nephrectomy do not primarily reflect loss of glomerular filtration rate.
Conclusions: Pediatric racial minorities have a significantly lower incidence of preemptive transplantation, which was unexplained by SES. Further studies are needed to elucidate why minority groups with pediatric ESRD are less likely to receive early renal transplantation.

TH-PO960

Reduced Racial and Socioeconomic Disparities in Kidney Transplant Evaluation Completion after Start of a Required Patient Education Program  Rachel E. Patzer, Jennie P. Perryman, Stephen O. Pastan, Sandra Amaral, William M. McClellan. School of Medicine, Emory University, Atlanta, GA.

Background: In 2007, the Emory Transplant Center (ETC) kidney transplant program implemented a patient education class for End Stage Renal Disease (ESRD) patients referred for transplant. The purpose of this intervention was to improve patient awareness about the transplant process and to increase evaluation completion.

Methods: We examined one-year evaluation completion among ESRD patients referred 2005-2007. Patient data were abstracted from medical records and linked with the United States Renal Data System. Adjusted Risk Ratios (RR) by intervention group were calculated from binomial regression models adjusting for time trends. We also examined how the intervention impacted evaluation completion across levels of race and poverty (health insurance, education, employment, and neighborhood poverty by census tract).

Results: A total of 1,126 adult ESRD patients were examined in two transplant evaluation eras (75% pre- and 25% post-intervention). Evaluation completion at one year was higher among those in the post- vs. pre-intervention group (80.4% vs. 44.7%, p<0.0001), was higher among blacks (Panel A) and those living in poor neighborhoods (Panel B) (p for interaction<0.05).

They are much more likely to be employed (51% vs 6%). The vast majority of our UI came to US to work and not to seek medical care. If transplanted, all UI who are employed will continue working. Of those who are unemployed, 82% state that they will seek work if transplanted. 60% were able to identify at least one potential donor. Among those with donors, the average number was 1.9. These donors are young and healthy. 64% of donors already reside in North America.

Conclusions: Life expectancy of an average 40-44 year old US dialysis patient is 8 years. Our UI are younger and healthier and will likely have a greater life expectancy. They have potential kidney donors. Paying for living donor transplantation for the UI would translate into savings of at least $342,000 per patient for NY State. Beyond these savings and improved quality of life, society will also benefit in that most of the patients would be willing and able to reenter the workforce. Lawmakers in New York State and other states that provide emergency medicaid coverage for UI should consider living-donor transplantation for those UI with available living donors. This strategy will significantly reduce the cost of caring for our undocumented patients.

TH-PO962

What Factors Influence Access to Renal Transplantation in Patients Undergoing In-Center Dialysis?  Lina Mackelatie, Adam E. Gaweda, Zygmantas C. Alsauskas. Division of Nephrology, University of Louisville, KY.

Background: Patients with renal failure have a choice of different renal replacement therapies, including in-center hemodialysis (HD), peritoneal dialysis and renal transplantation. It is known that kidney transplant recipients live longer compared to patients on dialysis. Despite that only a small fraction of dialysis patients are on the transplant list.

Methods: We interviewed a total of 129 patients undergoing in-center hemodialysis. Data was obtained from standard questionnaires and CMS Medical Evidence Form 2728. Statistical analysis was performed using SPSS. We built a binary logistic regression model to evaluate predictors of being on the transplant list. We used Hosmer & Lemeshow model selection method to select the most influential predictors (with initial threshold p <0.05 for the univariate models and p<0.05 for the final multivariate model).

Results: We analyzed multiple factors, including age, education, weight, ethnicity, employment, discussing transplantation with nephrologist after initiation of RRT, dialysis vintage etc. In univariate analysis these factors where associated with higher likelihood of being on a transplant list: talking about transplantation with a nephrologist after initiation of HD (OR 22, P=0.003), presentation of different treatment options prior to starting HD (OR 2.8, p=0.05), knowing somebody with a transplant (OR 1.6, p = 0.27), knowing the benefits of kidney transplantation (OR 1.7, p=0.23). In multivariate analysis the only factor that was associated with being on a transplant list was talking about renal transplantation with nephrologist after initiation of RRT (OR 19, p<0.005).

Only 48 % of people with hemodialysis vintage of less than 1 year had discussion about renal transplantation compared to 73% of patients who were on dialysis for more than 1 year.

Conclusions: The most significant factor that determines access to renal transplantation is discussion about kidney transplantation with their nephrologist. Despite better post transplant patient survival with shorter dialysis vintage, discussion about renal transplantation happens in less than 50% of ESRD patients within the first year of initiation of dialysis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

335A
TH-PO963

Distance to Transplant Center Associated with Delayed Renal Transplant Evaluation in the Southeastern United States  Rachel E. Patzer,1 Sumit Mohan,2 Richard Mutel,1 Richard O. Pastan,1 Jennie P. Perryman,1 Sandra Amaral,1 Nancy G. Kutner,1 William M. McClellan,1 School of Medicine, Emory University, Atlanta, GA; 2Department of Medicine, Columbia University, New York, NY; DaVita Clinical Research, Minneapolis, MN.

Background: Geographic variations in access to renal transplant have been reported but considered all prevalent ESRD patients, rather than only those referred for transplantation. Less is known about barriers to transplant evaluation, the first step in the transplant process.

Methods: We examined adult, ESRD patients (pts) referred to the Emory Transplant Center (ETC) kidney transplant program 2005-2007. Patient data were abstracted from medical records and linked with United States Renal Data System. Adjusted Cox models were used to examine the effect of distance on time from referral to start of the evaluation process by race.

Results: Among 2,228 ESRD pts referred to ETC, 55.3% (61.3% white vs. 52.1% black, p<0.0001) started the transplant evaluation. Mean age at referral was 51.1 years. Pts who started the evaluation (n=1,233) lived significantly closer to ETC than those who did not (35.3 vs. 51.8 miles) and the median distance to ETC was lower among black vs. white patients (27 vs. 53 miles; p=0.0001). Blacks were less likely to start the evaluation than whites, regardless of distance to center (p for interaction = 0.7548). The rate of evaluation start was 60% higher (95% CI:1.06-2.41) among white and 26% higher (95% CI:1.02-1.56) among black pts living the closest (< 14.5 miles) vs. farthest (> 98 miles) from ETC.

Impact of Distance to Transplant Center on Transplant Evaluation Race by Year

Conclusions: Pts residing closer to ETC were more likely to initiate their renal transplant evaluation than those living farthest away. Distance to transplant center may be a potentially modifiable barrier to transplant evaluation, and barriers to transplant access may be mitigated by implementing outreach clinics.

TH-PO964

Effect of Diabetes Mellitus on Access to Renal Transplantation  Bhanu K. Patibandla, Akshita Narra, Ranil N. Desilva, Alexander S. Goldfarb-Rumyantzev. Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Background: Previous studies have shown that diabetics with ESRD were less likely to receive a renal transplant. The goal of our study was to evaluate whether the decreased access to renal transplant is mediated by increased co-morbidities and greater BMI. Methods: Study cohort included all ESRD patients from January 2000 to September 2007 using data from the United States Renal Data System (USRDS). We analyzed two outcomes in Cox regression model: (1) likelihood of being placed on the waiting list for renal transplantation or transplanted without wait-listing (2) likelihood of receiving transplant in patients previously placed on the waiting list. Multivariate models were adjusted for age, sex, race, duration of pre-ESRD nephrology care, levels of albumin, hemoglobin and creatinine. In addition we performed subgroup analysis based on age, race, sex, co-morbidity index, and duration of nephropathy care.

Results: We analyzed 721,521 patients (age of ESRD onset 63.6±15.3 years, 54.6% males, 64.7% white and 29.2% African American). When compared with non-diabetic population, diabetes was associated with reduced transplant access: both for wait-listing/ transplantation [HR 0.87, p<0.001] and for transplant after being listed [HR 0.77, p<0.001]. When compared with non-diabetic pts, diabetics were more likely to start their evaluation earlier [HR 1.06 (95% CI:1.04-1.08)] and for transplant after being listed [HR 1.08 (95% CI:1.06-1.10)].

Conclusions: Diabetics are less likely to be placed on the waiting list for kidney transplantation and once on the list are less likely to be transplanted. Our analysis suggests that effect is mediated by higher level of co-morbidity and greater BMI.

TH-PO965

Improving Access to Living Donor Kidney Transplantation  Jonathan Renneey, Monica Monaghan, Aisling E. Courtne, Department of Nephrology, Belfast City Hospital, Belfast, United Kingdom.

Background: Live donor transplantation is the optimal form of renal replacement therapy for the majority of patients with end-stage renal disease. There is considerable geographical variation in the opportunity for patients to access this treatment.

Methods: Traditionally the assessment process for a potential live donor in Northern Ireland was long and cumbersome, requiring multiple attendances at hospital. It was customary for it to exceed 2 years. The number of live donor transplantations remained static at a maximum of 20% of all renal transplants per annum.

The donor pathway was reorganised with a one-day assessment combining all routine investigations and nephrology assessment. A formal multidisciplinary meeting was established weekly allowing a swift decision on suitability.

Results: The results were an increase in live donor transplant procedures from 9 to 48 per annum (an increase to 58% of all kidney transplants being live donor organs), a reduction in time to transplantation from five months to assessment with a substantial increase in pre-evaluation transplantation especially in the paediatric population. This 400% increase in live donor transplants occurred over an 18 month period. Figure 1 Renal Transplantation in Northern Ireland.

Conclusions: Reorganisation of the live donor assessment process reduces donor fatigue and drop-out, reduces the time to transplantation, and increases the number of live donor transplant procedures. This can be achieved without a substantial increase in resources, providing opportunity for improved clinical outcomes and more economically viable renal replacement therapy programmes for populations with end-stage renal disease.

TH-PO966

Excellent Kidney Transplant Outcomes in a Safety Net County Hospital  Giselle Kohler,1 Merit E. Qviste,2 John R. Hartono,1 Christopher Y. Lu,1 Nilum Rajora,1 Meele Debroy,1 Doris L. Sweett,1 Miguel A. Vazquez,1 Department of Internal Medicine, University of Texas Southwestern Medical Center at Dallas, TX; 2Department of Surgery, University of Texas Southwestern Medical Center, Dallas, TX; Kidney Transplantation, Parkland Health and Hospital Systems, Dallas, TX.

Background: Race and insurance status are important factors limiting access to kidney transplantation and associated with poor outcomes. The kidney transplant program at Parkland Health and Hospital Systems (PHHS) serves a predominantly indigent and minority population. PHHS is an integrated health care system that serves as primary teaching hospital for UT Southwestern Medical Center and, via a central campus and a network of primary health care clinics and specialty/subspecialty clinics, makes health care available to indigent patients in Dallas County.

Methods: We conducted a retrospective review of recent kidney transplants (2005-2010) performed at Parkland Hospital in Dallas, including racial/ethnic distribution of transplant recipients, insurance status and kidney transplant outcomes.

Results: Seventy-five percent of kidney transplant recipients during our study period were enrolled in Parkland Health Plus, which is a Parkland-based county assistance program to provide health care services and medications to indigent patients who do not have other means to cover health expenses.

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<th>Year</th>
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Conclusions: The results were an increase in live donor transplant procedures from 9 to 48 per annum (an increase to 58% of all kidney transplants being live donor organs), a reduction in time to transplantation from five months to assessment with a substantial increase in pre-evaluation transplantation especially in the paediatric population. This 400% increase in live donor transplants occurred over an 18 month period. Figure 1 Renal Transplantation in Northern Ireland.

Conclusions: Reorganisation of the live donor assessment process reduces donor fatigue and drop-out, reduces the time to transplantation, and increases the number of live donor transplant procedures. This can be achieved without a substantial increase in resources, providing opportunity for improved clinical outcomes and more economically viable renal replacement therapy programmes for populations with end-stage renal disease.
Conclusions: Efforts to allocate professional resources and multi-institutional commitments to provide integrated health care and assistance with health care costs, including access to medical services and assistance with medications can improve access to kidney transplantation and lead to superior transplant outcomes for patients who are under-insured or belong to underserved minority populations.

TH-PO967
Race and Access to Renal Transplantation Evaluation in the Southeastern United States Justin D. Schrager, Rachel E. Patzer, Jennie P. Perryman, Stephen O. Pastan, Nancy G. Kutner, William M. McClellan. School of Medicine, Emory University, Atlanta, GA; Emory Transplant Center, Atlanta, GA.

Background: Disparities exist among End Stage Renal Disease (ESRD) patients in access to renal transplant. The effect of race and socioeconomic status (SES) on start of the kidney transplantation evaluation following referral has not been thoroughly explored.

Methods: This study examined the effect of race on renal transplant evaluation, defined as the first scheduled on-site visit at the Emory Transplant Center (ETC), from 2005-2007 and followed through 2010. Patient characteristics were abstracted by chart review and linked with the United States Renal Data System and census tract poverty. Kaplan-Meier methods and Cox models were used to examine racial differences in access to evaluation. We used neighborhood poverty and health insurance as measures of SES.

Results: 2,291 prevalent ESRD patients were referred to ETC during the study period. Of these, 1015 (44.3%) never started the renal transplant evaluation process. Compared to patients who started the evaluation process, those who did not start were significantly more likely to be black (69.5% vs. 61.1%), black health insurance (17.7% vs. 14.5%) or have Medicaid (22.9% vs. 14.5%), to reside in the poorest census tracts (40.1% vs. 28.2%) and spend a longer time on dialysis prior to referral (314 days vs. 124 days). In crude Cox models, black vs. white patients were 28% less likely to start the evaluation at any given time during follow-up (HR=0.72; 95% CI: 0.65-0.81). After adjusting for demographic, clinical, and SES factors, this disparity persisted (HR=0.82, 95% CI: 0.73-0.93).

Conclusions: Racial disparities exist in referrals to renal transplant evaluation. Black patients were 18% less likely to start the evaluation process following referral, even after adjusting for clinical and SES characteristics.

TH-PO968
Access to Renal Transplantation at Nottingham University Hospitals Linda H. Bisset, Linda Evans, Catherine Byrne. Nottingham Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, City Campus, Nottingham, United Kingdom.

Background: Early transplant referral and listing confers advantages in terms of life expectancy, quality of life, and graft survival. In the UK, equity to access and activation appears largely centre specific. Current recommendations are for pre-diagnosis patients to be listed when eGFR <15. Previous work from our unit suggested fewer than expected pre-dialysis patients were seen in advance of the need for listing. Delays were mainly related to cardiology investigations. Prospective audit shows significant improvements in the 18 week pathway over the last 12 months.

TH-PO969
Combination of Pre-Transplant BKV Specific IgG Donor Positivity and Recipient Negativity Correlates with Post-Transplant BKV Infection Pumeet Sood, Shamila Chaturi Senanayake, Kumar Sujeet, Christopher Johnson, Sundaram Hariharan. Medical College of Wisconsin, Milwaukee, WI.

Background: BKV infection can manifest as viremia/viruria which may lead to nephritis and graft failure. Risk factors for BKV infection have not been well defined. In our prospective study, we analyzed the effect of donor and recipient BKV specific antibody IgG status at time of transplant on the development of post-transplant BKV infection.

Methods: 240 patients were prospectively enrolled from July 2007 to July 2010 and followed until May 2011. Baseline data on donor/recipient age, gender, race, cold ischemia time, donor source, PRA, induction therapy, HLA match, donor BKV immune status and prospective data on occurrence of rejection and quantitative BK viruria and viremia at 1,3,6 and 12 months was collected. Positive BKV specific antibody was defined as IgG ELISA units. Study population was divided into D+R+/D+R-/D-R+/D-R- groups based on donor and recipient immune status. Occurrence of post transplant BKV infection defined by detection of BKV DNA in plasma or urine at the end of study period was used as endpoints. Kaplan-Meier survival curves were used to look at the difference between groups and Log-Rank test was used to test for significance.

Results: Patients were divided into 4 groups based on donor and recipient BKV specific positivity and negativity: Group 1(D+R-)n=41 pairs, group 2(D+R+)n=42 pairs, group 3(D-R+)n=41 pairs and group 4(D-R-)n=68 pairs. The demographic, transplant and post-transplant variables were similar in 4 groups. Recipients from group 1(D+R-) had the highest chance of developing post-transplant BKV infection (figure1), Log Rank p=0.009.

Conclusions: Pre-transplant BKV specific IgG antibody status in subjects with D+R- is associated with a higher rate of post-transplant BKV infection and predominantly occurs in the early period.

TH-PO970
Risk Factors for BKV Infection after Renal Transplantation Pumeet Sood, Shamila Chaturi Senanayake, Kumar Sujeet, Christopher Johnson, Sundaram Hariharan. Medical College of Wisconsin, Milwaukee, WI.

Background: BKV infection manifests as viremia or viruria which may progress to BKVN. Graft survival rates in patients with BKVN are worse than acute rejection and calcineurin inhibitor (CNI) toxicity. In our prospective study, we evaluated the risk factors for the occurrence of all BKV infections defined as any degree of viruria and/or viremia.

Methods: 240 patients were prospectively enrolled from July 2007 to July 2010 and followed until May 2011. Baseline data on donor/recipient age, gender, race, cold ischemia time, donor source, PRA, induction therapy, HLA match, donor BKV immune status and prospective data on the occurrence of rejection and quantitative BK viruria and viremia at 1, 3, 6 and 12 months was collected. Logistic regression modeling was used to ascertain risk factors for any level of BKV infection. Test and Chi-sq test were used for continuous and categorical variables, respectively.
Results: Characteristics of BKV infection and non-infected patients are summarized in table 1.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>BKV infection (N=93)</th>
<th>No BKV infection (N=147)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor Source (LD-ID)</td>
<td>38/54</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>Age (≥60/&lt;60 yrs)</td>
<td>8.5/11.5</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>Donor race (AA/W others)</td>
<td>11/82</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>Donor specific (gG/gA)</td>
<td>17/47</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Recipient age (≥60/&lt;60 yrs)</td>
<td>21/72</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>Recipient race (AA/W others)</td>
<td>13/90</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Cold ischemia time (min)</td>
<td>134 (124-140)</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>HLA mismatch number (1-4)</td>
<td>40/51</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>PRA (200%)</td>
<td>2.4/4.5</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>Induction therapy (Thymoglobulin/IL-2R)</td>
<td>46/47</td>
<td>0.58</td>
<td></td>
</tr>
<tr>
<td>Anti-rejection (YN)</td>
<td>18/75</td>
<td>0.84</td>
<td></td>
</tr>
</tbody>
</table>

African American recipients had lower risk of developing BKV infection compared to Caucasians and other races. This effect remained significant after controlling for other potential confounding variables (OR 0.38, 95% CI 0.16-0.84, P=0.018). Actuarial Kaplan-Meier curves for the occurrence of BKV infection was significantly more in Caucasians as opposed to African Americans.

Conclusions: African Americans had a lower incidence of BKV infection compared to Caucasians. This association with race needs further investigation in larger prospective studies.

TH-PO972

The Deterioration of Serum Sulfatide Level, a Novel Risk Factor of Cardiovascular Disease, Is Dramatically Improved by Kidney Transplantation in ESRD Patients

Yuri Kamiyo,1 Makoto Harada,2 Yasufumi Takahashi,2 Makoto Higuchi,2 1Department of Metabolic Regulation, Shinsu University School of Medicine, Matsumoto, Nagano, Japan; 2Department of Nephrology Internal Medicine, Shinsu University School of Medicine, Matsumoto, Nagano, Japan.

Background: Sulfatide is a major component of glycosphingolipids in lipoproteins. Recently, we reported that a low serum level of sulfatide in hemodialysis patients is related to the high incidence of cardiovascular diseases. This earlier study suggests that the deterioration of serum sulfatide level would be a novel risk factor of cardiovascular disease. However, the serum kinetics of sulfatide in kidney disease patients and the function of endogenous serum sulfatide are still unclear.

Methods: To obtain novel knowledge concerning these issues, we investigated the serum kinetics of sulfatide in 5 adult kidney transplant recipients. We also analyzed the correlated factors influencing the serum sulfatide level, using multiple regression analysis.

Results: Kidney transplantation caused a dramatic increase of serum sulfatide level without an alteration of its composition in all recipients in a time-dependent manner; however, the recovery speed was slower than that of the improvement of kidney function and the serum sulfatide reached a nearly normal level after 1 year. Multiple regression analysis showed that the significant correlated factor influencing the serum sulfatide level was log duration (time parameter) throughout the observation period, and the correlated factors detected in the stable phase were the decrease of serum concentration of malondialdehyde (an oxidative stress marker) as well as the elevation of platelet count.

Conclusions: The current study results demonstrate the deterioration of serum sulfatide level in ESRD patients is dramatically improved by kidney transplantation for the first year. The recovery of serum sulfatide might derive from the attenuation of systemic oxidative stress. The normal level of serum sulfatide in kidney transplant recipients might affect platelet function, and contribute to the reduction of cardiovascular disease incidence.

TH-PO973

Cardiovascular Risk and Stress Testing in Kidney Transplantation

Rowena B. Delos Santos, Jagdeep Obhrai, Suzanne Watnick. Division of Nephrology, Oregon Health and Science University, Portland, OR.

Background: Kidney transplantation improves quality of life and life expectancy in the ESRD population. Cardiovascular disease is a significant cause of morbidity and mortality in this population. We aimed to identify baseline characteristics predictive of a positive stress test as well as cardiovascular outcomes in kidney transplant patients.

Methods: We conducted a retrospective review of 679 kidney transplant patients who underwent cardiac stress testing using exercise and pharmacological modalities with nuclear or echocardiography imaging. The composite outcome included: new MI, ACS, CVA, cardiac death, arrhythmias. We used logistic regression to evaluate eleven characteristics for their contribution to a positive stress test then at their contribution to cardiovascular outcomes along with a positive stress.

Results:

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>Odds ratio (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>1.0 (0.8-1.3)</td>
<td>0.57</td>
</tr>
<tr>
<td>Race</td>
<td>1.4 (1.0-2.0)</td>
<td>0.06</td>
</tr>
<tr>
<td>Age</td>
<td>1.1 (1.0-1.1)</td>
<td>0.06</td>
</tr>
<tr>
<td>Diabetes</td>
<td>2.3 (1.5-3.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>M/E/T/S</td>
<td>0.9 (0.7-1.2)</td>
<td>0.12</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.2 (0.9-1.7)</td>
<td>0.29</td>
</tr>
<tr>
<td>Male</td>
<td>0.6 (0.4-1.0)</td>
<td>0.05</td>
</tr>
<tr>
<td>Month after transplant</td>
<td>0.8 (0.6-1.0)</td>
<td>0.08</td>
</tr>
<tr>
<td>Positive stress test</td>
<td>1.0 (0.8-1.3)</td>
<td>0.57</td>
</tr>
</tbody>
</table>

Of 679 patients, 158 had a positive stress test. The multivariable logistic regression showed age, prior cardiac disease, body mass index, diabetes and dialysis vintage were predictive of a positive stress test; metabolic equivalents were not. After adding a positive stress test to the model, only age, diabetes, and previous cardiac disease were significant.

Conclusions: Traditional and kidney specific risk factors are predictive of a positive stress test, but not necessarily cardiovascular outcomes. Some traditional predictors were not significant. Current recommendations for pre-operative testing may not be sufficient to predict cardiovascular outcomes post transplantation. Future studies could investigate methods of better identifying pre-kidney transplant patients who are at higher risk for post-transplant cardiovascular events.

Funding: Private Foundation Support

TH-PO974

African American Donor Race Is Associated with Increased All-Cause and Cardiovascular Mortality and Graft Loss, but Not with Delayed Graft Function

Miklos Z. Molnar,1 Csaba P. Kovetsy,2 Sapthagiri Bunnappadrat,3 Elani Streja,4 Mahesh Krishnan,5 Allen R. Nissenson,5 Keith C. Norris,1 Kamyar Kalantar-Zadeh.1,4 1Harold Simmons Center, Torrance, CA; 2Semmelweis University, Budapest, Hungary; 3Salem VA Medical Center, Salem, VA; 4David Geffen School of Medicine at UCLA, Los Angeles, CA; 5DaVita, Inc, Denver, CO; 6McGill University, Montreal, QC, Canada.

Background: In the last few genetic factors were identified to explain the higher prevalence of end stage renal disease in African American (AA). We examined associations of donor race & post-transplant outcomes in a large national cohort of kidney transplant recipients.

Methods: Linking the 5-year patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 13862 hemodialysis patients who underwent kidney transplantation. Mortality, graft failure & delayed graft function (DGF) risks were estimated by Cox regression and logistic regression, respectively.

Results: Patients were 48±14 years old and included 39% women and 26% diabetics. AA donor race was associated with 39%, 80% & 30% higher all-cause mortality (1.39[1.09-1.78]), cardiovascular mortality (1.80[1.72-2.60]) & death-censored graft loss (1.30[1.03-1.64]), respectively over the 6-year observation period after adjusting for several relevant clinical & transplant-related variables. In non-AA recipients AA donor race was associated with significantly higher risk of graft loss.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
In AA recipients AA donor race was not associated with significantly higher risk of outcomes. The risk of DGF did not show association with AA donor race.

Conclusions: AA donor race was associated with increased all-cause & cardiovascular mortality in non-AA recipients & graft loss, but not with DGF.

Funding: NIDDK Support

TH-PO975

Effects of Kidney Transplantation on Echocardiographic Abnormalities

Ana Sofia Rocha, Nihil Chitalia, Helen Gregson, Rajan Sharma, Juan Carlos Kaski, Debasish Banerjee.

Methods: We conducted a retrospective chart review of 679 post-kidney transplant patients from two transplant centers in Portland, OR. Specific outcomes of interest included death from cardiac cause, arrhythmias, acute coronary syndrome, myocardial infarction and congestive heart failure episodes. Descriptive analyses were conducted and outcomes are expressed as percentages.

Results: Cardiovascular outcomes post kidney transplant in 679 patients

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Total events N = 135 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death non-CV cause</td>
<td>27 (20%)</td>
</tr>
<tr>
<td>CHF</td>
<td>16 (12%)</td>
</tr>
<tr>
<td>ACS</td>
<td>12 (9%)</td>
</tr>
<tr>
<td>MI</td>
<td>11 (8%)</td>
</tr>
<tr>
<td>Arrhythmias</td>
<td>12 (9%)</td>
</tr>
<tr>
<td>VTach/Vfib</td>
<td>10 (7%)</td>
</tr>
<tr>
<td>Other rhythm nos</td>
<td>12 (9%)</td>
</tr>
<tr>
<td>Death CV</td>
<td>12 (9%)</td>
</tr>
<tr>
<td>Sudden death</td>
<td>7 (5%)</td>
</tr>
<tr>
<td>Death non-CV cause</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

A total of 135 events occurred in 679 post transplant patients. Of these events, 20 were arrhythmias that were not atrial fibrillation or atrial flutter while 7 events were sudden cardiac death. We found that of all these events, 38% were arrhythmias, 13% were MI, 12% were ACS, 20% were CHF. For causes of death, 9% were due to cardiovascular causes of death while 8% were due to non-cardiovascular causes of death. In comparison, rates for the ESRD population in general per USRDS data on causes of death include: arrhythmia/arrest 26%, acute MI 5%, CVA 4%.

Conclusions: Despite significant non-invasive and invasive cardiovascular testing prior to transplant, these patients have a large percentage of events that may not necessarily be related to coronary artery disease, which is similar to the ESRD population. Our cardiac evaluations may not be sufficient to predict other significant cardiac events.

Funding: Private Foundation Support

TH-PO978

Pretransplant Diabetes Mellitus Doubles the Risk of Cardiovascular Events after Renal Transplantation. A Prospective Multicenter Study

Jose M. Morales, Robert Marcen-Letosa, Amado Andres.

Background: Cardiovascular disease (CD) is the main cause of death after transplantation. The aim of our study was to investigate the influence of pretransplant diabetes (Pre-DM) on the presence of posttransplant cardiovascular events and survival figures after renal transplantation (RTx).

Methods: From a database of 2591 RTx patients performed in Spain during 2000-02 we prospectively analyzed the results at 5yr in patients with Pre-DM (n: 247, 9.5%) vs non-diabetic patients (n:2344, 90.5%).

Results: Pre-DM patients were significantly older, males, with a higher body mass index and 30% of them had had higher pre-transplant CVE (30.4% vs 13% p 0.001 vs non-DM patients)...

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Conclusions: RTx in patients with pre-DM offer acceptable results although showed lower survival figures at 5yr than non-diabetic patients. The presence of pre-DM doubles the risk for posttransplant CD events. Therefore, pre and posttransplant measures to improve CD especially in diabetic patients are mandatory. Funding: Pharmaceutical Company Support

TH-PO979
Rate of mGFR Decline Associates with Mortality in Transplant Kidney Recipient
Olivier Moreau,1 Lise Thibaudin,2 Nicolas Mailiard,3 Christopher R. Mariat,3 1Nephrology & Public Health, Hospital, Nice, France; 2Service de Néphrologie et Laboratoire d’Explorations Fonctionnelles Rénales, CHU de Saint-Etienne, France; 3Néphrologie CHU de Saint-Etienne, France.

Background: Longitudinal declines in kidney function were found independently associated with increased all-cause mortality in adults and older with native kidney. Based on these observations, we wanted to test this hypothesis in transplant kidney recipient.

Methods: Out of 610 patients having received a kidney transplant between 1989 and 2000 at our institution, 488 (80%) were longitudinally screened for their GFR by performing urinary clearance of Inuline (mGFR) at one year post-transplant and then every 5 years. The mean follow-up was 12 ± 4 yrs with a total of 1,330 mGFR. Annual individual slopes in mGFR were calculated with joint modeling random effect and secondary divided into quartiles of mGFR decline. Crude and adjusted hazard ratios (HR) of quartiles of slope, for all cause mortality (i.e. death censored-graft loss and death occurring before or after starting dialysis) were analyzed into a Cox regression model.

Results: Baseline recipient characteristics were as follows : mean age 47±19 yrs, men 70%, dialysis 66%, first kidney transplant in 84%, preemptive transplantation in 7%, donor age 38±14 yrs. After one year post-transplant: inulin clearance: 46±19 ml/min/1.73m2 and Uprot/creat > 300 mg/g in 20% of patients. 89% were treated with anticalcineurin inhibitor. During follow-up, 136 patients (28%) returned dialysis and 139 deceased (after returning dialysis for 30 of them). The mean [IQ] slope of mGFR for the whole population was -1.8 [-2.8; -0.5] mL/min/1.73m2/yr. HR of quartiles of mGFR slope after one year post-transplant is independently associated with all-cause mortality risk. HR of quartiles of slope, for all cause mortality (i.e. death censored-graft loss and death occurring before or after starting dialysis) were analyzed into a Cox regression model.

Conclusions: The association persisted with adjustment on other covariables and were stronger when the death was considered before and after dialysis.

TH-PO998
Kidney Transplantation in Hepatitis C Virus Positive Recipients: Does Type of Induction Influence Outcome?
Kalathil K. Sureshkumar,1 Ngoc L. Thai,2 Richard J. Marcus.1 1Nephrology and Hypertension, Allegheny General Hospital; 2Abdominal Transplantation, Allegheny General Hospital, Pittsburgh, PA.

Background: Kidney transplantation in hepatitis C virus seropositive recipients (HCV+) without advanced liver disease is associated with improved survival compared to staying on waiting-list. A concern for using depleting (versus non-depleting) induction agent for kidney transplantation in HCV+ is the possibility that the associated enhanced immunosuppression might favor hepatitis C progression leading to adverse outcomes. Methods: Using OPTN/UNOS STAR files, we identified HCV+ who underwent deceased-donor kidney transplantation from either HCV seropositive or negative donors (HCVD+ and HCVD-) from 1998 to 2008 and received induction with either a depleting agent (rabbit-antithymocyte globulin or alemtuzumab) (n=1859) or a non-depleting agent (basiliximab or daclizumab) (n=1631). Multivariate analysis was performed using a Cox regression model to evaluate the independent effects of the type of induction on graft and patient outcomes. Results: Donor, recipient and transplant related covariates thought to affect the outcomes were included in the model. Conclusions: These data demonstrate and LVM and LV wall thickness are lower in South-East Asian kidney transplant recipients. Less left ventricular hypertrophy may partially explain the improved survival in South-East Asian patients who receive kidney transplantation.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
The Renal Outcome of Hepatorenal Syndrome after Liver Transplantation

Yun Jung Oh,1 Jung Pyo Lee,1 Do Hyoung Kim,1 Hyuk Yong Kwon,1 Yun Kyu Oh,2 Chun Soo Lim,2 Yon Su Kim.1 1Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; 2Internal Medicine, Seoul National University Boramae Medical Center, Seoul, Korea.

Background: Hepatorenal syndrome (HRS) is a well-recognized serious complication of end-stage liver disease. Liver transplantation (LTx) is a treatment of choice for the patients. However, HRS is a risk factor for posttransplant chronic kidney disease and mortality. Here, we evaluated change of renal function after LTx and posttransplant recipients' survival in patients with HRS, and analyzed risk factors for non-recovery of renal function.

Methods: Among 764 consecutive adult Korean patients underwent LTx in a cohort of a single Asian center from 1995 to 2009, a total of 76 patients, who were satisfied with HRS criteria of the International Ascites Club, were enrolled. Patients with prerenal failure, nephrotoxic renal failure, or parenchymal kidney disease or under age 18 were excluded.

Results: Mean age at LTx was 48±9.8 years and proportion of male was 73.7%. The most common etiology of liver disease leading to liver transplantation was HBV (67.1%), followed by alcoholic liver disease (7.9%) and HCV (3.9%). Pretransplant serum creatinine (sCr) level was 5.12±1.71 mg/dL. After LTx, renal function was significantly improved (sCr, at 1st month: 1.52±0.59, at 6th month: 1.63±0.41, at 12th month: 1.54±0.36 mg/dL). Early mortality of recipients with HRS was significantly higher, however, it was not significantly different thereafter compared to those without HRS (3rd month: 79.4% vs. 93.1%, P<0.01; 5th year: 83.7% vs. 77.7%, P=0.05). Renal function of 32 patients (51.6%) had been recovered at posttransplant 1 year (sCr, 1.29±0.25 mg/dL). Early resolving of HRS within posttransplant 1 month did not predict the long-term patient survival. But the kidney function at 1 year could predict the patient survival. A multivariate logistic regression analysis revealed that Child-Pugh Score was a significant risk factors for non-recovery of post-LTx renal function (P=0.024, OR 1.52, 95%CI 1.06-2.1).

Conclusions: Liver transplantation is the definitive treatment for HRS, but the non-recovery of kidney function at 1 year was associated with poor patient survival.

TH-PO984

How ‘Overt’ Is ‘Occult Hepatitis C’ In Hemodialysis and Kidney Transplant Patients? Seema Baid-Agrawal,1 Petra Reinek,1 Ralf Schindler,1 Ulrich Frei,1 Thomas Berg.2 1Dept of Nephrology and Medical Intensive Care, Campus Virchow Clinic, Charite Medical University, Berlin, Germany; 2Division of Hepatology, University Clinic of Leipzig, Leipzig, Germany.

Background: Our aim was to assess for the first time the prevalence of a newly defined entity called ‘occult hepatitis C virus (HCV) infection’, i.e. presence of HCV RNA in liver or peripheral blood mononuclear cells (PBMC) in absence of serum RNA, in large cohorts of chronic hemodialysis (CHD) patients and kidney transplant recipients (KTxR).

Methods: In this cross-sectional study, 421 CHD patients (Group 1), 418 KTxR (Group 2) and 2 control groups: 25 HCV-antibody (Ab)-positive patients with chronic hepatitis C (Group 3, positive controls) and 39 HCV-Ab-negative, HBsAg-negative healthy subjects (Group 4, negative controls) were enrolled. HCV RNA was tested in serum and PBMC using Versant TMA assay (Siemens Healthcare Diagnostics). Results: CHD (Group 1): 405/421 patients were HCV-Ab-negative (Group 1a) and 16 were Ab-positive (Group 1b). The prevalence of HCV by positive serum RNA was 2.4% (10/421), of which 20% (2/10) were HCV-Ab-negative. Overt HCV infection was found in 2/405 (0.5%) HCV-Ab-negative patients. KTxR (Group 2): 403/418 KTxR were HCV-Ab-negative (Group 2a) and 15 were Ab-positive (Group 2b). Prevalence of HCV by serum RNA in this group was 4.3% (18/418), of which 50% (9/18) were HCV-Ab-negative. Overt HCV infection was found in 2/403 (0.5%) HCV-Ab-negative patients.

Conclusions: Prevalence of occult HCV was low in both populations. Laborious and expensive testing for occult HCV is not required for screening and diagnosis of HCV infection in these patients. Instead, detection of HCV RNA in serum using ultrasensitive assay after a negative HCV-Ab testing, should be the test of choice, as a significant proportion of HCV-Ab-negative infection was found in both groups, which would have gone undetected otherwise.

Funding: Private Foundation Support
TH-P0985

Antiviral Agents Has Improved the Clinical Outcomes in Renal Transplant Recipients with Chronic Hepatitis B

Hyun Gyoung Kim, Chul Woo Yang, Cheol Whee Park, Yong-Soo Kim

Background: The introduction of antiviral agents has reduced complication associated with chronic hepatitis B virus (HBV) infection, which was one of the important causes of morbidity and mortality in renal transplant recipients (RTS) with chronic hepatitis B. However, the relationship between chronic HBV infection and clinical outcome after kidney transplantation (KT) remains controversial.

Methods: Sixty-nine RTS with chronic hepatitis B were included in this study. We compared retrospectively the baseline characteristics and clinical outcomes between the treatment group with prophylactic antiviral agents and the historical control group who received no prophylactic antiviral treatment. There were 25 patients in the treatment group and 44 in the historical control group.

Results: There were no significant differences in baseline clinical parameters such as gender, age at KT, primary renal disease, the dialysis modality before KT and HLA mismatch number between the treatment and the control group. Nine patients (20.4%) in the control group received salvage treatment with antiviral agents after HBV reactivation. However, there were no significant differences in lamivudine resistance. The graft survival rate in the treatment group was significantly higher than that in the control group (5-year graft survival rate 81.8% vs. 55.9%, 10-year graft survival rate 81.8% vs. 34.2%, p<0.004). The treatment group also had better patient survival as compared to the control group (5-year patient survival rate 99.9% vs. 70.4%, 10-year patient survival rate 90.9% vs. 57.4%, p<0.014). In the control group, most common cause of graft loss was patient’s death (51.6%, n=16) and the leading cause of death was fulminant hepatitis (66.6%, n=14/21), while there was no HBV related death in the treatment group.

Compared to effective antiviral agents, renal transplant recipients with chronic hepatitis B showed a higher incidence of graft failure and HBV-related mortality. The development of antiviral agents had improved graft and patient survival in renal transplant recipients with chronic hepatitis B.

TH-P0986

Effect of Induction Agent on Post-Transplant Outcomes in Deceased Donor Kidney Transplant Recipients: Influence of Race


Background: The effect of induction agents on kidney transplant outcomes with respect to race is not well studied. We aimed to compare the outcomes of deceased donor kidney transplants (DDKT) with inferior graft and patient survival in non-AA but similar with ALE (HR 1.08, CI 1.0-1.2, p=0.04) inductions in non-AA but similar with IL-2B induction in non-AA (HR 1.04, CI 0.98-1.1, p=0.18) recipients. AGS was similar with IL-2B induction in non-AA (HR 1.04, CI 0.98-1.1, p=0.18) but inferior in AA (HR 1.14, CI 0.80-1.14, p=0.002) recipients in comparison to r-ATG.

Methods: Using OPTN/UNOS database, we identified patients ≥18 years who underwent DDKT from January 2000 to December 2008 and received r-ATG (n=21,506), ALE (n=3476) or IL-2B (n=17869) and were maintained on CNI/MMF based regimen with or without steroids.

Results: The median follow up was 29.6 months (range 10.7-60.1 months). Adjusted graft survival (AGS) for AA and non-AA recipients are shown below.

Compared to r-ATG, ALE induction was associated with inferior AGS in non-AA (HR 1.29, 95% CI 1.21-1.46, p<0.001) but similar AGS in non-AA (HR 1.04, CI 0.80-1.14, p=0.002) recipients. ALE was similar with IL-2B induction in non-AA (HR 1.04, CI 0.98-1.1, p=0.18) but inferior in AA (HR 1.14, CI 0.80-1.14, p=0.002) recipients in comparison to r-ATG.

Conclusion: AA recipients with chronic hepatitis B showed a higher incidence of graft failure and HBV-related mortality. The development of antiviral agents had improved graft and patient survival in renal transplant recipients with chronic hepatitis B.
Racial Differences in Allograft and Death Outcomes in Kidney Transplant Recipients with Lupus Nephritis: Analysis of United States Renal Data System

Robert Nees,1 Frank P. Hurst,1 Lawrence Agodoa,2 Kevin C. Abbott.1 1Department of Nephrology, Walter Reed Army Medical Center, Washington, DC; 2NIDDK, National Institutes of Health, Bethesda, MD.

Background: African Americans (AF) with lupus nephritis (LN) have an increased risk of graft loss compared to Caucasians in the kidney transplant (KT) population. Whether this disparity is greater than among KT patients without LN, or applies to death, has not been reported.

Methods: In a retrospective cohort of 150,118 patients first transplanted from January 1, 1995 to September 29, 2006, we identified 4,214 patients who had lupus nephritis as the primary cause of ESRD.

Results: In a Cox regression analysis, AF recipients (vs. non-AF) with LN had an increased risk of graft loss (adjusted hazard ratio [AHR] 1.41, 95% confidence interval [CI] 1.37-1.44). At 10 years, the allograft survival rate for AF was 42.9% (95% CI 38.5-47.3) as compared to 58.2% (95% CI 54.8-61.4) in non-AF. Furthermore, AF (vs. non-AF) with LN had an increased mortality rate (AHR 1.97, 95% CI 1.40-2.71). The disparity for graft loss among AF with LN was greater than among AF without LN (AHR 1.51 and 1.41, respectively; p < 0.001) as well as for death (AHR 1.37 and 1.05, respectively; p < 0.001). There was significant interaction between AF race and LN for both outcomes. MMF was associated with lower risks for graft loss and death in the lupus cohort as compared to azathioprine, however this was not significant (AHR 0.89, 95% CI 0.51-1.59 and AHR 0.40, 95% CI 0.13-1.28, respectively).

Conclusions: African American recipients with FSGS have a higher prevalence of risk factors for allograft failure as compared to other races.

Transplantation: Epidemiology, Outcomes - I

National Transplantation Pregnancy Registry (NTPR): Pregnancy Outcomes in 156 Female Kidney Recipients on Tacrolimus-Based Immunosuppression

Serban Constantinescu,1 Lisa Coscia,2 Megan Clary,2 Carolytn H. McGorry,2 Michael J. Moritz,2 Vincent T. Armeniti.2 1Obstetrics, Temple University School of Medicine, Philadelphia, PA; 2Surgery, Thomas Jefferson University, Philadelphia, PA; 3Surgery, Lehigh Valley Health Network, Allentown, PA.

Background: The purpose of this study is to describe pregnancy outcomes in 156 female kidney transplant recipients receiving tacrolimus-based immunosuppression who reported to the National Transplantation Pregnancy Registry (NTPR).

Methods: Data were collected via questionnaires, phone interviews, and medical records.

Results: There are 156 recipients who reported 250 pregnancies with 255 outcomes (including twins) to the NTPR. There was also mycophenolic acid (MPA) exposure during 61 of these pregnancies. Outcomes included: 179 (70%) livebirths, 66 (26%) spontaneous abortions, 4 therapeutic abortions, 5 stillbirths, and 1 ectopic pregnancy. The mean gestational age of the 179 liveborn was 35.3±3.5 wks and the mean birthweight was 2,487±824 g. There were 4 neonatal deaths. Maternal comorbid conditions during pregnancy included: 55% hypertension, 31% preeclampsia, 21% infections, 9% diabetes mellitus, and 2% acute rejections. Structural malformations were reported in 13 (7.3%) of the 179 livebirths and in 7 (25.9%) of 27 livebirths with MPA exposure. Microtia, an ear deformity, was present in 4 newborn with MPA exposure. There were 30 (49%) pregnancies with MPA exposure that resulted in spontaneous abortions. In October 2007 the FDA category label changed from C to D for MPA. The majority of recipients, 122 (79%) reported adequate kidney function at last follow-up. Only 10 recipients were on dialysis, 6 with reduced/poor function, 5 had died, and 13 were lost to follow-up.

Conclusions: Female kidney transplant recipients continue to report successful pregnancies. There is an increased incidence of spontaneous abortions and a pattern of birth defects reported with exposure to MPA during pregnancy. Pregnancies in female kidney transplant recipients are high-risk and appropriate counseling and close follow-up are warranted.

Funding: Pharmaceutical Company Support

Ex Vivo Study of Transplantable Transfer of Tacrolimus in Renal Transplant Recipients

Marion Jobard,1 Noël Zbahr,1 Simasotchis Christelle,1 Alexandra Benachi,1 Marie-Catherine Lott,1 Laetitia Croux,1 Christophe M. Legendre,2,3,4 Eric Thervet,5,6,8 Sophie Gil.1 1Pharmacy, University Paris Sud 11, Chatenay Malabry, France; 2Pharmacy, Beclere hospital, Clamart, France; 3Pharmacology, Pitié Salpetrière APHP, Paris, France; 4Renal Transplantation, Necker Hospital, Paris, France; 5Université Paris Descartes, Paris, France; 6Fondation CENTAURE, Paris, France; 7Obstetrical, Beclere Hospital, Clamart, France; 8Nephrology, Hospital European Georges Pompidou, Paris, France.

Background: Pregnancies in renal transplant recipients (RTR) are associated with an increased incidence of complications e.g. prematurity, intrauterine growth retardation and low birth weight. The role of foetal exposure to immunosuppressive drugs is possible but no information exists for quantitative transplacental transfer. The aim of the study was to evaluate the transplacental transfer of tacrolimus (TAC), using an ex vivo human cotyledon perfusion model.

Methods: Placentas from TAC pregnant RTR (TAC, n=4) and from untreated pregnant women with uncomplicated full-term pregnancies (control, n=6) were collected immediately after delivery. Cotyledons were perfused with TAC. Maternal perfusion was validated by quantification of antipyrine as internal control. Target TAC concentrations (80ng.mL-1) were tested to investigate possible modifications of TAC transport. Main transfer parameters including Fetal Transfer Rate (FTR, foetal to maternal concentrations ratio) and Clearance Index (CI, FTR ratio of tacrolimus vs. antipyrine) were assessed. Antipyrine and TAC concentrations were determined by HPLC and mass spectrometry respectively.

Results: In the TAC group, FTR of tacrolimus was 20.5±4.9% and the CI was 0.53±0.09. In the control group, these values were 19.6±4.8% and 0.62±0.09 respectively. Difference of transfer was not significant (p = 0.15).

Conclusions: TAC transfer through placenta exists, leading to foetal exposure. Transfer is similar in both groups but kinetics are different. By increasing patient numbers, We are studying the possible mechanisms for these differences. The demonstration of the transfer questions the role of foetal exposure in the related foetal complications.

Funding: Pharmaceutical Company Support
Effects of Rapamycin Conversion on Cellular Immune Profile and Alloreactivity in Renal Transplant Recipients

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Background: Rapamycin (Rapa) is known to expand regulatory T cells (Tregs), but its effects on the generation and expansion of different subpopulations of T helper cells are not fully elucidated.

Methods: We defined pre-OLT RD as at least one of the following: serum creatinine (SCR) ≥2 mg/dL at time of registration, dialysis requirement at time of registration, or dialysis requirement at time of transplantation. Patients were excluded if SCR was ≤1.5 at time of transplant. Primary outcome was both recovery of renal function (SCR ≥1.5 mg/dL) at time of discharge and patient survival 2-29 days.

Results: There were 1997 cases of pre-OLT RD. Renal recovery occurred in 1016 cases (51%). Stepwise logistic regression analysis identified the following factors to be independently associated with renal recovery: higher estimated glomerular filtration rate (eGFR) at registration (p=0.0001), presence of asciates at transplant (p=0.009), and use of thymoglobulin induction (p=0.049). The following risk factors were identified with persistent RD: liver graft dysfunction (p=0.00001), male sex (p=0.00001), higher body mass index (p=0.001), donor age (p=0.045), and tacrolimus use (p=0.039).

Conclusions: Among ESLD patients with pre-OLT RD, a greater eGFR at registration and/or transplant may indicate a greater renal reserve and be predictive of renal recovery. While tacrolimus use was associated with persistent renal dysfunction, thymoglobulin induction may be associated with renal recovery.

This work was supported in part by Dept. HHS contract 231-00-0115. The content is the responsibility of the authors and does not reflect the views or policies or imply endorsement by the Dept. HHS or the US Gov.

Funding: Other NIH Support - This work was supported in part by Dept. HHS contract 231-00-0115. The content is the responsibility of the authors and does not reflect the views or policies or imply endorsement by the Dept. HHS or the US Gov.

TH-PO997

Differeential Impacts of Calcineurin and Mammalian Target of Rapamycin Inhibition on Alloreactive T Helper Cells

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Background: Immunosuppressive drugs calcineurin inhibitor (tacrolimus,TAC) and mTOR inhibitor (sirolimus,SRL) affect naïve T cell differentiation and memory T cell expansion; however, their effects on the generation and expansion of different subpopulation of T helper cells are not fully elucidated.

Methods: Alloreactive CD4 T cells generated in a MLR were enriched and re-stimulated with autologous APCs with anti-CD3 plus TAC, SRL or the combination of TAC/SRL. At the end of culture, cells were restimulated and intracellular staining was used for detection of IFN-gamma, IL-17, and FOXP3 expression.

Results: Conversion to Rapa led to a sustained increase in CD4+25+Foxp3+ Tregs (Fig. A; p<0.001), but had no effect on the frequency of direct alloreactive T cells (Fig. B). Despite Treg expansion, there was a transient increase in indirect alloreactive at 12mo, followed by a decrease at 24mo, a pattern mirrored by the cytokines IL-1β, IL-6 and TNF-α (Fig. C & D; all p<0.05). In contrast, subjects maintained on Tac displayed decreased direct and indirect alloreactive responses throughout the study (p≤0.01). The direct and indirect alloresponses were similar between the two groups at 24mo.

Conclusions: These data suggest that, despite Treg expansion, conversion to rapamycin results in a transient inflammatory response that concurs with increased indirect alloreactivity. We speculate that Tregs may initially be functionally impaired by the inflammatory milieu, but eventually succeed in attenuating the alloimmune response.

TH-PO999

Cellular Infiltrates and NF-κB Subunit Signaling in Kidney Allografts of Patients with Clinical Operational Tolerance

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Background: While rapamycin (Rapa) is known to expand regulatory T cells (Tregs), it has also been associated with inflammatory side effects. The aim of this study was to simultaneously characterize the Treg and donor-alloreactive T cell frequency and the cellular inflammatory profile in a cohort of renal transplant recipients enrolled in a randomized trial comparing treatment with tacrolimus (Tac) to Rapamycin (Raps) at 12 months post transplant.

Methods: Blood was collected in 30 subjects (Rapa n=18; Tac n=12) at 0, 6, 12 and 24mo post randomization. T cell subset frequency was measured by flow cytometry, alloreactivity by the IFN-γ ELISPOT assay and cell culture supernatant cytokines by Luminex. Generalized estimated m ti were used for analysis.

Results: Conversion to Rapa led to a sustained increase in CD4+25+Foxp3+ Tregs (Fig. A; p<0.001), but had no effect on the frequency of direct alloreactive T cells (Fig. B). Despite Treg expansion, there was a transient increase in indirect alloreactive at 12mo, followed by a decrease at 24mo, a pattern mirrored by the cytokines IL-1β, IL-6 and TNF-α (Fig. C & D; all p<0.05). In contrast, subjects maintained on Tac displayed decreased direct and indirect alloreactive responses throughout the study (p≤0.01). The direct and indirect alloresponses were similar between the two groups at 24mo.

Conclusions: These data suggest that, despite Treg expansion, conversion to rapamycin results in a transient inflammatory response that concurs with increased indirect alloreactivity. We speculate that Tregs may initially be functionally impaired by the inflammatory milieu, but eventually succeed in attenuating the alloimmune response.
TH-PO997

DR<sup>4+</sup>CD45RA<sup>-</sup>Tregs Disappear Excessively in Patients with Acute Kidney Rejection, Causing a Reduction in the Suppressive Activity of the Total Treg Pool

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Background: Recent studies show that regulatory T cells (Tregs) play an essential role in tolerance induction after organ transplantation.

Methods: In order to examine whether there are differences in the composition of the total Treg cell population between stable transplant patients and patients with biopsy proven rejection (BPR), we compared the percentages and the functional activity of the different Treg cell subsets (DR<sup>4+</sup>CD45RA<sup>-</sup>Tregs, DR<sup>4+</sup>CD45RA<sup>+</sup>Tregs, DR<sup>4+</sup>CD45RA<sup>-</sup>Tregs, DR<sup>4+</sup>CD45RA<sup>-</sup>Tregs). All parameters were determined during the three different periods of time after transplantation (G2: 0-30 days, G3: 31-1000 days, G4: >1000 days).

Results: Among 157 transplant patients, 38 patients suffered from BPR. Sorting and subsequent differential testing of all four Treg cell subsets revealed that the DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs, and to a slightly lower degree, the DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs had the highest suppressive activity within the total Treg pool. The significantly reduced suppressive activity of the total Treg cell pool obtained from transplant patients with BPR correlated both with a significantly reduced HLA-DR mean fluorescence intensity (MFI) of the DR<sup>4+</sup>CD45RA<sup>-</sup>-Treg subset and a significantly reduced percentage of DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs within the total Treg pool. Therefore, it could be assumed that DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs potentially affect the suppressive activity of the total Treg pool and that the disappearance of this Treg subset gives a strong indication for acute rejection processes.

Conclusions: Therefore, both the monitoring of its percentage within the total Treg pool and the monitoring of the HLA-DR MFI of the DR<sup>4+</sup>CD45RA<sup>-</sup>-Treg subset may be useful predictions for graft rejection.

Funding: Clinical Revenue Support

TH-PO998

Contrasting Effects of Immunosuppression on FOXP3<sup>+</sup> Treg Biology in Liver and Kidney Allograft Recipients


Children's Hospital of Philadelphia and University of Pennsylvania; Toronto Hospital for Sick Children; Cincinnati Children's Hospital Medical Center.

Background: The effects of calcineurin inhibitor (CNI) therapy post-transplantation (Tx) on FOXP3<sup>+</sup>Tregs function are not well characterized.

Methods: We analyzed Treg numbers, FOXP3 methylation and suppressive function (SF) in 12 adults (8 liver, 4 kidney with serial sampling) and 46 children (38 liver, 8 kidney) on CNI or rapamycin (RPM)-based immunosuppression.

Results: In liver Tx recipients, CNI use led to decreased Treg numbers, viability and SF compared to use of RPM, but no significant differences were seen post-kidney Tx. In children with long-term grafts (8.5±0.6 y after Tx) and on CNI, Tregs of liver Tx recipients had more FOXP3 methylation (p<0.05) and 2-fold weaker SF but 23% more Tregs vs. children with kidney Tx. In mice, the liver can reportedly promote Tx tolerance, and subsequent differential testing of all four Treg subsets revealed that the DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs had the highest suppressive activity within the total Treg pool. The significantly reduced suppressive activity of the total Treg cell pool obtained from transplant patients with BPR correlated both with a significantly reduced HLA-DR mean fluorescence intensity (MFI) of the DR<sup>4+</sup>CD45RA<sup>-</sup>-Treg subset and a significantly reduced percentage of DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs within the total Treg pool. Therefore, it could be assumed that DR<sup>4+</sup>CD45RA<sup>-</sup>-Tregs potentially affect the suppressive activity of the total Treg pool and that the disappearance of this Treg subset gives a strong indication for acute rejection processes.

Conclusions: Therefore, both the monitoring of its percentage within the total Treg pool and the monitoring of the HLA-DR MFI of the DR<sup>4+</sup>CD45RA<sup>-</sup>-Treg subset may be useful predictions for graft rejection.

Funding: Clinical Revenue Support

TH-PO999

Allogeneic organ transplantation stimulated donor specific antibody expression in spite of anti-CD20 therapy

These recovering B cells produced high concentrations of donor-specific antibodies. Even when anti-CD20 antibody was given with a goal to block the T cell response, evidence of vascular antibody deposition (C4d deposition) and vasculitis was observed.

TH-PO1000

The Effects of Desensitization Treatment with IVIG and Rituximab on Blood Gene Expression Profiles by Microarrays

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Einstein/Montefiore Kidney Transplant Program; Computational Genomics Facility, Albert Einstein College of Medicine, Bronx, NY.

Background: We aimed to investigate the effects of intravenous immune globulin (IVIG) and rituximab desensitization treatment on kidney transplant rate and blood gene expression profiles by microarrays.

Methods: We enrolled patients with PRA levels >50% and on the deceased-donor waiting list for 5 years. Patients received IVIG (2.0 g/kg) on day 0 and 30; and rituximab (375 mg/m²) on day 15. The antibodies with mean fluorescence intensity (MFI) values > 5,000 were reported to UNET as unacceptable antigens. The gene expression profiles of blood samples collected in PAXGene were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: Of the 415 patients (10%) on the waiting list were eligible for desensitization treatment and 11 completed the treatment. While 15 of the remaining 29 patients (52%) received a transplant without therapy, only 2 of the 11 desensitized patients (18%) received transplant during a median follow-up of 217 days. While there were no statistically significant difference in demographics, desensitized patients had higher C4d percentage values (97% vs. 77%, p=0.0005) and more number of unacceptable antigens (39 vs. 10, p=0.0001). There was no significant change in the mean number of unacceptable antigens (39 ± 22 versus 39 ± 23) or reduction in the mean MFI values (11,333 ± 3,133 vs 11,289 ± 3,386).

Analysis of genes chosen as significantly differentially expressed revealed downregulation of genes involved in B cells and immune system (CD79a, B and T lymphocyte associated transcript, B cell scaffold protein, CD22, CXCR5, fas apoptotic inhibitory protein). Gene set enrichment analysis using Pathogenesis Based Transcripts created by Edmonton Group demonstrated significant downregulation of B cell associated (p=0.04) and immunoglobulin transcripts (p=0.03).

Conclusions: Although, desensitization with IVIG and rituximab decreases the expression of B cell and immunoglobulin associated transcripts, it was not successful in increasing kidney transplant rate or in decreasing the number of unacceptable antigens.

TH-PO1001

De Novo Donor Specific Antibodies among Non-Desensitized Renal Transplant Patients Predicts Antibody Mediated Rejection but Does Not Predict Renal Function at 1 Year

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Background: Pre and post-transplant DSA is associated with poor kidney allograft survival in desensitized patients. The significance of routine monitoring of post-transplant de novo DSA in non-desensitized patients is not well known. We prospectively evaluated
When De Novo DSA Appears after Reducing Immunosuppressants?

Junichi Hoshino,1,2, Hugo Kamene,2 Matthew J. Everly,2 Paul I. Terasaki,2,3
1University of California Los Angeles, school of Public Health, Los Angeles, CA; 2Terasaki Foundation Laboratory, Los Angeles, CA; 3One Lambda Inc., Los Angeles, CA.

Background: Weaning of immunosuppression (IS) is a common practice in transplant today. Based on the humoral theory, adequate HLA antibody monitoring is the most appropriate. However, the risk of de novo donor specific HLA antibody (DSA) after weaning is still unclear. Here we examine the duration of de novo DSA appearance after reducing IS.

Methods: Of the kidney transplantation patients (pts) from IKDRC-ITS, India under clonal deletion protocol, 72 pts, without pre-formed DSA, identical donor, nor splenectomy, were monitored to develop de novo DSA by LABScreen Mixed/single antigen beads (one Lambda Inc.) for every month or every outpatient service. All pts had stable allograft functions and no DSA at the time of IS weaning. Positive DSA was defined as MFI >1,000.

Results: Within 72 pts (mean observation period, 12.4±8.1 months), 35 pts experienced de novo DSA. The half of them had class I DSA. To determine if the degree of reduction was important, we further evaluated patients into three groups: group 1 – 18 out of 26 pts on prednisone (Pred) alone after weaning, group 2 – 14 out of 24 pts on Pred≥10mg after weaning and group 3– 3 out of 22 pts on Pred≥10mg of prednisone (Pred) alone after weaning, Group 2 – 10% developed AMR whereas none of the patients without DSA developed AMR (p=0.02). There was no significant difference in the incidence of ACR among patients with DSA (p=0.05). Among those who developed DSA, baseline median creatinine was 1.29 (0.61-2.7) and at 1 year was 1.2 (0.6-4.1) which was not significantly different.

Conclusions: From our preliminary data development of post-transplant de novo DSA may be associated with higher incidence of AMR in non-desensitized kidney allograft recipient. However it is difficult to recommend routine monitoring of DSA in non-desensitized transplant recipients to predict AMR based on this study. DSA do not predict renal allograft function at 1 year. However larger studies with longer duration of follow up are needed to further evaluate the role of DSA.

TH-PO1002

Pretransplant Cellular Allosensitization as a Predictor of Transplant Rejection

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Background: Cellular alloreactivity is prevalent in dialysis patients and when present may relate to transplant rejection. It may occur independently from humoral allo sensitization. Pre-transplant anti-donor alloreactivity has been shown to correlate with acute cellular rejection (ACR) but donor cells are often difficult to obtain. In this study, we evaluated recipient third party cellular alloreactivity as a surrogate for anti-donor cellular alloreactivity to predict ACR.

Methods: We prospectively studied 62 kidney transplant recipients in whom we tested peripheral blood mononuclear cells against donor when available (n=35), and a panel of third party allologic B cell stimulators (Panel of Reactive T cells or PRT assay) by IFNγ ELISPOT assays at time of transplantation. The results were then correlated with ACR episodes.

Results: Mean age was 49±13 yo, 42% female, 30.6% AA. We found a correlation between anti-third party and anti-donor cellular alloreactivity (R2=0.17, p=0.014). Percent reactivity to the panel expressed was higher in patients with ACR than in those with no ACR (53±8 vs 22±3% respectively, p=0.003). Significantly more patients with ACR were found to be PRT positive (defined as >40% reactivity to the panel) compared to those w/o ACR (78% vs 25%, p=0.002). PRT positivity (with either PRA results) predicted ACR in 39% of the recipients while PRA positivity only predicted it in 11% (p=0.008).

Conclusions: Delta DSA at 3M and C4d staining in post-reperfusion biopsy are novel risk factors for AMR in sensitized patients undergoing Luminex based preconditioning regimens.

Funding: NIDDK Support

TH-PO1004

A Novel Risk Factor for Antibody-Mediated Rejection in Luminex Based Desensitization Strategies


Background: We sought to determine novel risk factors for acute rejection in Luminex-based desensitization strategies.

Methods: We performed a prospective analysis of 116 consecutive patients with a negative third party crossmatch. All patients were desensitized based on pre-transplant immunodominant DSA (SAB-Luminex one-Lambda=n=47, 12, 6, 16 and 35 in protocols D1, D2, D3, D4 and D5).

Desensitization Protocols

<table>
<thead>
<tr>
<th>Protocol</th>
<th>Donor</th>
<th>IDSA</th>
<th>Induction</th>
<th>PE + IVIG</th>
<th>TAC + MP A</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>Live</td>
<td>100-500</td>
<td>Simul ect</td>
<td>Days (-3, -1, 3)</td>
<td>Day -7</td>
</tr>
<tr>
<td>D2</td>
<td>Live</td>
<td>501-1000</td>
<td>Simul ect</td>
<td>Days (-3, -1, 3)</td>
<td>Day -7</td>
</tr>
<tr>
<td>D3</td>
<td>Live</td>
<td>1001-3000</td>
<td>Simul ect</td>
<td>Days (-3, -5, -3, -1, 3)</td>
<td>Day -7</td>
</tr>
<tr>
<td>D4</td>
<td>Deceased</td>
<td>500-1000</td>
<td>Thymo (5-7 mg/kg)</td>
<td>Day (-1, 3)</td>
<td>Day -7</td>
</tr>
<tr>
<td>D5</td>
<td>Deceased</td>
<td>1001-3000</td>
<td>Thymo (5-7 mg/kg)</td>
<td>Day (-1, 3)</td>
<td>Day -7</td>
</tr>
</tbody>
</table>

Results: Mean peak PRA and DSA at transplant were 40% and 894 MFI respectively. There was a significant association between DSA, PRA and flow crossmatch (Fig 1)

Figure 1. There was a linear correlation between DSA, peak PRA and positive FXM

Figure 2. Acute rejection rate at 3 months post-transplant.

Conclusions: This study highlights de novo DSA appears rapidly after weaning in low dose IS condition. Frequent HLA monitoring could be used to allow for early recognition of humoral activation when scheduled weaning is initiated.

TH-PO1003

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

346A
None of the PRA or PRT panel reactions did predict acute humoral rejection. This result was independent of induction or immunosuppressive therapy.

Conclusions: Pretransplant third party cellular alloreactivity can serve as a surrogate of anti-donor cellular alloreactivity. PRA can complement the information obtained by PRT to predict ACR but not humoral rejection.

Funding: Other NIH Support - NAID

TH-PO1005

Characteristics of Immune Profile in Renal Transplant Recipients with Long Term Allograft Acceptance Byung Hwa Chung, Yu Ah Hong, Chul Woo Yang. Department of Internal Medicine, Seoul St. Mary's Hospital, The Catholic University of Korea, Seoul, Korea.

Background: We evaluated the immunologic profile in patients with stable allograft function during long term follow up compared to patients with deteriorated allograft function.

Methods: Twenty-four renal transplant recipients (RTx) who have showed stable allograft function for more than 10 years were included (Long term stable group (LS group)). We compared the immunologic characteristics of these patients with age and post-transplant duration matched chronic rejection group (CR group). Patients with biopsy-proven acute rejection (AR, n=9), healthy group (HC, n=21) and end stage renal disease patients on hemodialysis (HD, n=23) was included as control group in this analysis as well.

Results: In effector T cell subset, the percentage of TH1 cell showed significant increase in CR group compared to LS group (P < 0.05). The percentage of TH1 and TH2 cell did not differ significantly between LS and CR group (P > 0.05). In the chemokine receptor analysis, CCR4/CCR6+ T cell and CCR4/CCR6+ T cell was significantly increased in the AR group and the proportion of interleukin-17 producing in those cells was significantly increased in AR group as well (vs. all other groups, P < 0.05). The percentage of naïve T cell was increased in LS group compared with CR and AR group (P < 0.05, respectively) and the value of LS group was similar with that in HC group. In contrast, memory T cell (effector memory T cell (TEM) and central memory T cell (Tcm)) did not show significant differences between LS and CR group. In B cell subset, memory B cell was increased in LS group. Immature B cell did not differ significantly among LS, CR and AR group. The percentage of IL-10 producing immature B cell was increased in CR and AR group compared with LS group.

Conclusions: In long transplant recipient who showed long term acceptance with minimal immune suppressant, the decrease of TH17 response was significantly dominant compared with patients with chronic rejection.

TH-PO1006

High Serum Soluble CD26 Levels in Acute Cellular Rejection in Recipients with Renal Transplant Sanjay Gupta,1 Ankit Saxena,2 Dipendra Kumar Mitra,2 Amit K. Dinda,3 Sandeep Gulera,3 Nephrology, All India Institute of Medical Sciences, India; 2Immunogenetics, AIIMS, India; 3Surgery, AIIMS, India.

Background: Cell mediated allograft rejection is one of the major causes of early renal graft dysfunction. Information regarding the presence of markers indicating activation of immune cells can help in early monitoring graft status. The T-cell activation marker, CD26 is an important dipeptidly peptidases which is known to activate some of the important chemokine ligands and immunocompetence are associated with the enzymatic activity. Also CD26 is an important dipeptidly peptides which is known to activate some of the important chemokine ligands (RANTES and CXCL10) that are involved in the recruitment of T cells in the rejected graft. Aim was to study any association of serum soluble CD26 levels with early clinical events of allograft viz acute cellular rejection (ACR), calcineurin inhibitor toxicity (CNI).

Methods: The serum sample was collected from the 44 renal transplant recipients: renal allograft dysfunction for the first time within first year n=22 and recipients with well functioning grafts (WF) (n=22). Patients were on Tacrolimus (Tac), Mycophenolate Mofetil and Steroids. None had induction with antibodies. Allograft biopsies performed for all the graft dysfunction cases showed ACR n=11 (Banff Grade I-A-2, IV-B-4, II-1, III-4) and CNI n=11. The serum soluble CD26 (sCD26) levels was analyzed by ELISA.

Results: Mean duration post transplant in ACR, CNI and WF was 3.0±2.5, 4.8±3.4, 5.6±0.4 months respectively and Tacrolimus levels in respective groups were 7.8±1.7, 8.3±3.7 µg/L. Significantly high serum sCD26 levels were observed among patients with ACR (1.92±0.9 µg/ml) in comparison to the non-immunological graft dysfunction cases CNI toxicity (1.08±0.4 µg/ml (p=0.008) and WF (1.05±0.3 µg/ml)(p=0.001). Tac levels did not correlate with sCD26 levels. All the 7 patients with sCD26 levels above 1.6µg/ml had ACR.

Conclusions: The high serum sCD26 levels are associated only with ACR suggesting T cell activation and not with CNI toxicity and well functioning graft. The sCD26 seems to be a promising biomarker to assess adequate immunosuppression in the early phase after kidney transplantation.

Funding: Government Support - Non-U.S. -

TH-PO1007

Polyclonal Immunoglobulin Free Light Chains Provide a Novel insight into Immunosuppressant Use in Renal Transplant Recipients Shazia Shabir,1 Anne Bevin,2 Paul Cockwell,1 Richard Borrows,1 Colin A. Hutchison.1 Renal Unit, University Hospital Birmingham, United Kingdom; 2The Binding Site Group Ltd, Birmingham, United Kingdom.

Background: Polyclonal free light (FLC) levels in patients with chronic rejection. The rapid clearance of FLCs (2-6h) compared to immunoglobulins (Ig) (5-21 days) highlights a potential for FLCs to provide real-time monitoring of immune activity and dosing anti-proliferative medication. We investigated whether FLC levels were affected by routine immunosuppressants used in renal transplant recipients.

Methods: Serum samples were studied in two renal transplant populations: a cross-sectional prevalent cohort (n=399) and an incident cohort (n=40) with serial samples. Creatinine, polyclonal FLCs (~κ, ~λ) and cystatin C were measured. FLC production rates were calculated. Results were compared to a non-transplanted CKD cohort (n=872).

Results: In the cross-sectional transplant cohort, total FLCs correlated with renal function, including eGFR (R=0.537), cystatin C (R=0.594) and creatinine (R=0.531), all p<0.001. Total FLCs, total FLC level(47.4mg/L, range 11.6-204.8) was lower than the CKD cohort, p=0.001 (62.5mg/L, 19.2-353.5), but stayed above the normal range (p<0.001). Patients receiving anti-proliferative therapy had lower total FLC (46.6mg/L, 11.6-262.0) than those not, p<0.001 (70.2mg/L, 23.2-451.0). Patients receiving Tacrolimus had lower FLC (45.0mg/L, 11.6-366.0) than those on Cyclosporin, (53.0mg/L, 12.3-431.0) p=0.002. There was no difference in FLCs between patients receiving prednisone or not (p=0.186). The intra-patient analysis showed a sharp decline in total FLC in 32/40 patients within 14 days of transplantation. FLC production was decreased at 2 weeks post transplant (p=0.001), total FLC production increased over the 12-month period (p=0.001). This was also true for IgG (2wk p=0.001, 12mth p=0.001) and IgA (2wk p=0.034, 12mth p=0.018). 26/32 patients had a gradual rise in FLC production between 2weeks and 12months as immunosuppression was reduced (p=0.001).

Conclusions: Polyclonal FLC levels vary with different immunosuppressant regimes and doses within the renal transplant population. Potentially FLC measurement could monitor immunosuppression in these patients.

TH-PO1008

In-Vitro Immunomodulatory Effect of Qu Mai (Dianthus Superbus) on Human Alloreactive T Cells Jessica A. Reid-Adam,1 Nan Yang,2 Ying Song,2 Peter S. Heeger,3 Xiumin Li.1 1Pediatric Nephrology, Mount Sinai School of Medicine, NY, NY; 2Pediatric Allergy & Immunology, MSSM, NY, NY; 3Nephrology, MSSM, NY, NY.

Background: Immunosuppression for transplantation and autoimmunity is suboptimal, supporting the need for discovery of novel agents. We have been evaluating immune effects of traditional Chinese herbs.

Methods: We initially tested the effects of ~50 herbs on human alloreactive T cell cytokine profiles using ELISPOT-based mixed lymphocyte cultures. This screening approach identified 4 candidate preparations that increased the IL-10/IFNγ ratio. Among these was Qu Mai (QM, Dianthus Superbus), an herb used to treat urinary tract disorders.

Results: We generated 3 fractions of QM with HPLC fingerprints and performed dose response curves for each fraction to assess effects on cytokine production in MLRs using PBMC from 12 normal volunteers. Flow cytometry was used to identify which cell types within the PBMC were altered by the therapy.

Results: Analyses showed that 1 of the fractions (QM-AD, which contains flavonoid-rich compounds) exhibited a dose dependent inhibitory effect on alloinduced IFNγ (median decrease of 72% at 200 µg/ml) while enhancing IL-10 production 40 fold (p<0.05 vs. controls for each) and resulting in a marked change in IL-10/IFNγ ratios (0.2:1 in untreated MLR to 17:1 at 200 µg/ml, p<0.05). No effect on T cell apoptosis was observed. Flow cytometry and intracellular cytokine staining showed that in CD4 cells (including memory CD4 cells) QM-AD directly prevented production of IFNγ (mean 2.16% vs. 1.07% in QM-AD treated MLR), increased production of IL-10 (median 3.98% vs. 6.17% in QM-AD treated MLR), and induced CD4 CD25 Fasp3 Treg cells (mean 2.77% vs. 3.36% in QM-AD treated MLR).

Conclusions: Our data demonstrate that Chinese herbs, specifically QM-AD, contain compounds that favorably alter naive and memory alloreactive T cell immunity toward an immunosuppressive/Treg phenotype in vitro. These novel findings support the continued isolation and characterization of the QM-derived flavonoid compounds and testing them in in-vivo models as primary or adjunct treatments of pathogenic immune responses, including autoimmunity and transplant rejection.

Funding: Other NIH Support - NCCAM - P01 AT002647-01A1, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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PARVG Gene Polymorphism May Be Associated with Operative Renal Allograft Tolerance. Eric Theret,1,2,3,4,5 Richard Danger,1,4 Marie-Lise Grisoni,1 Pierre Laurent-Puig,6 Annaïc Pallier,6 Delphine Le Corre,7 Christophe M. Legendre,1,4,5 Sophie Brouard.3,6 Renal Transplantation, Hôpital Necker, Paris, France; Nephrology, Hôpital européen Georges Pompidou, Paris, France; Université Paris Descartes, Paris, France; Fondation Centeaure, Paris, France; ITERT, CHU Hotel Dieu, Nantes, France; INSERM, INSERM UMR S937, Paris, France. Background: Drug-free operationally tolerant kidney recipients (TOL), with long term stable graft function and low-grade proteinuria in an immunosuppression-free environment, were characterized by a specific set of 55 genes with differential blood transcriptional expression compared to contrasted clinical situations and healthy volunteers. The aim of this study was to investigate whether these expressions could be influenced by genetic polymorphisms located in the corresponding genomic sequences and whether some of these single nucleotide polymorphisms (SNPs) could be associated with clinical status of kidney transplanted patients. Methods: 1152 candidate tag SNPs spanning the 55 genes were genotyped using a Golden Gate Illumina assay in a sample of 163 kidney transplant patients consisted in 11 TOL patients, 36 patients with antibody mediated chronic rejection defined by the last Banff classification (CR) and 116 patients with a stable graft function while under immunosuppressive treatment (STIA). We then analyzed gene expression and clinical status according to the different SNPs. Results: Among the genes demonstrating strong expression difference between TOL compared to CR & STIA patients, PARVG, which is a member of a family of actin-binding proteins associated with focal contacts, stands out with two SNPs, (rs139144 and rs7664592) explaining about 15% of the gene expression variability. Linkage disequilibrium analysis of these two showed the rs139144-GG genotype was associated with decreased PARVG expression and tended to be more frequent in TOL (60%) than in CR & STIA (28%) patients (p = 0.068). Conclusions: These preliminary results that should be confirmed in a larger population open new perspective of regulation pathways and hypothesis in operational tolerance mechanism.

Interventions in Purine Metabolism toward Immunosuppression. Oshri Naaman,1 Yair Cohen,2 Moshe Zlotnik,3 Amos Douvdevani.1,12 Department of Nephrology, Soroka Medical Center, Beer-Sheva, Israel; 2Department of Clinical Biochemistry, Ben-Gurion University of the Negev, Beer-Sheva, Israel. Background: Purine metabolites have potent immunoregulatory roles. ATp, mainly by its P2X7-receptor promotes inflammation and increases lymphocyte proliferation. ATP is degraded to adenosine which can also support inflammation by its Gi-coupled A1 receptor (A1R) or suppress inflammation and cellular immunity by its Gs-coupled A2A receptor (A2AR). We reported that activation (preconditioning) of the A2AR upregulates the anti-inflammatory A1R. Our aim is to modulate the purineergic network toward immunosuppression by upregulation of the anti-inflammatory A1R, conversion of ATP to adenosine and maintaining high adenosine levels. In addition we apply an innovative antiinflammatory purine derivative to attenuate T cell activation. Methods: Preconditioning was induced by injection of A2AR agonist (CCPA) at 24 h and 12 h before splenectomy. Splenocytes from preconditioned or control animals were stimulated with Con A, anti-CD3 or cultured in a mixed lymphocyte reaction in the presence of apyrase (ATPase), adenosine deaminase (ADA), ADA inhibitor (EHNA), inosine-monophosphate (IMP) alone or combined. T-cell activation was assessed by cell proliferation and interferon-gamma (IFN-gamma) secretion. Results: A decrease in proliferation and IFN-gamma secretion of lymphocytes from preconditioned animals was observed. ATP dephosphorylation by apyrase reduced spleenocytes proliferation (49%). Reduction of adenosine levels by ADA increased T-cell activation while elevation of adenosine by EHNA caused the opposite effect. Furthermore, we showed that IMP reduces T-cell activation in a dose dependent manner, and when combined with EHNA, T-cell proliferation was eliminated (95%) as effectively as with mycophenolic acid (CellCept®). Conclusions: Elevation of A1R, ATP dephosphorylation and adenosine elevation effectively deaccelerate T-cell activation. In addition, for the first time IMP was shown to function as an independent immunosuppressant agent. We believe that deeper understanding of the immuno-modulatory mechanisms in which purine metabolites participate, can provide a basis for further therapeutic developments. Funding: Private Foundation Support.

Targeted Inhibition of Renal Rho Kinase: A Novel Approach to Reduce Macrophage Infiltration and Lymphangiogenesis in Acute Renal Allograft Rejection. Fariba Poosii,1 Saleh Yazdani,2 Maria Emma Dolman,3 Robbert J. Kok,4 Jai Prakash,5 Jacob Van den Born,2 Jan-Luuk Hillebrands,1 Harry Van Goor,6 Martin H. De Borst.7 1Pathology & Medical Biology, UMCG, Groningen, Netherlands; 2Nephrology, UMCG, Groningen, Netherlands; 3Pharmacokinetics, UMCG, Groningen, Netherlands; 4Pharmaceutics, Utrecht University, Netherlands. Background: Renal allograft rejection is associated with lymphangiogenesis, a process at least in part driven by inflammatory cell infiltration. Rho is activated early in lymphangiogenesis and also plays a role in renal inflammation. We therefore investigated whether tubular cell-specific Rho kinase inhibition reduces lymphangiogenesis and macrophage infiltration in a rat model of transplant rejection. Methods: The renal kinase inhibitor Y27632 was chemically bound to lysozyme (LZM), allowing selective uptake of the compound (Y27632-LZM) by proximal tubular cells upon its systemic administration. Renal allografting (Fisher→Lewis, n=12 per timepoint) was performed. The contralateral kidney was left in situ. Rats were not treated with immunosuppressive drugs to induce acute rejection. Animals were treated daily with Y27632-LZM 10 mg/kg equivalent to 278 µg/kg of free Y27632) or placebo until sacrifice at 1 or 4 days post-transplantation. Kidney sections were examined for macrophage influx (ED1) and lymphangiogenesis (Podoplanin). Conclusions: Y27632-LZM strongly reduced interstitial macrophage accumulation at day 1 (placebo 13.3±2.7; Y27632-LZM 10.8±2.1 macrophages/tubulo-interstitial field, p<0.05) and day 4 (17.7±6.21 vs 10.1±4.70, p<0.05) after allograft transplantation. Similarly, Y27632-LZM reduced the numbers of lymph vessels at both day 1 (2.60.1 vs 2.0±0.2 lymph vessels/tubulo-interstitial field, p<0.05) and day 4 (4.2±0.4 vs 3.1±0.2, p<0.05) in allografts. Tubulo-interstitial macrophage and lymph vessel numbers were strongly correlated (r=0.476, p=0.001). Y27632-LZM did not affect blood pressure, suggesting local delivery of the compound. Conclusions: Tubular cell-specific Rho kinase inhibition decreased renal lymph vessel numbers which may be secondary to reduced macrophage influx. Renal Rho kinase inhibition may be a valuable approach to treat allograft rejection.

Effect of Haemodialysis Membrane Exposure on Anti-HLA Antibody Formation in Incident Dialysis Patients Awaiting Renal Transplantation. Claire Kennedy, Frank J. O Brien, Colm Magee, Peter J. Conlon. Department of Nephrology, Beaumont Hospital, Dublin, Ireland. Background: Pre-emptive renal transplant has superior outcomes to standard transplantation, particularly in terms of acute rejection rates. This suggests an immunological advantage for those patients who do not receive dialysis prior to transplantation. In patients awaiting heart transplantation, left ventricular assist device have been associated with increased anti-HLA-antibody formation. New exposure to a foreign haemodialysis membrane has not been compared to peritoneal dialysis (self) or no dialysis in terms of anti-HLA antibody formation. We assessed whether starting haemodialysis was associated with a subsequent increase in anti-HLA antibodies as opposed to starting peritoneal dialysis or not receiving any renal replacement therapy. Methods: A retrospective cohort study of all patients who had been listed for a pre-emptive first renal transplant in Ireland was performed. Anti-HLA antibody formation was measured over time in patients who subsequently started haemodialysis, to those that started peritoneal dialysis and to those that started neither modality. Sensitizing events (pregnancies and blood transfusions) were also recorded. We defined a significant increase in HLA antibodies as any increase in calculated PRA (determined by Luminex method) of 10% or more in 161 patients who were included. Of these, 31 (19%) subsequently started haemodialysis (with a mean time on haemodialysis of 23 months), 18 (11%) started peritoneal dialysis (with a mean time of 14 months) and 112 (70%) started neither (with a mean follow-up time of 20 months). Baseline cPRA’s were 19% and 24% respectively. There were no significant differences in age, gender, comorbidities, number of pregnancies or blood transfusions between the 3 groups. Rates of new anti-HLA antibody formation over time remained similar in all three groups: 22% of those who started haemodialysis, 16% of those who started peritoneal dialysis and 24% who started neither had a significant rise in cPRA. Conclusions: Starting maintenance haemodialysis (and exposure to haemodialysis membranes) is not associated with an increase in production of anti-HLA antibodies.

Increased Interleukin-17 Producing Effector T Cells during Early Post-Transplant Period. Byungha Chung. In O Sun, Hyun Gyoung Kim, Yu Ah Hong, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. Division of Nephrology, Department of Internal Medicine, Seoul St. Mary’s Hospital, The Catholic University of Korea, Seoul, Korea. Background: The change of precise immune profile during early post-transplant period has not been fully investigated. In present study, we investigated the response of immune cells during early post-transplant period in renal transplant recipients. Methods: Twenty-nine living donor renal transplant recipients were enrolled in this study. We used triple immune suppressant composed of tacrolimus, mycophenolate mofetil and steroid and performed induction therapy by basiliximab in those patients.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only. Underline represents presenting author.
We prospectively investigated the immune cell profile before and at 1 month after transplantation. **Results:** The total lymphocyte counts did not differ significantly before and after transplantation (P > 0.05). The percentage of Th1 cell (P > 0.05) and Th2 cell (P > 0.05) in CD4+ T cell significantly decreased after transplantation compared to before transplantation. In contrast, the percentage of Th17 cell did not reduce after KT compared to before KT (P > 0.05). The percentage of naïve T cell (Tem) and central memory T cell did not alter after KT (P > 0.05, respectively). In contrast, the percentage of effector memory T cell (Tem) significantly decreased after KT compared to before KT (P < 0.05). However, the proportion of TEM cell (P < 0.05) and central memory Tc cell (P < 0.05) increased after transplantation. In CD8+ T cell, the percentage of Tc decreases significantly as like in CD4+ T cell (P < 0.05), and the proportion of IL-17 producing T cell in Tem showed increased pattern, even though it did not reach statistical significance. In B cell subset, all of memory B cell mature B cell, immature B cell in CD19+ B cell did not show significant change after transplantation (P > 0.05, respectively).

**Conclusions:** In contrast with another helper T cell subset, Th17 cell response did not decrease and rather IL-17 producing TEM cell increased after transplantation. It suggest that current immune suppressant is not enough to suppress allo-immune responses by Th17 cell during early transplant period.

**TH-PO1014**

Spleen Tyrosine Kinase Activation in Human and Experimental Acute Renal Allograft Rejection

Hyeonseok Hwang, Byung Ha Chung,1 Nephrology, Monash Medical Centre, Clayton, Victoria, Australia; 2Medicine, Monash University, Clayton, Victoria, Australia.

**Background:** Syk is an adapter molecule involved in B cell receptor and Fcy-receptor signalling. Syk has also been implicated in neutrophil recruitment and platelet activation. These data suggest that Syk might play an important role in acute allograft rejection. To investigate this we examined Syk activation (phosphorylation of Tyr525/526 in the Syk activation loop) in human renal allograft rejection.

**Methods:** Three cases each of biopsy proven acute antibody-mediated rejection (AMR) and cell mediated rejection (CMR) of human renal allografts were analysed for p-Syk by immunostaining. A group of 3 Sprague-Dawley rats was wisterized with splast cell cells and three weeks later received an orthotopic Wistar renal allograft (one native kidney remained).Recipient rats were killed 7 days later.

**Results:** Normal human kidney shows no p-Syk immunostaining. In human AMR, p-Syk+ cells were prominent within glomerular capillary loops and in some interstitial areas. In CMR, p-Syk+ cells were seen in the interstitium, with only small numbers of p-Syk+ cells in glomeruli. Most p-Syk+ cells appeared to be infiltrating leucocytes. In the rat model, all allografts showed severe renal arterial occlusion, thrombosis and areas of infarction with severe tubular necrosis. Other areas showed severe glomerulopathy, peritubular capillaritis and tubulitis. Rat allografts also exhibited rat IgG and C3 deposition, indicating elements of both AMR and CMR. Many infiltrating cells were stained for p-Syk. Double immunostaining identified Syk activation in both neutrophils and macrophages. Furthermore, phospho-p38 staining (a downstream Syk target) was evident in infiltrating leucocytes in a pattern similar to that of p-Syk, possibly indicating Syk-dependent leucocyte activation.

**Conclusions:** Prominent activation of Syk signalling is evident in infiltrating leucocytes in human and experimental allograft rejection. These findings suggest that Syk is a potential therapeutic target in acute renal allograft rejection.

**Funding:** Government Support - Non-U.S.

**TH-PO1015**

Comparison of Antibody Monitoring System with Single Antigen Luminex Assay in Renal Transplant Recipient Hyoung Hyun Byung, Hyun Bong Chung, Bumsoon Choi, Yong-Soo Kim, Suk Young Kim, Chul Woo Yang. Division of Nephrology, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Seoul, Korea.

**Background:** The antibody monitoring system (AMS) is a recently developed ELISA crossmatch assay to detect donor-specific anti-HLA IgG antibodies (DSA). This study was performed to compare the AMS with DSA detected by single-antigen Luminex panel reactive antibody assay in renal transplant recipients.

**Methods:** One hundred and one sera were screened from 71 patients on the waiting list for kidney transplantation for the presence of DSA. When anti-HLA Ab was detected by Luminex assay and the matched donor had the corresponding mismatched HLA antigen, it was considered to indicate DSA. The results of AMS and Luminex were compared.

**Results:** Twenty-nine (28.7%) sera were positive for DSA detected by Luminex assay. The DSA was directed against HLA class I Ag in 12 (11.9%) sera, against HLA class II Ag in 17 (16.8%) sera, and against both class I and class II Ag in 6 (5.9%) sera. AMS assay showed that the number of compatible sera with DSA was 79 (78.2%) and it was a similar concordance rate compared to Luminex (97.99%, p < 0.0001). The sensitivity of the AMS assay for detection of DSA was 37.9%; the specificity was 97.2%; the positive predictive value was 84.6%, and the negative predictive value was 79.5%. Compared to complement dependent cytotoxic crossmatch test (CDC), AMS showed higher concordance rate than Luminex assay (k = 0.541 for AMS vs. k = 0.458 for Luminex). For flowcytometric crossmatch test, the concordance rate was similar between AMS and Luminex assay (k = 0.432 for AMS vs. k = 0.436 for Luminex). The estimated glomerular filtration rate at 12 months after transplantation was significantly lower in positive AMS patients than in negative AMS patients, respectively (46.8 ± 4.1 vs. 60.7 ± 25.4, p = 0.009). However, the proportion of Th17 cells did not reduce after KT compared to before KT (P > 0.05). The percentage of naïve T cell (Tem) and central memory T cell did not alter after KT (P > 0.05, respectively). In contrast, the percentage of effector memory T cell (Tem) significantly decreased after KT compared to before KT (P < 0.05). However, the proportion of TEM cell increased after transplantation. It suggest that current immune suppressant is not enough to suppress allo-immune responses by Th17 cell during early transplant period.

**Conclusions:** In contrast with another helper T cell subset, Th17 cell response did not decrease and rather IL-17 producing TEM cell increased after transplantation. It suggest that current immune suppressant is not enough to suppress allo-immune responses by Th17 cell during early transplant period.

**Funding:** Private Foundation Support

**TH-PO1016**

UGT2B7 -900 C>G Predicts Leucopenia in Pediatric Kidney Transplant Recipients

David K. Hooper,1 Barry L. Lawershaw,2 Tsuyoshi Fukuda,1 Cassie L. Kirby,1 Hiren P. Patel,1 Deepa H. Chang,1 Gina-Marie Barletta,1 Scott K. Van Why,1 Rene’ G. VanDeVoerde,1 Donald J. Weaver,1 Lisa Martin,1 Alexander A. Vinks,1 Jens W. Goebel,1 ‘Cincinnati Children’s Hospital; ‘Emory University; ‘Nationwide Children’s Hospital; ‘Akron Children’s Hospital; ‘Phoenix Children’s Hospital; ‘Medical College of Wisconsin; ‘Levine Children’s Hospital.

**Background:** Mycoplalonate Mofetil (MMF) causes leucopenia in a substantial proportion of kidney transplanted (KT) recipients, prompting empiric dose reduction which can increase rejection risk. Single nucleotide polymorphisms (SNPs) in genes encoding uridinediphosphate glucuronoyltransferases (UGTs) and multi-drug resistance protein (MDR) have been associated with altered exposure to MMF.

**Methods:** A case-control gene association study was performed to determine whether UGTs and MDR1 SNPs would predict MMF-related leucopenia in pediatric KT pts. Pts were identified retrospectively and matched by race, center, induction therapy, steroid duration and age. Pts experiencing MMF-related leucopenia prompting dose reduction within the first year were considered active cases, whereas controls received full-dose MMF for 1 year following KT without leucopenia. Pts with lupus, liver disease, acute nephritic syndrome, aminosaluentza membrane, and non-adherence were excluded. A paired t-test assuming an additive model was used to compare frequency of alleles between cases and controls.

**Results:** 54 of 225 (24%) pts qualified as cases, and 59 (26%) qualified as controls. 66 (29%) pts had transient leucopenia not requiring MMF dose change, 30 pts had other MMF-related side effects and 16 pts had leucopenia from other causes (e.g. cytomegalovirus). We enrolled 29 matched pairs for genetic analysis. The odds of UGT2B7 -900 G allele in cases was 2.4 times greater than in controls (p=0.03). SNPs at MDR1 3435T, UGT1A9 2152, and UGT1A9 -440 were not significantly associated with leucopenia (p=0.72, 0.16, 0.16 respectively).

**Conclusions:** MMF-related leucopenia prompting dose reduction was seen in 24% of pts. UGT2B7 -900 C>G mutant was associated with an increased risk of leucopenia. Larger studies are needed to confirm this result.

**Funding:** Other NIH Support - The Center for Environmental Genetics, University of Cincinnati (Sub Recipient; NIH/NIEHS), Centers for Education and Research in Therapeutics (Sub Recipient, CCHMC, AHRQ), Private Foundation Support

**TH-PO1017**

Racial Influence on ABCB1 Gene Expression in Peripheral Blood Mononuclear Cells in Stable Renal Transplant Recipients

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**Background:** Immunosuppressive therapy (IT) such as tacrolimus are influenced by p-glycoprotein (P-gp) which modulates cellular efflux of this drug. P-gp is present on peripheral mononuclear cells (PBMC) and is encoded by the ABCB1 gene. No data are available regarding the impact of race on ABCB1 gene expression in PBMCs post-transplant over IT dosing interval.

**Methods:** An observational study was completed in 20 African American (AA) and 11 Caucasian (C) stable renal transplant recipients (RTR) (ages 30-74 yrs) receiving tacrolimus (trough: 5 - 10 ng /ml), and enteric coated mycophenolate sodium. At time 0 (prior to IT) & 4, 8 and 12 hours after immunosuppression, PBMCs were collected for ABCB1 gene expression analysis by quantitative real-time-polymerase chain reaction (QRT-PCR). The target ABCB1 gene PCR product was cloned, and verified by sequencing. The cloned ABCB1 gene was used to establish standard curves (linear[K]) over 8 orders of magnitude; r2 >0.996 and assess PCR efficiencies. Total ABCB1 copies and normalized copies using Alien RNA were assessed.

**Results:** The normalized (p<0.0006) and non-normalized (p< 0.0001) ABCB1 gene expression was higher among Caucasians and at each time up to 12 hours. ABCB1 Gene Expression in PBMC

**Funding:** Other NIH Support - ARRA funded R21 grant: NIH-R21 DK077325-01A.
B Cell Depletion Synergizes with ECASI-Fixed Rat Splenocyte Infusions To Induce Concomitant Rat to Mouse Islet Xenotransplantation Tolerance Shusen Wang, Tabi Kheradmand, James Tasch, Jie Yang, Xun-Rong Luo.

Methods: ECASI-Infused B cells were co-cultured with recombinant adenovirus (Ad)-transfected donor splenocytes (SP) and treated with in vitro tolerization. The coculture was reinfused into irradiated recipients. Results: 1. ECASI-infused B cells do not induce antigen-specific immune responses and are not cytotoxic to donor SP. 2. ECASI-infused B cells and donor SP co-culture results in an in vitro expanded regulatory T cell population. 3. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro proliferation of donor SP. 4. ECASI-infused B cells and donor SP co-culture results in an increase in the in vitro apoptosis of donor SP. 5. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro cytokine production of donor SP. 6. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro chemokine production of donor SP. 7. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro adhesion molecule expression of donor SP. 8. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro adhesion molecule expression of recipient SP. 9. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro adhesion molecule expression of recipient SP. 10. ECASI-infused B cells and donor SP co-culture results in a decrease in the in vitro adhesion molecule expression of recipient SP.

Conclusion: ECASI-infused B cells synergize with ECASI-fixed donor splenocytes to promote islet xenotransplantation tolerance.

Clinical Revenue Support

Funding: Clinical Revenue Support

TH-POI020

Further Elucidation of Mechanisms Mediating Reduced Autoregulatory Tone in Diabetes

Type 2 diabetes is characterized by the presence of hyperfiltration and increased renal sodium and water reabsorption, which are mediated by an increased renal production of renin and angiotensin II (AngII). These adaptations are thought to contribute to the development of diabetic nephropathy and cardiovascular disease. The role of autoregulatory mechanisms in the regulation of renal hemodynamics in diabetes remains unclear.

Methods: We measured renal hemodynamics and autoregulation in Zucker fatty (ZFA) and Zucker diabetic fatty (ZDF) rats, which are models of type 2 diabetes and nondiabetic obesity, respectively. We measured renal hemodynamics using in vivo tonometry and examined autoregulation using a constant pressure perfusion model. We also measured the expression of autoregulatory proteins, such as RAS-related C3 botulinum toxin substrate 1 (Ras-related C3 botulinum toxin substrate 1, or RAS1B), in renal tissues.

Results: We found that renal autoregulation was impaired in diabetic ZDF rats, with a decrease in the renal perfusion pressure at which autoregulation was maximal (40 ± 5 mmHg vs. 100 ± 10 mmHg in ZFA rats, p < 0.05). This impairment was associated with a decrease in the expression of RAS1B in renal tissues (50 ± 15% vs. 100 ± 10% in ZFA rats, p < 0.05). We also found that the administration of an RAS1B inhibitor improved renal autoregulation in diabetic ZDF rats (80 ± 10% vs. 100 ± 10% in ZFA rats, p < 0.05).

Conclusion: Impaired renal autoregulation in diabetes is associated with decreased expression of RAS1B, and the administration of an RAS1B inhibitor improves renal autoregulation.

Clinical Revenue Support

Funding: Clinical Revenue Support

TH-POI018

The Impact of Gender on Arterial Stiffness and the Renin Angiotensin System in Healthy Humans

Background: Arterial stiffness is an important predictor of cardiovascular disease (CVD) and is influenced by gender. However, the mechanisms underlying gender differences in arterial stiffness and the renin-angiotensin-aldosterone system (RAAS) remain unclear.

Methods: We measured arterial stiffness using pulse wave velocity (PWV) and arterial compliance using transcutaneous impedance (TIC) in a population of healthy volunteers (n = 250, age 18-70 years) who were stratified by gender, age, and body mass index (BMI). We also measured plasma levels of renin, angiotensin II, aldosterone, and angiotensinogen.

Results: Women had higher PWV and lower arterial compliance than men, with significant differences observed in both young (age < 40 years) and older (age ≥ 40 years) subgroups. Women also had lower levels of circulating angiotensin II and aldosterone, with no significant differences in plasma renin activity (PRA) between genders.

Conclusion: Gender differences in arterial stiffness and the RAAS are evident in healthy adults and may be important for the development of CVD.

Clinical Revenue Support

Funding: Clinical Revenue Support

TH-POI019

Tolerance and Efficacy of Intravenous Bacle Calmette Guerin (BCG) Treatment in Non Muscle-Invasive Bladder Cancer after Renal Transplantation

Background: BCG is an effective therapy for non-muscle invasive bladder cancer (NMIBC), but its use is limited by the need for repeated intravesical instillations and the risk of severe side effects. We evaluated the efficacy and safety of a single-dose intravenous BCG (ECASI) in patients with NMIBC.

Methods: We conducted a single-center, open-label, randomized controlled trial of 60 patients with NMIBC who had undergone renal transplantation. Patients were randomly assigned to receive either ECASI (n = 30) or standard intravesical BCG (n = 30) as maintenance therapy. The primary endpoint was disease-free survival at 12 months.

Results: At 12 months, the disease-free survival rate was 83% in the ECASI group and 73% in the BCG group (p = 0.22). There were no significant differences in side effects or quality of life measures between the two groups.

Conclusion: ECASI is an effective and well-tolerated treatment for NMIBC in patients with renal transplantation.

Clinical Revenue Support

Funding: Clinical Revenue Support

TH-POI022

Protective Effect of AT1-Blockade on the Oxidative Stress and the Proinflammatory Response Produced in the Kidney of Normal Rats Fed with a High Salt Diet

Background: Oxidative stress and inflammation play a crucial role in the development of hypertension. AT1-receptor blockade has been shown to reduce oxidative stress and inflammation in experimental models of hypertension.

Methods: We fed 6-week-old male rats with a high salt diet (2% NaCl) for 12 weeks. Blood pressure, oxidative stress markers, and proinflammatory cytokines were measured.

Results: Rats fed a high salt diet had significantly higher blood pressure, increased levels of oxidative stress markers, and increased proinflammatory cytokines compared to control rats.

Conclusion: AT1-receptor blockade reduces oxidative stress and inflammation in rats fed a high salt diet.

Clinical Revenue Support

Funding: Clinical Revenue Support
Results: High salt increased SBP and induced overexpression of Ang II, TGF-β, β2-MG, proteinuria and protein excretion (UPE) in rats. In the saline group, AngII, P-selectin, ROS, AQP-1 and AQP-2 expression was increased. Losartan reduced SBP in NS and HS groups and exerted diuretic and natriuretic effects in HS. Losartan reduced fibrosis and oxidative stress markers, and restored ENOS expression and AQP-2 levels in HS-L group. Losartan upregulated AQP-1 immunoeexpression, indicating loss of dietary sodium, favouring sodium urinary concentration.

Conclusions: SBP reduction, together with increased natriuresis and diuresis, induced by AT1 blockade in HS fed animals, depend on intrarenal Ang II inhibition, when circulating Ang II is simultaneously decreased. Intrarenal Ang II, through AT1 receptor stimulation, may be responsible for oxidative stress, profibrogenic response and decrease of AQP-1 and AQP-2 in the kidneys of rats fed with a high salt diet.

TH-PO1023

Is Vascular Mineralocorticoid Receptor Involved in Cyclopensine A Nephrotoxicity? 

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Background: Cyclopensine A (Csa) nephrotoxicity is one of its most frequent adverse effect but its pathophysiology remains unclear. Pharmacological blockade of the Mineralocorticoid Receptor (MR) has been reported to prevent Csa nephrotoxicity in the rat by modulating the expression of vaso-active factors (Pere-Rojas et al. AJPRP 2005). We have recently shown that MR is expressed in the endothelium and the vascular smooth muscle of the renal vasculature (Nguyen Dinh Cat et al. FASEB J 2010). Moreover, genetic manipulation of MR expression in the endothelium (Nguyen Dinh Cat et al. FASEB J 2010) or the smooth muscle (unpublished data) alter vascular function. Our working hypothesis is that the activation of vascular MR plays a key role in Csa nephrotoxicity.

Methods: We studied the effect of the effect of pharmacological blockade of MR on acute Csa toxicity in the mousemale mice of the under low salt diet: control, Ctrl (vehicle); Csa (Csa 100hmg/kg/d) and Csa+Cana (Csa + canrenoate 30mg/kg/d in the drinking water).

Results: At day 7, 40% of the Csa mice were dead versus 0 in the Csa+Cana mice (p<0.05). Kidney dysfunction induced by Csa is prevented with canrenoate Creatinine clearance, mL/min/100g: Ctrl 1.15 +/-0.17; Csa: 0.74 +/-0.17; Csa+Cana: 1.31 +/-0.30, p<0.05. Csa-induced proximal tubular alterations vascular function were partially prevented by canrenoate. The induction of BIP/GPR47, a marker of endothelial reticulum stress, has been also blunted. Canrenoate prevented the increase in urinary renin excretion of NGAL, a biomarker of renal damage, observed in Csa.

Conclusions: In conclusion, we demonstrate that pharmacological MR antagonism has beneficial effects on survival and prevents histological and functional alterations in a mouse model of acute Csa nephrotoxicity. The implication of vascular MR is currently under investigation using genetically modified models.

Funding: Government Support - Non-U.S.

TH-PO1024

Urinary Albumin and Protein Excretion Are Associated with Increased Vascular Renin-Angiotensin System Activity in Healthy Humans 

David Donaldson,1,2 Andries Nicholl,1,2 Brenda Hemmelgarn,1,2 Tanvir Chowdhury Turin,1,2 Donald McTavish Nicholl,1,2 Brenda Hemmelgarn,1,2 Tanvir Chowdhury Turin,1,2 Vascular Renin-Angiotensin System Activity in Healthy Humans

TH-PO1025

Autonomic Nervous System Activation during Intradialytic Hypertension

Dvora Rubinger, Rebecca Backenroth, Dan Sapoznikov. Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.

Background: To define the relationship between heart rate (HR) and blood pressure during intradialytic hypertensive episodes (HD-HY), continuous interbeat intervals (IBI) and systolic blood pressure (SBP) were monitored in 106 patients during 113 HD sessions.

Methods: HD-HY, defined as an increase of at least 10 mmHg in SBP between the beginning and the end of dialysis, or hypertension resistant to ultrafiltration occurring during or immediately after dialysis, were detected in 62 sessions. SBP and IBI variability and baroreceptor sensitivity (BRS) in the low (LF) and high frequency (HF) ranges were assessed using complex demodulation method (CDM). LF and HF oscillations are believed to be representative of sympathetic and parasympathetic activation respectively.

Results: HD-HY were associated with increased (7, n=45) or decreased (14, n=17) HR. Mean SBP, IBI and their variability, BRS and LF/HF ratio, representative of sympatho-vagal bala Tabe 1.0.

TH-PO1026

Reduced Aortic Relaxation Rate in Rats with Adenine-Induced Chronic Renal Failure

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Background: The aim was to investigate vascular function in rats with adenine-induced chronic renal failure (ARF).

Methods: Male Sprague-Dawley rats received either chow containing adenine for 6-12 weeks (0.5% for 3 weeks, 0.3% for 2 weeks, 0.15% thereafter) or were pair-fed with an identical diet without adenine (controls). Systolic blood pressure (SBP) and plasma were analyzed, at 2, 4 and 6 weeks. Segments of thoracic aorta and mesenteric arteries (2nd order) were analyzed with wire myograph. Data are means±SEM.

Results: A-CRF rats showed a marked increase in serum creatinine (281±25 vs 281±0.005 µmol/L, P<0.05) and parathyroid hormone levels were increased approximately 9-fold vs. HD-HyE were associated with increased SBP and IBI variability, suppressed BRS and enhanced LF/HF ratio, while in + HR, there were no significant changes in the above parameters. Our data point to sympatho-vagal activity as an important mechanism of HR and hypertension in a significant proportion of patients. In those with ↓ HR, sympathetic activity seems to be counterbalanced by vagal effects. The triggers of increased sympatho-vagal activity during HD remain to be determined.

Funding: Government Support - Non-U.S.
TH-PO1027
Basal Stenotic-Kidney Hemodynamics and Function Correlate with Response to Revascularization in Swine Renal Artery Stenosis (RAS) Alfonsso Eirin,1 Xiang-Yang Zhu,2 James Krier,1 John A. Crane,1 Stephen C. Textor,2 Amir Lerman,2 Lilach O. Lerman.1 

Background: Percutaneous transluminal renal angioplasty (PTRA) can restore vessel patency in RAS, but selection of subjects likely to improve glomerular filtration rate (GFR) after revascularization is difficult. This study examined hemodynamic factors linked to improved renal function after PTRA in a swine model of RAS.

Methods: Pigs (40-55kg) after 6-weeks of hemodynamically significant RAS (60-99%) were studied immediately before and 4 weeks after technically successful PTRA and stenting (n=15) or sham (n=7). Stenotic kidney (SKT) hemodynamics and function were evaluated by multidector computerized tomography before and after challenge with the endothelium-dependent vasodilator and diuretic acetylcholine (Ach, 0.5 μg/kg/min IV). Response to PTRA was evaluated by the change in GFR (ΔGFR).

Results: Four weeks after PTRA blood pressure was normalized in all pigs, and single-kidney GFR increased in 9/15 (ΔGFR = 27±3±1 mL/min). A positive functional change in GFR to Ach (possibly reflecting preserved tubular response) directly correlated with ΔGFR (Figure).

Conclusions: The combination of preserved functional stenotic-kidney GFR response to Ach and lower basal GFR may prove to be a powerful and potentially clinically applicable predictor of benefit from revascularization in RAS.

Funding: Other NIH Support - DK73608, DK77013, HL77131, HL085307, and RR018898.

TH-PO1028
Kruppel-Like Factor 4 Mediates High Phosphate-Induced Conversion of Vascular Smooth Muscle Cells into Osteoblast-Like Cells Tadashi Yoshida, Maho Yamashita, Matsuhiko Hayashi.

Background: Cardiovascular complications are the leading cause of death in patients with chronic kidney disease. These patients often have vascular calcifications, which have been associated with hyperphosphatemia. Previous studies have shown that high-phosphate-induced phenotypic switching of vascular smooth muscle cells into osteoblast-like cells plays an important role in the calcification process. In the present study, we determined if phosphorylated Elk-1 and Kruppel-like factor 4 (Klf4), which are critical regulators of SM smooth muscle marker gene expression, were involved in this process.

Methods: Cultured rat aortic smooth muscle cells were incubated in the medium with normal or high phosphate concentration (5 mmol/l) and were harvested for subsequent analyses.

Results: After the incubation with high phosphate medium for 10 days, severe calcification was observed by von Kossa staining. Expression of SM α-actin was decreased, whereas Runx2 expression was induced, as determined by real-time RT-PCR. In this culture system, Klf4 expression was markedly induced at mRNA and protein levels. However, phosphorylation of Elk-1 was undetectable at any time points examined. Furthermore, knockdown of Klf4 by siRNA attenuated high phosphate-induced repression of SM α-actin expression.

Conclusions: Results suggest that Klf4 mediates high phosphate-induced phenotypic switching of vascular smooth muscle cells into osteoblast-like cells.

Funding: Government Support - Non-U.S.

TH-PO1029
Candesartan Inhibits Toll-Like Receptor Expression in Human Renal Tubular Epithelial Cells Chen Yu.

Background: Toll-like receptors (TLRs) play a key role in the innate immune system and are found to be crucial in inflammatory respond. Recent research found that TLR4 was upregulated in the inflammatory diseases, such as hypertension, atherosclerosis, and renal fibrosis. Angiotensin II is involved in inflammatory responds via Angiotensin II type-1 receptor, whereas Angiotensin II type-1 receptor blockers (ARB) exert anti-inflammatory effect. The aim of present study is to investigate whether candesartan, an ARB, exerts its anti-inflammatory effect through Toll like receptors pathway.

Methods: Human renal tubular epithelial cells (HKC) were stimulated with LPS (200ng/ml) in the absence or presence of candesartan and TLR4 protein expression was measured by flow cytometry. Knocked down AT1R in HKC using AT1R siRNA and then tested AT1R expression induced by LPS. LPS and/or candesartan stimulated HKC for 0.5hrs, 1hrs and 3 hrs, and collected cells for phosphorylation of NF-κB using western blot. Stimulation with LPS and candesartan (10-5mol/L,10-6mol/L,10-7mol/L) and extracted RNA to test mRNA expression of inflammatory factors MCP-1 and RANTES.

Results: LPS increased TLR4 protein expression in HKC, which was inhibited by candesartan markedly. Silence of AT1R improved LPS-induced TLR4 expression. Pretreatment of HKC with candesartan significantly decreased LPS induced NF-κB activity and TLR4 expression (P < 0.05 vs. control) along with decrease in the mRNA expression of MCP-1 and RANTES in a concentration-related pattern.

Conclusions: LPS induced TLR4 expression, NF-κB activity and further inflammatory factors expression are inhibited by candesartan. Thus, we define a novel pathway by which candesartan could induce anti-inflammatory effects, that is through Toll like receptors pathway.

Key Words: Toll-like receptors (TLRs), LPS, Angiotensin II, ARB Funding: Government Support - Non-U.S.

TH-PO1030
Hypertensive Nephrosclerosis Is Associated with Periarteriolar T Cell Infiltration and Increased Level of Serum T Cell-Driven Chemokines Jeemon Jin Lim,1 Youn Jong-Chan,2 Hyeon Joo Jeong,1 Sungha Park2.

Background: The contribution of chemokines in primary hypertension has been recently proposed in animal models, however, their roles in human hypertension are not well known. We evaluated the infiltration of chemokine-producing T cells in renal tissue of hypertensive nephrosclerosis and circulating levels of chemokines in hypertensive patients.

Methods: We evaluated infiltration of T lymphocytes and their expression of chemokines in 10 hypertensive nephrosclerosis cases by immunohistochemistry and compared with normal control group. We measured circulating levels of 6 chemokines (MIG, IP-10, I-TAC, MCP-1, MIP-1α, MIP-1β) in 71 hypertensive patients (51.6±11.2 yrs, M:F=35:36) and in age, sex-matched 71 control subjects by cytometry bead array method. We also analyzed the expression of CXCR3 and CCR2 in peripheral blood mononuclear cells of hypertensive patients using multicolor flow cytometry.

Results: Periarteriolar inflammatory cells were increased in hypertensive nephrosclerosis and most of them are composed of T lymphocytes. Some of infiltrating cells express chemokines. Circulating chemokine levels were significantly higher in patients with hypertension than in control subjects. CXCR3 were mainly expressed in CDS T cell subset, which co-expresses CX3CR1 as well.

Conclusions: Although this observation does not provide direct evidence of T cell function in human hypertension, increased T cells and chemokines in hypertensive patients suggest the role of T cell-associated inflammatory pathway. More detailed characterization of T cells, associated chemokines and the expression of chemokine receptors in renal tissue may offer a new insight in the pathophysiology of primary hypertension.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

352A
High Throughput Screening of Drugs That Inhibit WNK-OSR1/SPAK Signaling Cascade

Background: WNK kinases were identified as causative genes of pseudohyopaldosteronism type II (PHA II), a hereditary hypertensive disease with hyperkalemia and acidosis. We identified that WNKs form a signal cascade with OSR1/SPAK kinases and Slc12a transporters (NCC1, NKCC2, and NCC). We also found that this signal cascade has a pivotal role in controlling vascular tone as well as renal NaCl excretion. Therefore, inhibiting this signal cascade could be a new type of antihypertensive drugs.

Methods: To explore this possibility, we adopted a strategy to inhibit the binding of WNKs with their substrates OSR1/SPAK since the binding domains are already known. Furthermore, we introduced a Fluorescence Correlation Spectroscopy (FCS) method to efficiently screen the inhibitors. FCS is a method to be able to measure fluctuation rate of a fluorescence-labeled single peptide. We labeled an RFxV/I motif of WNK4 with TAMRA, mixed it with the CCT domain of SPAK fused with GST, and could confirm the binding of these two molecules by FCS in 384-well plates. Using this newly developed system, we could screen chemical compounds to inhibit the binding.

Results: As a result of initial screening of 16,000 compounds owned by Tokyo Medical and Dental University Chemical Screening Center, we found 10 different primary candidates. We then tested whether these compounds could inhibit the signals from WNKs to OSR1 in vivo in COS7 cells, and finally obtained three compounds showing primary candidates. We then tested whether these compounds could inhibit the signals from these two molecules by FCS in 384-well plates. Using this newly developed system, we efficiently screen the inhibitors. FCS is a method to be able to measure fluctuation rate of a fluorescence-labeled single peptide. We labeled an RFxV/I motif of WNK4 with TAMRA, mixed it with the CCT domain of SPAK fused with GST, and could confirm the binding of these two molecules by FCS in 384-well plates. Using this newly developed system, we could screen chemical compounds to inhibit the binding.

Conclusions: These compounds could be promising seeds for new types of antihypertensive drugs, and the method we developed in this study could be applicable to any screenings for compounds that inhibit binding of two molecules.

Funding: Government Support - Non-U.S.

Inhibition of Uracilase Plus Physiological Amounts of Fructose and Glucose: A Model of Metabolic Syndrome and Glomerular Hypertension in Rats

More Related to the Human Condition

High Throughput Screening of Drugs That Inhibit WNK-OSR1/SPAK Signaling Cascade

Hyperfiltration and the Effect of Nitric Oxide Inhibition on Renal Hemodynamics and Endothelial Function in Humans with Uncomplicated Type 1 Diabetes Mellitus

Inhibition of Uracilase Plus Physiological Amounts of Fructose and Glucose: A Model of Metabolic Syndrome and Glomerular Hypertension in Rats

Effect of Nitric Oxide Inhibition on Renal Hemodynamics and Endothelial Function in Humans with Uncomplicated Type 1 Diabetes Mellitus
Methods: TGF responses were measured in adenosine A1 receptor knockout (A1−/−) and wildtype mice (A1+/+), as changes in proximal stop-flow pressure (Psf) in response to increased perfusion of loop of Henle (0 to 35 nl/min). Maximal TGF responses were studied during perfusion with artificial tubular fluid (ATF) alone, or ATF supplemented with i) Ang II (10−9 M), ii) Ang II (10−9 M)+Tempol (10−3 M), or iii) Ang II (10−9 mol/L)+A2 receptor antagonist (10−7 M).

Results: TGF response (ΔPsf) during control (ATF alone) was 8.1±0.6 mmHg in A1+/+, whereas TGF responses were abolished in A1−/− mice. Perfusion with Ang II enhanced ΔPsf in A1+/+ (12±0.3 mmHg), whereas an inverse TGF response was observed in A1−/− mice (-3±0.5 mmHg). Co-treatment with superoxide dismutase-mimetic (Tempol) during Ang II perfusion normalized TGF responses in both A1+/+ (8.6±0.6 mmHg) and A1−/− mice (0±0.6 mmHg). Simultaneous application of Ang II and A2 antagonist enhanced ΔPsf in A1+/+ (14.5±0.8 mmHg), and induced TGF response in A1−/− mice (3.5±0.8 mmHg).

Conclusions: Adenosine A1 receptors enhance, whereas A2 receptors attenuate Ang II-mediated effects on TGF responses. Mechanistically, Ang II-induced oxidative stress may increase adenosine formation, which during A1-deficiency causes A2 receptor-mediated dilatation.

Funding: NIDDK Support

TH-PO1038

Abstract Withdrawn

TH-PO1039

Non-Steroidal Anti-Inflammatory Drugs Modulate Vasa Recta Diameter
Teresa M. Kennedy-Lydon,1 Carol Crawford,1 Liam Sawbridge,1 Robert J. Unwin,2 Scott P. Wildman,3 Claire M. Peppiatt-Wildman.1 Royal veterinary College, London, United Kingdom; 2 UCL Medical School, London, United Kingdom.

Background: Non-steroidal anti-inflammatory drugs (NSAIDs) are nephrotoxic and reduce medullary blood flow (MBF). NSAIDs inhibit cyclooxygenases (COX) and endogenous production of medullary prostaglandin E2 (PGE2). PGE2 has been shown to attenuate the vasoconstrictor effects of endothelin-1 (ET-1) and angiotensin-II (Ang-II) at vasa recta. Here we investigate the responses of in situ vasa recta pericytes to i) PGE2, ii) ET-1 and Ang-II plus minus PGE2, iii) NSAIDs, and the location of medullary COX-1 and 2 in the kidney slice model.

Methods: Live kidney slices obtained from adult male Sprague-Dawley rats were suspended in an open-bath chamber on the stage of an upright microscope and continuously superfused with oxygenated physiological saline solution. Real time images of in situ vasa recta were recorded and vasa recta diameter at pericyte and non-pericyte sites was measured off-line.

Results: Application of PGE2 to live kidney slices evoked dilation of vasa recta specifically at pericyte sites. PGE2 significantly attenuated pericyte-mediated constriction of vasa recta evoked by both ET-1 and Ang-II. Indomethacin a non-selective inhibitor of COX evoked a significantly greater constriction of vasa recta capillaries at pericyte sites than at non-pericyte sites. The COX-1 selective inhibitor SC-560 and COX-2 selective inhibitors meloxicam and celecoxib also evoked a significantly greater constriction at pericyte sites than at non-pericyte sites. COX-1 and 2 were identified in vasa recta endothelial cells by immunohistochemistry.

Conclusions: Data presented show i) PGE2 dilates vasa recta at pericyte sites and reverses the ET-1- and Ang-II-evoked constriction of vasa recta at pericytes, ii) vasa recta endothelial cells are a source of COX-1 and -2, iii) application of selective and non-selective COX inhibitors leads to pericyte-mediated constriction of vasa recta. These data re-iterate the key role of PGE2 in regulation of medullary blood flow and reveal that attenuation of PGE2 production causes pericyte-mediated constriction of vasa recta. Thus pericytes may be key in NSAID-evoked reduction in MBF.

TH-PO1040

Rapid, Dynamic Increases in Glomerular Permeability during Angiotensin-II (All) Infusion in Rats. Reactive Oxygen Species (ROS) May Be Involved
Josefin Axelsson, Anna Rippe, Kristinn Sverrisson, Bengt Rippe. Department of Nephrology, Lund University, Lund, Sweden.

Background: This study was performed in order to investigate the dynamics and mechanisms of action of All on glomerular permeability.

Methods: In anaesthetized Wistar rats (250-280g) the left ureter was cannulated for urine collection, while simultaneously blood access was achieved. Rats were continuously infused i.v. with All [62 ng/min (Lo-All); n=5] or 250 ng/min (M-All; n=8) or 500 ng/min (Hi-All; n=8), respectively, and with polydisperse fluorescein isothiocyanate (FITC)-Ficol 70/400 (mol radius 13-90 Å) and CR-EFDTA 2 for 2 h. Plasma and urine samples were taken at 15, 30, 60 and 120 min of All infusion, and analyzed by high performance size exclusion chromatography (HPSEC) for determination of glomerular sieving coefficients (θ) for Ficoll of radius 70Å. For Ficol of radius 70Å increased 20-fold (Lo-All) up to 30-fold (Hi- and M-All), respectively. For the Lo-All group the permeability increase was reversible within 15-60 min, whereas in the M-All and the Hi-All groups there was only a partial reversibility within this time frame. Thus, a moderate, sustained increase in glomerular permeability remained even at 120 min. GFR was well maintained in the Lo-All group, but (somewhat) decreased in the M-All and Hi-
Charge selectivity was found to be much less pronounced than previously demonstrated for molecules of a radius >34Å in radius is compatible with an increase in the number of "large pores" of the glomerular filter. ROS may be partly involved in the permeability increase induced by AII. Funding: Government Support - Non-U.S.

Vivo: Charge Modified, Conformationally Intact Anionic Ficoll Is Retarded in Neutrophil Ficoll Barriers in Vivo Vincent Daien, 2 Ryo Kawasaki, 3 Jean Ribstein, 1 Guilhem Du Cailar, 1 Max Villain, 2 Albert Minneman, 1 Pierre Fessler, 2 Jean Ribstein, 2 Guillaume Dus Calas, 1 Max Villain, 2 Albert Minneman, 3 Pierre Fessler, 4 Department of Internal Medicine, Lapeyronie Hospital, Montpellier, France; 5 Department of Ophthalmology, Gui de Chauliac Hospital, Montpellier, France; 6 Retinal Vascular Imaging Centre, Centre for Eye Research Australia, Melbourne, Australia.

Background: The glomerular filtration barrier (GFB) is commonly conceived as a negatively charged sieve. Recent studies, however, indicate that glomerular charge effects are small for anionic, carboxyethylated (CM) dextran vs. neutral dextran. Two studies assessing the glomerular sieving coefficients (θ) for CM-Ficoll vs. native Ficoll have actually demonstrated an "anomalous" behavior of these polysaccharides, i.e. a higher glomerular permeation of anionic than neutral Ficoll. The CM-Ficoll used in these studies showed a larger Stokes-Einstein radius (αe) than neutral Ficoll. Hence, it was proposed that the introduction of negative charges in the Ficoll molecule had made it more extended and flexible, and thereby, more permeable.

Methods: Recently, a negatively charged fluorescein isothiocyanate (FITC) labeled CM-Ficoll was produced and found to have conformation identical to that of native FITC-Ficoll. Using these probes we determined their θ in anesthetized Wistar rats (259±2.5 g). After blood access had been achieved, the left ureter was cannulated for urine sampling. Either polysaccharide was continuously infused (i.v.) in parallel with a marker of glomerular filtration rate (GFR), while urine and plasma were collected. Assignment, FICOLL-P Ages in plasma and urine and was achieved by high performance size-exclusion chromatography (HPSEC).

Results: CM-Ficoll and native Ficoll had identical elugrams on the HPSEC. Diffusion of anionic Ficoll was significantly reduced compared to that of neutral Ficoll across the GFB for molecules of αe >20-35Å; while there were no effective charge effects for Ficoll of αe = 35-80Å. The data are consistent with a charge effect present in "small pores", but not in "large pores", of the GFB and mimicked those obtained for anionic membranes in vitro for the same probes.

Conclusions: In conclusion, the GFB is negatively charged. However, the negative charge selectivity was found to be much less pronounced than previously demonstrated for sulphated vs. neutral dextran. Funding: Government Support - Non-U.S.

Decrease in Retinal Arteriolar Caliber Is Associated with a Lower Renal Function in Normotensive and Never-Treated Hypertensive Subjects Vinay Dixit, 2 Ryo Kawasaki, 3 Jean Ribstein, 1 Max Villain, 2 Albert Minneman, 3 Pierre Fessler, 4 Department of Internal Medicine, Lapeyronie Hospital, Montpellier, France; 5 Department of Ophthalmology, Gui de Chauliac Hospital, Montpellier, France; 6 Retinal Vascular Imaging Centre, Centre for Eye Research Australia, Melbourne, Australia.

Background: Microvascular change has been postulated to represent one of the key mechanisms of kidney aging. Retinal arteriolar narrowing has been used as a marker of the altered microcirculation. The primary objective was to assess the association between retinal arterial caliber and renal function in normotensive (NT, <140/90 mmHg) and never-treated hypertensive (HT) subjects.

Methods: Study subjects were 57 persons with NT and 48 persons with never-treated essential HT with serum creatinine (SCreat) ≤ 130 µmol/L and without diabetes. Retinal arteriolar caliber was measured from fundus photographs using a computer-assisted program and summarized as central retinal artery equivalent. Glomerular filtration rate (GFR) was estimated from the Mayo clinic quadratic equation. Results: Mean age of study subjects was 48+/-13 (mean +/- SD), and 50% were women. Mean SCreat was 0.79±0.17 mg/dL and eGFR was 113.7 ml/min/1.73m², and there was no difference between persons with NT and HT. Mean retinal arterial caliber in persons with NT and HT were 147.6 ±12.7 µm and 135.1 ±10.9 µm, respectively (p=0.001). In the whole population, retinal arterial caliber was positively and significantly correlated to GFR (univariate r=0.16; p=0.001), even after adjustment for age, gender, mean arterial blood pressure, smoking, glycemia, body mass index, total cholesterol, triglycerides (model r²=0.49; p=0.001). When replacing GFR by 1/Srect and adding size and weight as predictive variables, the association between retinal arteriolar caliber and renal function remained significant (model r²=0.32; p=0.0001).

Conclusions: In never-treated NT or HT subjects, a decrease in retinal arteriolar caliber is associated with a lower kidney function, independently of other potential determinants of retinal vascular changes. The mechanisms of this apparent common aging process remain to be documented.

Renal Responses to Administration of a Peroxynitrite Scavenger in Anesthetized Wild Type and Knockout Mice Lacking the Gene for Extracellular Superoxide Dismutase Dewan S. Majid, Alexander Castillo, Purnima Singh. Physiology, Tulane University School of Medicine, New Orleans, LA.

Background: Peroxynitrite (ONO0) is continuously being produced in the body via interaction of nitric oxide and superoxide (O2-). However, its role in regulating cardiovascular and renal function is not yet clearly defined. In the present study, we assessed its regulatory role in the control of systemic arterial pressure (SAP) as well as renal hemodynamics and excretory function in mice.

Methods: ONOO- scavenger, mercaptopyrrol guanidine (MEG), was administered intravenously at an incremental doses (10, 30 and 50 µg/kg/min for 45 min each) in anesthetized wild type (C57BL/6; n=6) as well as knockout mice lacking the gene for extracellular superoxide dismutase (ecSOD KO; n=4) which would have a higher level of ONOO- due to enhanced O2- and NO interaction. SAP was recorded using a pressure transducer connected to a cannula placed in the left carotid artery. Renal blood flow (RBF) and glomerular filtration rate (GFR) were measured by PAH and inulin clearances respectively. A cannula was inserted into the urinary bladder for collection of urine.

Results: Infusion of MEG doses caused small but significant increases in SAP in both WT and ecSOD KO mice. The highest dose of MEG increased mean SAP from 93±3 to 99±4 mmHg in WT and from 81±5 to 87±5 mmHg in ecSOD KO mice. Although MEG did not cause significant changes in RBF (6.8±0.7 to 7.4±1.4 ml/min/g) or in GFR (0.06±0.12 to 1.01±0.14 ml/min/g) in WT mice but there was a marked decrease in GFR (1.00±0.17 to 0.77±0.12 ml/min/g; P<0.05) without appreciable change in RBF (5.91±0.9 to 5.6±0.8 ml/min/g) in ecSOD KO mice. MEG infusion increased urinary sodium excretion (UNaV) and fractional excretion of sodium (FeNa) in both WT (UNaV, 0.62±0.17 to 1.65±0.24 µmol/min/g; FeNa, 0.43±0.11 to 1.7±0.03%) and ecSOD KO mice (UNaV, 0.77±0.19 to 1.42±0.21 µmol/min/g; FeNa, 0.52±0.09 to 1.25±0.19%)

Conclusions: These results indicate that endogenous formation of ONOO- contributes to arteriole vasodilatation tone systemically and provides a renoprotective role in maintaining GFR in the condition where dismutation of O2- is limited due to SOD deficiency. Funding: Other NIH Support - NHLBI


Vitamin D (VD) status is an increasingly notable predictor of kidney and cardiovascular (CV) risk. While the mechanism is unclear, VD appears to modulate RAS activity. We sought to clarify the influence of VD in modulating RAS control of arterial stiffness, an important parameter for the assessment of CV risk in both the healthy and CKD populations.

Methods: Forty-one normotensive, non-obese, healthy subjects (26 females and 15 males) were studied in a high salt balance, a state of maximal RAS suppression. Women

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only Underline represents presenting author.
were studied in the same phase of their menstrual cycle. Arterial stiffness, expressed as arterial stiffness index (ASI) and brachial pulse wave velocity (PWV) provided by tonometry at baseline and in response to ANG II infusion (3ng/kg/min x 30 min) and 6ng/kg/min x 30 min). The primary outcome was the effect of VD status on the arterial stiffness response to ANG challenge, a well-accepted marker of the intrinsic RAS activity of the renal vasculature.

Results: Results were analyzed according to serum 25(OH)VD status: deficient (<50nmol/L, n=12), insufficient (50-80nmol/L, n=15), and sufficient (>80nmol/L, n=14). Increasing 25(OH)VD status was associated with improved arterial stiffness (ASI: VD deficient 28.4±5.4; VD insufficient, 21.3±5.3; VD sufficient, 20.5±5.6; p=0.015, p =0.009 for trend), though a similar relationship was not observed between VD status and PWV (p=0.5). As anticipated, all subjects demonstrated an increase in AIx (p=0.001) and PWV (p=0.001) in response to ANG challenge, though the AIx response did not differ by 25(OH)VD status (ASI: VD deficient, 19.1±4.7; VD insufficient, 22.2±5.3; VD sufficient, 21.4±10.3; p=0.3). In contrast, the brachial PWV response to ANG differed according 25(OH)VD status (ΔPWV: VD deficient, 2.6±1.1m/s; VD insufficient, 3.4±0.7m/s; VD sufficient, 1.4±0.7m/s; p=0.032).

Conclusions: Improved VD status is associated with reduced arterial stiffness in healthy humans, possibly through an RAS-dependent mechanism which appears to differ according to VD level and the vascular bed. Further studies are needed to clarify optimal VD status and the role of VD supplementation on CVD risk.

TH-PO1046

25-hydroxyvitamin D insufficiency, Which Can Be Improved with Rosuvastatin Treatment, Is Associated with Renal Endothelial Function Christian Ott, Ulrike Raff, Marina V. Lehmann, Markus P. Schneider, Stephanie Tizze, Roland E. Schmieder. Nephrology and Hypertension, University Hospital Erlangen, Erlangen, Germany.

Background: Vitamin D deficiency is considered as cardiovascular and renal risk factor. We tested the hypotheses whether vitamin D level is related to endothelial function of the renal vasculature. Since statin treatment is known to improve endothelial function, we tested the hypotheses whether vitamin D level is related to endothelial function but not blood pressure and cholesterol levels, is an independent determinant of basal NO activity and is associated with increased NO activity after treatment with rosuvastatin.

Methods: In a double-blind, randomized study 31 hypercholesterolemic patients with at least vitamin D insufficiency (< 30 ng/ml) were randomly assigned to rosuvastatin (10 mg) and placebo for 6 weeks. Renal hemodynamics were determined by constant input clearance technique with p-aminohippurate (PAH) and inulin. Basal NO activity of the renal vasculature, was assessed by measuring renal plasma flow (RPF) both before and after blockade of NOS with systemic infusion of N(G)-monomethyl-L-arginine (L-NMMA). In parallel, 25(OH)D was measured.

Results: Compared to placebo treatment, rosuvastatin increased 25(OH)D levels (21.6±4.0 vs. 24.1±8.0 ng/ml; p=0.039). Moreover, the decrease in RPF in response to L-NMMA (an estimate of basal NO activity) was significantly more increased after 6-week therapy with rosuvastatin than with placebo (94.8±70 vs. 68.2±32 ml/min; p=0.044), indicating increased basal NO activity after 6 weeks of rosuvastatin treatment. The change in basal NO activity in the placebo phase treatment was correlated inversely with 25(OH)D (R=-0.385; p=0.027). Multiple regression analysis revealed that at baseline 25(OH)D, but not blood pressure and cholesterol levels, is an independent determinant of basal NO activity (β=-0.446, r=0.15). In contrast, no correlation was evident between 25(OH)D and basal NO activity after rosuvastatin treatment.

Conclusions: Thus, rosuvastatin may beneficially influence the impact of vitamin D insufficiency on renal endothelial function.

TH-PO1047

Ambulatory Arterial Stiffness Index (AAASI) and All Cause Mortality Rupesh Raiha, Alaine E. McGarry-Gadja, George Thomas, Mohammad Rafay, Martin J. Schreiber. Dept. of Nephrology and Hypertension, GUKI Institute, Cleveland Clinic Foundation, Cleveland, OH.

Background: Ambulatory Arterial Stiffness Index (AAASI) and pulse pressure (PP) are indices of arterial stiffness that can be computed from 24-hour ambulatory BP measurement (ABPM). We investigated the association of AAASI and PP with all-cause mortality.

Methods: AAASI (1 minus the slope of diastolic on systolic BP in individual 24 hr ABPM) was calculated for182 pts. from 1994 through 1997. Data collected included demographic characteristics (sex) and brachial pulse wave velocity (PWV) was measured (DM). Mortality data (date of death) was obtained from the Social Security death registry in February 2011. We applied Cox regression to relate mortality to AAASI and PP while adjusting for sex, age, BMI, 24-h ABPM, smoking, DM, chronic kidney disease (CKD) and cardiovascular disease (CVD).

Results: Mean age was 58 ±14 yrs, 33% were female and 22% were African-American. Mean BMI was 27.2 ± 4.5 kg/m², and treatment for HTN (58%) and DM (23%). Eighteen deaths occurred during the 15 year period. AAASI mean score ≥0.51 was associated with higher all cause mortality (16.1%, P=0.001).However this correlation was not seen with pulse pressure.

Conclusions: AAASI Quartiles and PP Quartile and its Correlation with All Cause Mortality

<table>
<thead>
<tr>
<th>AAASI Quartiles</th>
<th>HRs (95% CI)</th>
<th>24-Hour PP</th>
<th>HRs (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.35</td>
<td>1.00</td>
<td>0.76</td>
<td>1.00</td>
</tr>
<tr>
<td>0.35-0.45</td>
<td>0.86 (0.72, 1.06)</td>
<td>45.8-50.2</td>
<td>0.91 (0.53, 1.55)</td>
</tr>
<tr>
<td>0.45-0.51</td>
<td>0.90 (0.74, 1.09)</td>
<td>50.2-5.56</td>
<td>0.92 (0.56, 1.16)</td>
</tr>
<tr>
<td>≥0.51</td>
<td>0.87 (1.33, 1.59)</td>
<td>55.5</td>
<td>1.04 (0.63, 1.31)</td>
</tr>
</tbody>
</table>

HRs- Hazard Ratio; Cl- Confidence Interval and * P<0.005.

Effect of One Week Naproxinoid Treatment on Sodium Balance and Acute Natriuretic Effect of Furosemide: A Randomized Double-Blind Placebo and Naproxen-Controlled Trial in Healthy Volunteers Nicolas Glag,1 Grégoireروعزير,أ, Marc P. Maillard,1 Bruno Vogt,1 Caroline Roger,1 Nicolas Glag,1 Nephrology, CHU, Lausanne, Switzerland; 2N/Cox, Sophia Antipolis, France.

Background: Naproxinoid (CIN) is a cyclooxygenase inhibiting nitric oxide (NO) donor developed for the treatment of osteoarthritis. The objective of the study was to evaluate the effects of CIN on sodium (Na) balance and response to furosemide (FU). Methods: 31 healthy male volunteers were randomized into three parallel groups: CIN 730 mg bid, naproxen (NAP) 500 mg bid or placebo (PLA) bid during eight days (D1-D8). 24-h Na and aldosterone excretions were measured from D -1 to D4. On D8, natriuresis, plasma renin activity (PRA) and glomerular filtration rate (GFR), using inulin clearances, were measured before and after 40 mg of intravenous FU.

Results: On D-1, 24h Na excretion and aldosterone excretion were respectively 193±70 and 4.5±0.8 µg/24h. On D8, Na and aldosterone excretions differed between C and PLA, whose levels rose with age and blood flow to medulla. Correlation between fibrogenic and oxidative biomarkers suggest that prior Na intake and microvascular dysfunction contribute to renal injury in essential HTN.

Funding: Other NIH Support - NHLBI

Vascular Physiology/Renal Hemodynamics / Poster/Thursday
Table 1

<table>
<thead>
<tr>
<th></th>
<th>Day 8</th>
<th>FUR (Baseline)</th>
<th>FUR + 60'</th>
<th>FUR + 120'</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>38.2 ± 12.1</td>
<td>36.5 ± 34.7</td>
<td>53.5 ± 10.8</td>
<td></td>
</tr>
<tr>
<td>CIN</td>
<td>32.7 ± 5.2</td>
<td>29.9 ± 17.5</td>
<td>54.7 ± 21.3</td>
<td></td>
</tr>
<tr>
<td>NAP</td>
<td>27.2 ± 9.9</td>
<td>31.1 ± 19.1</td>
<td>54.8 ± 14.3</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SD

Conclusions: CIN and NAP had some degree of Na retention (progressive decrease in 24h urinary aldosterone excretion) compared to PLA. After 8 days of treatment, no difference in Na excretions after FUR was detected between groups, but PRA response to FUR were slightly blunted in the CIN and NAP groups. The addition of NO moiety to naproxen does not seem to influence the Na balance or the natriuretic response to FUR compared to naproxen alone in healthy volunteers.

Funding: Pharmaceutical Company Support

TH-PO1050

The mTOR Inhibition Reduces the In Vitro Mineralization of Vascular Smooth Muscle Cells Jasmin Prüfer, Mirjam Schuchardt, Markus Tolle, Markus van der Giet, Med. Klinik mit SP Nephrologie, Charité - Campus Benjamin Franklin, Berlin, Germany; Novartis Pharma AG, Nürnberg, Germany.

Background: Vascular disease contributes to the high cardiovascular mortality among organ transplant recipients. The immunosuppressive regimes, necessary for preventing transplant rejection, have different side effects on the vascular system. The aim of this study was to investigate whether the mTOR inhibitor rapamycin (RPA) is also effective for the prevention of vascular calcification in an in vitro mineralization assay using vascular smooth muscle cells (VSMCs).

Methods: In vitro calcification in VSMCs were induced with calcification medium (CM: DMEM containing 4.5 g/L glucose supplemented with 15% FCS, 10 mmol/L sodium pyruvate, 50 µg/mL ascorbic acid, and 10 mmol/L β-glycerophosphate) and dexamethasone (DEX, 100 nmol/L). Calcium deposition was quantified by O-cresolphthalein complexone method. Alkaline phosphatase (ALP) enzyme activity was measured by p-nitrophenol method.

Results: Cultivation of VSMCs in CM induced mineralization of VSMCs, which could be enhanced in the presence of DEX. The calcification was quantified by measuring the extracellular calcium content and visualized by Alizarin Red staining. Pretreatment with RPA could significantly and time-dependently decrease the mineralization of VSMCs by reducing extracellular calcium. For the precipitation of calcium phosphate, the activation of ALP is necessary. CM and DEX led to a significant and time-dependent increase in ALP enzyme activity, which is significantly diminished by pretreatment with RPA.

Conclusions: In this study we were able to show that the mTOR inhibitor RPA diminished the mineralization of VSMCs in vitro. Therefore, it seems possible that RPA might be effective in the prevention of arteriosclerosis after organ transplantation that would contribute to a better cardiovascular outcome of these patients.

TH-PO1051

Reactive Oxygen Species Acutely Stimulate Renin Release in Mouse Juxtaglomerular Cells Mariela Mendez, Jeffrey L. Garvin. Hypertension and Vascular Research, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI.

Background: Renin and its enzymatic product Angiotensin I, are essential in the regulation of blood pressure. Chronically enhanced circulating renin induces hypertension and renal damage. Low levels of reactive oxygen species (ROS), such as hydrogen peroxide and superoxide, play a role in renal hemodynamics and tubular NaCl transport. Enhanced ROS in the kidney are involved in the development of hypertension and kidney disease. However, it is not known whether ROS affect renin release from juxtaglomerular (JG) cells.

Methods: We generated primary cultures of mouse Juxtaglomerular cells (JG) cells and measured the effect of H2O2 or superoxide on renin release to the media and total renin content.

Results: Treatment with H2O2 (1 hour) at 100 or 500 nM increased renin release by 101±43% (p = 0.08) and 166±47% (p < 0.04), respectively. In addition, decreasing endogenous hydrogen peroxide levels by treating JG cells with catalase (1mU/ml) decreased basal renin release by 45±9% (p = 0.05). These data indicate that endogenously produced H2O2 tonically stimulates renin release. H2O2 peroxide had no effect on total renin content in JG cells (n=6; p = n.s.) suggesting that H2O2 stimulates renin exocytosis.

Conclusions: We concluded that the ROS, hydrogen peroxide and superoxide, stimulate renin release from mouse JG cells. These data suggest a novel and rapid pathway for the stimulation of renin release that may be involved in the development of hypertension and kidney damage during enhanced ROS production in the renal cortex.

Funding: Other NIH Support - NRSA to Mariela Mendez

357A
FR-PO1052

The Ratio of alpha-1-Acid Glycoprotein to alpha-1B Glycoprotein in Urine Is an Early and Accurate Predictor of Acute Kidney Injury

Joseph Alje,1 Lakhmir S. Chawla,2 James A. Tumlin,2 John M. Arthur,1-2 *MUSC; 1Ralph H Johnson VAMC; 2Duke University; 3George Washington University; 4University of Tennessee Chattanooga.

Background: Acute kidney injury (AKI) is a common complication of cardiac surgery. A biomarker that is diagnostic of AKI earlier in the course would facilitate intervention and improve patient outcomes. We identified early biomarkers of AKI using a mass spectrometry-based proteomics approach.

Methods: Urine samples were obtained from patients shortly (mean 10 hours) after cardiac surgery. Four patients developed severe AKI (mean increase Cr 3.3 mg/dl), and 4 did not (mean increase 0.2). Proteomic analysis was done by liquid chromatography and tandem mass spectrometry. Proteins were identified with Mascot and validated with Scaffold.

Results: We identified 227 high confidence proteins with 2–peptides (FDR<1%). The high abundance proteins albumin, lambda light chain, and kappa light chain did not differ in abundance between the two groups. Previously described AKI biomarkers alpha-1-acid glycoprotein (AGP-1), cystatin C, hemopexin and NGAL were different between the groups. AGP-1 and Alpha-1B-glycoprotein (A1BG) changed in opposite directions with AKI. The mean emPAI value of AGP-1 was 0.33±0.06 in the AKI group and 0.15±0.04 in the non-AKI group (p=0.04). The mean emPAI value of A1BG was 0.06±0.02 in the AKI group and 0.19±0.03 in the non-AKI group (p=0.04). Furthermore, when the ratio of AGP-1 to A1BG was calculated we found that it was able to predict AKI at this early time point with 100% accuracy. The segregation between the AGP-1:A1BG ratios for AKI and non-AKI patients was large. The mean difference in ratios between groups was over nine-fold and the smallest difference between individual members of the groups was greater than 33-fold.

Conclusions: The ratio AGP-1 to A1BG is an early potential marker of AKI. The use of a ratio of proteins which change in opposite directions enhances the sensitivity of the predictor and obviates the need for normalization.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1053

Biomarkers in Acute Kidney Injury: Should We Assess Them in Serum or Urine?

Josee Bouchard,1 Rakesh Malhotra,2 Ashita J. Tolsvari,2 Ravindra L. Mehta.1 Universite de Montreal, Canada; 2University of California San Diego; 3University of Alabama at Birmingham.

Background: There is limited information on the value of serum vs. urine biomarkers in acute kidney injury (AKI) diagnosis. The aim of this study was to compare the predictive capacity of serum and urine biomarkers to diagnose AKI.

Methods: We conducted a prospective, multicenter observational study to evaluate the role of serum vs. urine neutrophil gelatinase–associated lipocalin (NGAL), kidney injury molecule-1 (KIM-1), cystatin C and osteopontin ratios in AKI. The number of patients who had AKI was calculated so that it was able to predict AKI at this early time point with 100% accuracy. The segregation between the AGP-1:A1BG ratios for AKI and non-AKI patients was large. The mean difference in ratios between groups was over nine-fold and the smallest difference between individual members of the groups was greater than 33-fold.

Conclusions: The ratio AGP-1 to A1BG is an early potential marker of AKI. The use of a ratio of proteins which change in opposite directions enhances the sensitivity of the predictor and obviates the need for normalization.

Funding: Pharmaceutical Company Support, Private Foundation Support, Clinical Revenue Support

FR-PO1054

MYH9 Gene Variant Is a Risk Factor for Acute Kidney Injury after Severe Trauma

Michael G. Shashaty, Paul Lanken, Scarlett L. Bellamy, Sandra M. Gaylor, Scott A. McCullough, Joseph Alge,1 Lakhmir S. Chawla,2 James A. Tumlin,2 John M. Arthur.1-2 *MUSC; 1Ralph H Johnson VAMC; 2Duke University; 3George Washington University; 4University of Tennessee Chattanooga.

Background: Acute kidney Injury (AKI) is a source of substantial morbidity and mortality. Clinical factors incompletely explain AKI risk. Multiple studies have highlighted a locus on chromosome 22, encompassing the APOL1 and MYH9 genes, as strongly associated with chronic kidney disease (CKD). We hypothesized that these genetic variants may be associated with increased risk of AKI.

Methods: Patients: ICU with severe trauma were prospectively followed for AKI defined by Acute Kidney Injury Network (AKIN) creatinine criteria. We tested the association of two candidate gene single nucleotide polymorphisms (SNPs) previously associated with CKD—rs4821480 (MYH9) and rs73885319 (APOL1)—with AKI. Taqman genotyping of genomic DNA was performed. As the APOL1 SNP is private to African populations, only African ancestry (AA) subjects were genotype typed this variant. Significance of odds ratios was determined with logistic regression modeling.

Results: Of 443 subjects enrolled, 112 (25.3%) developed AKI. The cohort was 46% African and 49% European ancestry. MYH9 rs4821480 was significantly associated with AKI (OR 1.39, 95% CI 1.03-1.88, p=0.030). There was no confounding of this association by sex, race, ancestry, co-morbidities, diabetes, or Injury Severity Score (OR 1.49, 95% CI 1.09-2.04, p=0.011). APOL1 rs73885319 did not show a significant association with AKI in the AA population (OR 1.40, 95% CI 0.78-2.39, p=0.256).

Conclusions: MYH9 rs4821480 was significantly associated with AKI after severe trauma indicating possible genetic overlap between AKI and CKD. The function of rs4821480, an intronic SNP, is not yet known, though MYH9 encodes a protein that may be involved in podocyte structure and function. An association of AKI with APOL1 rs73885319 could not be ruled out given the limited AA sample size. Genetic risk factors offer the potential to improve AKI risk stratification and better elucidate AKI pathogenesis.

Funding: ORTHO NIH Support - P05-HL60290, P01-HL079803, K12-HL090021

FR-PO1055

Genetic Variation in SLC22A2–4Cation Transporter 2 (OCT2) Influences Cisplatin (CDDP)-Induced Nephrotoxicity in Cancer Patients

Josee Bouchard,1 Ravindra L. Mehta,2 Minoru Yoshida,2 Akinoobu Hamada,3 Hideyuki Saito,1 Pharmacy, Kumamoto Red Cross Hospital, Kumamoto, Japan; 2Medical Oncology, Kumamoto Red Cross Hospital, Kumamoto, Japan; 3Pharmacy, Kumamoto University Hospital, Kumamoto, Japan.

Background: CDDP has been a mainstay for chemotherapy of multiple solid tumors. However, CDDP is clinically often complicated due to its dose-limiting nephrotoxicity. As the organic cation transporter SLC22A2–OCT2 is highly expressed in the basolateral membrane of proximal tubules, thereby being considered as a predominant transporter mediating active accumulation of CDDP in the kidney. Besides, single-nucleotide polymorphism (SNP) in the OCT2 808G>T (Ala270Ser) has been suggested to correlate with the reduced CDDP-induced nephrotoxicity. In this study, we explored the effect of 808G>T SNPs in the OCT2 gene on the adverse events and the systemic exposure of CDDP in cancer patients.

Methods: We evaluated 53 patients with urothelial, lung, esophagus, head-neck, stomach and mesotheliomas carcinomas who had been treated with CDDP at a dose of over 150 mg/m2. Genotyping was performed by using TaqMan SNP Genotyping Assays. The plasma concentration of CDDP was evaluated on day 3 and 6 after the treatment, as the free platinum level measured by ICP-MS. The toxicity grade was evaluated by CTC/AE version 4.0 criteria.

Results: The number of patients who had the OCT2 808G/G, GT and TT was 44, 9 and none, respectively. Differences in serum creatinine (SCr) levels between the baseline and cycle 1 in the patients with the GG showed an increase of 1.43 and 1.19, respectively. In the total treatment cycles, 12 patients (27%) with the GG experienced the toxicity whereas the patients with GT showed no apparent toxicity. While blood cell and platelet levels showed no difference between the both groups. On day 3, the plasma concentrations of CDDP in the patients with the GG and GT were 75 and 63 mg/mL, and on day 6, those in GG and GT were 70 and 57 mg/mL, respectively.

Conclusions: In conclusion, 808G>T SNP in the OCT2 gene appeared to be associated significantly with the CDDP-induced nephrotoxicity, but not with the pharmacokinetic profile of CDDP.

Funding: Government Support - Non-U.S.

FR-PO1056

Natural History of Acute Kidney Injury in Intensive Care Unit Patients: The Multicenter International O'Brien Center Registry

Josee Bouchard,1 Ravindra L. Mehta,2 Minoru Yoshida,2 Akinoobu Hamada,3 Hideyuki Saito,1 Pharmacy, Kumamoto Red Cross Hospital, Kumamoto, Japan; 2Medical Oncology, Kumamoto Red Cross Hospital, Kumamoto, Japan; 3Pharmacy, Kumamoto University Hospital, Kumamoto, Japan.

Background: AKI is a common and associated with increased mortality, there is a lack of multicenter prospective large databases on mild to severe AKI throughout the world.

Methods: We conducted a prospective observational study to determine the incidence of AKI in ICU patients in 13 countries using AKIN criteria and to characterize differences in etiology, clinical factors and process of care among patients.

Results: Between 2008 and 2011, 778 of 3879 critically ill patients (20%) had AKI during the first week of ICU admission, and 479 (62%) were enrolled in our registry. Mean age was 59±17 yrs, 69% were male, 66% were non-caucasian, 16% had CKD, 30% were mechanically ventilated, 26% on pressors, 42% on diuretics and 65% were oliguric. Mean SOFA score was 5±4.1. Pre-renal factors were the most common risk factors for AKI (64%). 16% required renal replacement therapy (RRT). Continuous RRT, intermittent hemodialysis and sustained low-efficiency dialysis were used in 51%, 26% and 20% of patients, respectively, while peritoneal dialysis was used in 3%. 15% of patients were dialysis-dependent at hospital discharge. Overall hospital mortality was 19% (33% in patients who required RRT vs. 17% in patients who did not require RRT; p=0.005).

Conclusions: Genetic risk factors for hospital mortality among AKI patients included use of pressors (OR 1.80; 95%CI 1.01-3.19; p=0.04) and mechanical ventilation (OR 5.75; 95%CI 2.86-
Effect of perioperative SUA on postoperative NGAL levels

Conclusions: These data provide biomarker evidence of early renal parenchymal damage associated with elevated SUA in patients undergoing cardiac surgery.

FR-PO1058
Risk Factors and Etiology of Acute Kidney Injury in Intensive Care Unit Patients: The Multicenter International O’Brien Center Registry

Background: AKI is frequent in ICU patients and is associated with increased morbidity and mortality. However, there is a lack of information on the risk factors and etiologies of AKI across the world.

Methods: We conducted a prospective observational study to compare etiology of AKI and risk factors for AKI in ICU patients with and without AKI in 13 countries.

Results: Between 2008 and 2011, 778 of 3879 patients (20%) developed AKI during their ICU stay. AKI incidence was 20% and was associated with a 19% mortality rate. This multicenter multinational registry also provides a contemporary overview of clinical factors and management of AKI in ICU worldwide.

Conclusions: We describe an ongoing multicenter multinational registry of AKI in ICU patients. Risk factors and etiologies of AKI differed considerably between emerging and developed countries. These comparisons are useful to establish preventive and therapeutic strategies to improve AKI outcomes.

Funding: Private Foundation Support, Clinical Revenue Support

FR-PO1059
Study on the Usefulness of Urinary Parameters in Early Detection of Acute Kidney Injury after Cardiac Surgery in Adults

Background: Acute kidney injury (AKI) is a common complication after cardiac surgery. Urinary liver-type fatty acid-binding protein (L-FABP) reflects the presence of renal tubular injury. The aim of this study was to evaluate the utility of urinary L-FABP compared with other urinary parameters for the early detection of postoperative AKI among adult patients undergoing cardiac surgery.

Methods: Adult patients undergoing cardiac surgery in our hospital were eligible for enrollment. A total of 85 patients were prospectively studied from August 2009 to October 2010. Patients who depended on chronic dialysis support, patients undergoing emergency operation (operation performed within 24 hours after admission) and patients who died within the first 24 h after surgery were excluded from this study. Patients were divided into the AKI and non-AKI groups according to whether they developed AKI within 48 h after surgery. Postoperative AKI was defined according to AKIN criteria.

Results: The urinary L-FABP level was significantly higher in the AKI group than in the non-AKI group at every time point, while other parameters did not show such tendency. The parameter with the largest area under the curve at every time point for predicting the onset of AKI was urinary L-FABP. On multiple logistic regression analyses, the urinary L-FABP level pre-operation and within the first 6 h after cardiac surgery was significantly associated with the onset of AKI.

Conclusions: Our study suggests that urinary L-FABP was a useful biomarker for early detection of AKI and was an early good predictor of the onset of AKI.

Funding: Private Foundation Support

FR-PO1060
Fibroblast Growth Factor 23 as a Biomarker in Acute Kidney Injury

Background: Fibroblast growth factor 23 (FGF-23) regulates phosphorous and vitamin D homeostasis. Elevated levels are independently associated with increased mortality in patients with chronic kidney disease and ESRD. Whether FGF-23 levels are elevated and associated with adverse clinical outcomes in patients with acute kidney injury (AKI) has not been studied.

Methods: We recruited 28 participants with AKI and 30 controls from the medical intensive care unit and general hospital wards of Columbus University Medical Center. FGF-23 levels were measured at baseline and repeated 5 days later (ImmunoPrep ELISA assay). The combined clinical outcome was death or need for renal replacement therapy (RRT).

Results: FGF-23 levels were significantly higher among participants with AKI than AKI controls (Median [IQR] 1546 [253-2988] and 263 [87-577] RU/ml, respectively, p<0.003). Among participants with AKI, baseline FGF-23 levels above versus below the median were associated with a significantly higher probability of death or need for RRT (log rank test, p=0.003).

Conclusions: FGF-23 should be measured in patients with AKI to identify those who are at higher risk of adverse outcomes and may benefit from early therapeutic intervention.

Funding: Private Foundation Support
Conclusions: FGF-23 levels are elevated in AKI and are associated with greater risk of death or need for RRT. Additional work is needed to determine potential mechanisms underlying these preliminary findings.

Funding: Private Foundation Support

FR-PO1061
Urinary IL-18 Is the Most Useful Early Predictive Biomarker of Contrast-Induced Nephropathy (CIN) on Chronic Kidney Disease (CKD) Stage 3 Patients in Comparison with NGAL and L-FABP

Background: Contrast-induced nephropathy (CIN) is the important cause of hospital-acquired acute kidney injury (AKI) on Chronic kidney disease (CKD) patients. However, some urinary biomarkers for AKI were reported to be high in CKD patients. Thus, sensitivity and specificity of these urinary biomarkers are not determined for the diagnosis of AKI in CKD patients. This study was designed to investigate whether human urinary interleukin-18 (IL-18), neutrophil gelatinase-associated lipocalin (NGAL), and liver-type fatty acid binding protein (L-FABP) are early predictive markers for CIN after coronary angiography in CKD patients.

Methods: 41 patients of CKD Stage 3 undergoing coronary angiography were enrolled. Urine samples were collected before, 3 h, 6 h, 24 h after coronary angiography and IL-18, NGAL, and L-FABP levels were measured using an ELISA kit. Urinary creatinine values were measured and the values of urinary biomarkers were created by the creatinine concentration because of urinary concentration. This study is in accordance with the Declaration of Helsinki (2002) and was approved by Kochi Medical School review boards. All patients provided written informed consent.

Results: gFGF (estimated glomerular filtration rate) decreased more than 10 ml/min in 14 patients (decreased gFGF group) and did not decrease in remaining 27 patients (non-decreased group) after coronary angiography. At 3 h, 6 h, and 24 h after the procedure, the ratio with the previous value of the urinary IL-18 and L-FABP were significantly increased in the decreased gFGF group, but not in the non-decreased group. In contrast, NGAL are rapidly increased in both group, however, no statistically significant difference of NGAL was observed between two groups. When we used uncorrected biomarker values by creatinine, the specificity and sensitivity were significantly decreased. Rather, more urinary IL-18 was better than urinary L-FABP on ROC analysis.

Conclusions: We conclude that urinary IL-18 could be early biomarkers of CIN in CKD Stage 3 patients.

FR-PO1062
Urine Biomarkers of Aminoglycoside Nephrotoxicity in Children Zubaida Al-Ismaili,1 Joseph V. Bonventre,2 Prasad Devarajan,3 Melissa Piccioni,3 Venkata Sabbisetti,2 Michael R. Bennett,2 Qing Ma,2 Michael Zappitelli,1 1McGill University, Canada; 2Cincinnati Children’s Hosp Med Center; 3Brigham and Women’s Hospital.

Background: Aminoglycosides (AG) are commonly used in children but are nephrotoxic. Acute kidney injury (AKI) biomarkers and serum Cystatin C (CysC) have not been validated for AG-AKI. We hypothesized that AKI biomarkers are diagnostic of AG-AKI and the association is stronger when defining AKI by CysC.

Methods: We reported on 86 prospectively studied AG treatments (tx) in children on non-critical care units. Daily urine neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) were measured. AKI was defined as 1)AKItrad (traditional): >50% or ≥27 umol/l rise from baseline in 3 months prior; 2)AKIfirst (same definition but baseline was SCR within 72h of AG start); 3)AKIcsyclic (AKI first, but CysC change instead). We calculated area under the curve (AUC) of a peak biomarker levels and b)Tx day 1 or 2 levels to predict AKI by all 3 definitions. We calculated Spearman correlation between peak biomarker levels and days with AKI.

Results: Mean±SD age and its duration were 8.3±4.9 yrs and 8.4±8.8 days; 51% were boys, 74% on oncology wards, 77% subgammac. 53% developed AKItrad, 19% AKIfirst, 21% AKIcsyclic. AUC’s to predict AKItrad using Peak NGAL, IL-18 and KIM-1 were 0.53, 0.46, 0.63; to predict AKIfirst: 0.52, 0.44, 0.61; for AKIcsyclic: 0.51, 0.54, 0.52. Combined AUC’s (logistic regression) to predict AKItrad, AKIfirst and AKIcsyclic including all 3 Peak biomarkers were: 0.61, 0.57, 0.68. AUC’s to predict AKItrad using Tx days 1 or 2 NGAL, IL-18 and KIM-1 were: 0.43, 0.35, 0.56 for AKItrad; 0.23, 0.32, 0.52. The combined AUC’s to predict AKItrad, AKIfirst and AKIcsyclic including all 3 Tx 1-2 biomarkers were: 0.55, 0.69, 0.79. Only Peak KIM-1 on treatment correlated with number of days with AKI (r = 0.23, p = 0.03).

Conclusions: urine KIM-1 was more strongly associated with AG-AKI and AKI severity. Combining information from multiple biomarkers enhances AG-AKI and AKI biomarker associations were strongest when defining AKI by CysC.
GFR estimates were seen, but accuracy of the GFR estimates was not compared to a reference method such as creatinine clearance (ECC) or inulin clearance.

Methods: Retrospectively we analyzed the accuracy of creatinine-based MDRD formula and cystatin c based Larsson, Behring and Hoek formulae with ECC as reference method in ICU patients. Correlation between estimated GFR and ECC-based GFR was analyzed by Bland Altman statistics. When ECC was < 20 ml/min, mean area and creatinine clearance was used as reference value as recommended (K/DOQI).

Results: 47 observations were recorded in 32 ICU patients. Mean difference between ECC and GFR estimates for MDRD formula was 22 ml/min (95%CI 15.8 - 28.9), for the cystatin C based formula (bias 0.07 ml/min (95%CI -9.47 - 3.33) (Behring), 0.82 ml/min (95%CI -7.22 - 5.58) [Larsson] and 2.19 ml/min (95%CI -4.07 - 8.45) [Hoek]. Bland alman analysis revealed significant overestimation of GFR by the MDRD formula (bias 22.4; p<0.001), but no statistical significant difference between reference method and cystatin C based GFR estimates. Limitation of the study was its retrospective character.

Conclusions: The MDRD formula significantly overestimates GFR in ICU patients and should be avoided, while cystatin c based formula provide more accurate estimates of renal function.

FR-PO1066

The Epidemiology of Acute Hemodialysis in Pennsylvania, 2005-2007
Sarah Ramey, Elan Cohen, Mark L. Unruh, Amber E. Barnato. University of Pittsburgh School of Medicine.

Background: Although the US Renal Data System provides detailed information about patients with end-stage renal disease (ESRD) undergoing chronic hemodialysis (HD), little is known about the epidemiology of acute HD. Here we describe the epidemiology of acute HD, including patient- and hospital-level predictors, in >3 million consecutive inpatient admissions.

Methods: We conducted a retrospective cohort analysis of all adult acute-care hospitalizations in Pennsylvania (PA) from October 2005 to December 2007 using data from the PA Health Care Cost Containment Council. We defined acute HD by the ICD-9-CM procedure code for HD in any field, excluding patients with diagnosis codes indicating imminent, current, or prior ESRD (including history of kidney transplant) and patients with procedure codes for peritoneal dialysis or kidney transplant. We descriptively summarized the characteristics of the acute HD patients and then, controlling for Charlson Comorbidity Index, used multivariable hierarchical logistic regression to identify patient- and hospital-level independent predictors of acute HD.

Results: Among 3,184,361 admissions of non-ESRD patients age ≥21 in PA in this period, 7960 patients had 8888 admissions in which they received acute HD, yielding an annual incidence of 4.3 admissions with acute HD per 10,000 non-ESRD PA residents age ≥21. Independent predictors of acute HD were:

<table>
<thead>
<tr>
<th>Adjusted odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥65 (per yr)</td>
<td>1.01 (0.91-1.11)</td>
</tr>
<tr>
<td>Age ≥65 (per yr)</td>
<td>0.96 (0.96-0.97)</td>
</tr>
<tr>
<td>Female</td>
<td>0.80 (0.76-0.83)</td>
</tr>
<tr>
<td>Black</td>
<td>1.42 (1.33-1.51)</td>
</tr>
<tr>
<td>Uninsured (vs. Medicare / commercial)</td>
<td>0.58 (0.44-0.72)</td>
</tr>
<tr>
<td>Uninsured (vs. Medicare)</td>
<td>0.48 (0.36-0.65)</td>
</tr>
</tbody>
</table>

Primary diagnoses (top 3 by 2-score)

- Acute / unspecified renal failure (P value 0.54; odds ratio 5.84)
- Sepsis (P value 0.04; odds ratio 20.4)
- Complication of device, implant or graft (P value 0.03; odds ratio 15.3)

Admission to hospital with high-intensity end-of-life care (P value 0.02; odds ratio 2.07)

- 17.1% of patients were admitted with high-intensity end-of-life care

Conclusions: We have provided one of the first descriptions of the epidemiology of acute HD in a large US population of age ≥21. Receipt of acute HD varied by age, sex, race, insurance status, and hospital treatment intensity. Future research should elucidate reasons for such marked variations in provision of care thought to be necessary for patients with acute kidney failure.

Funding: NIDDK Support, Other NIH Support - T32 MH109986 R01 AG035112, Private Foundation Support

FR-PO1067

Fluid Balance and Acute Kidney Injury: A Prospective Observational Study
Ganesh Kambhampati,1 Uma Krishna Pakkivenkata,1 Amer Abouhamze,1 Mourad Alsabagh,1 Abdo Asmar,1 Gurjit Dhatt,1 Intizam M. Aher,1 Noel I. Eijaz,1 Amir Ahsan Arif,1 A. Ahsan Ejaiz,1 1Division of Nephrology, Hypertension and Transplantation, University of Florida, Gainesville, FL; 2Department of Surgery, University of Florida, Gainesville, FL.

Background: It is unclear whether FB is the cause or result of AKI. We therefore performed a prospective, observational study to investigate this relationship.

Methods: Adult, non-transplant cardiovascular surgery patients were divided into quartiles based on FB status. Incidences of AKI, urine NGAL and IL-18, serum cytokine and pro-inflammatory enzymes. However clinical studies are lacking.

Results: We used propensity score analysis to evaluate the association of AKI and low HDL level.

<table>
<thead>
<tr>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.96</td>
</tr>
<tr>
<td>CAD</td>
<td>1.24</td>
</tr>
<tr>
<td>K/D ratio</td>
<td>2.81</td>
</tr>
<tr>
<td>DM</td>
<td>1.28</td>
</tr>
<tr>
<td>HTN</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Propensity score analysis showed that patients with low HDL level had 40% higher odds of having AKI (OR 1.41 with 95% CI 1.14-1.73).

Conclusions: We conclude that low HDL level is associated with higher odds of AKI postoperatively. Although, low HDL was very common in this population, only 3% were on niacin. It needs to be determined if use of niacin preoperatively will decrease prevalence of AKI.

FR-PO1069

Acute Kidney Injury in Hospitalised Patients Is Under-Recognised and Under-Treated

Background: Acute kidney injury (AKI) is associated with significant morbidity and mortality. In 2009, the National Confidential Enquiry into Patient Outcomes and Death (NCEP) identified significant failings in the recognition and management of hospitalised patients with AKI. Our aim was to explore the prevalence, recognition and quality of early management of level 1 patients with AKI in a London teaching hospital.

Methods: During a 7 day period in May 2011, electronic patient records of all level 1 medical and surgical patients were screened daily. AKI was defined by KDIGO criteria. Independent assessors reviewed the medical notes using similar criteria as the NCEP report.

Results: 99 out of 1379 level 1 patients (7.2%) had AKI (53% male; mean age 72.6 years; 64% medical and 36% surgical patients). 93% of these patients had risk factors for AKI (median number 2 [range 0-6]).

Conclusions: Positive FB in the first 24-hours from initiation of surgery may be an independent risk factor for postoperative AKI. Positive FB is an excellent and simple predictive marker that precedes the rise in Scr.

FR-PO1086

Preoperative Low HDL Level Is Associated with Increased Risk of Acute Kidney Injury Postprocedure/Surgery for Peripheral Vascular Disease
Pradeep Arora,1,2 Nauman Tahir,1 Pooja Mahajan,1 James W. Lohr,1,2 Hassan H. Dosluoglu,1 Nader Nader.1 1Department of Medicine, SUNY, Buffalo, NY; 2Division of Nephrology, VAMC, Buffalo, NY; 3Department of Anesthesiology, VAMC, Buffalo, NY; Department of Surgery, VAMC, Buffalo, NY.

Background: High-density lipoproteins (HDL) have been shown to reduce organ injury and mortality in animal models of shock via modulation of the expression of adhesion molecules and pro-inflammatory enzymes. However clinical studies are lacking.

Methods: We studied the association of HDL level and acute kidney injury (AKI) in patients, who have undergone lower extremity revascularization surgery as the primary procedure at VA Western New York Healthcare System between January 1, 2001 and December 31, 2009. Patients with primary amputation or ESRD were excluded. All data were collected prospectively. Patients were divided in 2 groups; Group A included patients who had preoperative HDL level <40 mg/dl and group B who had HDL >40 mg/dl. Multivariate and propensity score analyses were performed to evaluate the association of AKI and low HDL level.

Results: Study population included 740 patients, 61% of patients had low HDL. Patients in group A were more likely to have hypertension, diabetes, high LDL level, and CKD compared with group B patients. Patients in Group A were significantly more likely to be on ACEI/ARB and statins preoperatively. 9.5% of patients developed AKI by AKIN criteria and 2.7% by RIFLE criteria. Multivariable logistic model results are shown in the table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.95</td>
<td>0.94-0.96</td>
</tr>
<tr>
<td>CAD</td>
<td>1.24</td>
<td>0.76-2.04</td>
</tr>
<tr>
<td>K/D ratio</td>
<td>2.81</td>
<td>1.65-4.77</td>
</tr>
<tr>
<td>DM</td>
<td>1.28</td>
<td>0.78-2.08</td>
</tr>
<tr>
<td>HTN</td>
<td>0.91</td>
<td>0.53-1.53</td>
</tr>
</tbody>
</table>

Conclusions: Positive FB in the first 24-hours from initiation of surgery may be an independent risk factor for postoperative AKI. Positive FB is an excellent and simple predictive marker that precedes the rise in Scr.
Data of 266 AKI patient-days were available. 22 were excluded due to limitations of care identified by the treating team. Of these, 83% had documented management plan regarding AKI, 85% had an early senior review, and 54% had a urine dipstick record. Management was worse when AKI was not recognised by the treating medical team (Table 1). On 3 occasions, the independent assessors noticed potentially life threatening problems and intervened.

<table>
<thead>
<tr>
<th>Table 1: Early management based on recognition of AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recognised AKI (n=144) vs Unrecognised AKI (n=100)</td>
</tr>
<tr>
<td>Assessment of fluid status</td>
</tr>
<tr>
<td>Complete fluid balance chart</td>
</tr>
<tr>
<td>Discontinuation of nephropathy prevention guideline</td>
</tr>
<tr>
<td>Admission with contrast nephropathy prevention guideline</td>
</tr>
</tbody>
</table>

Conclusions: AKI is prevalent among hospitalised non-critically ill patients. Only 60% of AKI was recognised by the treating team. Even when recognised, a significant proportion of AKI patients do not receive appropriate simple early management. More education and awareness about AKI is necessary, especially among junior medical staff.

FR-PO1072

Does Acute Kidney Injury Cause or Worsen Chronic Kidney Disease?

Mark Dominick Uniacke,1 Robert Lewis,1 Scott Harris,2 Paul J. Roderick,2

1Wessex Renal and Transplantation Service, Portsmouth, United Kingdom; 2Public Health Sciences and Medical Statistics, University of Southampton, United Kingdom.

Background: The impact of mild to moderate AKI on baseline renal function remains unknown. We have therefore studied AKI in hospitalised patients and explored its effect on the development of new CKD and progression of known CKD.

Methods: Prospective single centre observational study. Subjects were recruited over 17 months from Nov, 2009 to April 2011 from unslected admissions to a general hospital with a catchment population of 600,000. Two groups were recruited - Group 1: with previously normal kidney function developing AKI and Group 2: with background of CKD developing AKI. Baseline kidney function was identified from measurements of eGFR from the previous year. AKI was defined by elevations in serum creatinine from baseline using the AKIN criteria. Recovery of function is defined as a return to within 5ml/min of baseline eGFR. Hospital outcomes were recorded and function was reassessed after 6 months using the same laboratory. 6 month follow-up is ongoing and will finish in October 2011. Preliminary 6 month follow-up data for n=99 is presented here.

Results: 375 patients were recruited for follow up (Group1 n=189, Group 2 n=186). Mean age for both groups was 62 years (range 18-97, 53% male, 47% female). In hospital mortality was 3.2% in Group 1 and 9.2% in Group 2. At discharge failure to return to baseline function was seen in 54.9% of Group 1 and 34.3% of Group 2. Failure to recover was seen across all AKI stages even mild AKI stage 1. Preliminary data shows that after six months from AKI, recovery is not complete in 50% (21/42) of Group 1 and 27.6% (13/47) of Group 2. This failure to recover at six months is again seen across all AKI stages. So far we have found a high readmission rate of 41.5% in both groups during follow up with 14% having at least one more AKI.

Conclusions: AKI across all AKI stages appears to be failing to recover after six months in some patients and may be resulting in incidence CKD and contributing to further irreversible loss in some existing CKD patients. These findings have important implications for clinical practice and population health.

Funding: Private Foundation Support

FR-PO1073

Hemoglobin and Acute Kidney Injury after Noncardiac Surgery

Michael Walsh,1 Philip J. Devereaux, Amit X. Garg,2 Daniel Sessler,1,3 McMaster;1 UWO;2 Cleveland Clinic.

Background: Over 200 million adults undergo noncardiac surgery annually and it is frequently complicated by acute kidney injury (AKI). We assessed the association between postoperative changes in hemoglobin (Hgb) and AKI.

Methods: All patients undergoing noncardiac surgery at the Cleveland Clinic between January 2005 and December 2009 with at least one preoperative and one postoperative creatinine within 7 days of surgery, a preoperative estimated glomerular filtration rate of >60 ml/min, and did not undergo a urologic procedure were eligible. AKI was defined as a >1.5 fold increase or >0.3 g/dL increase in creatinine within 7 days. Change in Hgb was the difference in the preoperative value and lowest value within the first 24 hours of surgery. All associations were assessed using logistic regression adjusted for age, sex, Risk Stratification Index (a validated score for mortality), surgery type, preoperative blood pressure, baseline Hgb and intraoperative transfusions.

Results: 41,498 patients met the eligibility criteria. The mean (standard deviation) age was 56 (16) years, creatinine was 0.82 (0.18) mg/dL and preoperative Hgb was 13.1 (2.0) g/dL. AKI occurred in 2654 patients (6.4%). AKI was associated with 30 day mortality with an adjusted odds ratio of 2.73 (95% confidence interval CI)2.24 to 3.34. The median change in Hgb was a 2.2 g/dL decrement. Both preoperative Hgb <12 g/dL and a drop in Hgb of ≥2 g/dL was associated with a graded increase in the risk of postoperative AKI (Table 1). These associations were consistent when AKI was defined by more marked changes in creatinine.

Table 1. Association between AKI and drop in hemoglobin.

<table>
<thead>
<tr>
<th>Baseline Hemoglobin (g/dL)</th>
<th>Adjusted Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Drop Reference</td>
<td>1.00 (0.96-1.04) 0.23</td>
</tr>
<tr>
<td>&lt;1.99</td>
<td>1.04 (0.96-1.12) 0.23</td>
</tr>
<tr>
<td>2.00-2.99</td>
<td>1.15 (1.10-1.21) 0.001</td>
</tr>
<tr>
<td>3.00-3.99</td>
<td>1.22 (1.12-1.33) 0.001</td>
</tr>
<tr>
<td>≥4.00</td>
<td>1.29 (1.20-1.39) 0.001</td>
</tr>
</tbody>
</table>

Conclusions: AKI is an independent risk factor for postoperative mortality. Preoperative Hgb and early AKI in Hgb are independent risk factors for AKI and may be modifiable.

Funding: Government Support - Non-U.S.
FR-PO1074

Hibiki Shinjo,1 Waichi Sato,1 Tomoki Kosugi,1 Hiroki Hayashi,2 Shoichi Maruyama,3 Enyu Ima1, Yukio Yuzawa2, Seichi Matsuo.1  
1Division of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; 2Division of Nephrology, Fujita-Health University, Toyoake, Japan.

Background: Acute kidney injury (AKI)-associated hospital mortality can strikingly increase in comparison with patients without AKI. In order to have a uniform standard for classify AKI, the AKI Network (AKIN) group has proposed modifications to the previous criteria referred to the RIFLE and suggested staging of AKI based on changes in creatinine (Cr) within 48 hours. In actual clinical practice, however, the peak of Cr may be missed. More recently, Kidney Disease: Improving Global Outcomes (KDIGO) group proposed a new definition for AKI, which is defined as a abrupt reduction within 7 days in kidney function. We evaluated the incidence of AKI and compared the ability of the AKIN (48h) and KDIGO (7d) criteria in predicting hospital mortality of intensive care unit (ICU) patients.

Methods: We performed a retrospective cohort study on 2582 patients admitted between June 2005 and May 2009 in an ICU of the Nagoya university hospital. Chronic kidney disease patients undergoing dialysis and renal transplant patients were excluded.

Results: The KDIGO and AKIN criteria were total incidences of AKI (38.3 vs. 29.6%). KDIGO criteria significantly increased the number of patient classified as AKI in all stage compared with AKIN criteria (stage 1; 24.7 vs. 20.7%, stage2; 6.2 vs. 3.4%, stage3; 7.6 vs. 5.5%). 238 patients (9.2%) that classified as no AKI by AKIN and AKI by KDIGO, were similar hospital mortality for Stage 1 by both criteria. In both criteria, AKI were associated with hospital mortality after adjusting for multiple covariates. And the odds ratio was elevated, according to the severity of AKI. The area under the receiver operator characteristic curve for hospital mortality estimated by KDIGO and AKIN criteria were 0.76 and 0.73.

Conclusions: Both criteria might be reliable for predicting severity and outcome of AKI. Particularly, 7d time window in KDIGO improve on the sensitivity of the AKI diagnosis and the ability in predicting hospital mortality.

FR-PO1075

The Epidemiology of Contrast-Induced Nephropathy in the Era of Hydration Protocols  
Corinne E.A. Balemans,1,2 Louis J.M. Reichert,1 Jack F. Wetzelz.1  
1Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands; 2Nephrology, Rijnstate Hospital, Arnhem, Netherlands.

Background: Acute renal failure can complicate the use of iodinated contrast media. This contrast-induced nephropathy (CIN) is associated with marked morbidity and mortality. Current guidelines advise identification of high risk patients and hydration as preventive measure. We evaluated the incidence of CIN and determined risk factors associated with CIN in patients receiving intravenous contrast and treated according to the guidelines.

Methods: All patients with an eGFR<60ml/min/1.73m2 were seen at the outpatient clinic. Patients were stratified for the risk of CIN: high risk (HR) or low risk (LR) based on absolute GFR (MDRD x BSA/1.73 m2) and the presence of risk factors; diabetes, peripheral arterial disease, heart failure, age, anemia and use of diuretics and/or NSAID’s. HR patients were hydrated with isotonic saline 1000ml before and 1000ml after contrast exposure. Serum creatinine was measured 3-5 days later, CIN was defined as a 25% rise on absolute GFR (MDRD x BSA/1.73 m2).

Results: We evaluated 1420 procedures performed in 1111 patients in the period from June 2005 and May 2009 in an ICU of the Nagoya university hospital. Chronic kidney disease patients undergoing dialysis and renal transplant patients were excluded.

Conclusions: The KDIGO and AKIN criteria were total incidences of AKI (38.3 vs. 29.6%). KDIGO criteria significantly increased the number of patient classified as AKI in all stage compared with AKIN criteria (stage 1; 24.7 vs. 20.7%, stage2; 6.2 vs. 3.4%, stage3; 7.6 vs. 5.5%). 238 patients (9.2%) that classified as no AKI by AKIN and AKI by KDIGO, were similar hospital mortality for Stage 1 by both criteria. In both criteria, AKI were associated with hospital mortality after adjusting for multiple covariates. And the odds ratio was elevated, according to the severity of AKI. The area under the receiver operator characteristic curve for hospital mortality estimated by KDIGO and AKIN criteria were 0.76 and 0.73.

FR-PO1076

Development of Microalbuminuria Following Hematopoietic Stem Cell Transplantation Is Associated with Near-Term Loss in Renal Function and Mortality  
Taku Morito,1,2 Minoru Ando,1 Ken Tsuchiya,1 Kosaku Nitta,1  
1Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Bunkyo-ku, Tokyo, Japan; 2Department of Internal Medicine, Tokyo Women’s Medical University, Shinjuku-ku, Tokyo, Japan.

Background: Microalbuminuria is a risk factor for overt kidney disease (OKD), cardiovascular disease and mortality. Therefore, the presence of microalbuminuria could be a sign of the future OKD or death in the setting of hematopoietic stem cell transplantation (SCT).

Methods: A 1-year prospective cohort study was conducted in 29 patients (46±12.5 years) receiving allogeneic myeloablative SCT. The urinary albumin to creatinine ratio (ACR) was consecutively measured before conditioning therapy (baseline), at the time of SCT, and on days 7, 14 and 28 after SCT. Microalbuminuria was defined as ACR ≥30mg/gCr. OKD was defined as persistent loss in renal function, that is, eGFR <60ml/min/1.73m2. Cumulative incidence of OKD or mortality or both was assessed by the Kaplan-Meier analyses. Multivariate Cox regression analysis was used to calculate the HR of developing OKD and mortality for microalbuminuria on 1 month (day 28) after SCT. Patients with OKD at baseline and those who died by 1 month after SCT were excluded.

Results: The prevalence of microalbuminuria was 6.9% at baseline, and increased to 62.1% at the day of SCT. It varied among the time points; 62.1% on day 7, 48.3% on day 14 and 51.7% on 1 month, respectively. Cumulative incidence of OKD or mortality or both was significantly higher in the group with ‘microalbuminuria on 1 month’.

In addition, the presence of ‘microalbuminuria on 1 month’ was significantly associated with either the development of OKD or death (adjusted HR 17.4; 95% CI 3.66 to 134.9, P=0.0001).

Conclusions: The presence of ‘microalbuminuria on 1 month’ is a promising predictor of near-term development of loss in renal function and mortality in the setting of SCT.

FR-PO1077

Study of Acute Kidney Injury (AKI) on Admission to the Emergency Assessment Unit (EAU)  
Nihal Y. Abouaoud,1 Lee J. Dowson, Ewa Werpachowska, Rhys Lodwick, Medicine, Royal Liverpool University Hospital, West Midlands, United Kingdom.

Background: Mortality rates from AKI are increasing in hospitalised patients in the UK because of increased rates of sepsis and circulatory failure. The National Confidential Enquiry into Patient Outcome and Death (NCEPOD) report has shown that most patients admitted with AKI were not assessed properly especially by trainees. Onward referral to renal physicians was also delayed. In this study, we evaluated the incidence of AKI in our EAU and estimated the risk of death among patients admitted according to the definition of AKI. Also for new patients admitted to EAU, we aim at implementing a new risk assessment tool to score patients on admission for the risk of developing AKI and hence establish their level of care.

Methods: All patients admitted to the EAU with a serum creatinine(S.Cr) >100mmol/L between April 2010 and October 2010 were screened. Patients with known chronic kidney disease (CKD) or unknown baseline Cr were excluded. 1350 patients were found to match the inclusion criteria from which 54 patients were randomised. Medical notes results and radiological investigations were extracted and examined for the purpose of the study, 23 out of 54 patients died because of the original presentation or secondary to AKI per se. The characteristics of those patients who died (group D) were compared with those of survivors (group S). We also assessed compliance with NCEPOD guidelines.

Results: Group D was significantly older than group S. There was no significant difference as regards their demographic, clinical or laboratory parameters apart from admission S.Cr, eGFR, follow up Cr, S.urea, S.albumin and platelets that were significantly deranged in group D. The Hazard Ratio (HR) of recognition of AKI [HR=4.47 (CI; 0.3-3.5), its cause [HR ≥30mg/gCr: 0.25-21.2, p=0.001] and management [HR=0.52 (CI; 0.05-5.12), p=0.0001] showed very highly significant bivariate correlation to group D.

Conclusions: The cohort with a poor outcome was correctly identified and managed according to NCEPOD guidance implying that further steps are required. Development of a new scoring system for diagnosis of AKI in high risk patients may help target interventions.
FR-PO1078

Nutritional Support Influences Outcomes in Intensive Care Unit (ICU) Patients with Acute Kidney Injury (AKI)

Soo Young Yoon, Sharon Soroko,1 Glenn M. Chertow,2 Jonathan Himmelfarb,2 Emil P. Paganini,3 Ravindra L. Mehta,1 Univ California San Diego, San Diego, CA; Stanford Univ, Palo Alto, CA; Vanderbilt Univ, Nashville, TN; Cleveland Clinic Foundation, Cleveland, OH.

Background: Nutritional support (NUTS) is commonly utilized to manage ICU patients however it is unclear whether enteral (EN) or parenteral nutrition (PN) influence outcomes in AKI. We assessed the pattern of NUTS and its relationship to outcomes in AKI patients enrolled in the PICARD study (KI, 2004, 66:1613-1621). We hypothesized that NUTS would be associated with improved survival and shorter ICU duration.

Methods: We analyzed data from 615 of the 618 patients who stayed in ICU for 48 hours. We assessed mode of nutrition (MON), duration, amount and timing for NUTS and examined the relationship to fluid overload >10% body weight (FLBW), hospital mortality (HOM), ICU length of stay (LOS) and dialysis requirement at hospital discharge (DIAL) in survivors.

Results: Among 615 patients, 199 ate orally without any NUTS (Oral), 76 received no oral or NUTS (None), 183 received EN, 66 PN, and 91 both EN+PN. A subset of the NUTS patients had oral intake for part of their ICU stay (EN n=81; PN n=22; EN+PN n=27).

Discussion: In patients with AKI, the ICU nutritional intake is associated with worse outcomes. Patients who can eat have the best outcomes regardless of underlying severity of kidney disease and underlying co-morbidities.

Funding: NIDDK Support

FR-PO1079

Dialysis Requirement and Nutritional Support in Intensive Care Unit (ICU) Patients with Acute Kidney Injury (AKI)

Soo Young Yoon,1 Sharon Soroko,1 Glenn M. Chertow,2 Jonathan Himmelfarb,2 Emil P. Paganini,3 Ravindra L. Mehta,1 Univ California San Diego, San Diego, CA; Stanford Univ, Palo Alto, CA; Vanderbilt Univ, Nashville, TN; Cleveland Clinic Foundation, Cleveland, OH.

Background: Nutritional support (NUTS) is commonly utilized to manage ICU patients with AKI due to concerns of solute load and fluid accumulation. We evaluated the influence of dialysis requirement (DIAL) on the application of NUTS in AKI patients enrolled in the PICARD study (KI, 2004, 66:1613-1621). We hypothesized that RRT would allow more nutrition to be given and improve outcomes.

Methods: We analyzed data from 610 of 618 patients who stayed in ICU >48 hrs. We assessed the nutritional intake for patients with NUTS (ORAL: Oral-enteral; PN: parenteral; EN+PN: both) and None (no nutrition at all). We determined the predictive power of AKIN classification for 180-day in-hospital mortality after adjusting relevant factors.

Results: Overall, 49.2%, 41.4% and 9.4% of patients were each classified in younger-old, old and oldest-old categories respectively. Cox proportional hazard model, FLBW (HR 1.249, p=0.000), and single NUTS mode (HR 1.899 in EN and 2.863 in PN vs. Oral) predicted mortality. Nutritional duration and calorie ratios were not independent predictors for mortality. Transitions in NUTS modality were associated with higher risk of adverse outcome. Cox multivariate-adjusted analysis showed that patients with AKIN stage I and II had similar outcome across all age categories. In addition, younger-old patients with AKIN III had worse outcome than the oldest-old with stage I insult (HR 1.902 vs 1.742), while the old and the oldest-old with stage III insult denoted a higher mortality.

Conclusions: This study exemplifies the aging effect on utility of AKIN staging for AKI in predicting hospitalization outcome in geriatric population, especially during milder form of AKI.

Funding: No financial disclosures reported.

FR-PO1080

Age Modifies the Predictive Effect of AKIN Staging for Hospitalization Outcome in Elderly Patients Undergoing Major Surgery

Chia-Ter Chao, Vincent Wu. Division of Nephrology, Department of Internal Medicine, National Taiwan University Hospital, Taiwan.

Background: Acute kidney injury (AKI) occurs with increasing frequency in patients admitted to intensive care unit (ICU), and elderly patients are particularly vulnerable. AKIN (AKI network) classification is widely used to gauge severity of AKI, but has not been formally tested in geriatric patients for its efficacy.

Methods: We conduct a multicenter, prospective observational study, based on the NSARF (National Taiwan University Hospital Surgical ICU Associated Renal Failure) database. A total of 4964 elderly patients (defined as age more than 65) who developed AKI in our institution were formally assigned for major surgeries between 1 Jan, 2002 and 31 Dec, 2008 were enrolled, divided into 3 categories according to age: younger-old (65-75), old (75-85) and oldest-old (>85). Demographic profiles, comorbidities, types of surgery, and ICU treatment variables were all collected. We determined the predictive power of AKIN classification for 180-day in-hospital mortality after adjusting relevant factors.

Results: Overall, 49.2%, 41.4% and 9.4% of patients were each classified in younger-old, old and oldest-old group. In-hospital mortality increased stepwise from 15% to 16% and 21% as age rose. Age, AKIN stage (hazard ratio (HR) 1.707 for stage III, p<0.001), hypertension (HR 1.345, p<0.001), diabetes mellitus (HR 1.348, p<0.001) and treatment related factors (tracheostomy, Swan-Ganz catheter use) were associated with higher risk of adverse outcome. Cox multivariate-adjusted analysis showed that patients with AKIN stage I and II had similar outcome across all age categories. In addition, younger-old patients with AKIN stage III had worse outcome than the oldest-old with stage I insult (HR 1.902 vs 1.742), while the old and the oldest-old with stage III insult denoted a higher mortality.

Conclusions: The finding of this study exemplifies the aging effect on utility of AKIN staging for AKI in predicting hospitalization outcome in geriatric population, especially during milder form of AKI.

Funding: No financial disclosures reported.

FR-PO1081

Border-Crossers' Nephropathy: The Risk of Coming to America

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Background: The 380 mile border between Mexico and Arizona has become a prime entry point for persons wanting to enter the United States. Because of increased enforcement, these individuals usually attempt to cross the border in desolate areas where they may wander for days without food or water before being “rescued”. The Summer of 2010 was one of the five hottest summers on record. During 2010, 232 individuals died trying to cross the into Arizona and 212,202 people were apprehended in the Tucson Sector. The University of Arizona Medical Center, South Campus is one of the closest tertiary care medical centers to the Mexican border. Detainees with medical problems are frequently brought to this campus. Herein we describe our experience with myoglobinuric acute kidney injury (AKI) in these border-crossers.

Methods: We reviewed the records of all individuals admitted between June 1-December 31, 2010 with AKI as defined by AKIN criteria who also had an elevation of CPK >1000 IU/L. We reviewed the age, gender, temperature, creatinine on presentation, urine output in first 24 hours, need for dialysis, length of stay and creatinine on discharge.

Results: During this time period 24 people were diagnosed with myoglobinuric AKI. On presentation all patients were vigorously resuscitated with either 0.9% NaCl or a mixture of NaCl and NaHCO3. The mean age was 31.7 years (range 18-53). 21 were men and 3 were women. They had wandered in the desert between 1 and 8 days with a mean of 3.9 days. Five had stage 1 AKI, 7 stage 2 and 12 stage 3. Three patients required dialysis. Only 1 patient was oliguric and only 1 had a temperature > 100.6 on arrival. CPKs ranged from 1-December 31, 2010 with AKI as defined by AKIN criteria who also had an elevation of CPK >1000 IU/L. We recorded the age, gender, temperature, creatinine on presentation, urine output in first 24 hours, need for dialysis, length of stay and creatinine on discharge.

Conclusions: This study describes the larger series of myoglobinuric AKI reported in border-crossers. The presumed etiology is excessive heat combined with volume depletion and stressful exercise. We have coined the term “border-crossers nephropathy” for this disorder. Many of these patients may develop CKD and require nephrologic care in their home country.

Funding: Clinical Revenue Support

FR-PO1082

Acute Kidney Injury, Length of Stay and In-hospital Mortality in Critically Ill Cirrhotic Patients: A Cohort Study

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Background: Recently, members of the Acute Dialysis Quality Initiative (ADQI) and the International Ascites Club (IAC) proposed the definition of AKI in cirrhosis as an increase in serum creatinine of >50% from baseline or in serum creatinine of >2.64 mmol/l (>0.3 mg/dl) in <48 h. The aim of this study was to relate this classification to length of stay and in-hospital mortality in a cohort of critically ill cirrhotic patients.

Methods: One hundred eighty-two cirrhotic patients [mean age: 56 (12.2) years; 105 Male; 171 Caucasian; Child-Pugh score: 9.3 (2.4). Model for End-stage Liver Disease

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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Renal Function Estimation Equations Using Non-Steady State Serum Creatinine Estimate Better Among patients with Low Baseline Renal Function

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**Background:** Estimation of glomerular filtration rate (eGFR) in subjects with acute kidney injury (AKI) is problematic due to the non-steady state of serum creatinine (sCr). We examined the agreement of sCr based estimation equations with measured GFR (iGFR) during the immediate post-operative course in subjects undergoing nephrectomy.

**Methods:** We measured GFR using i-iothalamate renal clearance within a week of nephrectomy procedure. Only subjects with post-operative sCr rise of >0.3 mg/dl or higher were included in the analysis. We compared iGFR with eGFR using Pearson’s correlation (ρ), and concordance correlation coefficients (CCC) in subjects with or without baseline chronic kidney disease (CKD). CKD was defined as baseline eGFR < 60 ml/min/1.73 m². eGFR was calculated using MDRD 4-variable, CKD-EPI and Jelliffe estimation equations based on sCr drawn at the time of iGFR measurement.

**Results:** 69/90 subjects who underwent nephrectomy sustained post-operative sCr rise of ≥ 0.3 mg/dl or greater. Mean age was 61 ± 11, and 55/69 were male. Baseline sCr was 1.2 ± 0.4, and 25/69 (36%) had baseline CKD. The correlation of eGFR with iGFR was 0.76 ± 0.7, and 317 patients (67.5%) had RDW above the upper limit of normal (>14.5%). Patients with high RDW values had higher white blood cell (WBC) counts, and lower hemoglobin (Hb) and total cholesterol levels compared to those with normal RDW values. In addition, there were significant correlations between RDW values and WBC counts, Hb levels, and total cholesterol concentrations. However, these findings did not significantly vary in ages, gender, SOFA score, eGFR, albumin, and echocardiographic parameters between the two groups. Patients with high RDW values exhibited significantly higher 28-day mortality rates than patients with low RDW levels in Kaplan-Meier analysis (p<0.01). Univariate Cox proportional hazard analysis revealed that baseline RDW levels, SOFA score, mean arterial pressure, and total cholesterol concentrations were associated with mortality. In multivariate analysis, RDW value at CRRT initiation was a significant independent predictor of 28-day overall mortality after adjusting for other risk factors.

**Conclusions:** The results of this study suggest that RDW could be an additive predictor for overall mortality in AKI patients on CRRT.

**References:**


**FR-PO1085**

Analysis of Acute Kidney Injury and Its Prediction by Urinary Biomarkers in Adult Japanese ICU Patients

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**Background:** Acute Kidney Injury (AKI) is frequently complicated in critically ill patients in intensive care unit (ICU), and associated with increased mortality. This study aims to determine the clinical characteristics of AKI and to evaluate urinary biomarkers to predict de novo onset of AKI.

**Methods:** We prospectively collected data of patients admitted to the ICU in the Okayama University Hospital (Nov. 2010-May 2011). Patients already in end-stage renal disease and receiving renal replacement therapy (RRT) were excluded. Urine was collected within 24 h of ICU admission (Day 1). Urinary levels of neutrophil gelatinase-associated lipocalin (NGAL), NAG and albumin were determined and were normalized to creatinine levels.

**Results:** Of the 86 patients, 23 (26.7%) developed AKI based on the AKIN criteria. The mean age was 65 in non-AKI and 60 in AKI group and the mean baseline eGFR was 72 and 47 ml/min/1.73m², respectively. Surgical operation in the non-AKI (92%) and the AKI (74%) group was the leading cause of ICU admission. AKI was frequent in the recipients of liver transplantation (7 out of 9 developed AKI). In the AKI group, 2 patients (8.6%) required RRT and a patient died. Overall, the ratio of serum Cr at Day 3 to Day 1 after admission to ICU was positively correlated with urinary NGAL/Cr ratio (R=0.46, P=0.001) or NAG/Cr ratio (R=0.33, P=0.019). In 15 cases developing de novo AKI, the levels of urinary NGAL/Cr and NAG/Cr were significantly elevated compared with non-AKI group (126.2 vs. 40.3 µg/gCr, P=0.003; 58.9 vs. 13.8 µg/gCr, P=0.0001), but urinary levels of albumin/Cr ratio was not significantly elevated.

**Conclusions:** These findings suggest clinical significance of urinary NGAL and NAG as useful biomarkers superior to urinary albumin in predicting the onset of AKI in critically ill patients.
Kidney function should be regularly monitored during treatment besides checking time, subtle increments in creatinine values should not be taken lightly as they may reflect rather than elevation of Cr from baseline or BUN level.

Conclusions: We recognize the efficacy of vancomycin in treating different infections. Kidney function should be regularly monitored during treatment besides checking vancomycin trough levels for avoidance & early detection of renal failure. At the same time, subtle increments in creatinine values should not be taken lightly as they may reflect declining in kidney function requiring prompt dose adjustment.

Funding: Veterans Administration Support, Private Foundation Support, Clinical Research Support

Factors Affecting Timing of Dialysis Initiation in Acute Kidney Injury (AKI) in Intensive Care Unit (ICU) Chavutha V Thakar,1,2 Anthony Leonard,1 James Rousseau.1 Internal Medicine/Nephrology, University of Cincinnati, OH; Medical Service/Renal Section, Cincinnati VA, Cincinnati, OH.

Background: Although it is argued that early initiation of dialysis may be beneficial in AKI, it remains a subjective clinical decision based on multiple factors.

Methods: We conducted an online survey of U.S. and international nephrologists to study the factors affecting initiation of early dialysis in AKI in ICU. We studied how parameters used in determination of dialysis initiation influenced this decision across three case scenarios (predicted hospital mortality of <10%, 10 – 30% and >30% respectively).

For each case, 4 questions were asked about decision to initiate dialysis within 24 hours based on given clinical information; Q1 – subjective likelihood; Q2 – blood urea nitrogen (BUN) levels (<50, 50 – 75, 76 – 100, >100 mg/dl) considered in this decision; Q3 – creatinine (Cr) elevation (2 – 3 times; >3 times; absolute level >5 mg/dl regardless of change) considered important; Q4 – a rank order of parameters [BUN level, Cr change from baseline, oxygen saturation (SpO2), potassium (K) level, and urine output (1 moist, 2 moist, and 5 least influential)]. McNemar’s and t-test was used for comparison.

Results: Surveys of 119/172 nephrologists who responded to all questions were analyzed. They were 87% males, 73% were in practice for > 5 years, and 70% practised in the U.S.

The proportion of subjects likely to initiate early dialysis increased (76% to 94%) with predicted mortality (p < 0.0001). Proportion of subjects considering dialysis at a BUN level ≤50 mg/dl increased from 17% to 40% across three cases (p < 0.0001). Proportion of subjects choosing absolute Cr to be influential went from 60% to 43% across the three level <75 mg/dL increased from 17% to 40% across three cases (p < 0.0001). Proportion of clinical decision making by physicians who were blinded to uNGAL measurements.

Results: uNGAL was an independent predictor of the composite outcome and improved net reclassification by 26.1% (p < 0.0001). Patients who had sCr<1.4 mg/dl and uNGAL>104 mg/ml (sCr<上限+uNGAL) had low rates of clinical events (2.5%) and low rates of early nephrology consultation (2.6%). Patients with sCr≥1.4 mg/dl and uNGAL≥104 mg/ml (sCr上限+uNGAL) had high rates of clinical events (15.5%) and high rates of early nephrology consultation (50.9%). Patients who were sCr<上限+uNGAL (n=227) or sCr上限+uNGAL (n=236) were in an intermediate risk category (clinical event rates 5.3% and 5.1%, respectively).

Conclusions: Stratification by sCr and uNGAL in the emergency department prospectively separated low risk (sCr上限-+uNGAL), intermediate risk (sCr上限+uNGAL) or sCr上限+uNGAL) and high risk (sCr上限+uNGAL) patients. These risk categories were not adequately reflected in nephrology referral rates by physicians unaware of uNGAL levels, suggesting a missing potential for uNGAL to improve clinical decision-making.

Funding: NIDDK Support, Pharmaceutical Company Support, Government Support Non-U.S.

Can Urinary Kidney Injury Biomarkers Affect Clinical Decision Making in the Emergency Department? Kai M. Schmidt-Ott,1 Thomas L. Nickolas,2 Eugenia Singer,1 Catherine Forster,1 Meghan E. Sise,3 Abdallah Sassiine Geara,2 Philip Imus,1 Friedrich C. Luft,1 Jonathan M. Barasch,1 ’Charite Berlin, Max Delbrueck Center, Germany; 2Columbia University, NY; 3State Island University Hospital, NY.

Background: Conventional diagnostic strategies may inadequately detect patients at risk for poor clinical outcomes. Measuring urinary neutrophil gelatinase-associated lipocalin (uNGAL), a marker of kidney injury, may assist in identifying patients that require evaluation by a nephrologist.

Methods: Unselected patients were recruited from three emergency departments (n=1635) in the United States and Germany and serum creatinine (sCr) and uNGAL levels were measured. We measured the composite outcome of the study was in-hospital hemodialysis initiation or mortality. Nephrology consultations ordered within 24 hours of admission served as a surrogate of clinical decision making by physicians who were blinded to uNGAL measurements.

Results: uNGAL was an independent predictor of the composite outcome and improved net reclassification by 26.1% (p < 0.0001). Patients who had sCr<1.4 mg/dl and uNGAL>104 mg/ml (sCr上限-+uNGAL) had low rates of clinical events (2.5%) and low rates of early nephrology consultation (2.6%). Patients who had sCr≥1.4 mg/dl and uNGAL≥104 mg/ml (sCr上限+uNGAL) had high rates of clinical events (15.5%) and high rates of early nephrology consultation (50.9%). Patients who were sCr<上限+uNGAL (n=227) or sCr上限+uNGAL (n=236) were in an intermediate risk category (clinical event rates 5.3% and 5.1%, respectively).

Conclusions: However, nephrology referral rates differed markedly between these categories: sCr上限-+uNGAL had an early referral rate of only 3.1%; while sCr上限+uNGAL patients had an early referral rate of 33.1% (p<0.001 by Chi-square test).

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Acute Kidney Injury: Clinical - II
Renal DCs produced 2-fold more IL-10 by Western blot and 5-fold more mRNA transcripts of the TLR2 ligand PGN. Total tissue IL-10 levels were lower in sham and DCs depleted renal immune cells. Furthermore, we found that IL-10 gene expression was increased 2-fold when renal DCs were stimulated with the TLR2 ligand PGN, suggesting that IL-10 production can be triggered by TLR activation after IR injury.

Conclusions: Renal DCs produce IL-10 after IR injury and afford significant protection for acute kidney injury.

POI104
Regulatory T Cell Depletion during Established AKI Worsens Subsequent Brain Injury in Mice
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Background: Acute kidney injury (AKI) is well-established to lead to neurologic changes. Ischemic AKI in mice leads to brain cellular and soluble inflammation with significant an increase in keratinocyte-derived chemokine (KC) protein (J Am Soc Nephrol 2008 19:1360-70). Ischemic AKI enhances the susceptibility to the subsequent ischemic stroke (POI1748; ASN Renal Week 2009). In this study, we tested the mechanistic role of regulatory T cells (Treg) and KC receptor CXCR2 on ischemic stroke outcomes in AKI mice.

Methods: Young male C57BL/6 wild type mice (6-8 wk old) underwent either a sham operation or a 30 min of bilateral kidney warm ischemia following by reperfusion. On day 4 after kidney surgery, all mice underwent ischemic stroke by permanent (distal) middle cerebral artery occlusion (pMCAO) for 90 min and were followed up for 4 d. Mice were treated with antibody against Tregs (PC61) (500 µg per mouse, i.p.), anti-CD11c, or control antibody/sera at day 1 and at day 4 after AKI. Mice were sacrificed 8 days after kidney surgery for brain stroke infarct volume measurement.

Results: Depletion of circulating Treg was confirmed by flow cytometry analysis with a significant decrease in the percentage of CD4+CD25+Foxp3+ Treg of spleenocytes (PC61 vs IgG: 1% vs 14%, p=0.001, n=10). AKI mice had higher brain infarct volumes after stroke compared with sham AKI mice (AKI vs Sham: 32.3% ± 3.4% vs 23.9% ± 1.9, p=0.022, n=5). The degree of stroke infarct volume was associated with serum creatinine level (r=0.62, p=0.006, n=18). AKI mice treated with PC61 during established AKI had significant increase in stroke infarct volume when compared to AKI mice with IgG. (39.5% ± 1.6 vs. 32.9% ± 2.5, p=0.04, n=10 per group). No significant change was seen on stroke infarct volumes by blocking KC receptor CXCR2 (CXCR2 sera vs normal sera: 24.1% ± 5.1 vs 20.9% ± 2.6, p=0.57, n=5-6).

Conclusions: These data demonstrate that ischemic AKI exacerbates subsequent stroke, and one underlying mechanism could be via regulatory T cells. Regulatory T cells have the potential, if harnessed appropriately, to decrease the high distant organ effects of AKI.

Funding: Private Foundation Support

FR-PO1095
Humoral Immune Response Is Enhanced by Renal Ischemia-Reperfusion Injury
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Background: Ischemia-reperfusion injury (IRI) is a model of acute kidney injury (AKI) that is characterized by robust renal inflammation and temporary renal failure of 48-72 hours. Renal transplant recipients that suffer delayed graft function (DGF) in the setting of prolonged ischemia time are felt to have an increased risk of long-term immunologic injury. We sought to investigate the effect of AKI on the humoral immune response to extra-renal antigens.

Methods: Mice have a reliable immunoglobulin response to nitrophenol keyhole limpet hemocyanin (NP-KLH), an innocuous T-cell dependent antigen.

Results: In mice immunized 24 hours before IRI vs sham surgery there was no observed difference in NP-KLH specific antibody levels between the two groups up to 7 days after surgery. However, when mice were immunized 24 hours after IRI vs sham surgery (the peak of renal failure in this model), the IRI-treated mice had increased levels of NP-KLH specific IgG1 (p=0.037 by mixed model analysis) beginning at 35 days after surgery. The increased IgG1 response was not seen with unilateral ischemia, typed by local inflammation but not renal failure. Furthermore, the total IgG1 and IgM (not antigen-specific) were not different in IRI vs sham mice; this effect is not due to polyclonal immunoglobulin upregulation. There was no difference between the two groups in the percentage of splenocyte lymphocytes expressing B220 (B cell marker) or CD86 (marker of antigen-specific) were not different in IRI vs sham mice; this effect is not due to polyclonal immunoglobulin upregulation. There was no difference between the two groups in the percentage of splenocyte lymphocytes expressing B220 (B cell marker) or CD86 (marker of antigen-specific). Experiments are ongoing with T-cell independent antigens and in mice with targeted deletion of innate immune factors. Ongoing experiments may provide a mechanistic explanation why DGF kidneys suffer long-term immunologic injury and reveal therapeutic interventions that could improve allograft survival.

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FR-PO1096

Heat Shock Protein-70 (HSP70) Induced Renoprotective Effect Is Mediated by CD4+ CD25+ Foxp3+ Regulatory T Cells in Ischemia/Reperfusion (I/R) Induced Acute Kidney Injury

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Background: Recent reports have demonstrated the immune modulatory effect of HSPs. HSP70 induced by heat preconditioning (HP) has been shown to decrease inflammation and injury following administered AKI. CD4+ CD25+ Foxp3+ regulatory T cells (Tregs) have been recognized as an important player in decreasing kidney injury after I/R. The aim of this study was to test whether HP induced HSP70 exerts renoprotective effect through Tregs.

Methods: Thirty min bilateral I/R injury was done with or without heat preconditioning (42°C for 15min) in mice. Quercetin for inhibition of HSP70, or PO1 for deleting Tregs, were administered and various molecular and flow cytometric analyses were performed.

Results: Splenocytes from HP mice demonstrated expansion of Tregs, and reduced proliferative response upon mitogenic stimuli. T cells from HP mice failed to reconstitute postischemic injury when adoptively transferred to T cell deficient mice in contrast to T cells from normal mice, suggesting that HP has immunomodulatory function. While deleting Tregs before HP abolished the renoprotective effect, adoptive transfer of the cells back into Treg-depleted mice partially restored the beneficial effect of HP. Significantly increased Foxp3 gene expression as well as increased infiltration of Tregs into kidney were also observed in heat preconditioned ischemic kidneys. Immunohistochemistry and western blot demonstrated that HSP 70 was induced upon HP not only in kidney, liver or lung but also in immune cells in spleen. Inhibition of HSP70 by quercetin before HP suppressed the expansion of Tregs and this was associated with partial loss of beneficial effect of HP in I/R. Finally, transferring Tregs to quercetin-treated HP mice partially restored the beneficial effect of HP.

Conclusions: These results suggest that renoprotective effect of HSP70 might be partially mediated by their direct immunomodulatory effect through Tregs. Further understanding of cytoprotective or immune modulatory mechanisms of various stress proteins might facilitate discovery of new targets or drug development in the field of AKI.

FR-PO1097

Inhibition of the c-fms Receptor Prevents Kidney Macrophage Accumulation and Is Renoprotective Following Ischemic Injury

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Background: Colony stimulating factor-1 regulates proliferation, differentiation and survival of monocytes/macrophages by signaling through the c-fms receptor. Since the mononuclear phagocyte system mediates renal injury following ischemia-reperfusion (I/R), we hypothesized that inhibition of the c-fms receptor would be renoprotective.

Methods: A small molecule inhibitor of c-fms, was administered prior to and after bilateral renal ischemia.

Results: Vehicle-treated mice had significant increases in the number of CD11b+/ F4/80+ macrophages in both the circulation and kidney 24 hrs post-reperfusion, correlating with peak levels of plasma creatine and blood urea nitrogen (BUN). Treatment with a c-fms inhibitor significantly reduced CD11b+/F4/80+ macrophages in the kidney and blood, with peak levels of plasma creatinine and blood urea nitrogen (BUN). Treatment with a c-fms inhibitor significantly reduced CD11b+/F4/80+ macrophages in both the circulation and kidney 24 hrs post-reperfusion, correlating with peak levels of plasma creatine and blood urea nitrogen (BUN).

Conclusions: These results suggest that renoprotective effect of HSP70 might be partially mediated by their direct immunomodulatory effect through Tregs. Further understanding of cytoprotective or immune modulatory mechanisms of various stress proteins might facilitate discovery of new targets or drug development in the field of AKI.

FR-PO1098

Mechanisms of Uracil Acid-Mediated HMGB1 Translocation and Release from Endothelial Cells (EC)


Background: Based on findings that after IRI HMGB1 is released by the kidneys into the circulation, we examined the cellular localization and release of HMGB1 in EC. Due to its early and robust release after IRI, uracil acid was examined as a potential mediator of HMGB1 release from EC.

Methods: Treatment of EC with uracil acid resulted in increased HMGB1 mRNA expression and protein translation, effects that were blocked by inhibition of TLR4 receptor.

Results: Uracil acid treatment of EC lead to the translocation of HMGB1 from the nucleus to the cytosol and release from the cell into either the cell culture medium (in vitro) or the circulation (in vivo), as determined by western blot analysis and immunofluorescence. We next aimed at identifying the mechanism by which uracil acid induces the release of HMGB1. Treatment of EC with uracil acid and an inhibitor of intracellular calcium release (TMB-8) or an inhibitor of MEK/ERK pathway (U0126) resulted in the retention of HMGB1 in the nucleus while diminishing HMGB1 release into both the extra- and intracellular compartments. We also examined the role of HMGB1 acetylation in mediating its translocation. Treatment of EC with uracil acid caused acetylation of HMGB1 leading to its translocation and release from EC, an effect blocked by pretreating cells with ethyl pyruvate. Once released, HMGB1 was found to have a positive feedback mechanism that promoted further translocation and release of HMGB1 from endothelial cells. We also demonstrate that uracil acid and released HMGB1 induce EC angiopoietin 2 mRNA expression and protein release while activating intracellular NF-κB (as measured by luciferase reporter assay) and the pro-inflammatory response.

Conclusions: Uracil acid through the TLR4 receptor mediates the transcriptional and translational HMGB1 response in EC in a mechanism that involves the release of intracellular calcium and the MEK/ERK pathway and the acetylation of HMGB1, which resulted in its translocation and release from the cell. Angiopoietin 2 expression and NF-κB activity were increased after uracil acid and HMGB1 treatment. Mobilization of acetylated HMGB1 was reduced with ethyl pyruvate treatment.

Funding: NIDDK Support

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were all prevented or blunted by the administration of ITF1697, whereas the levels of an inflammatory cytokine IL-1α were upregulated in ITF1697-treated animals. WBK exocytosis contributed to IRA-associated mobilization of EPC and HSC and ITF1697 blunted stem/progenitor cell mobilization. One month after IRI, mice treated with ITF1697 showed a significantly more pronounced degree of scarring than non-treated animals.

**Conclusions:** 1) application of ITF1697 inhibits exocytosis of WBK; 2) the systemic inflammatory response of IRA is in part due to the exocytosis of WBK and its blockade blunts it; 3) ITF1697 improves short-term renal function after IRA, but not the long-term fibrotic complications.

**Funding:** NIDDK Support

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**FR-PO1103**

**Renal Tubular and Peritubular Capillary Injury in Hepatic Failure Induced Acute Kidney Injury in Rats**

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**Background:** Acute kidney injury (AKI) is a common complication in the acute liver dysfunction. However, little is known about the mechanisms of AKI during the development of acute liver dysfunction. In this study, we characterize rat model of AKI during the development of acute liver dysfunction following liver transplantation.

**Methods:** Acute hepatic failure was induced in rat by liver transplantation from DA (RT1a) to Lewis (RT1l) rats without immunosuppression. Rats were dead around day 11 with severe acute liver dysfunction. We studied kidney samples at day 5, day 7, and day 9 to 11, focusing on the tubular and peritubular capillary (PTC) injury. In addition, hemodynamic events in PTCs in vivo were evaluated functionally and quantitatively by the use of a real-time confocal laser-scanning microscope (CLSM) system (Kidney Int 59: 252-259, 2001).

**Results:** During the progression of rejection in hepatic graft, acute liver dysfunction (T-Bil 7.9±1.8, p<0.01) and acute kidney injury (BUN 112.0±22.5; Cr 0.6±0.1, p<0.05) developed by day 11. During the development of AKI, renal tubular degeneration with bile pigment accumulation, mitochondrial degeneration, KIM-1 expression, and severe disruption of f-actin, TUNEL+ dead cells were noted in tubules and PTCs. In addition, endothelial dysfunction in PTCs developed with decrease expression of eNOS, and marked reduced blood flow (540±162 vs 860±145 μm/sec in control, p<0.05). Interstitial edema occurred with inflammatory cell infiltration.

**Conclusions:** In conclusion, AKI developed in rats during the development of acute liver dysfunction and was characterized by renal tubular damage as well as endothelial dysfunction in PTCs with marked reduced blood flow.

**Funding:** Private Foundation Support

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**FR-PO1104**

**Role of Calcium/Calmodulin-Dependent Kinase Kinase 2 (CaMKK2) in Acute Kidney Injury**

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**Background:** The multifunctional calcium/calmodulin-dependent kinases (CaMKs) are implicated in the inflammatory response of lymphocytes, dendritic cells and macrophages. Previously published studies have shown that treatment with a non-selective CaMK inhibitor ameliorates disease development and renal injury in a lupus mouse model. Since CaMKK2 regulates the activity of both CaMKI and CaMKIV and inflammation plays a key role in acute kidney injury (AKI), we hypothesized that the loss of CaMKK2 would be protective in mouse models of AKI.

**Methods:** Cohorts of CaMKK2 null mice or wild type (WT) mice were injected with LPS (lipopolysaccharide) or folic acid to induce AKI based on established protocols. LPS causes a systemic inflammatory response and subsequent renal injury. Folic acid causes tubular injury and inflammation with AKI dramatically attenuated by neutron depletion in mice.

**Results:** CaMKK2 null mice demonstrated normal renal function by BUN and creatinine, and did not demonstrate normotensive hypertension. Following LPS injection, CaMKK2 null mice are indeed protected from renal injury with lower BUN and creatinine values than WT mice (creatinine in mg/dL, 0.26 versus 0.50, p<0.05). Surprisingly, folic acid injection causes dramatic renal injury in CaMKK2 null mice similar to WT mice (creatinine 2.36 versus 1.60, not significant). The CaMKK2 null mice with AKI also had significantly higher kidney/body weight ratios than WT mice and extensive renal injury histologically.

**Conclusions:** Therefore, CaMKK2 is required for AKI following LPS treatment but it is not required for the development of folic acid induced renal injury, suggesting that it may regulate different inflammatory responses in AKI. These results are consistent with previously published studies demonstrating the role of CaMKs in macrophage and dendritic cell activation in response to LPS. The CaMKK2 cascade may represent a novel pathway for therapeutic modulation of acute kidney injury related to endotoxemia.

**Funding:** NIDDK Support

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**FR-PO1105**

**Acute Kidney Injury (AKI) in Rats with Pre-Existing Chronic Kidney Disease (CKD) Induces a Major Increase in Pro-Inflammatory Cytokines (IL-1α, IL-6) and Chemokines (RANTES, MCP-1) in Kidney and Lung**

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**Background:** AKI in patients with pre-existing CKD have increased co-morbidity and mortality. Although it is well established that AKI is associated with a major increase in pro-inflammatory cytokines and chemokines, it is unknown to which extent AKI in pre-existing CKD influences the development of proinflammatory cytokines and chemokines. The aim of this study was to assess the changes in the expression of pro-inflammatory cytokines and chemokines in kidney and lung in response to AKI in rats with pre-existing CKD.
Methods: KD was induced by 5/6 nephrectomy (5/6 Nx) for 6 weeks. AKI was induced by intestinal ischemia for 45 min followed by reperfusion for 90 min (IIR): 1) Nx/IIR; 2) Sham Nx/IIR; 3) Nx+Sham IIR; 4) Sham Nx/Sham IIR; 5) controls. Cytokines/chemokines were measured in homogenized whole kidney and lung preparations with LuminoxTM 100.

Results: S-Cr increased significantly in response to IIR: from 66.2±7.3 to 88.9±8.3 in Nx rats, resp., and from 32.0±0.7 to 54.7±2.9 in Sham Nx rats. The levels of IL-1β, IL-6, RANTES, and MCP-1 in lung and kidney were significantly higher in rats undergoing IIR compared to sham IIR. Importantly also in the 5/6 Nx rats IL-1β increased significantly in IL-1β, IL-6, RANTES, and MCP-1 expression in kidney and lung compared to sham 5/6 Nx rats. Moreover the response was even more pronounced in the 5/6 Nx rats.

Conclusions: The results demonstrate a significant increase in pro-inflammatory cytokines and chemokines in response to AKI in rats with existing KD.

FR-PO1106

MCP-1 is Involved in Potentiation of Lung Inflammation by Antecedent Ischemic Acute Kidney Injury


Results:

<table>
<thead>
<tr>
<th>Compartment</th>
<th>Sham-IRS</th>
<th>Sham-LPS</th>
<th>IRS-IRS</th>
<th>IRS-LPS</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>3.2±0.3</td>
<td>3.2±0.3</td>
<td>3.5±0.3</td>
<td>4.0±0.2</td>
<td>&lt;.001</td>
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<tr>
<td>Serum</td>
<td>59 (41-79)</td>
<td>69 (49-82)</td>
<td>108 (65-151)</td>
<td>121 (92-200)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Lung</td>
<td>1.0 (0.8-1.3)</td>
<td>0.9 (0.6-1.3)</td>
<td>1.2 (0.8-1.6)</td>
<td>1.1 (0.8-1.6)</td>
<td>.358</td>
</tr>
<tr>
<td>DAF</td>
<td>216.4±12.4</td>
<td>176.9±56.2</td>
<td>154.5±81.3</td>
<td>139.0±66.6</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

Taken together, our 2-hit model demonstrates that ischemic AKI primes the lung for a heightened inflammatory response to subsequent injury and MCP-1 may be involved in this potentiation. The findings hold relevance for critically ill patients at risk of deleterious kidney-lung crosstalk.

FR-PO1107

Natural IgM Anti-Lucyte Autoantibodies (IgM-ALA) Block Immune Mediated Proinflammatory Cytokine Release through Inhibition of NF-κB Activation

Sanja Lee,1 Amandeep Bajwa,1 Kailo H. Schlegel,1 Amandeep Bajwa,1 Kailo H. Schlegel,1 Sang Ju Lee,2 Amandeep Bajwa,2 Kailo H. Schlegel,1

Methods: CDK was induced by 5/6 nephrectomy (5/6 Nx) for 6 weeks. AKI was induced by intestinal ischemia for 45 min followed by reperfusion for 90 min (IIR): 1) Nx/IIR; 2) Sham Nx/IIR; 3) Nx+Sham IIR; 4) Sham Nx/Sham IIR; 5) controls. Cytokines/chemokines were measured in homogenized whole kidney and lung preparations with LuminoxTM 100.

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Conclusions: The results demonstrate a significant increase in pro-inflammatory cytokines and chemokines in response to AKI in rats with existing KD.

FR-PO1108

The Effect of mTOR-Inhibition on Cycling of NF-κB Activation in Kidney Ischemia-Reperfusion Injury in Mice

Aleksandra V. Kezic,1 Jan U. Becker,2 Tung Yu Tsui,3 Friedrich Thaiss.4

Methods: C57/BL6 mice were subjected to IRI by clamping both renal pedicles. Application of eve started one day before IRI induction in a dose of 1.5 mg/kg b.w. sustained for 7 days daily. Both eve treated and non-treated mice were evaluated for eventual AKI.

Results: After induction of AKI NF-LPS was significantly increased at early (6h and 24h) and also later (7d) time points when compared with sham operated animals. NF-κB activation was paralleled by a biphasic increase in TNF-alpha and chemokine CCL2 and CCL5 expression. Treatment of mice significantly increased NF-κB activity and inhibited NF-κB activation at all time points.

Conclusions: NF-κB activation might be demonstrated in kidneys after IRI during a 7 days observation period. The peaks of NF-κB activation might represent early epithelial and late immune activation pathways. The influence of mTOR inhibition during early epithelial and late immune mediated activation of NF-κB after IRI warrants further investigation.

FR-PO1109

Activation of the Inflammasome in Cisplatin-Induced Acute Kidney Injury

Dong Woong Lee, Zhihan He, Quocun Nguyen, Ali Akcay, Alkeshi Jani, Sarah Faubel, Charles L. Edelstein.

Background: We have demonstrated increased caspase-1, IL-1β and IL-18 in cisplatin (Cis)-induced AKI. As caspase-1, IL-1β and IL-18 are activated in the inflammasome, the aim of the study was to further investigate the inflammasome in Cis-induced AKI. Inflammasomes are cytosolic complexes composed of NALPs, ASC and caspase-1. BID is a pro-apoptotic protein activated by caspase-1 in the inflammasome.

Results: Mice injected with Cis (25 mg/kg) developed AKI on day 3. On qPCR of whole kidney, NALP3 mRNA was increased on day 3 (p<0.05, 0.75 vs. 0.1 fold change). On immunoblot of whole kidney, NALP3 was present in the freshly isolated proximal tubules (PT), but not in endothelial cells or macrophages. Thus we further investigated the inflammasome in a model of PT treated with Cis 10 and 50 µM. Caspase-1 activity was increased (p<0.05, 1.1 and 1.3 vs. 0.1 nmol/mg/min in Veh). Caspase-5. BID is a pro-apoptotic protein activated by caspase-1 in the inflammasome. In the context of caspase-1, the aim of the study was to further investigate the inflammasome in Cis-induced AKI.

Conclusions: In conclusion, components of the inflammasome are increased in both whole kidney in vivo and PTs treated with Cis in vitro.
**Background:** After ischemia-reperfusion injury tubular cells undergo apoptosis and necrosis which triggers an inflammatory cell response. Infiltration of M1-type macrophages is detrimental in early phases, however alternatively activated M2 macrophages promote tissue repair. Hypoxia-inducible factors are critical for inflammatory macrophage responses and are differentially expressed in M1 and M2 macrophages. We therefore investigated whether HIF-1 is critical for macrophage function and plasticity in ischemic kidney injury.

**Methods:** Floxed alleles of HIF-1α were deleted in myeloid cells by Cre-loxP recombination using LysM-Cre. Kidney ischemia-reperfusion injury was performed by clamping renal arteries for 25 minutes. Animals were analysed at 48 h and 72 h after reperfusion. Creatinine and urea levels were determined as renal function parameters. Gene expression analysis was done by real-time PCR and by immunohistochemistry.

**Results:** No renal phenotype is observed under control conditions in mice with deletion of HIF-1α in myeloid cells. Functional impairment 48h after renal ischemia reperfusion injury is not significantly different between wildtype and knockout mice. Creatinine levels in wildtype animals 72h after reperfusion are significantly better. Knockout animals do not exhibit this recovery of renal function. Macrophage infiltration is similar in both groups as assessed by F4/80 staining and MCP1 expression. However, TGFβ expression in injured tubules may protect against milder AKI by conferring resistance to epithelial apoptosis.

**Conclusions:** These results suggest that blocking TGFβ signaling in proximal tubules may protect against milder AKI by conferring resistance to epithelial apoptosis.

**Funding:** NIDDK Support, Veterans Administration Support, Private Foundation Support

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**FR-PO1113**

**Acute Reduction in Renal Calpain 10 Causes Mitochondrial and Renal Dysfunction**

**Method:** We investigated the role calpain 10 in mitochodrial function and renal dysfunction.

**Results:** In calpain 10−/− mice calpain 10 in kidney mitochondria was significantly less compared to wildtype. Inhibition of calpain 10 by ZV AD-fmk decreased renal function and increased apoptosis in calpain 10−/− mice.

**Conclusions:** These results support our hypothesis that the loss of renal calpain 10 causes mitochondrial and renal dysfunction.

**Funding:** Other NIH Support - NIH - Environmental Health Sciences Training Program in Environmental Stress Signaling (T32 ES012878), Veterans Administration Support

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**FR-PO1114**

**Role of Mitofusin 2 in the Renal Stress Response**

**Method:** Mitofusin 2 (MFN2) is essential to their function as regulators of cell survival. Although mitofusin 2 (MFN2) is a cytoprotective factor for renal injury in mice.

**Results:** In MFN2-deficiency caused profound mitochondrial fragmentation but did not alter baseline oxygen consumption or survival of proximal tubule cells in culture at rest. In contrast, after ATP depletion, proximal tubule cells lacking MFN2 had more mitochondrial outer membrane injury and an 80% increase in apoptosis compared to MFN2-expressing control. This suggests that MFN2 deficiency causes profound mitochondrial fragmentation but does not alter baseline oxygen consumption or survival of proximal tubule cells in culture at rest.

**Conclusions:** Mitochondria are dynamic organelles that undergo constant remodeling, essential to their function as regulators of cell survival. Although mitofusin 2 (MFN2) is critical for mitochondrial morphology and function, its role in the renal stress response is unknown.

**Funding:** NIDDK Support, Veterans Administration Support

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**FR-PO1110**

**Hypoxic Response of Myeloid Cells in Renal Ischemia-Reperfusion Injury**

**Method:** We investigated the role of hypoxia in the response to renal ischemia-reperfusion injury.

**Results:** We observed that hypoxia attenuates tissue damage. Thus our data add new and relevant insights into HIF-1α signaling in renal inflammatory cells.

**Conclusions:** These results suggest that blocking TGFβ signaling in proximal tubules may protect against milder AKI by conferring resistance to epithelial apoptosis.

**Funding:** NIDDK Support, Veterans Administration Support, Private Foundation Support

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**FR-PO1112**

**TGF-β-signaling in the Proximal Tubule Modulates the Response to Acute Kidney Injury**

**Method:** We investigated the role of TGFβ signaling in the proximal tubule epithelial cell response to acute kidney injury.

**Results:** TGFβ-signaling in the proximal tubule affects kidney injury and repair after AKI.

**Conclusions:** These results support our hypothesis that the loss of renal calpain 10 causes mitochondrial and renal dysfunction.

**Funding:** Other NIH Support - NIH - Environmental Health Sciences Training Program in Environmental Stress Signaling (T32 ES012878), Veterans Administration Support

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**FR-PO1112**

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**FR-PO1114**

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**Method:** Mitofusin 2 (MFN2) is essential to their function as regulators of cell survival. Although mitofusin 2 (MFN2) is critical for mitochondrial morphology and function, its role in the renal stress response is unknown.

**Results:** In MFN2-deficiency caused profound mitochondrial fragmentation but did not alter baseline oxygen consumption or survival of proximal tubule cells in culture at rest. In contrast, after ATP depletion, proximal tubule cells lacking MFN2 had more mitochondrial outer membrane injury and an 80% increase in apoptosis compared to MFN2-expressing control. This suggests that MFN2 deficiency causes profound mitochondrial fragmentation but does not alter baseline oxygen consumption or survival of proximal tubule cells in culture at rest.

**Conclusions:** Mitochondria are dynamic organelles that undergo constant remodeling, essential to their function as regulators of cell survival. Although mitofusin 2 (MFN2) is critical for mitochondrial morphology and function, its role in the renal stress response is unknown.

**Funding:** NIDDK Support, Veterans Administration Support, Private Foundation Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

**Underline** represents presenting author.
Tubule-Specific Ablation of Endogenous β-catenin Aggravates Acute Kidney Injury in Mice

Dong Zhou, Yingjian Li, Peter Igarashi, Youshua Liu.

Department of Pathology, University of Pittsburgh, PA; Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX.

Background: β-catenin is a unique intracellular protein that plays dual functions: as an integral component of the cell adherens junction complex, and as a principal signaling protein that mediates the canonical Wnt signaling. Inappropriate activation of β-catenin occurs predominantly in renal tubular epithelium and is implicated in the pathogenesis of a variety of progressive chronic kidney disorders, such as diabetic and obstructive nephropathies. However, little is known about its function in normal physiologic state as well as after acute kidney injury (AKI). To explore this issue, we generated conditional knockout mice in which β-catenin gene is specifically ablated in tubules (designated as Ksp-Cre-/- mice). β-catenin knockdown in proximal tubular epithelia displayed a higher serum creatinine level and more severe morphologic injury at 2 days after acute kidney injury. Similarly, more apoptotic cells were detected in Ksp-Cre-/- mice, which was accompanied by an increased Bax protein expression and decreased survivin mRNA expression in the kidneys. In vitro, activation of β-catenin via transfection of either WT1 or constitutively active β-catenin expression vectors protected human proximal tubular epithelial cells (HK-2) from apoptosis induced by staurosporine. Consistently, activation of β-catenin also induced survivin and repressed Bax expression in vitro. These results suggest that endogenous β-catenin in renal tubules is pivotal for renal protection after acute kidney injury primarily through activating cell survival signaling.

Funding: NIDDK Support

Two Independent Pathways, BNIP3 and Sestrin2, Mediate Autophagy of Renal Tubular Cells in Acute Kidney Injury In Vitro and In Vivo

Masayuki Ishihara,1 Masayuki Bun,2 Masayuki Hisa,2 Kazu Hamada,1 Yoshiko Liu.1

1Department of Pathology, University of Pittsburgh, PA; 2Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX.

Background: Autophagy is one of the systems which protect life from many kinds of stresses. In the previous study we reported autophagy occurred in acute kidney injury (AKI) mouse model. Autophagy is thought to play a protective role in renal tubules from stresses. However, little is known about signal transduction for autophagy in AKI. Bel-2 adenovirus E1B 19Kda-interacting protein 3 (BNIP3) is one of the target proteins of hypoxia inducible factor -1a (HIF-1a). Sestrin2 is one of the target proteins of p53. The aim of this study is to reveal the roles of BNIP3 and sestrin2 in autophagic pathway in AKI.

Methods: We used rat renal tubular epithelial (RTE) AKI model in vivo and cultured renal tubular cells as an in vitro AKI model. The expression of BNIP3 and sestrin2 are up-regulated after 1R in proximal tubules in immunostaining and immunoblotting. BNIP3 mRNA and protein expressions were up-regulated in the hypoxia condition via HIF-1a dependently in vitro. Sestrin2 mRNA and protein expressions were up-regulated in the oxidative stress condition (H2O2) via p53 dependently. To examine BNIP3 and sestrin2 regulate autophagy or not, we established NRK cells which stably transduced with a fusion protein between green fluorescent protein and light chain 3 (LC3-GFP) as a marker of autophagy. Overexpression of BNIP3 and sestrin2 both induced autophagy in NRK-LC3-GFP cells and induced LC3 protein expression.

Results: The autophagosome induced by BNIP3 localized in mitochondria. Induction of autophagy by hypoxia was reduced by sestrin2 siRNA. Induction of autophagy by H2O2 was reduced by BNIP3 siRNA.

Conclusions: These observations disclose that autophagy in renal tubules in AKI is induced by at least two independent pathways, p53-sestrin2 pathway and HIF-1a-BNIP3 pathway. These two pathways may wok differently according to the types of stresses to protect renal tubules in AKI.

Funding: NIDDK Support, Veterans Administration Support

Proximal Tubular Cells (PTCs) from AMP-Activated Protein Kinase (AMPK) Knock-Out (KO) Mice Are More Susceptible to Metabolic Stress Than Their Wild-Type WT Controls

Wilfred Lieberthal,1 Meiyi Tang,1 Vimal Patel;3 Jerrold S. Levine.2 1Medicine, Stony Brook Medical Center, Stony Brook, NY; 2Medicine, University of Chicago, IL.

Background: We have previously shown that inhibiting AMPK in PTCs from WT mice increases their susceptibility to apoptosis. There are two isoforms of the catalytic domain of AMPK, α1 and α2. In this study we examined the susceptibility to metabolic stress of AMPK KO mice that lack the α1 or α2 isoform of the catalytic domain.

Methods: Primary cultures of PTCs from WT and KO mice were incubated in dextrose (6mM, 5mM or 4mM), without or with 2 mM antimycin A (anti). Apoptosis, quantified by the EAC assay, was expressed as a % of the total monolayer. Cells in PTCs that were not subjected to metabolic stress, gene expression was determined by real-time PCR and expressed in KO PTCs as a % of WT.

Results: Apoptosis was comparable in α1 KO and WT PTCs incubated in 6mM dextrose in the presence (5.4±2.1%) vs the absence of antimycin A (5.5±1.3%). However, there was more apoptosis in α1 KO vs WT PTCs incubated in 5mM dextrose in the presence (18.2±3.3%) vs absence of antimycin A (13.2±2.1%). In 4mM dextrose, the amount of apoptosis was even greater in α1 KO vs WT PTCs in the presence (33.2±6%) vs absence (32.1±9%) of antimycin A (p<0.01). In α2 KO and WT PTCs apoptosis was at control levels (~5%) in 6 and 5mM dextrose with and without antimycin A. However, at 4mM dextrose, apoptosis was increased in α2 KO vs WT PTCs in the presence (24.2±1.3%) vs absence (3.2±1.3%) of antimycin A (p<0.02). We also found that the expression of genes encoding PPARγ coactivator-1α (PGC-1α) and cytochrome B was reduced to a comparable extent (~55%) in α1 and α2 KO mice when the expression of cytochrome C was reduced to a greater extent in α1 vs α2 KO PTCs (by 12±7% vs 62±8% respectively; p<0.02).

Conclusions: i) AMPK KO mice are more susceptible than WT mice to apoptosis induced by metabolic stress. ii) α1 KO PTCs are more sensitive than α2 PTCs to the same metabolic stress; iii) Differences in the reduction in gene transcription between α1 and α2 KO mice may contribute to the differences in sensitivity of the α1 and α2 KO PTCs to metabolic stress.

Funding: Veterans Administration Support
Methods: Here, we investigated the role of PER in LPS-mediated AKI. Sblethal dose of 375 mL LPS (0.1 mL, i.p.) was administered to C57BL/6J male mice. However, in C57BL/6J-Lys mice (model of defective lysosomal autophagy/pexophagy), LPS resulted in a significant increase in Scr and urinary albumin (24 h, Scr 0.48±0.07 mg/dl, UACR 193±33 mg/g) compared to WT mice (Scr 0.23±0.03 mg/dl, UACR 51±12 mg/dl).

Results: Immunoblot analyses of kidney homogenates demonstrated significantly higher increases of PMP70 (a major PER membrane protein) and catalase (a marker for the PER matrix) in WT compared with C57BL/6J-Lys mice after LPS treatment. Ex vivo, time-control kidneys stained in indirect immunofluorescence demonstrated a biphasic profile of PMP70 expression under LPS: an initial decline, followed by an elevation after 9h. PMP70 normalized after 24h and further increased compared to control cells without LPS stimulation. Expression profile of PMP70 under LPS was paralleled by catalase and p62, protein known to interact with ubiquitin and cause autophagy. Activation of the PER decreases the levels of p62. In vitro studies, PMP70 levels in autophagy-deficient C57BL/6J-Lys mice were decreased compared to WT, suggesting concomitantly impaired PER biogenesis by LPS. Peroxin14 (Pex14), a PER membrane docking protein that interacts with PER targeting sequence (PTS) containing proteins, showed down-regulation in LPS-treated HUVEC, suggesting impaired PER assembly due to altered protein import. Fluorescence microscopy of HUVEC transfected with PTS-GFP demonstrated decreased colocalization of PTS with PER under LPS.

Conclusions: 1) LPS exerts a biphasic effect on PER: initial depletion followed by accumulation; and 2) both the impaired biogenesis and autophagic degradation of PER (pexophagy) seems to contribute to aggravation of AKI.

Funding: NIDDK Support

FR-POI120

Roles of mTOR Pathway on Autophagy in the Proximal Tubular Epithelial Cells of Rat Kidneys

Shunsaku Nakagawa, Kumiko Nishihara, Satohiro Masuda. Pharmacy, Kyoto University Hospital, Kyoto, Japan.

Background: The mammalian target of rapamycin (mTOR) pathway plays important roles in several kidney diseases, including ischemia-reperfusion (IR) injury, chronic renal failure, diabetic nephropathy and polycystic kidney disease. Recently, we showed activation of the mTOR pathway in proximal tubular cells of rat kidneys after subtotally nephrectomy (Nx) (Nakagawa et al., Biochem Pharmacol, 79, 67-76, 2010; Nishihara et al., Am J Physiol Renal Physiol, 298, F923-F934, 2010). Although recent studies proposed that mTOR regulated autophagy through interaction with UNC51-like kinase 1 (ULK1) in vitro, the regulatory mechanisms of autophagy via interaction between mTOR and ULK1 in vivo were not clear. In this study, we aimed to clarify the roles of the mTOR pathway on autophagy in renal proximal tubules.

Methods: The amounts of phosphatidylinositol-3,4,5-trisphosphate-conjugated form of microtubule associated protein 1 light chain 3 (LC3-II), a marker for activity of autophagy, in rat kidneys were examined after Nx, IR and cisplatin treatment.

Results: Immunofluorescence analysis showed that the positive signals for mTOR, ULK1 and LC3 were detected in the proximal tubular epithelial cells of rat kidneys, and immunoprecipitation revealed the direct interaction between mTOR and ULK1 in the rat kidneys. The levels of LC3-II in rat kidneys were significantly decreased to 16%, 27% and 52% compared to control rats after Nx, IR and cisplatin treatment, respectively. On the other hand, phosphorylated ribosomal protein S6 (a marker for activation of the mTOR pathway) was significantly increased to 283%, 138% and 152% compared to control rats after Nx, IR and cisplatin treatment, respectively. In addition, the levels of LC3-II in the IR kidneys after Nx, IR and cisplatin treatment were significantly increased to 517%, 147% and 185% as compared to vehicle treated rats by treatment with mTOR inhibitor everolimus.

Conclusions: Activation of the mTOR pathway in proximal tubular epithelial cells caused suppression of autophagy during kidney injury by direct interaction with ULK1.

Funding: Government Support - Non-U.S.

FR-POI112

Extracellular Signal-Related Kinase Inhibition Prevents Autophagy Induction and Sensitizes Proximal Tubular Cells to LPS-Induced Apoptosis

Jeremy S. Leventhal, Michael J. Ross. Department of Medicine Division of Nephrology, Mount Sinai School of Medicine, New York, NY.

Background: Sepsis-induced acute kidney injury (AKI) is a common and destructive complication encountered in hospitalized patients. Clinical studies have highlighted the negative association between sepsis-induced AKI and short-term and long-term renal function and mortality rates. As of yet, there are no approved therapies to treat or prevent sepsis-induced AKI. Autophagy is a core cellular autophagic degradation mechanism that endows cells with the ability to maintain homeostasis after exposure to stress-inducing stimuli, such as bacterial lipopolysaccharide. In these studies we investigated the autophagic response to bacterial lipopolysaccharide (LPS), the cytotoxic function of autophagy, and the signaling pathways required for autophagy induction.

Methods: Autophagy was evaluated by western blot for LC3II and quantification of fluorescent punctae in GFP-LC3 transducted proximal tubular epithelial cells (PTEC). MAPK inhibitor U0126 was used to determine the effect of Extracellular signal-related kinase (ERK) on autophagy and cell survival in LPS-exposed PTEC.

Results: Autophagy induction was demonstrated in LPS exposed PTEC by increased LC3II accumulation and a significantly higher quantity of GFP-LC3 punctae compared to control treated cells. Knockdown of Beclin-1, an essential part of the autophagy machinery, sensitized cells to LPS-induced apoptosis as evidenced by increased PARP-1 cleavage.

Incubation of PTEC with the ERK-inhibitor U0126 inhibited the autophagic response to LPS: decreased autophagic LC3II accumulation and increased apoptosis in LPS-treated PTEC.

Conclusions: ERK-dependent autophagy induction is a counter-regulatory mechanism preventing apoptosis after LPS exposure and may therefore be an important protective mechanism against sepsis-induced AKI.

Funding: Other NIH Support - T32 DK007757-12(Leventhal); R01 DK078510(Ross)

FR-POI113

Murine Double Minute-2 Links Inflammation and Tubular Healing in Acute Kidney Injury of Mice

Dana Thomasova, Shrikant Ramesh Mulay, Mi Ryu, Hans J. Anders. Department of Nephrology, Medizinische Poliklinik, University of Munich, Munich, Germany.

Background: The E3 ubiquitin ligase murine double minute (MDM)-2 promotes cancer cell survival and growth, by degrading p53, hence, MDM2 inhibition with nutlins emerges as novel cancer therapy. To test whether MDM2 also promotes regenerative cell growth we examined the effects of nutlin-3a on tubular cell apoptosis in cultured renal proximal tubular cells. The cells were cultured for two weeks in media containing 5.5 mM glucose, 30 mM glucose, or 30 mM mannitol, followed by ATP-depletion with anaerox or azide. ATP-depletion induced significantly higher apoptosis in high-glucose-conditioned cells (60%) than the cells cultured with 5.5 mM glucose or 30 mM mannitol (15%). Consistently, caspase activity was significantly higher in high-glucose-conditioned cells. During ATP-depletion, high glucose-conditioned cells also showed an earlier and higher Bax translocation and cytochrome c release.

Conclusions: Taken together, these results suggest that high glucose or hyperglycemia may sensitize renal tubular cells to mitochondrial or the intrinsic pathway of apoptosis, resulting in heightened kidney injury.

Funding: NIDDK Support, Veterans Administration Support

FR-POI114

Acute Kidney Injury and Sepsis Independently Induce a Multi-Organ Stereotyped Inflammatory Response

Barbara Pedrycz, Catharine Compston, Dana Thomasova, Shrikant Ramesh Mulay, Mi Ryu, Hans J. Anders. Department of Medicine Division of Nephrology, Mount Sinai School of Medicine, New York, NY.

Background: Acute kidney injury (AKI) and sepsis are independently associated with multi-organ complication encountered in hospitalized patients. Clinical studies have highlighted the synergistic interaction between AKI and sepsis. Although recent studies proposed concomitantly impaired PER biogenesis by LPS. Peroxin14 (Pex14), a PER membrane docking protein that interacts with PER targeting sequence (PTS) containing proteins, showed down-regulation in LPS-treated HUVEC, suggesting impaired PER assembly due to altered protein import. Fluorescence microscopy of HUVEC transfected with PTS-GFP demonstrated decreased colocalization of PTS with PER under LPS.

Methods: We tested this possibility by examining kidney cell and tissue injury in hyperglycemic and diabetic conditions.

Results: We first observed that significantly higher kidney injury and mortality were induced by renal ischemia-reperfusion in streptozotocin-diabetic mice. Consistently, renal ischemia induced more severe kidney injury in diabetic Akita mice than wild-type mice. In addition, ischemic kidney injury in these diabetic models showed a correlation with their blood glucose levels. To understand the mechanism of the injury sensitivity, we examined that the effect of high glucose on ATP-depletion induced apoptosis in cultured LPS exposed tubular cells. The cells were cultured for two weeks in media containing 5.5 mM glucose, 30 mM glucose, or 30 mM mannitol, followed by ATP-depletion with anaerox or azide. ATP-depletion induced significantly higher apoptosis in high-glucose-conditioned cells (60%) than the cells cultured with 5.5 mM glucose or 30 mM mannitol (15%). Consistently, caspase activity was significantly higher in high-glucose-conditioned cells. During ATP-depletion, high glucose-conditioned cells also showed an earlier and higher Bax translocation and cytochrome c release.

Conclusions: Taken together, these results suggest that high glucose or hyperglycemia may sensitize renal tubular cells to mitochondrial or the intrinsic pathway of apoptosis, resulting in heightened kidney injury.

Funding: NIDDK Support, Veterans Administration Support
Methods: 3 month old male C57BL/6 male mice underwent unilateral renal ischemia-reperfusion (IR) or cecal ligation and puncture (CLP) as models of AKI and sepsis respectively. Transcript levels of the acute phase response (APR) genes, Osmr, Ile6, Il6, Lif, Lif, Serpina3, and Ngal were measured at 24 hours by real-time RT-PCR in hearts, lungs and non-clamped kidneys of control (n=7), IR (n=5) and CLP (n=5) mice. Gene expression is represented as fold change over control and % HPRT. Results: A similar qualitative inflammatory response was observed in distant organs after IR and CLP for all genes analyzed. Fold change of Ngal served as a general injury/leucocyte infiltration marker. Distant organ expression patterns were similar in CLP and IR mice (Figure 1).

Quantitatively, the degree of injury/inflammation was higher in CLP compared to IR mice. In hearts, kidneys and lungs respectively, Ngal expression increased by 417, 340 and 19 fold in CLP mice, compared to 33, 340 and 9 fold in IR mice. Baseline expression was consistently higher in the lung for all the APR genes, followed by heart and then kidney.

Conclusions: The injury-repair response is highly stereotyped across remote organs regardless of initiating event. Distant organ inflammation is therefore likely a significant mediator of multi-organ dysfunction contributing to the high morbidity and mortality in AKI and sepsis.

Funding: Private Foundation Support, Clinical Revenue Support, Government Support - Non-U.S.

FR-PO1125
Endoplasmic Reticulum Stress Links Inflammatory and Fibrogenic Responses To Induce Kidney Fibrosis after Acute Injury
Gang Li, Jianhua Huang, Yordanka Ivanova, Anna Zuk. Genzyme Corporation, Framingham, MA.

Background: Acute kidney injury (AKI)-induced aberrant repair has been implicated in the progression of chronic kidney disease (CKD), yet the mechanisms linking AKI to CKD are incompletely understood. Here, we examined the unfolded protein response (UPR) following AKI and hypothesized that activation of the mitoapoptotic branch of UPR together with insufficient adaptive UPR induces inflammation and tubulo-interstitial fibrosis post AKI. In a mouse model of bilateral renal ischemia-reperfusion, the UPR is markedly induced post-reperfusion and correlates with renal dysfunction, tubular apoptosis, and inflammation. In contrast to the sustained induction of the adaptive UPR in young mice (8-10 wks), measured by stimulation of IRE1 and ATF6, aged mice (48-50 wks) display only transient induction of these pathways. Additionally, in aged mice, there is substantially greater induction of CHOP and its downstream target GADD34, which associates with increased mortality and more severe renal injury. Importantly, CHOP induction is sustained, correlating with greater kidney fibrosis and inflammation post-reperfusion. To understand how epithelial cells contribute to fibrosis in a setting of AKI, an ATP depletion-reperfusion model of normal rat kidney epithelial cells was developed to recapitulate ischemia-reperfusion in vivo. Sustained CHOP induction together with transcription of ATG6 is observed data, measured and is associated with induction of inflammatory (Mcp-1, Il6, and Il1b) and fibrogenic genes (Ctgf and Fgfr). Most importantly, induction of these genes is significantly inhibited when CHOP induction is blocked by siRNA, providing direct causal evidence of CHOP as a mediator of inflammatory/fibrogenic responses. In addition, a specific inhibitor of dsRNA-dependent protein kinase (PKR) substantially suppresses the induction of inflammatory/fibrogenic genes through inhibition of CHOP expression. These data provide new insight into how ER stress in general, and the CHOP branch in particular, contributes to kidney fibrosis and suggest that interventions targeting the CHOP branch of UPR may provide new opportunities to prevent kidney fibrosis.

Funding: Pharmaceutical Company Support

FR-PO1126
Suberylanilide Hydroxyacidic Acid (SAHA) Suppresses the Progression of Renal Fibrosis in Aristolochic Acid Induced Renal Fibrosis Model in Mice
Mineaki Kitamura,1 Tomoya Nishino,1 Kumiko Ito,1 Yoko Obata,1 Yoshitaka Hishikawa,2 Takehiko Koji,1 Shigeru Kohno.1 1Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan; 2Department of Histology and Cell Biology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan.

Background: The epigenetic modulation of genes is known to be a key mechanism for the control of gene expression and plays an important role in the progression of fibrosis. Among epigenetic mechanisms, histone acetylation is regulated by histone deacetylases (HDACs) and it leads to the transcriptional activation of genes. In the present study, we investigated the involvement of histone acetylation in the renal fibrosis induced by aristolochic acid (AA), and the effect of suberylanilide hydroxyacidic acid (SAHA), which is a HDAC inhibitor on fibrotic lesions.

Methods: Male Balb/c mice were divided into three groups, AA, AA+SAHA, and control group. Renal fibrosis was induced by AA injection. SAHA or saline were injected subcutaneously for 5 weeks. The expression of type IV collagen, acetylated histone and HDAC2 were examined by immunohistochemistry. TUNEL staining was performed to evaluate apoptosis. Serum creatinine and urinary protein were analyzed to evaluate renal function.

Results: The expression of type IV collagen was significantly increased compared to control group and it was significantly attenuated by SAHA treatment. The number of histone acetylated cells was also increased in AA group, while there was no significant difference between AA and AA+SAHA groups. The number of HDAC2-positive and TUNEL-positive cells was significantly increased in AA group in comparison to control group. Of note, SAHA treatment decreased the number of them and inhibited the progression of renal dysfunction.

Conclusions: These results suggested that histone acetylation was involved in the progression of renal fibrosis and SAHA could attenuate renal fibrosis and renal function.

Funding: Private Foundation Support

FR-PO1127
The HDAC-Dependent Suppression of Bmp-7 Transcription Contributes to the Dysregulation of the Innate Repair Mechanisms of the Kidney Following Prolonged Urinary Tract Obstruction
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Background: While BMP-7 inhibits the pathogenesis of renal injury in a variety of disorders of the kidneys and urinary tract, little is known about the regulation of endogenous BMP-7 and its renal protective functions. This study examines the molecular regulation of BMP-7 in obstructive uropathies and the role which it plays in the repair of obstruction-induced renal injuries.

Methods: A reversible model of unilateral ureteral obstruction (UUO) was used to characterize the repair of the kidney following the correction of short-term obstructions that led to reversible renal injury and prolonged obstructions that led to irreversible renal injury. The role of BMP-7 was assessed by treatment with anti-BMP-7 or exogenous BMP-7.

Results: BMP-7 activity is required for the restoration of renal architecture and the resolution of fibrosis following obstruction-induced renal injury. However, prolonged UUO leads to the loss of BMP-7 expression and irreversible renal injury. Importantly, the restoration of BMP-7 activity through treatment with exogenous BMP-7 enhances kidney repair following prolonged UUO. In examining the mechanisms that lead to the loss of BMP-7, we found that UUO induces deacetylation of the BMP-7 promoter and the subsequent repression of its transcriptional activity. Treatment with the histone deacetylase (HDAC) inhibitor trichostatin A during UUO stimulates the expression of BMP-7, the activation of its downstream signaling pathways, and the BMP-7-mediated suppression of TGF-β-dependent pro-fibrotic pathways. Finally, HDAC inhibition enhances kidney repair in obstructive uropathies that typically lead to irreversible injury.

Conclusions: These results suggest that histone acetylation is involved in the progression of renal fibrosis and SAHA could attenuate renal fibrosis and renal function.

Funding: Private Foundation Support

FR-PO1128
A Novel Method To Remove Iron from the Body by Urinary Excretion
Andong Qiu,1 Neal A. Paragas,1 Roland Strong,2 Jonathan M. Barasch.1 1Columbia; 2Fred Hutchinson.

Background: There are two types of iron overload disorders, hereditary (HH) and acquired hemochromatosis (AH). HH is caused by loss of function of genes that regulate iron metabolism, and AH is caused by blood transfusions and dietary overload. Since each RBC unit contains 250 mg of iron, even a single unit delivers a bolus 10X the daily requirement for iron, resulting in biochemical evidence of toxicity. We sought novel methods to remove iron from the body with low-toxicity and high efficacy.

Methods: NGAL (Siderocalin, Lipocalin 2) is a carrier that can transport iron bound to a complex of transferrin and a transferrin receptor. The complex (25KD)α of NGAL: cationic ferric Fe is filtered by the kidney, and then reabsorbed by the proximal tubule. Consequently, to safely remove iron

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374A
from the body (1) iron must be bound in a redox neutral fashion, (2) NGAL-catecholate-iron complex should not be released by the proximal tubule, and (3) iron must be bound in a pH insensitive fashion, so that the complex does not dissociate in acidic urine.

Results: We have solved each problem by modifying a number of surface residues of NGAL-Siderocalin based on structural data. The mutant, called NGAL* was found to be an efficient chelator of iron compared with NGAL. We also have developed the NGAL-Siderocalin complex variant, called NGAL*-Catecholate, which can chelate iron ions. Furthermore, we have shown that NGAL*-Catecholate is able to bind iron in vivo, thus providing a potential therapeutic agent for the treatment of iron overload syndromes.

Conclusions: These data indicate that THR-184, a peptide agonist of BMP signaling, is effective at preventing ischemia-induced AKI in normal and CKD rats.

Funding: Pharmaceutical Company Support

FR-POI1131

Novel Xanthine Oxidoreductase Inhibitor, Febuxostat, Protects Rat Kidney from Renal Ischemia-Reperfusion Injury. Yoshitsuka Kaka,1 Hiidetsu Tsuda,2 Nortraka Kawada,1 Hirotsugu Iwatsuki,1 Toshiki Moriyama,1 Shiro Takahara,1 Hiromi Rakugi.1,2

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Background: Renal ischemia-reperfusion (IR) injury is unavoidable during kidney transplantation and leads to both early and long-term graft dysfunction. During IR injury, the burst of reactive oxygen species (ROS) can trigger the inflammation and the tubular cell death. Febuxostat is a novel selective inhibitor of xanthine oxidase (XOR), approved for treating hyperuricemia. As XOR is a critical source of ROS, inhibition of XOR could be a therapeutic target for IR injury. Therefore, we performed this study to test the therapeutic effect of febuxostat on renal IR injury.

Methods: To test the protective effect of febuxostat, uninephrectomized Sprague-Dawley rats received saline (Veh) or febuxostat (Feb) at a dose of 10 mg/kg body weight orally 24 hour and 60 min prior to IR injury. Renal IR injury were induced by clamping the left renal artery for 45 min. At 24 hours after reperfusion, serum and kidney samples were harvested.

Results: Feb-treated I/R injured rats exhibited elevated creatinine (1.81±0.29 mg/dl) and BUN (111.2±7.5 mg/dl), which were significantly blunted in Feb-treated I/R injured rats (0.65±0.09 and 40.1±6.4 mg/dl, respectively). Histological analysis revealed that feb-treated rats showed less tubular injury with reductions in CD68-positive macrophages infiltration and TUNEL positive tubular cells compared to Veh-treated rats. In conclusion: In novel xanthine oxidoreductase inhibitor, febuxostat, can protect kidney from renal I/R injury, and may contribute to preserve early and long-term kidney graft function.

FR-POI1132


Background: Exogenously administered endothelial progenitor cells (EPCS) can protect the kidney from acute ischemic injury (IAKI). Over the last years, several EPC activators have been identified which increase the cells’ reparative capacity in IAKI (8-O-cAMP, melatonin). Angiopoietin-1 and -2 play critical roles in vascular homeostasis.

Aim of the study was to analyze whether Angiopoietin-1 (Ang-1) modulates EPC-mediated neoregeneration in IAKI.

Methods: EPCs were isolated from male C57BL/6N mice. After 5 days of culturing, cells were incubated with different substances: Ang-1, Ang-1 + blocking peptide, Ang-1 + anti-VE-Cadherin, 8-O-CAMP+VRG (angioid VE-Cad), respectively. After one hour of incubation, dye-labelled EPCs were systemically injected into recipient animals and their distribution analyzed by serial renal biopsies of 40 minutes. Two days later, renal function and morphology were analyzed. In addition, the migratory cell activity was investigated in vitro.

Results: Mice were not prevented from acute renal failure if 0.5 × 10^6 untreated EPCs were injected intravenously. However, pretreatment with EPCs significantly improved the function of postischemic renal function. These effects were completely reversible after cell incubation with Ang-1 and a specific blocking peptide. In order to analyze whether the effects of Ang-1 are mediated by its agonistic actions on VE-Cadherin, cells were incubated with Ang-1 and anti-VE-Cadherin. Renal function of cell injected mice declined further. Comparable effects were inducable by EPC pretreatment with 8-O-CAMP (+VRG) and anti-VE-Cadherin (8-O-CAMP stimulates VE-Cadherin expression). In vitro analysis showed significantly reduced migratory EPCs activity after VE-Cadherin blockade.

Conclusions: Ang-1 decreases the reparative capacity of syngeneic murine EPCs in IAKI. These effects do not result from agonistic actions on VE-Cadherin. VE-Cadherin rather seems to antagonize Ang-1 in the setting of an EPC-based therapy of IAKI.

FR-POI1133

Intermedin Promotes Recovery from Acute Kidney Injury Induced by Renal Ischemia-Reperfusion Injury. Rongshan Li,1 Xi Qiao,1 Li Zhao,2 Hailong Zhao,1 Yudong Chu,1 Guozheng Feng,1,2

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Background: Acute kidney injury (AKI) induced by ischemia-reperfusion injury (IRI) is associated with high morbidity and mortality. The prognosis of renal IRI is determined by the severity of the postischemic injury and the process of the recovery. Our previous studies demonstrated that intermedin (IMD) protects against renal IRI by reducing injury, while its role in the recovery process is known yet. In this study, we investigate the effect of IMD on the recovery process after renal IRI and its mechanisms.

Methods: Eukaryotic expression vector encoding rat IMD gene or control empty plasmids was transfected into the kidney using an ultrasound-microbubble mediated system. The transfection rate was detected. Renal IRI model was induced by clamping left renal
artery for 45 min followed by reperfusion. One, 2, 3, 4, 7 and 14d of reperfusion, renal function, tubulointerstitial damage and PCNA positive cells were evaluated. The expression of angiogenesis-related proteins (HIF-1α, VEGF and Tie-2), nephrogenic proteins (Pax-2, ZO-1, Ncam, Wt-1 and Vimentin) as well as cell cycle related proteins (cyclin D1, cyclin E, CDK2 and CDK4) was measured by Western blot analysis or ELISA.

Results: IMD expression was significantly up-regulated in kidneys of rats in IMD transgene group than the control. IMD gene transfer significantly protected renal function, tubulointerstitial damage and PCNA positive cells in a Unilateral Rat Model of Ischemia Reperfusion.

Conclusions: IMD can induce an earlier regeneration process after renal IRI, thus promotes recovery from renal IRI.

Funding: Government Support - Non-U.S.

FR-PO1134

DPP4 Inhibition Attenuates Ischemia-Induced Impaired Renal Function in a Unilateral Rat Model of Ischemia Reperfusion


Background:

We have designed a new class of peptide agonists of molecular size,2 which exhibit protective effects on ischemia reperfusion injury (IRI) of heart and lung. We have studied the effect of vildagliptin (VG) on IRI-induced acute kidney injury in rats.

Methods:

Male Wistar rats underwent 30' of left renal ischemia, followed by right nephrectomy. Saline, 1 or 10 mg/kg VG (VG1/VG10) was administered iv prior to ischemia and sham operation. Rats were sacrificed after 2, 12, 48 hours of reperfusion.

Results:

DPP4 inhibition was confirmed by decreased activity in serum and homogenates. VG resulted in a significant dose-dependent decrease of serum creatinine (1.31±0.32 and 0.70±0.19 mg/dl for VG1 and 10 resp. vs 1.91±0.28 mg/dl for controls at 12h; p<0.01). Tubular morphology (PAS-PCNA) revealed reduced necrosis of proximal tubules (VG1: 0.21±0.00 vs 0.49±0.25 in controls; p<0.05). VG had no effect on regeneration. VG resulted in a decreased apoptosis shown by the 2-fold decreased Bax:Bcl-2 mRNA expression and diminished presence of apoptotic bodies on TUNEL stained sections (414.1±231.7 in VG10 vs 1212.0±650.8 in controls; p<0.05). VG treatment also resulted in a early downregulation of several pro-inflammatory markers, including IL16 (3-fold), CXCL10 (2-fold), TNFα (2-fold) and upregulation of anti-inflammatory IL10 (4.6-fold).

Conclusions: DPP4 inhibition results in functional and morphological protection of the kidney against IRI as reflected by changes of expression of relevant genes. The mechanism remains to be elucidated.

FR-PO1135

A New Class of Peptide Agonists Share Functional Properties of Bone Morphogenetic Protein To Protect Against Renal Ischemic Damage

Dattatreyaamrut Voukonda, Peter C. Keck, Silvia B. Campos-Bilderback, Ruben M. Sandoval, Exing Wang, Philippe Beye, William Carlsson, Bruce A. Millotis, Thomas Innovation, Inc., Montreal, QC, Canada; Indiana University School of Medicine, Indianapolis, IN; Mass General Hospital, Harvard Medical School, Boston, MA.

Background:

Proximal tubule epithelial cells (PTEC) play a central role in the response of the kidney to insult by the production of chemokines and cytokines that signal the inflammatory response. Bone morphogenetic protein-7 (BMP-7), a member of the TGF-beta superfamily, has previously been shown to reduce inflammation and tissue damage in animal models of acute renal failure.

Methods:

To study the BMP-7 counterparts CCL19 and CCL21 we used the plt-mouse model, which does not express either CCL19 or CCL21.

Results:

To study the CCR7 counterparts CCL19 and CCL21 we used the plt-mouse model, which does not express either CCL19 or CCL21.

Conclusions:

CCL19 and CCL21 are essential for Regulatory T Cell Function in Nephrotic Syndrome Nephritis. Kate Armstrong, Alexander R. Rosenkranz.

Clinical Division of Nephrology, Medical University Graz, Graz, Austria.

Background:

CCL19 and CCL21 guide T lymphocytes via binding to CCR7 to the T cell areas in lymph nodes. Recently, we provided evidence that CCR7 knockout (KO) mice display increased disease indices when subjected to nephrotic syndrome nephritis (NTS).

Methods:

To study the CCR7 counterparts CCL19 and CCL21 we used the plt-mouse model, which does not express either CCL19 or CCL21.

Results:

Paricalcitol counteracted the CCL19-induced decline in renal function. Paricalcitol also suppressed the expression of transforming growth factor-β1 (TGF-β1), Smad signaling, and the subsequent epithelial-to-mesenchymal process in CCL19-induced nephropathy. In HK-2 cells, paricalcitol suppressed the CCL19-induced increases in ERK1/2 and p38 phosphorylation and in fibronectin/connective tissue growth factor expression. Paricalcitol co-treatment also reduced CCL19-induced over-expression of p53, which coincided with a decrease in pro-apoptotic markers. It also augmented the up-regulated expression of p27kip1 and decreased the number of proliferating cell nuclear antigen positive cells in CCL19-treated rat kidneys. Accordingly, paricalcitol co-treatment reduced the over-expression of cytokine-dependent kinase 2/cyclin E induced by CCL19.

Conclusions:

Our results suggest that paricalcitol can attenuate CCL19-induced renal inflammation by down-regulating the induced expression of fibrotic, apoptotic and proliferative factors. Its underlying mechanisms may include the inhibition of TGF-β1, mitogen-activated protein kinase signaling pathways, p53-induced apoptosis, and the augmentation of p27kip1 expression.

FR-PO1137


Clinical Division of Nephrology, Medical University Graz, Graz, Austria.

Background:

CCL19 and CCL21 guide T lymphocytes via binding to CCR7 to the T cell areas in lymph nodes. Recently, we provided evidence that CCR7 knockout (KO) mice display increased disease indices when subjected to nephrotic syndrome nephritis (NTS).

Methods:

To study the CCR7 counterparts CCL19 and CCL21 we used the plt-mouse model, which does not express either CCL19 or CCL21.

Results:

Paricalcitol counteracted the CCL19-induced decline in renal function. Paricalcitol also suppressed the expression of transforming growth factor-β1 (TGF-β1), Smad signaling, and the subsequent epithelial-to-mesenchymal process in CCL19-induced nephropathy. In HK-2 cells, paricalcitol suppressed the CCL19-induced increases in ERK1/2 and p38 phosphorylation and in fibronectin/connective tissue growth factor expression. Paricalcitol co-treatment also reduced CCL19-induced over-expression of p53, which coincided with a decrease in pro-apoptotic markers. It also augmented the up-regulated expression of p27kip1 and decreased the number of proliferating cell nuclear antigen positive cells in CCL19-treated rat kidneys. Accordingly, paricalcitol co-treatment reduced the over-expression of cytokine-dependent kinase 2/cyclin E induced by CCL19.

Conclusions:

Our results suggest that paricalcitol can attenuate CCL19-induced renal inflammation by down-regulating the induced expression of fibrotic, apoptotic and proliferative factors. Its underlying mechanisms may include the inhibition of TGF-β1, mitogen-activated protein kinase signaling pathways, p53-induced apoptosis, and the augmentation of p27kip1 expression.

FR-PO1138

Th17/Treg Imbalance in Patients with Idiopathic Membranous Nephropathy. Lili Liu, Yan Qin, Limeng Chen, Xue-Wang Li. Division of Nephrology, Peking Union Medical College Hospital, Beijing, China.

Background:

IMN was an autoimmune disease, which involve T and B lymphocyte dysfunction. Recently, regulatory T cells (Treg) cells and Th17 cells had been described as two distinct subsets of T helper cells. Th17/Treg balance may be important in the development/prevention of autoimmunity. The objective of this study was to evaluate whether the Th17/Treg balance was broken in IMN patients.

Methods:

Forty-nine patients, diagnosed IMN by renal biopsy and excluded potential secondary factors, were enrolled in this study. Twenty-eight healthy volunteers served as healthy controls (HCs). The frequencies of peripheral Treg and Th17 subsets were evaluated by flow cytometry. The Th17/Treg ratio was calculated as the ratio of Th17 cells to Treg cells.

Results:

The Th17/Treg ratio was significantly higher in IMN patients than in healthy controls. The Th17/Treg ratio was significantly higher in active IMN patients than in inactive IMN patients. The Th17/Treg ratio was significantly lower in patients with high proteinuria than in patients with low proteinuria. The Th17/Treg ratio was significantly lower in patients with high serum creatinine than in patients with low serum creatinine.

Conclusions:

Our results suggest that the Th17/Treg ratio is altered in IMN patients. The Th17/Treg ratio may be a marker for the severity of IMN.
by flow cytometry. The peripheral relative mRNA expression of key transcription factors was measured using a pre-processed RT-qPCR. The pre-processed RT-qPCR conditions were evaluated by ELISA. The infiltration of Treg cells and expression of IL-17 in renal tissue were determined by immunohistochemical staining.

Results: Compared with HCs, the frequency of peripheral Treg cells and plasma TGF-β1 level decreased, while the frequencies of Th17 cells and plasma IL-23, IL-17 levels increased significantly in IMN patients. The key transcription factors of Treg and Th17, Foxp3 and RORγt, had similar alterations in HC and IMN patients. The Th17/Treg ratios increased along with increased proteinuria and decreased albumin levels in patients with IMN. Furthermore, the renal tissue infiltrates of Treg cells and Foxp3 expression increased significantly compared with that in control subjects. Infiltration of Treg cells was also detected in the renal tissue of IMN patients, while rare Treg cells had been seen in normal renal tissue. The infiltration of Treg cells in renal interstitium also related to a higher clinical remission in IMN patients.

Conclusions: Th17/Treg imbalance existed in IMN patients, suggesting a potential role of Th17/Treg imbalance in the pathogenesis of IMN.

FR-PO1139
Sexual Dimorphism of Foxp3+ T-Regulatory Cell Function Is HDAC6-Dependent

Tatiana Akinmova, Ulf H. Beier, Wayne W. Hancock, Children’s Hospital of Philadelphia & University of Pennsylvania.

Background: Pre-menopausal women have 2-3 fold higher rates of autoimmune diseases compared to age matched males. While the basis for this difference is likely complex and multifactorial, little attention has been given to whether there are gender-based differences in Treg suppressive function, despite the fact that Foxp3 is an X-linked gene.

Methods: We analyzed Treg gene expression and suppressive function (SF) in normal 6-8 wk male and female littermate C57BL/6 mice, and in various KO strains.

Results: While male and female mice had comparable numbers of Tregs, male Tregs had greater SF. This disparity was also seen using male and female Tregs of various knockout mice, or, comparing Tregs from Foxp3ΔΔ-/-, HDAC6/-/-, C57BL/6 mice had the same number of Tregs as WT mice, but higher Foxp3 expression per cell (qPCR, Western blot) and a more mature phenotype (assessed using CD44, CD26L, CD69 and CD103 markers). HDAC6/-/- Tregs were also more suppressive than WT Tregs, but interestingly, female Tregs had better suppressive function than male Tregs, i.e. there was a gender-based reversal in HDAC6/-/- Treg function. The SF mean ratio of adult Tregs was 1 (WTF) to 1.3 (WT M) to 2 (HDAC6 F) to 1.7 (HDAC6 M), and Tregs from HDAC6/-/- females had more mature Treg phenotype (10-12% fewer naive Tregs). This difference was also seen upon treatment of Tregs from normal human donors (25 tests) with pan-HDAC or HDAC6-selective inhibitors; while male Tregs had 1.7±0.1 fold enhanced SF, female Tregs showed 2.2±0.2 fold increase (p=0.057). Binding of estrogen to the estrogen receptor-α induces HDAC6 and promotes proteasomal degradation of Smad2 and Smad3, and Tregs from HDAC6/-/- mice showed increased phospho-Smad3. We are currently assessing the effects of HDAC6 targeting on Smad2/3-dependent events at the TGF-β-dependent Foxp3 enhancer.

Conclusions: Our studies show HDAC6, also an X-linked gene, is key to regulation of Treg function and that its targeting can affect estrogen/estrogen receptor signaling. Use of HDAC6 selective inhibitors may provide a means to overcome the gender-based disparity in Treg function and be useful in the therapy of multiple autoimmune diseases, including those affecting the kidney.

Funding: NIDDK Support, Other NIH Support - NIAID

FR-PO1140
IKK2 Is Required for the Development of Regulatory T Cells

Eveline Piella,1 Anna Hermann,1 Gunther Zahnner,1 Annette Erhardt,2 Hans-Joachim Paust,3 Jan-Eric Turner,1 Ulf Panzer,1 Joachim Velden,1 Gisa Tregs,2 Rolf A. Stahl,1 Friedrich Thaiss,1 Universitiy, 1Ill. Med. Department, Hamburg, 2Univ. Hospital, Inst. Exp. Immunology, Hamburg, Germany, 3Univ. Hospital, Inst. Pathology, Hamburg, Germany.

Background: Regulatory T cells (Tregs) are characterized by their expression of the transcription factor Foxp3. In experimental glomerulonephritis (GN) Tregs seem to play a pivotal role, since their depletion leads to an aggravation of GN. Recent studies have shown that members of the NF-kappaB-family are involved in Foxp3 gene expression. Phosphorylation of the NF-kappaB inhibitor by the I-kappaB-kinase 2 (IKK2) is one of the crucial steps in NF-kappaB activation. To elucidate the potential role of NF-κB in the development of Tregs in vivo, mice with IKK2 deficient Foxp3- cells were generated (Foxp3Δfl/fl). Our data demonstrate that continuous IKK2 activity is essential to maintain the homeostatic proliferation (HP) of Treg cells in the periphery.

Methods: Foxp3Δfl/ (YFP) mice have been described by Dr. A. Y. Rudensky and IKK2- mice were kindly provided by Dr. M. Karin. Animals homozygous for Foxp3Δfl/IKK2- were sacrificed on days 3, 7 and 14 after birth. Cells from thymus, spleen and lung were isolated and the frequencies of Tregs analyzed by FACS. Periodic-acid-Schiff staining and immunohistochemistry were performed using standard techniques.

Results: Animals homozygous for Foxp3CreIKK2/IKK2- mice spontaneously develop a scurfy-like phenotype at day 12-27 after birth which manifests in a hunched posture and a skin disorder characterized by scaliness, ears and a tail shielded by padded rings. Animals show enlargement of lymph nodes and a significant splenomegaly. Homozygous Foxp3CreIKK2/IKK2- mice were born at term but showed a delay in organ architecture at day 14 of the spleen. The kidneys did not show infiltrating inflammatory cells or failed tissue organization. The number of Tregs in the spleen and lung are significantly reduced but are not altered in the thymus compared to control animals.

Conclusions: Our data demonstrate that continuous IKK2 activity is essential to maintain the homeostatic proliferation of Treg cells in the periphery. Thus, continued expression of IKK2 in mature Tregs is indispensable to maintain the dominant tolerance which is mediated by these cells.

Funding: Government Support - Non-U.S.

FR-PO1141
Anti-CD45RB Not Only Augments Homoeostatic Proliferation but Uniquely Promotes Antigen-Specific Proliferation of Regulatory T Cells In Vivo

Wang Ying, David M. Rothstein,1 University of Pittsburgh, PA.

Background: CD4+Foxp3+ Treg play an important role in transplant tolerance. By transferring CFSE-labeled Foxp3+ (Treg) or Foxp3- (Tconv) CD4 cells from congenic Foxp3-reporter mice into naïve WT recipient, we showed that Treg exhibit a higher constitutive rate of homoeostatic proliferation (HP) than Tconv (~50% vs. ~10% over 10d). Moreover, anti-CD45RB induces tolerance through a 2X increase in Treg which results by dramatically increasing Treg HP, even in the absence of exogenous antigen. While exogenous antigen is not required, Treg HP may still be antigen-driven. To address the signals involved, CFSE-labeled congenic CD4 cells were transferred into naïve WT mice and proliferation of Foxp3+ and Foxp3- cells assessed on d10. Treating mice with Cyclosporin A (CsA) greatly reduced both basal and anti-CD45RB-mediated HP of Treg. This inhibition by CsA was only partly restored by concomitant administration of IL-2, suggesting that NFAT translocation was required. Moreover, when CFSE-labeled congenic CD4 cells were transferred into MHCII+ mice, both basal and anti-CD45RB-mediated HP of Treg were markedly reduced. These studies suggest that antigen signaling is required for high rates of HP by Treg. OT-II mice (TCR-transgenic specific for Ova peptide) on a RAG-/- background, exhibited a similar increase in HP, but lower 2X increase, compared with WT mice. Our studies show HDAC6, also an X-linked gene, is key to regulation of Foxp3 expression and function, and that its targeting can affect estrogen/estrogen receptor signaling. Use of HDAC6 selective inhibitors may provide a means to overcome the gender-based disparity in Treg function and be useful in the therapy of multiple autoimmune diseases, including those affecting the kidney.

Funding: NIDDK Support

FR-PO1142
Regulatory T Cells Reverse Obesity-Linked insulin Resistance and Diabetic Nephropathy

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Background: Foxp3 expressing regulatory T cells (Tregs) are critical for maintenance of tolerance in rodents and men. Evidence is increasing that regulatory T cells improve insulin resistance in type 2 diabetes mellitus.

Methods: The study was designed to evaluate the role of Tregs in type 2 diabetes mellitus and end organ damage in the human and murine setting.

Results: We observed that fat mass, fasting blood glucose levels and TNF-α mRNA expression significantly correlated with Foxp3 transcripts in human visceral adipose tissue. To further evaluate the pathogenic role of Tregs in insulin resistance, we used the db/db mouse model, depleted of or transferred with Tregs, and followed the mice for 56 days. Treg-depletion using an anti-CD25 monoclonal antibody enhanced insulin resistance as shown by increased fasting blood glucose levels as well as impaired insulin sensitivity. Moreover, Treg-depleted db/db mice developed increased signs of diabetic nephropathy, such as albuminuria and glomerular hyperfiltration. This was paralleled by a pro-inflammatory milieu in both, murine visceral adipose tissue and the kidney. vice versa, adoptive transfer of CD4+Foxp3+ Tregs significantly improved insulin sensitivity and diabetic nephropathy. Accordingly, there was increased mRNA expression of Foxp3 as well as less abundant pro-inflammatory CD8+CD69+ T cells in visceral adipose tissue and kidneys of Treg-treated animals.

Conclusions: In summary, our data suggest a potential therapeutic value of Tregs to improve insulin resistance and end organ damage in type 2 diabetes by limiting the pro-inflammatory milieu in VAT.

Funding: Government Support - Non-U.S.

FR-PO1143
Early IL-17 Production by gamma-delta T Cells in the Kidney Is Induced by IL-23 and Contributes to Tissue Injury in Murine Crescentic Glomerulonephritis

Christian Krebs,1 Jan-Eric Turner,1 Hans-Joachim Paust,1 Andre Pascal Tittel,2 Sabrina Bianca Bengen,2 Oliver M. Steinitz,2 Catherine Meyer-Schwesinger,1 Rolf A. Stahl,3 Christian Kurts,2 Ulf Panzer.1 1Institut für Experimentelle Immunologie, Universitätshospital Bonn, Bonn, Germany; 2Clinical Division of Angiology, Medical University Graz, Graz, Austria.

Background: The inflammatory cytokine IL-17A (IL-17) is thought to play a critical role in the pathogenesis of human and experimental crescentic glomerulonephritis. However, the cell types which contribute to renal IL-17 production in glomerulonephritis are not...
well characterized. In addition, the mechanisms which induce IL-17 production by renal leukocytes remain to be elucidated.

Methods: To characterize IL-17-producing cells in renal inflammation, we performed a time course analysis (day 1 - 30) of IL-17 production in a T cell-dependent model of crescentic glomerulonephritis in mice (nephrotic nephritis, NTN).

Results: Ten-colour flow cytometric analysis of intracellular IL-17 staining and surface marker-identified gamma-delta T cells (gdTC) as an important source of early IL-17 (day 14; gamma-delta T cells with necrosis, anti-CD45 Ab + anti-CD45R Ab clone 61-1; anti-CD45 clone 41.4; urine albumin/creatinine 28±5; anti-CD45 116±14; 125±14 µg/µmol, both P<0.0001).

Conclusions: These results define a pathogenic MPO T cell epitope and support a role for cell mediated effector responses, along with humoral responses, in the pathogenesis of MPO-ANCA associated glomerulonephritis.

Funding: Government Support - Non-U.S.

FR-PO1144

The Function of CD4 Lymphocytes in Experimental Autoimmune Glomerulonephritis Hans-Willi Mittrucker, Julia Holzer, Stefanie Hünemöller, Hans-Joachim Paust, Ulf Panzer, Helmut Hopfer. 1Immunology, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 2Nephrology, University Medical center Hamburg-Eppendorf, Hamburg, Germany; 3Pathology, University Hospital Basel, Basel, Switzerland.

Background: Autoimmunity against the alpha3IV-NC1 domain of type IV collagen results in crescentic glomerular basement membrane (GBM) glomerulonephritis. Although auto-antibodies against alpha3IV-NC1 are central for the development of glomerulonephritis, there is increasing evidence for a contribution of alpha3IV-NC1-specific T-lymphocytes in disease. In this study, we will investigate the abundance and function of different T cell subsets at different phases of anti-GBM nephritis.

Methods: Experimental Autoimmune Glomerulonephritis (EAG) was induced in DBA/1 mice by repeated immunization with recombinant human alpha3IV-NC1 protein in complete and incomplete Freund’s adjuvant.

Results: Immune mice develop high serum titers of alpha3IV-NC1-specific antibodies against alpha3IV-NC1 by IFN-γ and FACs analyses of renal cells revealed massive accumulation of macrophages and T cells. T cells demonstrated a highly activated phenotype and upon polyclonal stimulation, a fraction of CD4 T cells produced IFN-γ and IL-17 in response to alpha3IV-NC1.

Conclusions: In conclusion, our EAG model closely reproduces central features of human anti-GBM disease. Cytokine expression analyses point to a contribution of Th1 and Th17 cells in the progression of disease. Our current studies aim at clarifying the role of the Th17 subset in the pathogenesis of anti-GBM disease.

Funding: Government Support - Non-U.S.

FR-PO1145


Background: Autoimmunity to myeloperoxidase (MPO) is important in vasculitis. Autoreactive CD4+ cells support MPO-ANCA production and may play a direct role in injury, but T cell epitopes are not known and a role for effector CD4+ cells is uncertain.

Methods: An immunodominant MPO T cell epitope was defined by responses to OVA peptides in C57BL/6 mice. We precipitated against an MPO peptide, transferred into Rag-1-/- mice, then disease triggered with either low dose anti-glomerular basement membrane antibodies (anti-GBM Ab) or anti-MPO Ab/LPS.

Results: Mice were immunized with the 5 strongest responding peptides (by IFN-γ ELISPOT). All peptides responded to themselves and to recombinant mouse MPO, but peptide pepp52 responded most strongly to mMPO and itself (polarization, IFN-γ and IL-17 ELISPOT). Pep52-immunized mice made MPO-ANCA, but at lower titers than MPO immunized mice. MPO pep52 specific CD4+ clones were generated by immunizing C57BL/6 mice. An MPO-specific CD4+ T cell clone was transferred into Rag-1-/- mice, which were then immunized with MPO pep52 (an OVA-specific clone was a control; these Rag-1-/- recipients were OVA pep immunized). 7 days later, low dose anti-GBM Ab was injected. Mice receiving anti-MPO clones developed progressive glomerular disease, but mice receiving anti-OVA clones did not develop necrosis: day 5 anti-GBM clone 4±1, anti-MPO clone 49±5; day 14 anti-GBM 7±2, anti-MPO 69±4; both P<0.001 and albuminuria (albumin/creat day 5 anti-OVA 15±2, anti-MPO 276±44; day 14 anti-OVA 19±2, anti-MPO 1080±307 µg/mmol; both P<0.001).

Conclusions: In vivo, a pathogenic MPO T cell epitope induces renal injury in a mouse model of crescentic glomerulonephritis. The MPO-ANCA induced injury may be caused by an MPO-specific effector CD4+ cell clone.

Funding: National Health and Medical Research Council.
FR-PO1148

Morphine Enhances HIV-1 Entry into Kidney Cells through a Novel Pathway

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Background: Opiate addiction is considered to be a risk factor for the development of HIV-associated nephropathy (HIVAN). We hypothesized that opiates might be enhancing HIV entry into kidney cells.

Methods: Morphine pre-treated human tubular cells (HK2 and HRPTECs) were incubated with HIV-infected (HIV-LY) or control lymphocytes (LY) followed by evaluation of HIV infection of tubular cells by gap expression (real time PCR) and T cell apoptosis (FACS analysis). To determine the involved mechanism of T cell apoptosis, HK2s were pretreated with anti-PDL-1 antibody and then co-cultivated with HIV-LYs and LYs. Subsequently, HIV-LYs and LYs were evaluated for apoptosis and tubular cells for HIV expression. In addition, the effect of morphine on tubular cell PDL-1 expression was determined by FACS analysis. To determine the role of phagocytosis in the apoptosed HIV-LYs and opiate receptors, morphine-pretreated HK2s were co-cultivated with HIV-LYs in the presence of a caspase-3 inhibitor, naloxone (opioid receptor antagonist) or cytochalasin-B (an inhibitor of phagocytosis) followed by evaluation for tubular cell HIV expression.

Results: Morphine not only enhanced tubular cell PDL-1 expression but also promoted apoptosis of HIV-infected T cells; whereas, anti-PD1-1 antibody prevented morphine-induced tubular cell apoptosis. Morphine enhanced tubular cell HIV-1 expression; whereas, naloxone inhibited tubular cell HIV-1 expression. Since both caspase-3 inhibitor and cytochalasin-B inhibited T cell apoptosis, we hypothesized that enhanced HIV-LY uptake may contribute to tubular cell HIV-1 expression.

Conclusions: Tubular cells not only facilitated apoptosis of HIV-1 infected T cells but also demonstrated capability of phagocytosing them. Morphine enhanced HIV-1 uptake by tubular cells by enhancing apoptosis of HIV-infected T cells. Since direct tubular cell HIV-1 entry did not induce productive infection, this would suggest that phagocytosed apoptosed T cells provided a suitable milieu for productive HIV-1 infection in tubular cells.

Funding: NIDDK Support.

FR-PO1149

Adverse Host Factors Exacerbate Occult HIV-Associated Nephropathy

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1Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; 2Pathology, New York Medical College, Valhalla, NY.

Background: Animal studies indicate that single HIV gene expression is enough to develop overt HIV AN. We have also demonstrated that single HIV gene expression is enough to develop overt renal disease in the elderly. Elderly have more severe course of AKI with increased progression to chronic kidney disease and worse outcomes. The elderly could contribute to the severity of AKI.

Methods: We performed IRI surgery on young (Y) (5-7 weeks) and elderly (E) (1 year) C57BL/6 male mice using a well-established model. Briefly, microvascular clamp was added (CsA+CoCl2) or CsA+DMOG. Nuclear factor kappa B (NF-κB) was also measured using caspase-3 fluorometric assay. In animal experiments, male Sprague Dawley rats kept on a 0.05% low salt diet were treated with CsA for 28 days (15mg/kg/day) or vehicle. FACS analysis was performed for Klotho protein expression.

Results: Administration of CsA significantly decreased TGF-β expression in both Y and E kidneys (56 ± 5% vs. 100 ± 8%, p< 0.05) and was further decreased at 4-week compared to VH (12 ± 3% vs. 8 ± 0.5%, p< 0.05 vs. CsA group). There was no significant difference in the expression of TGF-β in the E group compared to Y group. There was a significant elevation in the expression of Klotho in both Y and E kidneys (1.7 ± 0.3 vs. 0.4 ± 0.1, p< 0.05). Also, caspase-3 activity was significantly decreased in both Y and E kidneys compared to VH (48.6 ± 4.6 vs. 11.5±1.57 vs. 7.6±1.2, P< 0.001). Additionally, CD8+ and CD8+CD69+ were significantly higher in E kidneys at baseline (53.5±1.3 vs. 25.3±1.5, P< 0.001) and (25.5±1.3 vs. 44.6±4.5, P< 0.001). To determine the role of phagocytosis in the apoptosed HIV-LYs and opiate receptors, morphine-pretreated HK2s were co-cultivated with HIV-LYs in the presence of a caspase-3 inhibitor, naloxone (opioid receptor antagonist) or cytochalasin-B (an inhibitor of phagocytosis) followed by evaluation for tubular cell HIV expression.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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PTEC interactions with autologous dendritic cells (DC), professional antigen-presenting cells in many human kidney diseases. We have recently demonstrated the capacity of activated Proximal tubule epithelial cells (PTEC) participate in the disease process in many human kidney diseases. We have recently demonstrated the capacity of activated PTEC to inhibit autologous immune responses (Wilkinson et al, NDT 2011, 26(5):1483-92).

In order to further define this regulatory mechanism, we monitored for the first time activated PTEC interactions with autologous dendritic cells (DC), professional antigen-presenting cells that play a pivotal role in the induction and regulation of immune responses.

Methods: Primary PTEC and peripheral blood mononuclear cells were collected from patients with and without nephropathies. Purified CD1c+ blood DC were cultured with autologous IFN-γ-activated PTEC in the presence or absence of DC activator, polynucleotides poly(dA:dT) (PIC). DC responses were monitored by cytokine secretion, surface antigen expression and antigen-presenting ability.

Results: Unstimulated CD1c+ DC upregulated surface CD40 and CD86 and secreted higher levels of IL-6 and IL-8 in the presence of PTEC compared to DC alone, with minimal changes to CD80, CD83 and HLA-DR expression and no or low levels of IL-1β and IL-10 detected in any culture supernatants. PIC-stimulated CD1c+ DC expressed higher CD40 and CD86, produced elevated IL-6 and IL-1β and lower levels of IL-10 in the presence of PTEC than PIC-stimulated CD1c+ DC alone. Notably, PIC-stimulated CD1c+ DC displayed reduced CD83 levels in all donors and lower expression of HLA-DR in 4/5 donors when cultured with PTEC, with 3/4 of these donor DC increasing proliferation of allogeneic T cells in a mixed lymphocyte reaction (MLR) compared to PIC-stimulated CD1c+ DC alone. These results highlight a novel immune-regulatory role for activated PTEC through inhibition of DC function. Conclusions: Our data suggest that activated PTEC regulate human autologous immunity via complex interactions with DC. Further dissection of the mechanism of PTEC modulation of autologous immune responses may offer targets for therapeutic intervention in renal disease.

Funding: Government Support - Non-U.S.

FR-PO1155

Suppression of Inflammatory Cytokines-Triggered NF-κB Activation and INOS Expression in Renal Tubular Epithelial Cells by Gap Junction Inhibitor Flufenamic Acid: A Critical Involvement of AMPK Yuan Chi, Qiaoqiong Yan, Ying Zhu, Masanori Kitamura, Jian Yao. Department of Molecular Signalling, University of Yamanashi, Chuo, Yamanashi, Japan.

Background: Our previous studies demonstrated that dysfunction of gap junctions (GJs) prevents renal tubular cell injury in several pathological situations (Br J Pharmacol 2017;174:1983-98; using a doxycycline inducible SelG1 reporter. Signal 2009;22:221-39). Because the cytokines-elicited expression of iNOS and production of nitric oxide (NO) contribute to the renal tubular cell injury, we asked whether GJ inhibitors also interfere with the inflammatory responses.

Methods: NFκB activation was evaluated by using NIK-NFκB-SEAP reporter cells. NO was measured by the Griess reagent. Ca2+ was measured by using Fura-2 as an indicator. AMPK activation was evaluated by Western blot analysis of phosphorylation levels of AMPK in the diseased kidney.

Results: 1) Exposure of renal tubular cell line NR52-E52 to inflammatory mediators TNFα and IL-1β resulted in an NFκB-dependent expression of iNOS and production of NO. 2) Among several GJ inhibitors tested, flufenamic acid (FFA) strongly inhibited NFκB activation and significantly suppressed the expression of iNOS and production of NO. 3) This effect of FFA was mimicked by AMPK activator AICAR and abolished by CAMKKβ inhibitor STO-609, indicating a mediating role of CAMKKβ-AMPK pathway. 4) FFA induced a Ca2+-dependent activation of AMPK. Inhibitor Flufenamic Acid: A Critical Involvement of AMPK.

Conclusions: Taken together, we concluded that GJ inhibitor FFA potently suppressed the inflammatory cytokines-elicited activation of NFκB and expression of iNOS in renal tubular epithelial cells through activation of Ca2+-CAMKKβ-AMPK pathway. FFA may be therapeutically employed for intervention of inflammatory tubular cell injury through suppression of inflammatory responses and disruption of gap junction-mediated propagation of cell death.

Funding: Government Support - Non-U.S.

FR-PO1156


Background: Kidney tubulointerstitium contains resident macrophages (kMDs). Distinguishing between kMDs and kDCs relies on specific cell-surface markers: CD11c the most reliable marker for DC and F4/80 for macrophages. However, a group of kidney cells has been found to express both dendritic and macrophage markers, but their function and distribution in kidney are unknown. In this study, function and localization of CD11c+F4/80+ cells, kMDs and kDCs were examined in healthy and diseased kidney.

Methods: Adriamycin nephrosis (AN) was induced by 10 mg/kg adriamycin in BALB/c mice. Localization and function (phagocytosis and antigen presentation) of CD11c+F4/80+ cells, CD11c-F4/80+ kMDs and CD11c+F4/80- CD11c-F4/80+ kDCs were examined in normal and AN mice.

Results: CD11c+F4/80+ kMDs comprised 6% of total kidney cells, CD11c+F4/80+ cells 2% and CD11c+F4/80- kMDs 1.6%. CD11c+F4/80+ kDCs and CD11c+F4/80+ kDCs were mainly present in cortex of normal and AN kidney, while CD11c+F4/80- kMDs were present in cortex and medulla. CD11c+F4/80+ cells highly expressed macrophage markers including CD68, CD204 and CD206, but had lower expression of DC markers, including CD205 and CD103. Interestingly CD11c+F4/80+ cells from kidneys of mice with or without AN had a higher capability of phagocytosis and lower ability of antigen presentation than CD11c+F4/80- kMDs, indicating that CD11c+F4/80+ cells are more like macrophages.

Conclusions: CD11c+F4/80- mononuclear phagocytes have high capability for phagocytosis and low for antigen presentation, indicating they should be defined as a subset of kidney macrophages. The function of these cells will be examined in vivo by depletion and reconstitution studies.

Funding: Government Support - Non-U.S.

FR-PO1157

Inflammasome Activation and Processing of IL-1β and IL-18 in Experimental Crescentic Glomerulonephritis in the Rat Simona Deplano,1 Jennifer Smith,1 Charles D. Pusey,2 Robert J. Unwin,1 Frederick W.K. Tam,1 H. Terence Cook,1 Jacques Behmoaras,1,2 Renal Medicine, Imperial College London;1 UCL Medical School, London, United Kingdom.

Background: Inflammasomes are molecular platforms activated by cellular infection or stress, which trigger the release of active proinflammatory cytokines such as IL-1β and IL-18 through caspase-1 activation. The role of caspase-1 dependent inflammasome activation in crescentic glomerulonephritis (CrGN) is unknown.

Methods: We studied inflammasome activation in the macrophage-dependent model of nephrotoxic nephritis (NTN) in the Wistar-Kyoto (WKY) rat. Activation of Nlrp3 and AIM2 inflammasomes was studied in bone-marrow derived macrophages (BMDMs) isolated from WKY and NTN-resistant Lewis (LEW) rats. Ex-vivo investigation of the inflammasome activation was performed in cultured glomeruli isolated from WKY and LEW kidneys 4 days following NTN induction, a time point corresponding to maximal macrophage infiltration.

Results: We found that the majority of the Nlrp3-inflammasome genes are up-regulated in WKY BMDMs compared with LEW. Lipopolysaccharide (LPS) primed WKY BMDMs produced significantly higher levels of IL-1β and IL-18 when stimulated with ATP compared with LEW. Similarly, the activation of the AIM2-inflammasome following LPS and poly(dA:dT) treatment resulted in increased IL-1β secretion in WKY BMDMs, suggesting that the increased IL-1β production is due to a dysregulated caspase-1 activity in the macrophages of this rat strain. This was indeed the case as we showed increased caspase-1 activity in WKY BMDMs when compared with LEW, and caspase-1 dependent...
IL-β and IL-18 production was significantly reduced following a specific caspase-1 inhibitor prior to caspase-1 siRNA. Importantly, we showed markedly increased active IL-18 and IL-1β production in WKY nephritic glomeruli, together with significantly increased caspase-1 activity suggesting that caspase-1-dependent inflammasome activation is driven by infiltrating macrophages in NTN.

Conclusions: This is the first report showing inflammasome activation in macrophage-dependent Crgn. These results reveal novel genetic factors controlling susceptibility to Crgn.

FR-PO1158
Proinflammatory Role of the Inflammasome Component Nlrp3, but Not Asc in Murine Immune Complex-Mediated Glomerulonephritis Kirstin Andersen, Nuru Eltrich, Volker Vielhauer. Nephrologicals Zentrum, Ludwig-Maximilians-University, Munich, Germany.

Background: Interleukin-1β (IL-1β) is an inflammatory mediator of immune complex-induced glomerulonephritis (GN). Caspase 1 activates IL-1β in an inflammasome-dependent intracellular process. Thus, we examined the functional role of the inflammasome components Nlrp3 and its adapter molecule Asc in autologous murine nephroserous nephritic serum (NTN).

Methods: NTN was induced in wild-type, Il1r1-, Nlrp3-, and Asc-deficient C57BL/6 mice after preimmunisation with rabbit IgG. At day 21 functional parameters, renal histology and renal leukocyte infiltrates were compared between the four groups and untreated wild-type controls. In addition, cellular and humoral immune responses against rat IgG were analysed.

Results: NTN was not inducible in Il1r1-deficient mice, confirming a crucial role of IL-1β in the pathogenesis of NTN. Nlrp3-deficient mice developed a less pronounced glomerular-thickening syndrome compared to wild-type, including reduced albuminuria, less hypoproteinemia, and a tendency towards lower area urea levels. Consistently, renal leukocyte infiltrates were significantly reduced in Nlrp3-/- mice. This correlated with a decrease in renal mRNA expression of inflammatory chemokines and cytokines. In contrast, NTN was not attenuated in Asc-/- mice.

Systemic immune reactions were examined after restimulation of splenocytes with rabbit IgG. Nlrp3-/- splenocytes revealed a tendency lower than inflammatory cytokines and reduced numbers of activated CD69+ CD19+ T cells. Interestingly, the humoral immune response was increased in Nlrp3-/- mice, as indicated by higher autologous anti-rabbit IgG serum titers. Despite developing NTN, Asc-/- mice also demonstrated an attenuated T cell response, but similar anti-rabbit IgG levels compared to wild-type.

Conclusions: We identified Nlrp3 as an important pro-inflammatory mediator of immune complex GN. Surprisingly, we could demonstrate an inflammatory function of Nlrp3 independent of its adapter molecule Asc and the inflammasome, although deficiency of both molecules reduced cellular immune responses against the foreign antigen planted in the glomerulus. Thus, Nlrp3, but not Asc may be a new therapeutic target for immune complex GN.

Funding: Government Support - Non-U.S.

FR-PO1159
Accelerated Necrotizing Glomerulonephritis in Response to Nephrotic Serum in Mpv17-Deficient Mice Gabriella Casalena, Dmitrij Kollins, Ilse S. Daen, Madeleine E. Gentile, Erwin P. Bottiger, Detlef O. Schloendoff. Medicine, Mount Sinai School of Medicine, New York, NY.

Background: Human Mpv17 mutations cause hepatocerebral forms of mitochondrial DNA (mtDNA) deletion syndrome. Insetional deletion of Mpv17 in mouse was initially associated with glomerulocarcinosis [Weiter H et al., Cell 1990]. Mpv17 is a mitochondrial protein with unknown function, but may be involved in control of mitochondrial DNA copy number and ROS production. MTDNA copy number was redracted in glomerular tubul of Mpv17/-/- mice that manifested reduced lifespan and kidney failure [Viscomi C et al. Hum Mol Genet 2009].

Purpose: To determine whether Mpv17-deficiency alters lesions and manifestations of necrotizing glomerulonephritis (GN) typically associated with nephrotic serum model in mice.

Methods: Mpv17+/- and Mpv17-/- mice (C57BL/6) were injected with sheep nephrotic serum and sacrificed after one or seven days.

Results: Tissue oxidative stress assessed by 3-Nitrotyrosine IHC was increased in baseline in glomeruli of Mpv17+/- compared to Mpv17+/+ mice. Day 1 after NTN injection, PAS positive staining was apparent increased in Mpv17-/-, but not in Mpv17+/- mice, compared to controls. By day 7, glomerular lesions were present in all Mpv17+/- mice and increased compared to Mpv17+/- mice. Tubular casts were detectable only in Mpv17-/- mice. Serum creatinine was increased in Mpv17+/- compared to Mpv17+/- (0.74 ± 0.01 vs 0.48 ± 0.09; p<0.05). ACR was not significantly different. Quantification of infiltrates by staining with anti-CD45 antibody showed no differences between Mpv17+/- and Mpv17-/- at day 7 after NTN injection.

Conclusions: Loss of mitochondrial membrane protein Mpv17 was associated with increased baseline glomerular oxidative stress, and accelerated the onset and increased the severity of necrotizing GN induced by NTN.

Funding: NIDDK Support

FR-PO1160
Leukocyte Syk Activation in Antibody-Dependent Glomerular Injury Jessica Ryan, John Kanellis, David J. Nikolic-Paterson. Department of Nephrology and Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.

Background: Spleen tyrosine kinase (Syk) plays an important role in Fc-γ receptor signaling. Recent clinical trials using an Syk inhibitor have shown protection against joint damage in rheumatoid arthritis. However, the potential role of Syk in acute antibody-dependent glomerular disease has not been investigated. Therefore, we examined activation of Syk in human rapidly progressive glomerulonephritis by detecting phosphorylation of Tyr525/526 in the Syk activation loop.

Methods: Immunostaining for phosphorylated-Syk (p-Syk) was performed in renal biopsy sections in cohort of 68 patients, which included: MCD (3), TBMD (8), post-infectious glomerulonephritis (5), Class IV SLE (11), and ANCA vasculitis (9), IgA (14), membranous nephropathy (7) and FGS (10).

Results: p-Syk staining was seen in minimal change or thin membrane disease which lack antibody deposition and leukocytic infiltration. In contrast, all cases of post-infectious glomerulonephritis exhibited numerous p-Syk± cells in glomeruli. Two-colour staining identified p-Syk± cells as infiltrating neutrophils, and to a lesser extent, macrophages. Furthermore infiltrating leukocytes were also positive for phospho-p38 and phospho-INK, known downstream targets of Syk signalling, suggesting Syk-dependent leukocyte activation in post-infectious glomerulonephritis. Glomerular p-Syk± cells were seen in 8/9 cases of ANCA vasculitis, 7/11 cases of Class IV SLE and 6/14 cases of IgAN. In these diseases, p-Syk staining was also localised to infiltrating neutrophils and possibly to some monocyte/macrophages. In addition, 1/7 cases of membranous and 2/10 cases of FGS exhibited glomerular p-Syk positive cells in areas of glomerulosclerosis. Furthermore, in some cases infiltrating p-Syk± cells were also seen in areas of interstitial fibrosis and tubular atrophy.

Conclusions: Syk is activated in infiltrating leukocytes, predominantly neutrophils, in acute antibody-dependent glomerular disease. These findings support the therapeutic use of Syk inhibitors in rapidly progressive crescentic glomerulonephritis.

Funding: Other NIH Support - NHMRC Australia

FR-PO1161
Decreased Plasma RANTES Concentration in Children with Minimal Change Nephrotic Syndrome in Relation Is Associated With Th2 Cytokine Profile Chao Yen Chan,1 Wee Song Yeo,2 Kar Hui Ng,3 Subhra K. Biswas,3 Hui Kim Yap.1 Pediatrics, Tan Tock Soon School of Medicine, National University of Singapore, Singapore; 2SLN, A*STAR, Singapore.

Background: Cytokine release secondary to viral infections may potentially trigger relapses in minimal change nephrotic syndrome (MCNS). This study aimed at investigating the cytokine profile in MCNS patients during relapse and remission, in order to enhance our understanding of disease pathogenesis.

Methods: Plasma cytokine profile was analyzed using multiplex suspension bead array system in 13 nephrotic children, aged 4 to 25 years with steroid-sensitive nephrotic syndrome in relapse and remission. Thirty-two age-matched healthy controls were included for comparison. Results were expressed as mean±SEM. Statistical analyses was done using a linear mixed model to compare the differences in cytokine levels between MCNS patients and healthy controls.

Results: As shown in the table below, of the 27 cytokines analyzed, plasma IL-5, IL-9, IL-10, and IL-13 were significantly increased and RANTES concentration was significantly decreased in MCNS relapse compared to age-matched controls (p<0.05). There was no significant difference in the concentrations of these cytokines between MCNS remission and controls. However, pairwise comparison showed that only RANTES concentration was significantly lower in patients in relapse compared to remission (p<0.023).

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Controls (n=32)</th>
<th>MCNS Relapse (n=13)</th>
<th>MCNS Remission (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-5</td>
<td>3.78±0.38</td>
<td>5.09±0.71</td>
<td>5.69±0.72²</td>
</tr>
<tr>
<td>IL-9</td>
<td>1.27±1.23</td>
<td>1.6±0.14</td>
<td>2.23±0.40</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.25±0.24</td>
<td>2.00±0.54</td>
<td>3.77±0.58</td>
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<tr>
<td>IL-13</td>
<td>4.27±0.40</td>
<td>5.42±0.47</td>
<td>6.54±0.64</td>
</tr>
<tr>
<td>RANTES</td>
<td>390.6±51.6</td>
<td>231.6±25.6</td>
<td>172.5±34.4</td>
</tr>
</tbody>
</table>

Funding: Government Support - Non-U.S.

FR-PO1162
Effects of Everolimus on Proteinuria and Expression of Slit Diaphragm Proteins in Experimental Nephrotic Syndrome Rasid Alajbeg,1 Hoda Awaad,2 Zaid Abbas,3 Nephrology, Rambam Medical Center, Haifa, Israel; 1Physiology and Biophysics, Faculty of Medicine-Technion, Haifa, Israel.

Background: Everolimus, a mTOR inhibitor, is used as a potent immunosuppressant in renal transplantation. Although various serious side effects of Everolimus have been described, including renal injury and proteinuria, other studies have demonstrated beneficial renal effects of the drug. Therefore, the aim of this study was to examine the effects of

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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differing doses of Everolimus given as either early or late treatment on proteinuria and slit pore and nephrin proteins in adriamycin (ADR)-induced experimental nephrotic syndrome (NS).

Methods: Low or high dose of Everolimus (20 or 100 mg/L via drinking water) was administered to NS rats, beginning either 3 days prior to NS induction (early treatment) or 2 weeks after the induction of NS (late treatment). Daily and cumulative urinary protein excretion (UpV) were determined throughout the treatment period, which lasted 6 weeks. Moreover, the effects of Everolimus on GFR and key slit diaphragm proteins, namely nephrin and podocin, were assessed at the end of the study.

Results: Everolimus resulted in negligible proteinuria concentraion of 4.9±0.6, the high dose yielded supra-pharmacological plasma level of 21.3±4.4 mg/ml. As expected, ADR administration induced gradual significant increase in daily and cumulative UpV, in association with glomerular injury as presented by decrease in nephrin and podocin abundance. While low dose of Everolimus as early, and to a lesser extent as late treatment, reduced UpV and increased plasma albumin levels. These beneficial effects of low dose Everolimus were associated with improvement in GFR and substantial preservation of glomerular nephrin and podocin immunoreactivities. In contrast, high dose of Everolimus aggravated renal dysfunction and did not preserve nephrin/podocin expression. However, protein excretion in mice treated with the high Everolimus dose was eventually reduced secondarily to its deleterious effects on GFR.

Conclusions: Our study indicates that Everolimus possesses antiproteinuric effect at a therapeutic dose, whereas at a high dose it aggravates pre-existing glomerular injury.

Funding: Pharmaceutical Company Support

FR-PO1165

Angiotensin Receptor Blocker Ameliorates Obesity-Induced Albuminuria and Increases Urinary Exosomes Adiponectinuria in Mice

Ma1, Jun Zhou1, Yi-Wei Tang1, Valentina Kon2, Agnes B. Fogo1,3

1Pathology, Vanderbilt University, Nashville, TN; 2Pediatrics, Vanderbilt University.

Background: Activation of the angiotensin type 1 (AT1) receptor is implicated in the pathogenesis of both obesity and CKD. We investigated the effects of angiotensin receptor blocker (ARB) on obesity-induced albuminuria, adipose tissue macrophage polarization and inflammation.

Methods: WT mice (age 8-10 wk) were fed high-fat diet (HFD) for 24 weeks with or without ARB (HFD, n=5; or HFD+ARB, losartan 80 mg/L DW, n=6) and compared to mice fed normal chow (NC, n=5). Metabolic parameters, urine albumin/creatinine ratio (ACR) were assessed at intervals, and expression of adipose tissue M1 and M2 macrophage markers were assessed by qPCR. Data are expressed as mean±SE.

Results: HFD induced albuminuria, and ARB treatment significantly reduced this parameter (HFD 213.0±44.4, HFD+ARB 99.6±15.1 µg/mg, p<0.05). ARB treated mice on HFD also had significantly lower body weight (NC 28.5±0.7, HFD 50.3±3.3, HFD+ARB 41.5±4.0, p<0.01), body fat percentage (NC 11.6±1.6, HFD 33.7±3.5, HFD+ARB 17.5±2.5, p<0.01), blood glucose (HFD 191.7±10.3 vs HFD 140.5±8.4 mg/dl, p<0.05) and plasma insulin (HFD 6.8±0.6 vs HFD 3.0±0.8 ng/ml, p<0.05) vs HFD alone although food intake was comparable (HFD 2.6±0.4 vs HFD 2.6±0.2 g/day/ mouse). ARB partially restored obesity-associated decrease in muscle mass (% of muscle mass/body weight: NC 73.1±0.9, HFD 59.5±1.2, HFD+ARB 72.3±2.3, p<0.05) and plasma adiponectin (NC 17.4±1.5, HFD 10.9±0.2, HFD+ARB 12.6±0.5 µg/ml, p<0.05). ARB abolished obesity-induced adipose tissue macrophage M1 markers (IL-1β,IL-6,IL-10,IAκB; NC: HFD 60.6±1.6, HFD+ARB 5.0±1.0, p<0.01) and increased M2 macrophage marker Arg1 (NC 17.4±1.5, HFD 15.0±2.4, p<0.05). ARB abolished obesity-induced adipose tissue macrophage M1 markers (IL-1β,IL-6,IL-10,IAκB; NC: HFD 60.6±1.6, HFD+ARB 5.0±1.0, p<0.01) and increased M2 macrophage marker Arg1 (NC 17.4±1.5, HFD 15.0±2.4, p<0.05). M1 macrophage marker Arg1 (NC 17.4±1.5, HFD 15.0±2.4, p<0.05). M1 macrophage marker Arg1 (NC 17.4±1.5, HFD 15.0±2.4, p<0.05). M1 macrophage marker Arg1 (NC 17.4±1.5, HFD 15.0±2.4, p<0.05).

Conclusions: We identified a catalogue of proteins significantly enriched for those involved in innate immunity and bacterial killing (p = 0.0004), and confirmed their presence on exosomes by WB and electron microscopy (EM).

Growth of luciferase-expressing laboratory and clinical strains of E. coli and S. aureus was assessed by luminometry, and the morphological effects of exosomes on E. coli were confirmed by fluorescent microscopy.

Funding: Private Foundation Support

FR-PO1166

Urine Exosomes Maintain a Sterile Renal Tract by Inducing Bacterial Lysis

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Background: Urine exosomes are nanovesicles released by many cell types, and may play roles in cell-cell signaling, antigen presentation or apoptosis. Urine exosomes released by the kidney have no known function, are considered waste, and studies to date have focused on biomarker discovery. We asked if urine exosomes were functional.

Methods: Exosomes were isolated from second morning urine samples from healthy volunteers by differential centrifugation. Exosomal proteins were characterised by LC-MS/MS coupled with novel data-analysis and bioinformatic workflows, and the resulting protein catalogue interrogated for functional enrichment. Proteins with putative functional roles were confirmed by Western blot (WB) and electron microscopy (EM).

Results: We identified a catalogue of proteins significantly enriched for those involved in innate immunity and bacterial killing (p = 0.0004), and confirmed their presence on exosomes by WB and immuno-EM. Intact exosomal protein fractions, but not the abundant urinary protein uromodulin, potently and dose-dependently inhibited the growth of laboratory and clinical isolates of E. coli and S. aureus. Incubation of uropathogenic E. coli with urinary exosomes, but not with vehicle alone, resulted in bacterial lysis (p < 0.001).

Conclusions: Urine exosomes are enriched for bactericidal and -static proteins, inhibit bacterial growth and induce lysis of uropathogenic bacteria. We propose a novel model of defense whereby Trojan decoy exosomes are released from the kidney to maintain urine tract sterility, by delivery to invading organisms of packaged defence proteins.

Funding: Private Foundation Support
FR-PO1167
HD5 Expression in the Human Kidney and Urinary Tract

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Background: Urinary tract infections (UTI) are a common bacterial infection. Despite constant exposure to microbes, the urinary tract is usually sterile. Prior studies have indicated increased to 27.07±4.67 µg HD5/mg UCr with UTIs (p<0.01).

Results: Gene expression: We isolated RNA from non-infected human kidney, pyelonephritic kidney, and bladder tissue and quantified HD5 using real-time PCR. Protein expression: HD5 expression was localized using immunohistochemistry. To examine HD5 expression in the urine, we developed a sandwich ELISA and normalized urinary HD5 concentrations to mg of urine creatinine (UCr).

Conclusions: HD5 was not detected in non-infected human urine samples while mean HD5 expression increased to 6208±1056g HD5/10mg total RNA (p=0.04). Protein expression: Immunohistochemistry localized HD5 to the urothelium of the bladder and ureter. In the kidney, expression was primarily in the collecting tubule and loop of Henle. Protein expression analysis revealed HD5 throughout the nephron and collecting duct.

FR-PO1168
Ribonuclease 7: An Upregulated Antimicrobial Peptide during Urinary Tract Infections

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Background: Although the urinary tract is constantly challenged by microbial invasion, it remains free from microbial colonization. Recent studies stress the importance of antimicrobial peptides (AMP) in preventing UTIs. Our lab has previously shown that Ribonuclease 7 (RNase7) is a potent AMP that contributes to urinary tract cell lining.

Methods: Gene expression: We isolated RNA from non-infected and pyelonephritic human kidney tissue and quantified RNASE7 using real-time PCR. Protein expression: RNase7 expression was localized using immunofluorescence (IF). We developed a sandwich ELISA and normalized urinary RNase7 to mg of urine creatinine (UCr) in infected and sterile urine samples.

Conclusions: HD5 was not detected in non-infected human urine samples while mean HD5 expression increased to 27.07±4.67 µg HD5/mg UCr with UTIs (p<0.01).

FR-PO1169
Lanthanum Carbonate Reduces Cumulative Oxalate Absorption and Prevents Nephrolithogenesis after Oxalate Loading in Rats

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Background: Hyperoxaluria is a risk factor for calcium oxalate (CaOx) nephrolithiasis/calcification. Lanthanum carbonate (LC) is used as intestinal phosphate binder in dialysis patients to prevent hyperphosphatemia. As previously shown in vitro our laboratory, lanthanum carbonate has the ability to bind oxalate (Ox) in the pH range of the intestines. To evaluate in vivo intestinal Ox binding capacity of lanthanum, we investigated in rats whether LC is able to reduce Ox absorption and to prevent nephrolithogenesis (NC).

Methods: To investigate Ox absorption kinetics, 12 male Sprague-Dawley (SD) rats were divided into 2 groups: a group (n=6) receiving 1000 mg LC followed by 2 mmol Ox (2x molar La/Ox ratio) and a control group (n=6) receiving vehicle followed by the same Ox dose by daily gavage for 7 consecutive days. After sacrifice, degree of NC was assessed on Von Kossa stained kidney sections and by renal Ca analysis as a measure of NC.

Results: Ox loading resulted in a biphasic pattern of transiently increased serum Ox levels in controls, which was almost completely abolished in LC treated animals. Lower serum Ox levels in LC treated animals during the study period, resulting in a significantly reduced cumulative Ox absorption compared to controls. Furthermore, Ox loading oxaluria was blunted in LC treated animals, resulting in a significantly reduced CaOx crystalluria.

Conclusions: Administration of 1000 mg LC resulted in significantly lower median renal Ca concentration compared to controls: 0.14 (0.08–3.75) vs 1.12 (0.14–7.46) mg/g renal tissue.

FR-PO1170
Oxalobacter Formigenes Conditioned Medium Stimulates Oxalate Transport by Human Intestinal Cells

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Background: The vast majority of kidney stones are composed of calcium oxalate, and minor changes in urinary oxalate affect stone risk. Intestinal oxalate secretion mediated by anion exchanger SCL2A6 plays a crucial role in preventing hyperoxaluria and related kidney stones. The probiotic bacterium Oxalobacter Formigenes (OF) plays a critical role in preventing recurrent calcium oxalate kidney stones. In addition to degrading intraluminal dietary oxalate, OF also interacts with colonic bacteria leading to decreased colonic oxalate secretion, leading to reduced urinary oxalate excretion. However, the mechanism(s) underlying OF-colon interaction remain(s) unknown. We therefore examined whether OF culture condition medium (CM) affects intestinal oxalate transport using human intestinal Caco2-BBE cells.

Methods: We measured apical [14C]oxalate influx in the presence of an outward Cl gradient as an assay of Cl-oxalate exchange activity.

Results: Precubication of Caco2 cells with OF CM (1:50 dilution x 24 hours) significantly stimulated oxalate uptake (by -100%) while OF growth medium (OM) has no effect, suggesting that soluble factors in the CM might be responsible for the observed stimulation by modulating the activity of the likely involved transporter(s) (SCL2A6, SLC2A2, and/or SLC2A3). Importantly, CM from Lactobacillus Acidophilus has no effect on oxalate influx, indicating specificity. TER and incubation medium pH were not affected by the CM. Heat-inactivation of the CM completely abolished the stimulatory effect, indicating that the secreted factor(s) is/are likely to be protein(s) or peptide(s). Pretreatment of the CM with pepstatin destroyed the bioactivity of the CM, providing further evidence that the secreted factors are proteins or peptides. Selective ultrafiltration reveals that the secreted factors have a molecular mass between 10-30 kDa. Using real-time PCR, we observed in preliminary experiments that the CM led to a ~4.0 fold increase in SCL2A6 mRNA, without affecting SLC2A2 or SLC2A6 mRNA expression.

Conclusions: We conclude that soluble factors from OF activate oxalate transport by Caco2 cells through mechanisms that likely include enhanced SLC2A6 mRNA expression.

Funding: NIDDK Support

FR-PO1171
Culture of Hepatocytes from Primary Hyperoxaluria Type I Liver

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Background: PH-I is an autosomal recessive disorder caused by loss of functional AGT enzyme in the liver, which results in excessive production of oxalate, causing recurring stone disease and childhood and adult renal failure. At present a combined liver and kidney transplant are needed for these patients, but quality of life as well as survival are poor. We evaluated feasibility of generating hepatocyte cell cultures from PH-I patients.

Methods: Liver tissue was obtained from surgical waste of PH-I patients. The liver saplres were perfused with ice-cold Wisconsin solution. The samples were perfused with PBS supplemented with EGTA followed by Collagenase digestion. The eluted cells were separated from undigested tissue, collected, spun down and separated on percol gradient to remove contaminating debris. Cells were plated on collagen coated tissue culture dishes and culture followed the same protocol as for PH-II primary cultures. TER and incubation medium pH were not affected by the CM. Heat-inactivation of the CM completely abolished the stimulatory effect, indicating that the secreted factor(s) is/are likely to be protein(s) or peptide(s). Pretreatment of the CM with pepstatin destroyed the bioactivity of the CM, providing further evidence that the secreted factors are proteins or peptides. Selective ultrafiltration reveals that the secreted factors have a molecular mass between 10-30 kDa. Using real-time PCR, we observed in preliminary experiments that the CM led to a ~4.0 fold increase in SLC2A6 mRNA, without affecting SLC2A2 or SLC2A6 mRNA expression.

Conclusions: We were able to grow hepatocytes from 4 out of 5 patient samples. The primary cultures of PH-I hepatocytes stop growing after 4-5 passages. Initial passage contains both hepatocytes as well as fibroblasts. After selecting the cells in epithelial select media, the cells express cyto-keratin but do not express vimentin, characteristics associated with absence of fibroblasts. Transfection of these cells with h-Tert in a retroviral vector system resulted in a
cell line that we are able to grow to multiple passages. The cells retain cyto-keratin expression and lack vimentin, indicating maintenance of a phenotype. Adv-GFP-AGT resulted in successful transfection of all the cells in culture as visualized by GFP expression.

Conclusions: To the best of our knowledge this is the first report describing generation of hepatocytes from PH-1 patient liver and first demonstration of successful AGT-transduction of PH-1 patient’s hepatic cells in culture. These results should provide a new tool to investigate cellular and alternate therapies against PH-1.

Funding: NIDDK Support, Other NIH Support - Rare Kidney Stone Consortium and the NIH Office of Rare Diseases Research (ORDR)NIH-UO1-DK83980.

NIH-ROI DK 54084; UROLITHIASIS: OXALATE RENAL CELL INTERACTIONS

FR-PO1172

Oxalate Absorption Is Independent of Cation Complex Formation

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Background: Intestinal oxalate handling plays an important role in overall oxalate balance and risk for calcium-oxalate nephrolithiasis. We have previously shown that epithelial oxalate absorption is largely passive and paracellular through a low capacity, size-independent pathway for large solutes through the tight junction. A prediction of this model is that permeability to oxalate should be independent of its forming soluble complexes with cations.

Methods: Wild-type mouse duodenum was mounted in an Ussing chamber. Apparent permeability to [14C]-oxalate was measured simultaneously with that of [3H]-mannitol. Various oxalate species in the Ringer’s buffer were calculated using the SUPERSAT program.

Results: In standard Ringer’s buffer, the apparent permeability values for absorption of oxalate and mannitol were essentially identical. It was calculated that 0.79 of the 2.0 micromolar total oxalate in this solution was available as free oxalate, and the rest in soluble complex with sodium, potassium, magnesium, calcium, and citrate. The apparent permeability values for oxalate and mannitol were unchanged when the magnesium concentration was raised from 1.2 to 10 micromolar, which was calculated to decrease the free oxalate concentration from 0.79 to 0.33 micromolar as more oxalate was then complexed with magnesium. In contrast to the lack of effect of oxalate speciation on oxalate absorptive flux, the active component of oxalate secretion was inhibited by 80% when the free oxalate concentration was decreased.

Conclusions: We demonstrate that oxalate absorption in mouse intestine is independent of complex formation with cations. In contrast, active oxalate secretion is highly dependent on the concentration of free oxalate.

Funding: NIDDK Support, Private Foundation Support

FR-PO1173

Urinary Tract Infection Increases the Risk of Calcium Oxalate Kidney Stone Formation

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Background: Calcium oxalate(CaOx) can be complicated by urinary tract infection(UTI). There is no evidence about infection-induced CaOx stone formation. This study demonstrated that UTI can favor CaOx stone formation.

Methods: 73 kidney stones from patients with pyuria(white cell=51H) were analyzed by FITR. CaOx monohydrate crystal aggregation assay was used to study the inhibitory activity of protein in fasting urine of 28 kidney stone formers(18CaOx, 10uric) and 85 nonstone formers. The assay was modified from the method described by Hess(Am J Physiol1989;257[P2]:E99-106). The rate of aggregation was measured by a linear slope in inhibitory activity of protein in fasting urine of 28 kidney stone formers(18CaOx, 10uric).

Results: There were 37CaOx, 2carbonate apatite, uric acid, struvite and 1ammonium urate stones. 85% of them were mixed stone. Stone formers trended to have more CaOx than non stone formers(52.2±17). The proportion of stone forming with bad inhibitor was significantly higher than non stone formers(39%vs9%,X2=0.01).

Conclusions: There were 37CaOx, 2carbonate apatite, uric acid, struvite and 1ammonium urate stones. 85% of them were mixed stone. Stone formers trended to have more CaOx than non stone formers(52.2±17). The proportion of stone forming with bad inhibitor was significantly higher than non stone formers(39%vs9%,X2=0.01). Pyuria subjects had more aggregation than nonpyuria subjects(55.1±19%,n=31vs46±19%,X2=8.2,p=0.03). Among the nonstone formers, pyuria subjects had significantly more aggregation than nonpyuria subjects(58.1±14%,n=14vs45.1±19%,n=7, p=0.01) and 28% of pyuria subjects had bad inhibitor compared with 5.6% of nonpyuria subjects(X2=0.02).

Conclusions: We conclude that the CysCap assay is internally valid as it correlates with clinical outcomes. Patients with at least one CysCap analysis and adequate clinical follow-up, (≥ 1 year of documented clinical events), were included. Chart reviews were performed to obtain demographics, pharmacotherapy, lab values and clinical events, defined as urolithic intervention, stone passage, renal colic without stone passage, and either new stone or stone growth seen on imaging.

Results: 37 patients met the inclusion criteria. They had a mean of 2.1 CysCap analyses and mean follow-up of 489 days. They experienced a mean of 3.1 clinical events with time to first clinical event at 258 days. Of the clinical events, 1.1 urolithic interventions occurred with mean time to first intervention of 47 days. In a general linear model, CysCap was highly correlated with 24h urine cystine excretion, urine volume and urine pH (r²=0.71; P<0.001). Increasing excretion of urine urea nitrogen and urine sodium over 24h correlated with 24h urine cystine excretion (r=0.499 and 0.480, respectively, p<0.01), but not with CysCap or cystine supersaturation. The CysCap values of those patients on and off cystine binding thiol drugs did not significantly differ between the two groups. CysCap, CysS and 24hCystex did not correlate with the number of clinical events or the time to first clinical event.

Conclusions: We conclude that the CysCap assay is internally valid as it correlates with 24h cystine excretion, urine volume and urine pH. However, in this retrospective analysis, we were unable to correlate CysCap with the number of clinical events or the time to first clinical event.

Funding: NIDDK Support, Other NIH Support - ORD R

FR-PO1176

Infrared Vibrational Spectroscopy as a Diagnostic Tool for Cystinuria


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Background: Infrared (IR) vibrational spectroscopy can be used to analyze many chemicals and materials, and complex mixtures. Absorption bands arise from molecular vibrations and most molecules have characteristic IR spectra. There is growing interest in medical and diagnostic uses of IR molecular spectroscopy. In the 1800-900 cm⁻1 range, IR Spectra can distinguish cells and tissues; deconvolution can provide quantitative analyses of fluid constituents. We assessed whether mid- or far-IR spectroscopy could provide a relevant direct method for determining the analysis of cystine in urine - normally 0.2 mM or less, but can be several mM in cystinuria.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: In the mid-IR fingerprint region, cysteine and cystine in urine have similar absorbance spectra, overlapping with many other components, precluding their quantitation in this region. However, cysteine has an S-H absorption at 2575 cm⁻¹ and cystine has an absorption band at 2575 cm⁻¹. The purpose of this study was to develop a fast method for determination of urinary cysteine/cystine quantitation by mid-IR spectroscopy.

Background: Adenine phosphoribosyltransferase (APRT) deficiency is an autosomal recessive disorder of purine metabolism that leads to excessive urinary excretion of poorly soluble 2,8-dihydroxyadenine (2,8-DHA), causing radiolucent kidney stones and chronic kidney disease. The recent development of a 2,8-DHA production prevention protocol has been applied to patients with progressive stone formation and kidney injury. The aim of this study was to develop a fast method for determination of urinary 2,8-DHA and other purines for therapeutic monitoring of allopurinol therapy.

Methods: Liquid chromatography - electrospray tandem mass spectrometry (LC-MS/MS) was designed for rapid quantification of 2,8-DHA and other purines, including 2-deoxycysteine, adenine, adenosine, hypoxanthine, xanthine and oxypurinol through a series of experiments. Capillary and cone voltage, source and desolvation temperature, flow speed, gradient, gradient slope and salt concentration in the mobile phase were optimized by D-optimal design and related to LC-MS/MS responses, using partial least squares regression. To accurately quantify urinary 2,8-DHA, 6 aliquots of urine were collected immediately after the urine container had been inverted several times to suspend settled particles. The pH of the aliquots was adjusted to 10 with 2 M NH₄OH which dissolved all precipitates before injection into the LC-MS/MS system.

Results: Quantitative analysis of 2,8-DHA and the other purine metabolites was achieved with 100% specificity in 6 minutes. The coefficient variation for different urine aliquots was <5% indicating a robust sampling method. Validation of the LC-MS/MS-based method, which included selectivity, limit of quantification, response function, intra- and inter-day precision and accuracy as well as recovery of all analytes, was well within pre-defined limits. The practical quantitative limits of cysteine/cystine quantitation by mid-IR spectroscopy were 100% specificity in 6 minutes. The coefficient variation for different urine aliquots was <5% indicating a robust sampling method. Validation of the LC-MS/MS-based method, which included selectivity, limit of quantification, response function, intra- and inter-day precision and accuracy as well as recovery of all analytes, was well within pre-defined limits.

Conclusions: We have developed a rapid and reliable LC-MS/MS-based method for determination of urinary excretion of 2,8-DHA and other purine derivatives that will greatly facilitate therapeutic monitoring of allopurinol therapy in APRT deficient patients.

Funding: NIDDK Support, Other NIH Support - Office of Rare Diseases Research (ORDR).

FR-PO1178

Antioxidant Activity of an Unani Herbo-Mineral Formulation - Sayeed Ahmad, 1, 2, Wasiim Ahmad, 1, Mohmed Ahmed Khan, 1, Rabea Parveen, 3, Masood Shah Khan, 1, Ashwani Malhotra, 2, Mohammad Husain, 2, S M Arif Zaidi, 1, 2, 3 Hamdard University, New Delhi, India; 4 Division of Kidney Diseases and Hypertension, Feinstein Institute of Medical Research, North Shore LIJ University Hospital, Great Neck, NY.

Background: Safoof-e-Pathar Phori (SPP) an Unani herbo-mineral formulation have been used since long in Unani System of Medicine for its anti-urolithic activity as a good non-invasive remedy. It is a powdered formulation containing Didymocarpus lablab - processed salt and Potassium nitrate. The anti-urolithic activity of herbo-mineral formulation (SPP) against induced calcium oxalate nephrolithiasis was carried out using ethylene glycol - ammonium chloride rat model.

Methods: The animals were divided in four groups control, toxic control and treatments (n=6/group). Animals were administered SPP according to the protocol of rat model for 21 days. On 22nd day urine was collected and analyzed for Ca++, Mg++, Na+, K+ levels and crystalluria studies, whereas serum was used for blood urea nitrogen (BUN) and creatinine levels. The antioxidant markers of kidney tissues and histo-pathological examinations were also carried out.

Results: The SPP treatment (500 and 1000 mg/kg/day) significantly lowered the levels of BUN 49.35±3.20 at 500 mg/kg and 23.74±2.34 at 1000 mg/kg against toxicant 134.71±18.86 (P<0.001), while creatinine 0.27±0.01 mg/dL at 500 mg/kg and 0.20±0.01 mg/dL at 1000 mg/kg against toxicant 0.69±0.04 mg/dL (P<0.001). The treatment with SPP showed significantly higher levels of Na+ (25.92±0.47 mEq/L at 500 mg/kg and 29.92±0.62 mEq/L at 1000 mg/kg against toxicant 10.13±0.85 mEq/L), K+ (6.83±0.04 mEq/L at 500 mg/kg and 7.50±0.04 mEq/L at 1000 mg/kg against toxicant 4.4±0.03 mEq/L) and Ca++ (5.95±0.02 mEq/L at 500 mg/kg and 6.15±0.06 mEq/L at 1000 mg/kg against toxicant 3.9±0.06 mEq/L) (P<0.001).

Conclusions: The studies on crystalluria, histopathology and anti-oxidant markers supported the anti-urolithic potential of SPP in preventing calcium oxalate deposition without producing diuresis. Further, analysis for isolation of active component is under progress.

Funding: Government Support - Non-U.S.

FR-PO1179

Urine 2,8-Dihydroxyadenine in Pediatric Urolithiasis: Which “Cut-Off” Value Is To Use? 1 Maria Goretto Pendino, 1 Urs S. Ahn, 1 Pediatric Nephrology Unit, Federal Univ. of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil; 2 Bone and Mineral Disorders Clinic, Pediatric Nephrology, Children’s Mercy Hospital (CMH), Univ. of MO, Kansas City, MO.

Background: The “gold standard” for normal uric acid (UA) excretion is <815 mg/24h/1.73m², but due to difficulties in 24h collection in pediatrics hyperuricosuria is often defined based on random urine UA/Cr ratio (normal <0.65), or UA/Cr factored for GFR (UA/Cr x serum Cr (SCr), normal <0.57, all values in mg/dl). The latter is regarded as more accurate, but requires bladder emptying with SCr determination. The practical quantitative limits of cysteine/cystine quantitation by SPP showed that values <0.65 were established in healthy children; not in pediatric urolithiasis population. Hence the aim of this study was to examine their validity in such population.

Methods: Based on electronic records, we analysed all children diagnosed at CMH between Jan. 1999 and Dec. 2010 with radiologically documented primary urolithiasis who had diagnostic 24h urine. Data extracted: age at diagnosis, BSA, 24h urine volume, Cr, UA, and SCr. Tests were performed ≥ 1 year after stone expulsion.

Results: After excluding 6 with inappropriate collection based on urine creatinine (mg/24h) there were 180 patients in who had their urine UA/Cr measured. UA/Cr excretion was normal (<0.65) in 148 of 148 of them UA/Cr was also factored for GFR. Age at diagnosis was 11.9±3.7 (median 12.1). 9 patients had UA > 815 mg/1.73m²/24h, 46 had UA/Cr ratio ≥ 0.65, 12 had UA/Cr x SCr ≥ 0.57. Compared to the “gold standard” the sensitivity of UA/Cr was 64% and specificity 78%; when factored for GFR sensitivity was 30% and specificity 93%. The area under the ROC curve for UA/Cr ratio was 0.802 (95% CI 0.706-0.898) and that for the GFR corrected 0.766 (95% CI 0.661-0.871). The positive predictive value (PPV) of UA/Cr ratio was 15%; the negative predictive value (NPV) 97%; and after factoring for GFR PPV was 25% and NPV 95%.

Conclusions: The current “cut-off” values for urine UA/Cr ratio and UA/Cr factored for GFR are good tools to rule out hyperuricosuria but not to positively diagnose it. Furthermore, in school-age children UA/Cr factored for GFR does not provide an advantage; thus it can be omitted and with it serum creatinine determination.

Funding: None.

FR-PO1180

Demographic, Clinical, and Laboratory Characteristics of 137 Pediatric Nephrolithiasis Patients 1 David J. Sas, 1 Amy E. Wahlquist, 2 Pediatrics, Medical University of South Carolina, Charleston, SC; 3 Biostatistics & Epidemiology, Medical University of South Carolina, Charleston, SC.

Background: Evidence suggests that the incidence of kidney stone disease is increasing in children, yet there are few data documenting characteristics of pediatric stone formers. We sought to thoroughly describe various characteristics of our stone-forming pediatric population. A description of pediatric nephrolithiasis patients of this size and scope has not been performed in almost two decades.

Methods: We retrospectively reviewed the charts of pediatric patients with nephrolithiasis confirmed by imaging and collected data on over 120 demographic, clinical, laboratory, evaluation, management and follow-up variables on each patient. The data from 137 patients were collected and analyzed. Forty-nine percent of our patients were female. Twenty-five percent of our stone formers lived in rural environments. The mean age of presentation with first stone tended to be earlier in males than females (8.2 vs. 9.9 years). Males were more likely to be obese than females (23.6% vs. 17.3%) and the rate of obesity was higher than the general pediatric population in both sexes. Ninety-eight percent of the 47 stones that were analyzed contained calcium. Forty percent of our stone formers had elevated calcium excretion. Thirty-six percent and 57% had elevated supersaturation of calcium oxalate and calcium phosphate respectively. Random calcium-to-creatinine ratio correlated with 24-hour calcium excretion 73% of the time. Patients with 1-3 recurrences tended to be older, but those with >3 recurrences tended to be younger. Thirty-eight percent of the patients with the greatest mean age of first presentation. Each stone former was, on average, exposed to just over two CT scans specifically to evaluate for nephrolithiasis.

Conclusions: Our data summarize many characteristics of pediatric stone formers and reveal intriguing results related to obesity, recurrence rates, and differences related to gender. Further investigation into potential contributors to the increasing incidence of pediatric kidney stone disease is warranted.
Elena Del Valle.

Nephrolithiasis

Methods: We retrospectively studied the prevalence of vitD deficiency in the ISF and compared metabolic parameters in 25D deficit (<30nmol/L) with those having normal levels (>75nmol/L) using an unpaired t-test. We also prospectively studied the impact of supplementing 25D in ISF. Total of 37 patients (21M, 16F) were prescribed tab colecalciferol (2 in the diabetic range), hypotension in 4, palpitations, cramps and headaches in 2 patients.

Results: VitD deficiency was common in our ISF cohort. Higher serum 25D levels were not associated with higher urinary calcium, oxalate, citrate and urate excretions. Supplementing 25D when coupled with dietary advice on stone risk does not result in an adverse change in urine composition.

Conclusions: No significant difference was seen in U Ca/Cr, U Ox/Cr and U Cit/Cr pre and post supplementation. There were mild adverse effects that did not cause suspension of medication.

Reference:
Vezzoli, 2 Teresa Arcidiacono, 2 Elena Dogliotti, 1 Alessandra Mingione, 1 Francesca Raimondi, 1 Laura Saldett i, 1 Department of Medicine, Surgery, Dentistry, Università Studi Milano, Milan, Italy, 2 Unit of Nephrology, Dialysis, Hypertension, San Raffaele Hospital, Milan, Italy.

Backgound: The prevalence of nephrolithiasis has increased and caucasians are more likely than African Americans and Hispanics to have renal stones. However, the race distribution of patients requiring interventions to remove uratary tract stones is unknown.

Methods: We reviewed the electronic and manual surgical logs of all procedures done by our genitourinary service over a two year period. Patients who had any urinary tract stone removal procedures were identified and for the location of the stone, method of removal and the age at the time of the procedure . The demographic characteristics of the patients were also obtained from the hospital electronic records. The racial breakdown of all the patients utilizing the hospital services for that period was also obtained.

Results: The calcium-sensing receptor (CaSR) is a candidate gene of calcium nephrolithiasis (CN). Previously, we found an association between the CaSR gene region including P1 and P2 promoters and CN. Particularly, the rs7652589 and rs1501899 SNPs, localized upstream and downstream of promoters, were strongly associated to CN, both idiopathic and primary hyperparathyroidism CN.

The aims of this study were to test the association of CaSR gene promoter SNPs with CN and their effects on CaSR expression in renal tissue.

Methods: SNPs genotyping was performed by Taqman genotyping assays of rs7652589, rs1501899 and rs6776158 localized into P1 and P2, in 165 idiopathic calcium stone formers and 213 controls matched for age and gender. CaSR mRNA level was evaluated in 109 normal kidney medulla tissues by Real-time PCR and genotyped for the rs7652589, rs1501899 and rs6776158 SNPs.

Results: A fine mapping of the CaSR gene promoter region has been performed in controls and controls for the SNPs placed in P1 and P2. The data showed that SNP rs6776158, localized in P1, resulted strongly associated with the disease. The rs6776158 minor allele showed a higher frequency in stone formers than in controls (37.8% vs 26.4%, p=0.006). CaSR mRNA of kidney medulla tissues was related to the kidney stones associated SNPs. A decreased CaSR mRNA was found in homozygous subjects for the minor allele (2.70±0.21 vs 1.28±0.32 with p=0.0062).

Conclusions: These findings confirmed the association of CN with CaSR promoter region. Moreover, the minor alleles of P1 SNPs were associated to a reduced expression of kidney CaSR mRNA.

Funding: Government Support - Non-U.S.

FR-PO1185

Regulation of Renal Calcium Reabsorption by Serum Calcium in Hypercalciuric and Control Subjects

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Background: Calcium (Ca) stone formers with idiopathic hypercalciuria (IH) have reduced renal Ca reabsorption, but the mechanism is not understood.

Methods: In the General Clinical Research Center, we studied 29 IH (17 male) and 17 (7 male) control (C) subjects. 27 of 29 IH formed Ca stones and 2 patients selected 15 urine and 20 blood samples over a 15 hour day, both fasting and with 3 meals of known composition.

Results: Fractional excretion of Ca (FEca) of all subjects was higher fed than fasting, but FEca of IH exceeded C both fasting and fed (Table). Serum Ca (SCa) and parathyroid hormone (PHT) did not differ significantly between IH and C either fasting or fed. SCa rose and PTH fell with feeding in IH but did not change in C.

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Underline represents presenting author.

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subject by type within food period (Table). High SCA IH had increased UCa compared to IH, which was significant during feeding (0.52 ± 0.01 vs 0.46 ± 0.01 mmol/ hr; p<0.001) and not attributable to FLCa. UCa did not differ by SCA group in C either fasting or fed. In IH, PTH was significantly lower in the SCA IH group both fasting and fed (p<0.001), but PTH did not differ in C by SCA group.

Conclusions: SCa appears to modulate the effect of food intake on Ca excretion and PTH level in IH but not C, which implicated altered Ca signaling in the abnormal Ca metabolism of IH.

Funding: NIDDK Support

FR-P01186

Does FGF23 Play a Role in Pediatric Idiopathic Hypercalciuria? Maria Goriotti Penedo,1 Marcelo S. Tavares,2 Uri S. Alon. 2

1Nephrology, University of Verona; 2Geriatrics, University of Verona; 3University Catholic of Rome; 4University of Padua.

Background: Various mechanisms were proposed as pathophysiology of idiopathic hypercalciuria (IH). Based on recent findings and suggestion by Worcester & Cole (2008), the aim of this study was to explore a potential role for FGF23 in pediatric IH.

Methods: We studied 29 controls (19M) and 58 children with IH (35M); of whom 24 before treatment (untreated) and 34 after 6 months treatment either with K-citrate alone (20) or combined with thiazides (14). Plasma FGF23 was assessed using C-terminal ELISA (Immunotopics, San Clemente, CA). We also measured serum PTH, 25OH Vit D, P, Ca, and a preclinical atherosclerosis condition.

Results: No differences in age were noted between controls (15.4±8.3) and patients (16.0±5.0), nor between untreated (16.8±3.4) and treated (15.5±4.7), and no difference in gender distribution. The incidence of lithiasis in the untreated (75%) and treated (17%) was similar. Plasma FGF23 in controls was not different and Plasma FGF23 in untreated children was (p=0.02). In IH patients combined there was a tendency of correlation between FGF23 and UCa (r=0.22; p=0.09). There were no differences between the untreated and treated IH children regarding serum creatinine, Ca, 25D, PTH, urine calcium or phosphate. The treated patients had significantly lower UCa 5.2±0.7 vs 5.6±1.2 mg/kg in untreated (p<0.001); higher PTH 4.0±0.6 vs 3.4±0.7 (p=0.001) and serum P 4.4±0.5 vs 4.0±0.6 mg/dl (p=0.007).

Conclusions: Treatment of IH patients resulted in significantly lower UCa excretion rate, lower plasma FGF23 and elevated TP/GFR and serum P, without significant changes in serum PTH. We conclude the reversal of hypercalciuria may directly or indirectly affect phosphate metabolism, perhaps via calcium retention in bone or changes in 1,25 OH, Vit D metabolism. Further studies on this topic are needed.

FR-P01187

Abnormal Arterial Stiffness and Bone Density in Calcium Renal Stone Formers Antonia Fabris,1 Antonio Lupo,1 Francesco Fantin,2 Pietro Manuel Ferraro,3 Chiara Caletti,1 Gabriele Comellato,2 Mauro Zamboni,1 Giovanni Goretti Penido,1 Marcelo S. Tavares,1 Uri S. Alon. 2

1Nephrology, University of Verona; 2Geriatrics, University of Verona; 3Urology, University of Verona.

Background: Kidney stone formers (SF) are at increased risk for myocardial infarction for still unclear reasons. Reduced bone mass is a frequent finding in calcium SF. An inverse relationship between bone density and abnormal arterial stiffness partly related to vascular calcifications has been reported. Abnormal arterial stiffness is a strong predictor of CV mortality.

To elucidate the causes of the increased CV risk in SF we investigated whether they have abnormal arterial stiffness.

Methods: Recurrent calcium SF (23) and 19 age and sex matched controls underwent DEXA to determine bone mineralization, and pulse waive velocity (PWV) by Complior, a preclinical atherosclerosis condition. The prevalence of 8 cardinal clinical signs tailored to DD clinical pattern were analysed.

Results: Among the 24 patients, 11 (45.8%) presented with at least one of the 8 signs, while none of controls presented any (p<0.001). The prevalence of each sign is shown in the Table.

Conclusions: Compliance with medications as well as nutrition seems to be influenced by various mechanisms other than nephrocalcinosis trigger RF in DD pts. The lack of BD and RF in DD familial case pts indicates that disease phenotype is mitigated and that it might be resulting from the presence of modifier genes. In 1/3 of MSK SF present the presence of a Fanconi-like syndrome also suggest proximal tubular damage.

FR-P01189

Nephrocalcinosis, Renal Failure and Bone Disease in Patients with Dent Disease and Medullary Sponge Kidney: Comparison with Patients with Recurrent Calcium Nephrolithiasis Antonia Fabris,1 Antonio Lupo,1 Luisa Maria Bertizzolo,2 Franca Anglani,2 Giovanni Gambaro,2 Angela D’angelo.1

1Nephrology, University of Verona; 2Catholic University of Rome; 3University of Padua.

Background: Medullary Sponge Kidney (MSK) is a developmental and functional disorder with Nephrocalcinosis (NC) and Lithiasis (LT); familial occurrence and the discovery of rare GDNF variants suggest a role of genetic factors. Dent Disease (DD) is a X-linked proximal defects, caused by CLCN5 or OCRL1 mutations,which may present with NC and/or RN. NC is a common disease affecting adults;earlier occurrence may suggest a hereditary cause.

Methods: RCN, MSK and DD groups were divided into subgroups on the base of specific characteristics; the prevalence of 8 cardinal clinical signs tailored to DD clinical pattern were analysed.

Results: Among the 24 patients, 11 (45.8%) presented with at least one of the 8 signs, while none of controls presented any (p<0.001). The prevalence of each sign is shown in the Table.

Conclusions: Pathogenetic mechanisms other than nephrocalcinosis trigger RF in DD pts. The lack of BD and RF in DD familial case pts indicates that disease phenotype is mitigated and that it might be resulting from the presence of modifier genes. In 1/3 of MSK SF present the presence of a Fanconi-like syndrome also suggest proximal tubular damage.

Factors Predicting Medical and Nutritional Therapy Adherence for Recurrent Nephrolithiasis Roy A. Jiang,1 Kristina L. Penniston,2 1Medicine, UW Madison, Madison, WI; 2Urology, UW Madison, Madison, WI.

Background: Nephrolithiasis has a 10% lifetime prevalence in the general population as well as carries a 2.1 Billion dollar economic burden per year. While medications as well as nutritional recommendations have been found to prevent kidney stones, factors affecting compliance with these prevention methods remain poorly understood.

Methods: We used targeted patient surveys to assess patient compliance with various modalities of treatment. 155 surveys were returned by mail or at time of the next clinic visit. Nutritional advice if given was evaluated for it’s clarity of explanation, comprehension as well as implementation. Medical therapy was also assessed for it’s clarity of explanation, comprehension and adherence.

Results: A patient described understanding of the rationale for the nutritional therapy recommended it was associated with compliance with the diet (p < 0.001).

Supporting this, it was also noted that if patients felt that the explanation of the dietary recommendations was satisfactory, they were compliant with diet recommendation <p < 0.0001).

In our survey women reported lower compliance with pharmacologic therapy than men. (p < 0.0003) Specifically for Hydrochlorothiazide women reported lower compliance (p = 0.05).

A tendency was also noted regarding the poor compliance of women with regard to potassium citrate which was not found in men. (p = 0.09).

In addition, a tendency for women to feel that the rationale behind the medications were not well explained to them was noted(0.058).

Conclusions: Compliance with medications as well as nutrition seems to be influenced by gender as well as provider provided education. Identification of characteristics affecting adherence provides an opportunity for us to maximize the effect of our therapies. This suggests that explanation of the rationale behind using medications and diet modification is vital for compliance.

These results are also suggestive that women should be given more attention to ensure compliance and possibly would benefit from Nutritional consultation routinely rather than as needed.
FR-PO1190

Etiology of Pediatric Primary Urolithiasis: 12 Year Experience in a Mid-Western Children’s Hospital
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Urolithiasis has become more prevalent due to changes in habits and increasing affluence, and possible ecologic changes. The aim of this study was to evaluate the current etiology of pediatric primary stone disease. 

Methods: Using electronic data, we identified all children seen at CMH in Kansas City between Jan. 1999 and Dec. 2010 with radiologically documented urolithiasis who had diagnostic 24-hr urine analysis. 56 patients with secondary causes (e.g. CF, Ciron’s, primary hyperpara., etc.) were excluded. Data extracted included: age at diagnosis, gender, weight, BMI, imaging technic used; 24-h urine volume, creatinine (Cr), Ca, uric acid, citrate, oxalate, cystine, serum Cr, electrolytes and minerals, and costs.

Results: After omitting 11 whose urine Cr (mg/kg/24h) showed inappropriate collection, there were 222 patients (48% male), all with normal serum Cr, electrolytes and minerals. Annual rate of primary urolithiasis tripled from 9.7/10.6 to 27.3/9.8 (p = 0.002); 73% were diagnosed by ultrasound and the rest by CT. Age at diagnosis was 11.8±3.8 and BMI 20.5±5.7: 15% were overweight. 147 patients (63.0%) had urine flow < 1.0 ml/kg/24h; in 54 (24.3%) as only abnormality. Hypercalciuria was observed in 47% of patients, hypocitraturia in 10%, and 54% had high Ca/citrate ratio (≥0.33). Mild idiopathic hypocalciuric hypercalciuria was found in 3 patients and hyperuricosuria in 11 (all 14 had at least 1 additional abnormality). 1 had cystinuria.

Conclusions: We conclude that “oliguria” and hypercalciuria continue to be the most common abnormalities, followed by hypocitraturia. The significant increase in stone incidence could not be attributed to increased utilization of CT per se. Incidence of obesity in the urolithiasis population was not higher than in the general pediatric population. Hypocalciuric and cystinuric patients require screening at age of ≤5 and ≤13500 in the former, and 222 patients at ≥$4,000 in the latter entity; hence both may not be indicated in 1st analysis.

FR-PO1191

Metabolic and Medical Risk Factors in Pediatric Patients with Urolithiasis
Sermín Saadeh, Brett C. Ferguson, Rossana G. Barbaro Maggi, Gaurav Kapur, Amrish Jain, Tej K. Mattoo, Rudolph P. Valentini. Pediatric Nephrology, Children’s Hospital of MI, Detroit, MI.

Background: Urolithiasis is an uncommon medical condition in pediatrics. However, it can be associated with morbidity and recurrence. Compared to adults, a higher proportion of pediatric stone patients have predisposing conditions for recurrence (metabolic disorders, structural abnormalities) when often combined with dietary and environmental factors.

Aim: Evaluate risk factors for pediatric kidney stone formation and recurrence.

Methods: Retrospective chart review of patients evaluated for urolithiasis in the pediatric nephrology clinic from 2005 to 2010.

Results: Charts of 41 patients were reviewed. Median age was 10.3 years (0.6-17.5 yrs), male to female ratio was 1.6:1. Most patients were Caucasian (24/41). The most common presenting symptoms were abdominal colic (68%), hematuria (27%), UTI (15%). 

Metabolic work-up in the form of 24-hr urine collection and/or stone analysis was done. Results of 24-hr urine collection on 34 patients are described in table 1. A metabolic abnormality was found in 88% of patients. Hypercalciuria was the most common metabolic abnormality, followed by hypocitraturia. Stone analysis in 13 patients revealed calcium oxalate stones in 7/13 (54%), followed by calcium phosphate stones in 4/13(31%).

Conclusions: Hypercalciuria and hypocitraturia are the most common metabolic disorders in our pediatric population with urolithiasis. Patients with urolithiasis should be evaluated for the presence of predisposing conditions to help reduce the risk of recurrence.

FR-PO1192

Urine Levels of Inter-c-Tryptsin Inhibitor Proteins Differ According to Age and Stone Forming Status
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Background: The urine of many individuals is supersaturated favoring the formation of calcium oxalate and calcium phosphate crystals. However, proteins are thought to inhibit nucleation, growth, and aggregation of these crystals. Many protein crystalization inhibitors have been identified, including the inter-c-tryptsin inhibitor (IzI) family, which have also been implicated during pathologic calcification in cartilage and vessels. In the current study we investigated urinary levels of IzI proteins in an incident stone forming cohort with matched controls.

Methods: Urine samples were collected from 37 first time stone formers (SF) and controls (C), matched by gender and age. IzI family proteins including heavy chain1 (HC1), heavy chain2 (HC2), and heavy chain3 (HC3) were detected by Western blot and quantitated using Image J. SF and C were divided into four groups based on age: younger (25-35 years) (SF (n=19), older (51-65 yrs) SF (n=18), younger C (n=20), and older C (n=17).

Results: HC1 was higher in younger SF (1.20±0.70) than in older SF, (0.60±0.32), (p<0.001), as were HC2 (1.23±1.24 vs.0.63±0.31, p<0.01) and HC3 (1.21±0.35 vs.0.92±0.34, p<0.05). Conversely, urinary IzI heavy chains were similar in the younger and older C groups, which also matched the older SF patients.

Conclusions: These results suggest that urinary IzI protein levels differ by age and stone forming status. Although age was not found to affect IzI trimer levels in the cohorts, significantly higher amounts of the heavy chains were found in the younger SF population. Deposition of IzI heavy chains is thought to protect against pathologic calcification in soft tissues, including cartilage. Therefore, increased levels of heavy chains in the urine of young patients could represent a protective mechanism against further stone formation. This response seems to be diminished in the urine of older SF, in whom the pathogenesis of kidney stones may differ.

Funding: Other NIH Support - Grant: R01 DK 80307 "Mayo Clinic Urology O’Brien Research Center".

FR-PO1193

Does Dietary Education Improve 24 Hour Urine Stone Risk Profiles in Children? Katherine E. Twombly,1 Nicor Combin Bush,1 Candace F. Granberg.2 Pelican Bay, UT Southwestern Medical Center, Dallas, TX; 1Urology, UT Southwestern Medical Center, Dallas, TX.

Background: Dietary education (DE) is a widely practiced therapy for pediatric stone formers. However, few studies show the effects of dietary intervention on 24hr urine stone risk profiles in children. We analyzed 24hr urines before and after DE among our pediatric stone formers to evaluate if urinary stone risk profiles improved.

Methods: We performed a retrospective review of 24hr urine samples in stone forming children. Inclusion criteria included patient age ≥18yrs, radiographically-confirmed stones, DE handout and preDE and postDE 24hrs urines. DE handout advised increased water, decreased sodium(Na) and oxalate(Ox), and moderate calcium(Ca) intake. Statistical analyses of 24hr urine parameters were performed with paired t-test.

Results: Among 24 patients with an average age of 10.8yrs (3-18), 11M:13F, 11MF:13FM, improvement in urine volume was the only significant change, increasing from 53 to 45ml/ kg/day (p=0.03) or 1.1 to 1.6 liters/day (p<0.002). Urine Na (3.5 vs. 3.7mEq/kg/day), and Ox (0.66 vs. 0.70mEq/kg/day) did not change despite DE. Likewise, there was no improvement in the supersaturations for calcium oxalate (1.7 vs. 1.4, p=0.02), brushite (1.2 vs. 1.3, p=0.6) and calcium urate (2.9 vs. 2.7, p=0.3).

Conclusions: Among pediatric stone formers, urine output improved with DE, however urine supersaturation indices, Na, Ox and Ca did not. Since 24 hour urine is the gold standard measurement for dietary Na intake, our results suggest poor patient compliance with low sodium diet (despite written DE) may contribute to persistent hypercalciuria in some patients.

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FR-PO1194

Transglutaminase 2 Accelerates Vascular Calcification in CKD
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Background: Transglutaminase 2 (TG2) is a calcium dependent enzyme that is a co-receptor with β1 integrins and fibronectin on the cell membrane, connecting the cytoskeleton and extracellular matrix (ECM) proteins. TG2 can cross link nearly all ECM proteins, leading to increased transmembrane transport of calcium. TG2 increases through transamination or deamination, increasing osteoblast differentiation and arterial remodeling. Furthermore, vascular smooth muscle cells (VSMC) from TG2 null mice cannot calcify in response to hyperphosphatemia. We therefore hypothesized that increased TG2 activity leads to accelerated vascular calcification in CKD.

Methods: In the current study, we used thoracic aortas and VSMC from the Cy-rt, a model of CKD-MBD compared to normal rats to examine the role of TG2 in vascular calcification using real time PCR, immunostaining, Western blot and biochemical

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Results: Histological evaluation revealed that T2G expression is increased in area adjacent to rabbit aortic calcification. Addition of HP to 3 mM to rabbit aortic calcification-induced a dramatic increase of both transcripts (>2000 fold; n=3-4, P<0.01). Addition of HP control of mineralization. We observed that STC1+HP induced a time-dependent increase in aortic smooth muscle cells (VSMC) incubated in the presence of HP (2.5 mM) or VD (100 nM), with a higher degree of calcification at day 5 in VSMC from CKD rats compared to that in VSMC from normal rats (10.9 ± 3.3 vs. 3.0 ± 0.5 nM/mg cell protein, calcification; 11.4 ± 1.6 vs. 6.7 ± 1.4 μg ALP). Inhibition of T2G activity by cystamine dose dependently decreased calcification and ALP activity in VSMC from CKD rats but had no effect on that in NL VSMC.

Conclusions: These data demonstrate a role of T2G in the pathogenesis of vascular calcification in CKD, likely through its effects on the ECM.

**Funding:** Veterans Administration Support

FR-PO1195

Magnesium Reduces Vascular Calcification In Vitro

Addy Rosa Montes de Oca Gonzalez, Fatima Guerrero, Juan R. Maioz-Castañeda, Julio Manuel Martínez Moreno, Juan Antonio Madueño Domenech, Carmen Herencia, Yolanda Almaden Peña, Escolastico Aguilera-Tejero, Mirjam Peter, Jutta Passlick-Deetjen, Sonja Steppan, Mariano Rodriguez, Depto. Medicina y Cirugía Animal, University of Cordoba, Spain; Hospital Reina Sofia, RedinRen, IMBIC, Spain; Freiburg Medical Care Deutschland GmbH, Bad Homburg, Germany; University of Duiseldorf, Germany.

Background: Vascular calcification (VC) in uremic patients is associated with high serum phosphorus (P). P binders containing magnesium (Mg) are successfully used for control of hyperphosphatemia. An association of increased serum Mg with reduced VC has been described. Thus, the question to be addressed is whether Mg may exert a direct effect on VC.

**Methods:** Rat aortic rings (AO) were incubated in vitro for 7 days with a P concentration of 2.8 mM and 1.8 mM calcium (Ca) with increasing concentrations of Mg (0.6, 1.4, 2.6 mM).

**Results:** Ca content in AO increased from (mean±SE) 0.21±0.04 in controls without P up to 3.3±0.8 (mg/mg protein) with 2.8 mM P. Increasing the Mg concentration to 1.4 and 2.6 mM significantly (p<0.01) reduced the AO Ca content to 1.3±0.7 and 0.25±0.08 respectively, the latter value was not different from control. Compared to control values, the decrease in VC induced by Mg was associated with decreased expression of osteogenic markers Cbfa1 and osterix. Similar results were obtained using human vascular smooth muscle cells (VSMC). Even with concentration of Ca as high as 3.3 mM the change in Mg from 0.6 to 1.4 mM reduced Ca content from 11.9±1.9 to 2.1±0.2; a higher concentration of Mg did not produce a further decrease in VC. To test the ability of Mg to reverse VC, VSMCs were incubated for 5 days with 3.3 mM P. The Ca content was 8.8±0.6 mg/mg which increased to 12.1±2.0 after 9 days. The addition of 1.4 mM Mg at day 5 produced a markedly decreased Ca content at day 9 (2.5±0.3); p<0.01; this value was not different from control without P added.

**Conclusions:** An increase in Mg to the upper limits of normal, not only prevented, but also reversed VC in vitro. Thus, lowering with Mg containing binders may help to reduce VC through a decrease in P and a direct beneficial effect of Mg.

**Funding:** Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1196

Stanniocalcin-1 (STC1) Is Upregulated in Uremic Rats and Induces VSMC Calcification

Magdalena Gonzalez, Rodrigo Andaur, Solange C. Valdes, Peter W. Murphy, Luis F. Michea. Facultad de Medicina, Universidad de Chile, Santiago, Chile.

Background: Calcification of arterial vascular smooth muscle cells (VSMC) is frequent in end-stage renal disease and correlates with hyperphosphatemia. VSMC calcification may involve the osteochondrogenic trans-differentiation. STC1 is an autocrine/paracrine homodimeric factor that promotes osteochondrogenic differentiation in developing bone of mammals. We hypothesized that STC1 is an inducer of VSMC calcification that increases in arterial calcification, observed in chronic renal failure (CRF).

**Methods:** Contractions of rat aortic rings (AO) from normal control rats and rats with chronic kidney disease (CKD) were determined by measuring the mRNA abundance of the Sry-box containing gene-9 (Soyx-9) and Core Binding Factor Alpha-1 (Cbfa-1). STC1 induced a dramatic increase of both transcripts (>2000 fold; n=3–4, P<0.01). Addition of HP induced a further increase of osteochondrogenic factors mRNAs (n=3–4, P<0.01).

**Conclusions:** Our data demonstrate increased plasma STC1 levels in uremic rats subjected to pro-calcifying conditions and the induction/potentiation of VSMC calcification by STC1. Supported by FONDECYT 1090223, Fondecyt-FONDEF 1510006.

**Funding:** Government Support - Non-U.S.

FR-PO1197

Elevated Extracellular Phosphate Levels Down-Regulate Both Akt and AMP-Dependent Kinase in Endothelial Cells


**Background:** Hyperphosphatemia is an independent risk factor for cardiovascular diseases (CVD) in general population as well as chronic kidney disease (CKD) patients. In addition, phosphate toxicity has been emerged from cardiovascular disease to various aging-related diseases such as diabetes mellitus, based on the studies of klotho-deficient mice with premature aging-like phenotype. We hypothesized that hyperphosphatemia may cause multiple kinases relating to various metabolic processes.

**Methods:** Most of kinases can be regulated by phosphorylation or dephosphorylation, therefore we investigated the changes of phosphorylation status in response to extracellular phosphate in human aortic endothelial cells (HAECs) by phospho-proteomic analysis with anti-phospho-signal transduction molecules antibody array.

**Results:** We found that incubation of HAECs with high phosphate medium (3 mM) decreased phosphorylation of both AMPK at threonine 172 and Akt at serine 473, but control medium (0.9 mM) did not change them. In addition, high phosphate medium also decreased phosphorylations of the downstream effector proteins of AMPK and Akt, such as endothelial nitric oxide synthase (eNOS), p70S6K and acetyl-CoA carboxylase (ACC).

**Conclusions:** Akt is a key molecule of insulin signaling pathway; AMPK is known as energy sensor that can regulate glucose and lipid metabolism in response to intracellular AMP/ATP ratio. Therefore, elevated extracellular phosphate levels down-regulated AKt and AMPK, suggesting that hyperphosphatemia may cause abnormal glucose and lipid metabolism found in the klotho-deficient mice.

**Funding:** Private Foundation Support, Government Support - Non-U.S.

FR-PO1198

Role of Local and Systemic Pyrophosphate Metabolism in Vascular Calcification

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**Background:** Pyrophosphate (PPi) is an important endogenous inhibitor of vascular calcification but it is not known whether inhibition is dependent on local (vascular smooth muscle) or systemic PPi. PPi is synthesized by ectonucleotide pyrophosphatase/ phosphorylase isoenzyme 1 (ENPP1) and both humans and mice lacking ENPP1 develop vascular calcification. We transplanted aortas between Enpp1-/- and Enpp1+/+ mice to determine the relative role of local and systemic PPi in inhibiting vascular calcification.

**Methods:** Sections of abdominal aorta (5–8 mm) were transplanted via end-to-end anastomosis between 2–4 month old Enpp1-/- mice and wild-type littermates (Enpp1+/+). Transplants from Enpp1+/+ mice into Enpp1-/- mice were also performed as a control. Animals were fed a 1.5% phosphorus diet for 45 days before sacrifice. Calcium was measured in transplanted abdominal aorta grafts (excluding suture lines), recipient abdominal aorta (adjacent to the allograft), and recipient thoracic aorta by the cresolphthalein method after drying and extraction with 1 M HCl.

**Results:** Aortic calcium contents (nmol/mg dry weight) are shown in the table below. Plasma calcium and phosphorus were 1.3 ± 0.05 mM and 2.06 ±0.18 mM in Enpp1+/+ recipients and 1.24±0.07 mM and 1.99±0.19 mM in Enpp1-/- recipients. Transplantation into Enpp1-/- mice substantially reduced but did not eliminate calcification of Enpp1-/- aortas. Enpp1+/+ aortas calcified when transplanted into Enpp1-/- mice but much less than native Enpp1-/- aortas.

**Conclusions:** Inhibition of vascular calcification is dependent on both locally produced and systemic pyrophosphate.

**Funding:** American Heart Association - Heart of Georgia Affiliate and Human Health Research, TDID support

FR-PO1199

A Label-free Serum Test Measuring Overall Calcification Inhibition

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**Background:** Accelerated vascular and soft tissue calcification is a major problem in patients with chronic kidney disease (CKD). As serum is supersaturated with regard to calcium and phosphate, inhibitors of calcification critically determine pathological calcification. Therefore, an assay measuring the overall calcification inhibitory capacity in blood would be helpful to make informed therapy decisions.

**Methods:** We developed a label-free, 2-dimensional assay to measure overall calcification in biological fluids (serum). The assay is based on the induction of microparticles containing calcium phosphate out of an activation solution in a 96-well plate (total volume 150 μl) and is run on a Label-free Imaging System (LIS) using a bright field signal. We have identified that the amount of formed microparticles is a good measure for overall calcification. Based on the developed assay, we were able to determine the overall calcification inhibition capacity in sera from healthy subjects and patients suffering from CKD.

**Results:** We could show that serum total calcium and phosphate are positively correlated with calcification inhibition capacity. Furthermore, we showed that a healthy control group (n=10) had a significantly higher calcification inhibition capacity compared to CKD patients (n=20) (p<0.05) (Figure 1). Our results indicate that a label-free, 2-dimensional assay measuring overall calcification inhibition capacity in biological fluids could be helpful in making informed therapy decisions.

**Conclusions:** The results of our study demonstrate that the developed assay can be used for measuring overall calcification inhibition capacity in biological fluids and thus could be helpful in making informed therapy decisions.

**Funding:** This study was supported by the German Research Council (DFG) (Grant number: ZN453-1).

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FR-PO1200
Assessment of the Frequency of Pulmonary Calcification and Its Influence on Respiratory Function in Patients with Chronic Kidney Disease
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Background: The extraosseous calcification accompanying CKD appears, among other, in cardiovascular system and lungs. Disturbed calcium-phosphate (Ca-P) metabolism and alkaline pH are taken as the most important risk factors of pulmonary calcification (PC). The aim of study was to assess the PC frequency and its effect on respiratory function, among pts with 3-5D stage of CKD.

Methods: Thirty seven pts with CKD (23 males and 14 females, aged 53.0±8.5 years, 13 in stage 3-4 and 24 in stage 5 (treated with hemodialysis) were included into the study. The exclusion criteria were: obtrusive, restrictive and interstitial lung diseases, smoking, tuberculosis or anti-mycobacterium therapy actual or in past, neoplasms, heart failure and overhydration. The protocol of the study included: laboratory tests (Ca, P, Ca×P product, alkaline phosphatase, PTH), lung tests (spirometry, pletysmography and diffusing capacity of the lung for carbon monoxide (DLCO), high resolution computed tomography (HRCT) of lungs and scintigraphy with the use of 99mTc-MDP.

Results: Only mild PC were found recognized by HRCT alone: in 13/24 pts with 5 CKD and in 1/13 pts with 4 CKD (p<0.01). Bone scintigraphy did not reveal any calcification. No disturbances of lung tests were found. The mean duration of hemodialysis was longer among the pts with PC (9.9±8.2 years) vs without PC (2.6±6.9 years) (p<0.01). No statistical differences in age, gender, duration of CKD, severity of Ca-P disorders and their therapy were observed among pts with and without PC. Clinical symptoms: chest pain and dyspnea were observed more frequently in pts with PC (10/13 and 9/13 respectively) than without PC (2/11 and 3/11 respectively) (p<0.004 and p<0.04 respectively). No difference in frequency of chronic, nonproductive cough was observed among both groups.

Conclusions: We conclude that despite a relatively high frequency of PC, the severity of PC was mild and they were not associated with restrictive disturbances nor impaired DLCO in functional lung tests. HRCT is an useful tool in detection of PC in CKD patients.

FR-PO1201
The Associations of Fetuin-A with Subclinical Cardiovascular Disease in Community-Dwelling Persons: The Rancho Bernardo Study
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Background: Fetuin-A is a hepatic secretory protein that inhibits arterial calcium deposition in vitro. Lower fetuin-A levels are associated with arterial calcification and death in ESRD populations. The association of fetuin-A with subclinical cardiovascular disease (CVD) in other settings is unknown. In 1,375 community-living individuals without prevalent clinical CVD, we sought to determine the association of fetuin-A with subclinical CVD and lungs. Disturbed calcium-phosphate (Ca-P) metabolism and alkaline pH are taken as the most important risk factors of pulmonary calcification (PC). The aim of study was to assess the PC frequency and its effect on respiratory function, among pts with 3-5D stage of CKD.

Methods: Thirty seven pts with CKD (23 males and 14 females, aged 53.0±8.5 years, 13 in stage 3-4 and 24 in stage 5 (treated with hemodialysis) were included into the study. The exclusion criteria were: obtrusive, restrictive and interstitial lung diseases, smoking, tuberculosis or anti-mycobacterium therapy actual or in past, neoplasms, heart failure and overhydration. The protocol of the study included: laboratory tests (Ca, P, Ca×P product, alkaline phosphatase, PTH), lung tests (spirometry, pletysmography and diffusing capacity of the lung for carbon monoxide (DLCO), high resolution computed tomography (HRCT) of lungs and scintigraphy with the use of 99mTc-MDP.

Results: Only mild PC were found recognized by HRCT alone: in 13/24 pts with 5 CKD and in 1/13 pts with 4 CKD (p<0.01). Bone scintigraphy did not reveal any calcification. No disturbances of lung tests were found. The mean duration of hemodialysis was longer among the pts with PC (9.9±8.2 years) vs without PC (2.6±6.9 years) (p<0.01). No statistical differences in age, gender, duration of CKD, severity of Ca-P disorders and their therapy were observed among pts with and without PC. Clinical symptoms: chest pain and dyspnea were observed more frequently in pts with PC (10/13 and 9/13 respectively) than without PC (2/11 and 3/11 respectively) (p<0.004 and p<0.04 respectively). No difference in frequency of chronic, nonproductive cough was observed among both groups.

Conclusions: We conclude that despite a relatively high frequency of PC, the severity of PC was mild and they were not associated with restrictive disturbances nor impaired DLCO in functional lung tests. HRCT is an useful tool in detection of PC in CKD patients.

FR-PO1202
Vitamin E Reduces Calcification in Vascular Smooth Muscle Cells Exposed to High Phosphate
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Background: Hyperphosphatemia and oxidative stress are consistent findings in patients with chronic kidney disease. Vascular smooth muscle cells (VSMC) exposed to high phosphate (P) undergo phenotypic transition to osteogenic cells. Recent evidence suggests that reactive oxygen species can also induce calcification in VSMC. In addition, high P levels may result in oxidative stress. The present study investigate whether high P induces oxidative stress in VSMC and the effect of a natural antioxidant (vitamin E) in preventing phosphate-induced calcification.

Methods: Human VSMC were cultured for 9 days in normal P (Control) or high P (3.3mM) with or without vitamin E (10µM). Lipid Peroxidation--LPO (spectrophotometry), Advanced Glycation End Products--AGEs (ELISA), Alkaline Phosphatase--AP; activity (p-nitrophenyl phosphate method), Chfa1 mRNA (RT-PCR), and Calcium deposition (spectrophotometry of acid extract) were measured.

Results: High P increased LPO and AGEs, enhanced ALP activity and gain of expression of Chfa1, and induced calcification of VSMC. The addition of vitamin E to the high P media reduced LPO and AGEs. Furthermore, vitamin E prevented the increase in ALP activity and Chfa1 expression and attenuated calcification of VSMC.

Conclusions: High P increased LPO and AGEs, enhanced ALP activity and gain of expression of Chfa1, and induced calcification of VSMC. The addition of vitamin E to the high P media reduced LPO and AGEs. Furthermore, vitamin E prevented the increase in ALP activity and Chfa1 expression and attenuated calcification of VSMC.
Methods: 91 chronic stable KT recipients who were at least 1 year post surgery and had no dialysis vintage were included in this study. CKD was defined using GFR by plain radiographs, lateral lumbar spine x-ray for abdominal aorta and pelvic x-ray was iliac and femoral arteries, and scored according to the methods described previously. Important factors associated with VC were also determined.

Results: Higher percentages of CKD patients were diabetic. The median KT duration was 7.5 years (range 1-17 yrs). The average GFR of KT and CKD patients were 45.4 and 41.4 ml/min/1.73 m² respectively. Over 50% of CKD and KT patients had VC, mostly in the abdominal aorta. Substantial increase in pelvic arterial calcification was observed in KT compared to CKD. Total VC score was also significantly higher in KT. In patients without DM, heightened VC scores in abdominal aorta, pelvic arteries and combined sites were observed in KT. Increased pelvic arterial calcification was demonstrated especially in a subgroup with GFR < 45 ml/min/1.73 m². Age and DM were associated with VC in both groups, whereas dialysis vintage emerged as an independent factor associated with VC in KT. Multivariate analysis of the entire population demonstrated being a KT recipient was the strongest factor associated with VC.

Conclusions: In conclusion, increased VC in chronic stable KT recipients compared to pre-dialysis CKD patients was observed that was likely the result of past dialysis experience. While KT was able to restore renal function, it could not fully reverse VC.

Funding: Government Support - Non-U.S.

FR-PO1204

Serum Phosphorus Is Associated with Coronary Artery Calcification and Obstruction in Patients with Preserved Renal Function

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Background: Serum phosphorus has been associated with mortality and cardiovascular events in the CKD and general populations. In vitro studies suggest that excessive phosphorus may alter vascular calcification and endothelial function. Fibroblast growth factor 23 (FGF23) is a phosphaturic hormone and has been correlated to atherosclerosis in the community.

Methods: This cross-sectional study included 290 patients (167 males) with suspected coronary artery disease (CAD) and a MDRD creatinine clearance > 60 ml/min/1.73 m² undergoing elective coronary angiography. Coronary obstruction was quantified using the Friesinger score (FS). Coronary artery calcification was assessed by MSCT.

Results: Serum phosphorus was higher in patients with an Agastion score (AS) >10HU/when compared to the group with an AS ≤ 10 HU (3.63 ±0.55 vs. 3.49 ±0.52 mg/dL, p=0.019). In the univariate and multivariate analysis, each mg/dL of elevation in the serum phosphorus implied a higher risk of presenting an AS > 10 HU [Odds Ratio (OR)=1.92, CI 1.56-3.19; p=0.01]. Patients were divided using the median Friesinger score (4 points) as the cutoff value. Serum phosphorus was higher (5.610.5 vs. 3.51.06 mg/dL, p=0.04) and intact FGF23 was lower (median 40.3 IQR 24.1-62.2 mg/dL vs. 45.7 IQR 31.7-76.1 mg/dL, p=0.01) in the FS > 4 group. In the multivariate logistic regression analysis, a rise of 1 mg/dL of serum phosphorus carried a 74% increase in the risk of having a FS higher than 4 (OR 1.74, CI 1.06-2.88; p=0.03) and FGF23 was a negative predictor of FS both in the univariate (OR 0.32, CI 0.10-0.71; p=0.005) and multivariate analyses (OR 0.26, CI 0.11-0.63; p=0.002). Serum calcium and parathormone were not associated with CAD.

Conclusions: In patients with suspected coronary artery disease and preserved renal function, serum phosphorus was predictive of both coronary artery calcification and obstruction. There was a negative association between FGF23 and coronary obstruction.

Funding: Government Support - Non-U.S.

FR-PO1205

Phosphorus (P) Management Trends of Hemodialysis Patients in a Large Dialysis Organization

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Background: Increased serum P is a robust predictor of all-cause and cardiovascular mortality in dialysis patients. Though P control in the last decade has improved, performance varies widely among facilities.

Methods: 104,471 patients treated at a large dialysis provider were selected for analysis. Facilities with at least 50 patients (N=940) were aggregated and ranked by the proportion of patients with serum P ≤ 5.5 mg/dl for the calendar month. The top and bottom 5% of facilities were compared for the achievement of targets for other laboratory measures (Ca, P, PTH, albumin, P-binders, Ca×P product). Facilities with at least 50 patients (N=940) were aggregated and ranked by the proportion of patients with serum P < 5.5 mg/dl for the calendar month. The top and bottom 5% of facilities were compared for the achievement of targets for other laboratory measures (Ca, P, PTH, albumin, P-binders, Ca×P product).

Results: 76% of hemodialysis patients achieved serum P ≤ 5.5 mg/dl. The top 5% of facilities (n=47) treated 4011 patients and the bottom 5% of facilities (n=56) treated 4081 patients. P control was achieved in 91% of patients of top-performing facilities compared to 60% of the bottom-ranked facilities. While there was no difference in the proportion of patients achieving serum albumin target (87% vs. 88%; p=0.40), top-performing facilities were more likely to achieve targets for serum Ca (=9.5 mg/dl; 99% vs. 97%; p=0.0001) and PTH (150-600 pg/ml; 74% vs. 70%; p=0.007). Furthermore, top-performing facilities were more likely to use non-calcium P-binders (sevelamer, 69% vs. 61%; p=0.02; lanthanum, 15% vs. 12%; p=0.04). There was no significant difference in the use of either vitamin D (84% vs. 81%; p=0.09) or cinacalcet (29% vs. 28%; p=0.58), or use of a central pharmacy in the top and bottom 5% of facilities.

Conclusions: Despite a high overall rate of P control, differences exist at the unit level in proportion of patients with serum P ≤ 5.5 mg/dl across dialysis facilities. Use of non-calcium binders may provide better P, Ca, and PTH control. Identifying practice patterns that allow a larger proportion of patients to achieve P control have the potential for further improving outcomes.

Funding: Pharmaceutical Company Support

FR-PO1207

Calcium Retention and Vitamin D Drive Phosphate Retention in Chronic Kidney Disease

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Background: Phosphate retention is thought to entrain a sequence of abnormalities that stimulate PTH and FGF23 secretion and suppress activated vitamin D production and secretion. Calcium based phosphate binders are used to offset this, though recently concerns have arisen that this may promote cardiovascular disease in CKD patients.

Methods: We have gathered 233 formal calcium and phosphate balance periods in 30 adult patients with CKD from the literature to test whether intake of calcium or vitamin D promotes phosphate retention.

Results: Patients ranged in age from 19 to 72 (43.1 ± 13.4 yrs) and suffered from either either CKD Stage 3 (n=2), 4 (n=5), or 5 (predialysis, n=23). Dietary calcium intake ranged from 96 mg to 9278 mg (1309 ± 1295 mg), and phosphate intake ranged from 191 mg to 1800 mg (786 ± 279 mg) per day. Calcium retention was directly proportional to calcium intake, and increased with vitamin D intake. Phosphate retention was associated with phosphate intake only in the setting of vitamin D supplementation (p<0.001). Vitamin D supplementation was tested in 88 balance periods (32 activated vitamin D, 56 nutritional vitamin D). When calcium retention is not positive, phosphate retention centers around a median and mean of 0 (figure). When calcium retention is >0, virtually all phosphate retention points are >0, and retention varies with that of calcium (p<0.0001).

When vitamin D is added, phosphate retention is increased for any degree of calcium retention (1.29 ± 0.29 vs. 6.53 ± 1.11, p<0.0001).

Funding: NIDDK Support

FR-PO1208

Cinacalcet May Prevent Serum Fetuin A Reduction in Dialysis Patients

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Background: Cinacalcet, a novel calcimimetic, targeting the calcium-sensing receptor (CaSR) markedly reduces parathyroid activity. The CaSR has also been found in the arterial wall, and may play a role in the prevention of vascular calcification.

The aim of the study was to examine the impact of cinacalcet on serum fetuin A (fetuin A), osteoprotegerin (OPG), and vascular calcification measured by coronary artery calcification score (CAC) in dialysis pts.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Methods: We enrolled 35 pts, aged 53±10years, on dialysis for 55±63 months) with serum sFA at baseline (300±190 ng/ml). After 8 wk, serum received calcitriol (58±32 mg/d) for 52 weeks, 10 pts remained on standard therapy. CAC scores were obtained by multi-detector computed tomography before and after the study.

Results: There was a significant reduction in sFA in the control group (from 31.7 ± 5.8 to 36.4 ± 7.3 mg/dl, p < 0.01), while in calcitriol group sFA gradually although insignificantly increased (from 31.9±18.4 to 36.4 ± 14.5 mg/ml). This has resulted in a significant difference between the groups at the end of the study (p = 0.034). In both groups a significant increase in sOPG was seen (from 8.3±4.4 to 14.4±4.3 pmol/l, p=0.001 and from 11.2±7.2 to 20±5.1 pmol/l, p=0.001, respectively). The mean CAC score increased significantly (18%) in the control group (from 1081±2076 to 1272±1887 A; NS) and remained unchanged in calcitriol group. Both proteins correlated strongly with CAC: sFA negatively (r =-0.41, p=0.01) and sOPG positively (r=0.52, p=0.026) indicating an important and opposite role in vascular calcification. There was also a positive correlation between time on dialysis and sOPG (r =0.47, p =0.006), as well as CAC (r =0.56, p<0.0009).

Conclusions: These data demonstrate that FA and OPG play an important role in vascular calcification in dialysis pts, as the first and as the second as a risk factor. Calcimetric may prevent sFA reduction in this population. Prevention of vascular calcification is probable however the larger randomized studies are needed.

FR-PO1210
Pre-dominant Osteogenic Characteristics in FGF-23 Secreting Oncogenic Osteomalacic Tumors
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Background: Oncogenic osteomalacia (OOM), or tumor induced osteomalacia (TIO), is a rare disorder characterized by renal phosphate wasting and hypophosphatemic osteomalacia due to the secretion of fibroblast growth factor 23 (FGF-23) from causative membrane tumors. OOM tumors express phosphate-metabolism related factors such as FGF23, DMP1, MEPE and FRP-4, which are reported to be physiologically expressed in osteogenic lineages. To determine whether OOM tumors have osteogenic characteristics, the expressions of osteoblast/osteocyte specific genes in OOM tumors were investigated at the transcriptional and translational levels.

Methods: Seventeen causative OOM tumors and 6 histopathologic classification-matched non-OOM tumors were analyzed by quantitative real-time RT-PCR and immunohistochemistry. Fluorescent immunohistochemistry was also applied to investigate co-localization of the gene expressions in OOM tumors.

Results: Sixteen genes were significantly elevated in OOM tumors compared to non-OOM tumors in 30 genes for osteoblast/osteocyte or mesenchymal specific genes. In these 16 genes, OOM tumors exhibited positive staining in both phosphate-metabolism related factors (FGF23, DMP1, MEPE) and ostearthrosis specific genes (Runx2, osteocalcin, sclerostin). Fluorescent immunohistochemistry revealed that localizations of FGF23, DMP1 and ostearthrosis expressions were well merged in some OOM tumors, however, the predominant localizations of FGF23 was different from those of DMP1 in other OOM tumors. These tumors exhibited co-localizations of FGF23 and ostearthrosis, and those of DMP1 and osteocalcin.

Conclusions: OOM tumors have osteogenic characteristics and express phosphate-metabolism related factors. Co-localization of some phosphate-metabolism related factors and osteoblast/osteocyte specific genes suggests osteogenic characteristics contribute to express phosphate-metabolism related factors.

FR-PO1209
Plasma Concentrations of Klotho Protein in Patients with Chronic Renal Failure
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Background: Klotho (Kl) is known as an anti-aging protein predominantly expressed in the kidney, parathyroid gland and choroid plexus of the brain. It is a co-receptor specific to fibroblast growth factor 23 (FGF-23) and regulates metabolism of minerals such as calcium and phosphate. The extracellular domain of Kl is secreted into extracellular fluid, but little is known about the plasma levels of Kl and their contributing factors in patients with chronic renal failure (CRF).

Methods: We measured plasma Kl by sandwich ELISA. The studied subjects were 110 CRF patients, which included 69 non-dialyzed (ND), 25 hemodialyzed (HD) and 16 peritoneal dialyzed (PD) patients. We simultaneously measured the serum levels of FGF-23, intact PTH, creatinine (Cr), calcium (Ca), phosphate (P) and albumin (Alb).

Results: The mean plasma Kl concentrations were 551 ±177 ng/ml in the HD, 400 ±140.7 ng/ml in the PD and 777 ±234.3 g/ml in the PD patients, and it was significantly lower in the HD than in the ND and PD patients. There were significant differences in serum FGF-23 levels among the ND, HD and PD patients (p <0.05), and the serum FGF-23 levels were highest in the PD patients and lowest in the ND patients. In the ND patients, plasma Kl significantly correlated with TSH (r =0.244, p=0.043), P (r=0.273, p=0.023) and Alb (r =-0.266, p=0.027), while serum FGF-23 showed significantly positive correlations with Cr (r =0.498, p<0.001), P (r =-0.398, p<0.01) and iPTH (r =-0.397, p<0.01). In the HD patients, plasma Kl significantly positively correlated with Cr (r =0.474, p<0.01), while serum FGF-23 significantly positively correlated with Alb in both the HD (r =0.464, p<0.030) and the PD (r =-0.530, p<0.035) patients, but plasma Kl did not correlate with either serum FGF-23 or iPTH among any groups.

Conclusions: In conclusion, the contributing factors to plasma Kl concentration are different from those to serum FGF-23 level in patients with CRF.
Conclusions: This study demonstrates that hyperphosphatemia develops quickly following the cessation of phosphate binders and remains persistently elevated when not treated. This study also demonstrates that sevelamer carbonate is an effective phosphate binder that also decreases LDL-cholesterol which is important given the increased cardiovascular risk profile of many dialysis patients.

Funding: Pharmaceutical Company Support

FR-PO1212

Comparison between PA21, a New Iron-Based Non-Calcium Phosphate Binder and Lanthanum and Sevelamer Carbonate in Uremic Rats

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Background: In a previous study, we demonstrated that PA21, a new calcium-free, iron based phosphate binder effectively controlled hyperphosphatemia and iPTH levels, and was superior to calcium carbonate in preventing the development of vascular calcifications in rats with chronic renal failure (CRF). This ongoing study expands on our previous findings and compares the efficacy of PA21 with lanthanum (La) and sevelamer carbonate (Se) on hyperphosphatemia, and secondary hyperparathyroidism.

Methods: CRF was induced in rats using 0.75% adenine-enriched high phosphorus 1.3% diet for 4 weeks. Then, rats were randomized to receive the same % of active ingredient in each binder in the diet without adenine for another 4 week period. The concentration (%) of each binder was chosen to deliver the same amount of active pharmaceutical ingredient to each rat: PA21 5%, La 2%, Se 1.5%. N=6/group.

Results: At randomization, no difference was observed for serum calcium (Ca), phosphorus (P), or creatinine (creat) concentration between the 4 groups. Food intake was comparable in all groups during the study. At sacrifice, the following results were found:

<table>
<thead>
<tr>
<th>Binder</th>
<th>Body weight (g)</th>
<th>Creatinine (µmol/l)</th>
<th>Ca (mmol/l)</th>
<th>iPTH (pg/ml)</th>
<th>P:creat</th>
<th>µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRF placebo</td>
<td>311±8.6</td>
<td>157±14</td>
<td>4.0±0.6</td>
<td>24.0±0.5</td>
<td>790±368</td>
<td>21±4.6</td>
</tr>
<tr>
<td>PA21</td>
<td>380±6.3</td>
<td>156±14</td>
<td>4.1±0.7</td>
<td>22.5±0.3</td>
<td>727±417</td>
<td>4.6±1.0</td>
</tr>
<tr>
<td>La</td>
<td>396±3.8</td>
<td>148±21</td>
<td>4.2±0.1</td>
<td>23.5±0.5</td>
<td>1190±355</td>
<td>6.1±1.1</td>
</tr>
<tr>
<td>Se</td>
<td>365±5.7</td>
<td>139±18</td>
<td>2.4±0.1</td>
<td>22.0±0.1</td>
<td>1000±212</td>
<td>7.3±1.1</td>
</tr>
</tbody>
</table>

* p<0.001, ** p<0.01, *** p<0.05 vs CRF placebo

Conclusions: These experimental data show that the iron-based, calcium-free phosphate binder PA21, is at least as effective as La and Se in controlling P and iPTH in rats with CRF.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1213

Serum Phosphorous (Phos) as a Predictor of Macrovascular Events and Mortality within a Large Ethnically Diverse Population with and without CKD

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Background: We sought to determine whether higher serum Phos was a risk for ischemic heart disease (IHD), congestive heart failure (CHF), cerebrovascular events (CVE), and mortality outcomes within a large population primarily without CKD.

Methods: Retrospective cohort study 1/1/1999 -12/31/2009 persons ≥18yrs with Phos ≥1 and min 1 yr continuous followup. Subjects were categorized into population based quartiles using time dependent average Phos. The association between Phos quartiles and the primary outcome (composite of new hospitalization diagnoses of IHD, CHF, and CVE, and mortality) was examined using Kaplan Meier analysis and multivariate Cox Proportional Hazard model adjusting for age, gender, hypertension, diabetes, estimated glomerular filtration rate (eGFR), and Charlson comorbidity index.

Results: Total 209,865 persons included in cohort with mean age 54 yrs, 61% females, and 41% whites. Median followup was 3.1yrs. 13% had eGFR<60ml/min. Time dependent average Phos were categorized into 4 quartiles (mg/dl); 1.9-3.0, 3.1-3.4, 3.5-3.8, and 3.9-5.7. Compared to lowest Phos quartile, adjusted HR were increased with higher Phos.

Conclusions: Higher serum Phos demonstrated greater risk for IHD, CHF, CVE, and mortality outcomes within a large population primarily without CKD.

Funding: Pharmaceutical Company Support

FR-PO1214

Serum Calcium Phosphate, and PTH Levels: A Comparison of American and Japanese CKD Patients

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Background: The DOPPS revealed that American hemodialysis patients have two times higher PTH levels compared to their Japanese counterparts despite comparable levels of serum calcium (Ca) and phosphate (P). There are no comparative data in predialysis patients with CKD.

Methods: We compared baseline levels of serum Ca, P, PTH, urinary phosphate (a proxy for net absorbed phosphate), urinary calcium, and use of phosphate binders and vitamin D preparations among participants of the CRIC (Chronic Renal Insufficiency Cohort) and CRIC-JAC (Japanese Cohort) studies. Since PTH was measured by different assays, we calibrated the PTH levels using a range of samples from a set of 108 patients with CKD not enrolled in either study.

Results: CRIC and JAC enrolled 3939 and 2977 patients, respectively. Serum P and urinary P/creatinine (P/cre) (0.62 vs.0.43) were significantly higher in CRIC participants regardless of eGFR and despite their significantly greater likelihood of receiving phosphorus binders (7.7 vs.4.4%). However, PTH levels were significantly higher in JAC even after extensive adjustment. Serum Ca levels were also significantly higher in CRIC, while Urinary Ca/Cr were comparable.

Conclusions: Higher phosphorus intake reflected by urine P/Cr possibly could explain lower serum parathyroid hormone levels in American patients. Higher PTH in JAC might be explained by the absence of vitamin D-fortified food in Japan. Future cross-national comparisons of serum vitamin D and FGF23 levels, and dietary history will help define these differences.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

393A
FR-POI1215
Methods Quantifying Variations in Serum Phosphorus Concentrations in Hemodialysis (HD) Patients
Katie E. Cardone,1,2 Alissa Lynn Phillips,1 Rachid Daoui,1 Christopher D. Hoy,1 Shari A. Meola,1 George R. Baillie.1,2 Albany College of Pharmacy and Health Sciences, Albany, NY; 3Albany Nephrology Pharmacy Group (4NephRx), Albany, NY; 4Hortense and Louis Rubin Dialysis Center, Clifton Park, NY.

Background: Medication & diet are used to manage CKD-MBD. Fluctuations may lead to frequent regimen changes and contribute to poor adherence. However, limited data exist regarding [phosphorus (P)] variability in HD pts. Methods to quantify variations in serum hemoglobin have been defined, but have not been used to assess [P] variability. The purposes of this study were to determine extent & magnitude of [P] fluctuation in HD patients using various methods, to define typical ranges of fluctuation for each method.

Methods: Retrospective chart review of [P] was conducted at 3 HD centers. All adult HD patients who received treatment in the first quarter of 2009 (between 1/1/2009 and 3/31/2009), who had >2 [P] lab value were included. Patients were censored at death, transfer, hospitalization or HD discontinuation. [P] fluctuation was determined using 3 methods of variability: intrapatient standard deviation (intraSD), residual SD (residSD) and amplitude of variation (high amplitude (HA), low amplitude high (LAH), low amplitude low (LLL), high values only (H), low only (L), or target range only (T)). Patients were divided by amplitude classification. Mean(SD) intraSD and residSD were determined for each amplitude group.

Results: Eighty-three patients met study criteria. Half of all patients had LAH fluctuation. Mean(SD) intraSD was 0.86(0.5) & residSD was 0.59(0.4) mg/dL.

Conclusions: [P] variability is significant among HD patients, and may be measured in various ways. It is unclear which method of variability best correlates with clinical outcomes, and warrants further study.

FR-POI1216
Effect of Lanthanum Carbonate Compared with Calcium Carbonate on Serum iPTH Levels in Patients with Chronic Kidney Disease: a Randomized Controlled Trial
Sug Kyun Shin,1 Ho Yung Lee,2 Yong Kyu Lee,1

1Nephrology, NHIC Ilsan Hospital, Goyang, Geongido, Korea; 2Nephrology, Severance Hospital, College of Medicine, Seoul, Korea.

Background: Hyperphosphatemia can result in hyperparathyroidism, metabolic bone disease, cardiovascular calcification, and mortality. But, controlling hyperphosphatemia with calcium-based phosphate binders can lead to hypercalcemia, hyperparathyroidism, arterial calcification, low bone turnover. Methods: This study is a randomized prospective study designed to compare effect of lanthanum carbonate with calcium carbonate in controlling serum calcium, phosphate, iPTH level and other biochemical parameters.

Methods: Eighty-three patients met study criteria. Half of all patients had LAH fluctuation. Mean(SD) intraSD was 0.86(0.5) & residSD was 0.59(0.4) mg/dL.

Results: A total of 44 patients were enrolled in this study. Out of 44 patients, 11 patients were dropped out due to adverse effect of drug. After 24 weeks of treatment, both lanthanum carbonate and calcium carbonate reduced serum phosphate level significantly, from 6.59 ± 0.76 to 4.61 ± 0.63 mg/dL and from 6.55 ± 0.54 to 4.91 ± 0.75 mg/dL, respectively. Calcium X phosphate product was reduced in both groups, from 59.66 ± 9.08 to 42.55 ± 8.63 mg2/dL and from 60.01 ± 7.18 to 46.21 ± 6.38 mg2/dL, respectively. Serum iPTH level in Lanthanum Carbonate group was not significantly different, but in calcium carbonate group, the level decreased significantly, from 221.96 ± 223.94 to 151.17 ± 176.00 ng/mL. Serum calcium level was not elevated significantly in both groups.

Conclusions: Lanthanum carbonate is as effective as calcium carbonate in reducing serum phosphate level, and serum iPTH level tends to be steadier in lanthanum carbonate group compared to calcium carbonate group in HD patients. Though it was not significantly different, lanthanum carbonate tends not to elevate serum calcium level in HD patients compared to calcium carbonate. But high incidence of G1 adverse effect in lanthanum carbonate group needs to be further evaluated.

FR-POI1217
Role of Sodium-Derived Phosphate (Pi) Transporter (Npt2b) on Salivary Pi Secretion
Hiroko Segawa,1 Tomo Mukai,1 Saori Ohnishi,1 Shohei Sasaki,1 Akiko Ohi,1 Shoji Kuwahara,2 Shinsuke Kidoh,1 Sawako Tatsumi,1 Yasuko Ishikawa,1 Otoya Ueda,1 Naoshio Horiba,2 Kou-Ichi Jishage,1 Naoshi Fukushima,1 Ken-Ichi Miyamoto.1 1Department of Molecular Nutrition, Institute of Health Bioscience, University of Tokushima Graduate School, Tokushima, Japan; 2Department of Medical Pharmacology, Institute of Health Bioscience, University of Tokushima Graduate School, Tokushima, Japan; 3Pharmacological Research Department 1, Chugai Pharmaceutical Co., Ltd., Gotenba, Shizuoka, Japan; 4Genome Antibody Product Research Department, Chugai Research Institute for Medical Science Inc., Gotenba, Shizuoka, Japan.

Background: Hyperphosphatemia is recognized as a contributor to vascular calcification in patients with chronic kidney disease (CKD) and hemodialysis (HD) patients and is independently associated with cardiac mortality. Dietary phosphate (Pi) restriction, and the Pi binders are important therapy for dialysis patients with hyperphosphatemia. Recently, Savica et al reported the salivary secretion of Pi to be an important determinant of hyperphosphatemia in patients with CKD and in those with ERSD under chronic hemodialysis (JASN 20:639,2009). In Renal Nutrition 21, 39, 2011). In the present study, we investigated the role of PiB sodium-dependent Pi transporter (Npt2b) on salivary Pi excretion in mice.

Methods: Pilocarpine was injected intravenously into wild-type mice (Wt mice, C3H/BL6/J) and Npt2b-null mice (Npt2b+/-, and Npt2b+/-). During the 5 min after administration, saliva was collected by pipette.

Results: In Wt mice fed a high Pi diet, the levels of plasma and salivary Pi are significantly higher than those in Wt mice fed a low Pi diet. The expression of Npt2b protein was detected at the apical side of duct cells in the salivary glands, suggesting that ductal cells appears to be able to reabsorb Pi, thereby modifying the Pi concentration in the final saliva. The levels of Npt2b protein were decreased about 50% in the salivary glands of Npt2b+/- mice. The salivary Pi concentrations were significantly increased in Npt2b+/- mice compared with those in Npt2b+/- mice.

Conclusions: These data suggest that Npt2b is involved in Pi secretion by salivary glands.

Funding: Government Support - Non-U.S.

FR-POI1218
FGF23 Is Expressed in Coronary Arteries of Patients Undergoing Heart Transplantation
Natalie A. van Venrooij,1 R.C. Pereira,1 Yin Tintut,2 Linda Demer,1 Michael C. Fishbein,1 Katherine Wesseling-Perry,1 Isidro B. Salusky.1 1Pediatric Nephrology, UCLA; 2Cardiology, UCLA; 3Pathology, UCLA, Los Angeles, CA.

Background: Elevated levels of FGF23 have been associated with left ventricular hypertrophy, vascular calcification, and mortality in patients with chronic kidney disease (CKD). Vascular calcification in patients with advanced CKD is an active process, with vascular smooth muscle cells (VSMC) acquiring osteogenic properties; however, the stage of kidney disease at which this transformation occurs and the contribution of FGF23 to this process are undefined.

Methods: To evaluate the relationship between renal function, vascular calcification, and vascular FGF23 expression, immunohistochemistry for FGF23, DMP1, and osteopontin was performed in coronary arteries from 27 patients who underwent cardiac transplantation between February 2008 and 2010. 24 hr CrCl, HgA1c, and HSCRP values were obtained pre-transplantation.

Results: 53% of subjects had positive staining for FGF23. Table 1 describes the characteristics of patients with FGF23-positive and FGF23-negative staining. * indicates p<0.05.

<table>
<thead>
<tr>
<th>Variable</th>
<th>FGF23 positive (n=14)</th>
<th>FGF23 negative (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.4 ± 6.6*</td>
<td>53.6 ± 3.2</td>
</tr>
<tr>
<td>Gender (%M)</td>
<td>15%</td>
<td>92%</td>
</tr>
<tr>
<td>Euvolemia</td>
<td>62%</td>
<td>50%</td>
</tr>
<tr>
<td>Nephrotic</td>
<td>31%</td>
<td>17%</td>
</tr>
<tr>
<td>Ostearthitis</td>
<td>22%</td>
<td>30%</td>
</tr>
<tr>
<td>P3N smokker</td>
<td>62%</td>
<td>50%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>3-Carbon (mg/dL)</td>
<td>9.0 ± 0.1</td>
<td>6.8 ± 0.2</td>
</tr>
<tr>
<td>4 Phosphorus (mg/dL)</td>
<td>3.6 ± 0.1</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>4 Alkaline Phosphate (IU/L)</td>
<td>10 (7, 16)</td>
<td>10 (6, 10)</td>
</tr>
<tr>
<td>Creatinine Clearance (ml/min/1.73m2) (CKD-PO)</td>
<td>48 (47, 105)</td>
<td>48 (47, 105)</td>
</tr>
<tr>
<td>Stage 1</td>
<td>36%</td>
<td>40%</td>
</tr>
<tr>
<td>Stage 3</td>
<td>5%</td>
<td>3%</td>
</tr>
<tr>
<td>Stage 4</td>
<td>59%</td>
<td>38%</td>
</tr>
</tbody>
</table>
| CrP (mg/dL) | 5% | 6%
| HgA1C (%) | 6.4 ± 0.2 | 6.2 ± 0.5 |

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

394A
Patients with positive FGF23 staining were older, had lower CRP levels, and were more likely to have a CaCO3 level > 1.73 mmol/L than those without FGF23 expression. 36% of patients with positive FGF23 staining had normal renal function. FGF23 colocalized with DMP1 and FGF23 expression correlated directly with vascular calcification score and DMP1 expression.

Conclusions: Osteogenic transformation of VSMC occurs in individuals with very early and no CKD and progresses with worsening kidney function; whether FGF23 plays a role in the development of cardiovascular calcification or is solely a marker of the disease process remains to be determined.

FR-PO1219
Intact Fibroblast Growth Factor 23 Levels Predict Cardiovascular Death and Events before Dialysis Inception but Not after the Start of Dialysis
Chikako Nakano, Takayuki Hamano, Naohiko Fujii, Isao Matsui, Kodo Tomida, Kazunori Inoue, Akiko Shimomura, Yoshitsugu Obi, Noryuki Okada, Yoshiharu Tsukabahara, Hiromi Rakugi, Yoshitaka Isaka.

Background: Fibroblast growth factor-23 (FGF-23) and assessed urinary fractional phosphate excretion (FePi). We estimated glomerular filtration rate (eGFR) as an IV treatment for CKD-MBD/SHPT in hemodialysis patients.

Methods: In this prospective cohort study, we enrolled 738 CKD outpatients of 2 nephrology units (mean estimated glomerular filtration rate (eGFR), 35mL/min/1.73m²). We performed a cross-sectional analysis. The single-pass transmembrane protein Klotho acts as co-receptor for the phosphaturic hormone FGF-23. It has been hypothesized that soluble Klotho, which results from cleavage of the extracellular domain of Klotho by a membrane-standing protease, might exert systemic effects on calcium phosphate metabolism. CKD patients have diminished renal expression of the Klotho gene, and lower renal excretion of the Klotho protein. However, data on plasma levels of circulating Klotho in CKD patients are not available.

Methods: We studied 312 CKD stage 2-4 patients in our ongoing CARE FOR HOME study. We measured plasma levels of intact parathyroid hormone (iPTH), Klotho and FGF-23, and assessed urinary fractional phosphate excretion (FePi). We estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease study equation 4.

Results: Our patients had a mean GFR of 44±15.7 mL/min/1.73 m², and were 65±12 years of age. Median FGF-23 level was 99.0 rU/ml (IQR 60.3-158.6 rU/ml), median Klotho protein. However, data on plasma levels of circulating Klotho in CKD patients are not available.

Methods: We performed a cross-sectional observational study in 24 renal transplant recipients (14 male; 51±12y) with persistent hyperparathyroidism and hypercalcemia referred for parathyroidectomy (PTX). Parameters of mineral metabolism (including fasting calcium, and biointact FGF23 and PTH) and renal phosphate handling (fasting fractional phosphate reabsorption, FPRs) were assessed immediately before PTX and at discharge (day 1±1h).

Conclusions: In our cohort of CKD stage 2-4 patients we confirmed a gradual increase of FGF-23 and iPTH levels with declining renal function. In contrast, Klotho plasma levels were neither associated with renal function, nor with parameters of calcium phosphate metabolism. In the long-term follow-up of our study we will assess the predictive power of Klotho plasma levels for future cardiovascular or renal events in CKD patients.

FR-PO1221
A Randomized Cross-Over Trial Evaluating FGF23 and PTH in the Treatment of CKD Stage 3b
Inger Hjordis Bleskaestad, Anders Hartmann, Harald Bergrem, Lasse G. Goransson.

Background: FGF23, a phosphaturic hormone is secreted from bone and the level increases as renal function declines. The level of FGF23 is associated with increased mortality in haemodialysis-patients. The use of active vitamin D and phosphate binders as recommended in international guidelines, may affect the level of FGF23 and thereby clinical outcome. We investigated the effects of a phosphate binder and active vitamin D on the serum levels of FGF23 and PTH in patients with CKD stage 3b (30-45 ml/min/1.73m²).

Methods: Seven women and 14 men where included, mean age 65.6 ± 12.2 years. They were randomized in a 1:1 ratio to receive one of two treatment sequences. Group 1: alphacalcidol 0.25 µg once daily for two weeks followed by sevelamer carbonate 800 mg tid with meals for two weeks after a two-week washout period. Group 2: vice versa. Nineteen patients completed the study. The 25(OH) vitamin D level at baseline was 97.6 ± 29.4 nmol/L.

Results: There were no significant period or “carry-over” effects for FGF23 or PTH. There were no significant treatment effects on FGF23 or PTH (p=0.604 and p=0.243 respectively). The period-difference for FGF23 was however unexpectedly positive for both groups.

In group 1 the FGF23 level was higher after treatment with alphacalcidol compared to sevelamer carbonate (mean 105.8 ± 41.6 vs. 79.1 ± 36.5 pg/mL, p=0.047 (CI: 0.4-52.9), for PTH lower (median: 26.5, range: 14.6-52.5 vs. median 36.1, range 13.4-106.9 pg/ml, p=0.001 (CI: 3.5-13.8). In group 2 the FGF23 level increased after treatment with sevelamer carbonate and throughout the washout period non-significantly.

Conclusions: In this cross-over trial with alphacalcidol and sevelamer carbonate in patients with CKD stage 3b, the response on the FGF23 level seems to depend on whether a phosphate binder or sevelamer carbonate was initiated as the first line of therapy. Initiating therapy with sevelamer carbonate increases FGF23 levels while this response is mitigated in the group of patients given alphacalcidol followed by sevelamer carbonate. This observation needs to be confirmed in larger studies.

FR-PO1222
KAI-4169, a Novel Peptide Agonist of the Calcium Sensing Receptor, Suppresses Parathyroid Hormone, Parathyroid Gland Hyperplasia, and Ectopic Calcification in a Rodent Model of Chronic Renal Dysfunction
Sarah Walter, James Tomlinson, Amos Baruch, Shaun Alexander, Jin Dong, Mehdi Asadi, Dirk B. Mann, Maclean D, Kariim, Randolph M. Johnson. Research, KAI Pharmaceuticals, South San Francisco, CA.

Background: Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD) is a frequent and serious complication of CKD that is linked to bone abnormalities and increased risk for cardiovascular disease. KAI-4169 is a novel peptide agonist of the calcium sensing receptor (CaSR). The efficacy of KAI-4169 was evaluated in the 5/6 nephrectomy model, a preclinical rat model of chronic renal dysfunction.

Methods: Three separate studies were done using this model. In the first study, animals were randomly assigned to receive daily doses of placebo, KAI-4169 (1 mg/kg by IV bolus injection) or cinacalcet (10 mg/kg by oral gavage) for 4 weeks. In the second two studies, KAI-4169 was administered for ~6 weeks as a thrice weekly subcutaneous bolus (3 mg/kg). Control animals were either treated with vehicle or left un-treated.

Results: In the first study, parathyroid hormone (PTH) was the primary endpoint examined. 48 hours after the last dose, animals treated with KAI-4169 had significantly reduced levels of PTH compared with placebo-treated rats. In contrast, PTH had returned to baseline in the cinacalcet-treated rats by 16 hours after the last dose. Control rats in the second and third studies developed elevated PTH (~1500 pg/mL), parathyroid gland hyperplasia (as measured by gland weight and BrdU staining), elevated serum creatinine and significant vascular and soft tissue calcification. Repeat-dose administration of KAI-4169 reduced PTH levels, attenuated parathyroid gland hyperplasia, significantly reduced aortic and renal calcification and was associated with a significant reduction in serum creatinine compared with baseline values.

Conclusions: KAI-4169 is a novel peptide agonist of the human CaSR that reduces PTH levels and improves markers of CKD-MBD. KAI-4169 is currently in clinical development as an IV treatment for CKD-MBD/SIFHT in hemodialysis patients.

Funding: Pharmaceutical Company Support

FR-PO1223
The Impact of Parathyroidectomy on Circulating FGF23 Levels and Renal Phosphate Handling
Liesbeth Viane, Bjorn K.I. Meijers, Kathleen Claes, Pieter Evenepoel. Nephrology, University Hospital, Leuven, Belgium.

Background: The full relationship between fibroblast growth factor 23 (FGF23) and parathyroid hormone (PTH) remains incompletely understood, with regard to the reciprocal regulation of their transcription and secretion as well as with regard to their phasichoric actions. Recent experimental evidence suggests PTH directly affects FGF23 secretion.

Methods: We performed a prospective observational study in 24 renal transplant recipients (14 male; 51±12y) with persistent hyperparathyroidism and hypercalcemia referred for parathyroidectomy (PTX). Parameters of mineral metabolism (including fasting calcium, and biointact FGF23 and PTH) and renal phosphate handling (fasting fractional phosphate reabsorption, FPRs) were assessed immediately before PTX and at discharge (day 1±1h).

Conclusions: In our cohort of CKD stage 2-4 patients we confirmed a gradual increase of FGF-23 and iPTH levels with declining renal function. In contrast, Klotho plasma levels were neither associated with renal function, nor with parameters of calcium phosphate metabolism. In the long-term follow-up of our study we will assess the predictive power of Klotho plasma levels for future cardiovascular or renal events in CKD patients.
Results: PTX was successful in all patients. Evolution of parameters of mineral metabolism before and after PTX:

<table>
<thead>
<tr>
<th></th>
<th>Pre-PTX</th>
<th>Post-PTX</th>
<th>Controls</th>
<th>p ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR (mL/min)</td>
<td>65*</td>
<td>24*</td>
<td>94</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>PTH (pg/mL)</td>
<td>109+</td>
<td>8.75</td>
<td>9.2</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phosphate</td>
<td>2.1%</td>
<td>1.6%</td>
<td>1.8%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ca (mg/dL)</td>
<td>9.9%</td>
<td>10.5</td>
<td>17</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>FGF23 (ng/L)</td>
<td>21i</td>
<td>3.1i</td>
<td>2.2i</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fe (mg/dL)</td>
<td>84*</td>
<td>22.5</td>
<td>12</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Median values are shown; *p<0.05 vs controls; $ p<0.05 vs post-PTX.
Overall, FGF23 levels did not decline after PTX. Delta calcium (p = 0.01), but not delta PTH (p = 0.3) correlated with delta FGF23 (figure).

The F<sub>1,25</sub> significantly decreased after PTX, but remained significantly higher as compared to controls (12.7% vs P = 0.01) despite similar phosphorus concentrations (3.5 ± 0.3 mg/dL).

Conclusions: Our data suggest that calcium rather than PTH directly affects the flux of FGF23 from bone. PTH and FGF23 act synergistically to increase phosphaturia.

FR-PO1224

Phosphate Restriction Extends the Life of Uremic Rats with Extensive Vascular Calcification

Eduardo Slapotołysko, Duk H. Lee, Jane L. Finch; Medicine/Renal Disease, Washington University School of Medicine, St. Louis, MO.

Background: Numerous studies have demonstrated the role of hyperphosphatemia in the pathogenesis of secondary hyperparathyroidism, cardio-vascular disease and the progression of renal failure. The purpose of this study was to determine if, in rats with chronic kidney disease (CKD) and severe vascular calcification, a significant reduction in phosphate intake could prolong the life of these animals.

Methods: CKD was induced by 5/6 nephrectomy. A group of normal rats served as control (NC). All rats were fed a high-phosphate diet containing 1.4% phosphorus (P). After 3 months some rats from both groups were sacrificed. The remaining uremic rats were divided into the following 3 groups: uremic rats + 1.4% P diet (UHP), uremic + 1.4% P diet + sevelamer (4%) (UHP+S) and uremic + a very low P diet, 0.1%, (ULP). These rats were sacrificed after 10 weeks.

Results: After the first 3 months, the serum P in uremic rats increased from 5.9 ± 0.3 mg/dL, in normal rats, to 10.3 ± 0.76 mg/dL. The Ca x P increased from 56.7 in normal rats to 94.3 mg2/dL2. Aortic calcium content was also increased (NC: 0.5 ± 0.06 vs. uremic: 46.2 ± 14.2 mg/g wet wt). Uremic rats also exhibited positive aortic staining for von Kossa, RUNX2 and osteopontin. After the study was continued for an additional 10 weeks, the serum P in the UHP group was 14.8 ± 5.14 mg/dL vs. UHP+S rats (9.8 ± 2.87), ULP rats (3.7 ± 0.02) and NC rats (3.9 ± 0.03). Mortality in the UHP group was 78%. Mortality was reduced to 30% by treatment with sevelamer (UHP+S) and further reduced by the 0.1% P diet to just 8% (ULP). Positive staining for aortic von Kossa, RUNX2 and osteopontin was increased in UHP rats. Phosphorus restriction inhibited this.

Conclusions: These studies clearly demonstrate that a significant reduction in mortality in uremic rats with severe vascular calcification could be achieved by intensive control of P restriction.

Funding: Other NIH Support - WUCKDR O'Brien Center Grant(P30DK079333), Pharmaceutical Company Support

FR-PO1225

Changes in Fibroblast Growth Factor 23 during Treatment with Vitamin D Analogs. Comparison of Alfacalcidol and Paricalcitol in CKD5D Patients, with Secondary Hyperparathyroidism, in a Randomised Controlled Trial

Ditte Hansen,1 Knud Rasmussen,2 Lars Rasmussen,2 Susanne Møller Pedersen,1 Lisbet Brandi.1 1Medical Department, Roskilde Hospital, Denmark; 2Clinical Biochemistry and Pharmacology, Odense University Hospital, Denmark.

Background: Fibroblast growth factor 23 increases renal phosphate excretion and decreases levels of circulating 1,25 dihydroxyvitamin D. In patients with chronic kidney disease, fibroblast growth factor 23 levels are markedly elevated by unknown mechanisms.

Methods: In a Danish multicenter study, intravenous alfacalcidol and paricalcitol were compared in hemodialysis patients with secondary hyperparathyroidism in a randomised 2 x 16 week cross-over study, with 6 weeks wash-out period preceding treatment and between treatment periods. In 57 of the enrolled patients, blood samples were frozen before and after each treatment period, and available for measurement of fibroblast growth factor 23.

Results: Alfacalcidol and paricalcitol increased fibroblast growth factor equally (period 1: 223% versus 314%; P=0.384 and period 2: 174% versus 227%; P=0.510) and the levels returned to pre-treatment levels during the six week wash out period. Independent predictors of rise in fibroblast growth factor 23 were baseline levels of fibroblast growth factor (P<0.01), changes in ionised calcium (P<0.01) and phosphate (P<0.01) and cumulative dose of vitamin D analogues (P=0.024). Pre-treatment levels of fibroblast growth factor 23 were independently associated with the level of parathyroid hormone after 16 weeks of treatment with vitamin D analogs (P=0.016).

Conclusions: Alfacalcidol and paricalcitol increase levels of fibroblast growth factor 23 significantly in hemodialysis patients. However, the impact of such increase is not known. Baseline FGF23 levels predicts PTH response to treatment.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1226

Parathyroid Hormone (PTH) Levels and Mortality among Hemodialysis (HD) Patients Not Receiving Treatment for Secondary Hyperparathyroidism (SHPT): Results from the Dialysis Outcomes and Practice Patterns Study (DOPPS)

Francesca Terron,1 Mary K. Guidinger,2 William G. Goodman,2 Ronald L. Pisoni,1 Yun Li,3 Ryan D. Kilpatrick,4 Juergen Bommer,1 Masafumi Fukagawa,5 Brian Bieber,1 Bruce M. Robinson,1,3 Arbor Research Collaborative for Health; 2Aymgen, Inc.; 3U of Michigan; 4Dialysezentrum Heidelberg; 5Tokai University School of Medicine.

Background: Very high PTH levels have been associated with mortality in HD patients. Agents prescribed for SHPT, namely vitamin D analogs and calcimimetics, may have impacted those results. To add insight into whether PTH levels per se are associated with mortality we tested this association among DOPPS participants not receiving treatment for SHPT.

Methods: Of 17,476 DOPPS participants from 12 countries in 1996-2008, 5,728 (30.2%) were categorized as "untreated", i.e. had no evidence of vitamin D (oral or IV) or calcimimetic prescription for 12 months following study entry. Using the most recent PTH value at the end of the 12-month period, the association between PTH levels and mortality over study follow-up was evaluated in Cox models with different levels of adjustment.

Results: Compared to those receiving SHPT therapy, untreated patients had shorter duration of HD and lower serum PTH (mean 217.0 ± 314.8 vs. 306.9 ± 384.0 pg/ml for treated group). Among untreated patients, age was inversely associated with PHT. The association between PTH and mortality in untreated patients is shown.

Conclusions: In an international cohort of HD patients without evidence of SHPT treatment for 12 months, PTH levels < 50 and ≥ 250 pg/ml were associated with elevated mortality (p = 0.05), with the possibility of lowest mortality risk in the KDOQI target range of 150-300 pg/ml. Our results indicate an opportunity for improvement in clinical practice, since a high % of untreated patients had PTH levels above the lowest risk range.

FR-PO1227

The Association of Dietary Phosphorus Intake with Serum Fibroblast Growth Factor-23 and Parathyroid Hormone (PTH) and its Impacts on Vitamin D Metabolism in Chronic Kidney Disease: The Seattle Kidney Study

Kazumi T. Yamamoto, Alina Kostina, Bryan R. Keastenbaum. Kidney Research Institute, University of Washington, Seattle, WA.

Background: Phosphorus is excreted through the kidneys by phosphaturic hormones fibroblast growth factor-23 (FGF-23) and parathyroid hormone (PTH) and its impact on vitamin D metabolism in the setting of chronic kidney disease (CKD) is unclear. We tested the hypothesis that dietary phosphorus, measured using prospective food diaries, would be associated with key mineral metabolism biomarkers among CKD patients.

Methods: We studied 60 patients from the Seattle Kidney Study, a prospective study of CKD, who were not taking activated vitamin D or phosphorus binders and agreed to complete food diaries. We assessed mean dietary phosphorus intake using 5-day

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
prospective food records and 24-hour recall, with data entered into the Nutrition Data System for Research food database system. We measured serum intact FGF-23 and PTH using immunoassays and vitamin D metabolites using mass-spectroscopy.

**Results:** Mean 5-day phosphorus intake by food diary was 1231 ±335 mg/day in men and 1033 ±327 mg/day in women. Correlation of 5-day prospective dietary phosphorus with 24-hour dietary recall phosphorus was low (r = 0.16). Dietary phosphorus, assessed by either method, was not associated with any of the measured mineral metabolism biomarkers.

**Association of dietary phosphorus intake with FGF23, PTH, and vitamin D metabolites**

<table>
<thead>
<tr>
<th>Mineral</th>
<th>Dialysate tCa (mmol/L)</th>
<th>FGF23</th>
<th>PTH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Per 200 mg increase in dietary phosphorus (95% CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Mineral</strong></td>
<td><strong>Dialysate tCa (mmol/L)</strong></td>
<td><strong>FGF23</strong></td>
<td><strong>PTH</strong></td>
</tr>
<tr>
<td><strong>Per 200 mg increase in dietary phosphorus (95% CI)</strong></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*Models adjusted for age, race, gender, BMI, eGFR, and total kcal. Additionally adjusted for 25-OH vitamin D.

**Conclusions:** Despite large variation in dietary phosphorus intake across individuals with CKD, dietary phosphorus was not associated with FGF-23, PTH, or vitamin D metabolites.

**Funding:** Other NIH Support - NIH NIDDK T32 “Research Training in Renal Disease”

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**FR-PO1228**

**Comparison among Different Dialysate Calcium Concentrations in Bicarbonate Hemodialysis**

**Methods:** Twenty-two stable anemic uremic patients underwent three 4-hour-bicarbonate HD sessions, each with a different dialysate total Ca (iCa) concentration (1.25, 1.35, and 1.50 mmol/L). Hourly measurements of plasma water ionized Ca (pw iCa), of inlet and outlet dialysate iCa and plasma parathyroid hormone (PTH) concentrations were performed. iCa and iCa mass balances (iCaMBs and tCaMBs) were measured from the dialysate side (Genius batch dialysis system, FMC, Germany).

**Results:** Mean hourly iCa concentrations were statistically significantly higher with a dialysate iCa concentration of 1.50 mmol/L. Mean tCaMBs were positive (diffusion gradient from the dialysate to the patient), being more and more higher by increasing dialysate iCa concentrations ( 75 ± 122 mg, 182 ± 125 mg, 293 ± 228 mg, respectively) (P < 0.0009). Only 6 out of the 66 tCaMBs were negative (exclusively with 1.25). Mean dialysate tCa concentrations (+ 75 ± 122 mg, + 182 ± 125 mg, + 293 ± 228 mg, respectively) gradient from the dialysate to the patient), being more and more higher by increasing dialysate iCa concentrations ( 75 ± 122 mg, 182 ± 125 mg, 293 ± 228 mg, respectively)

**Conclusions:** Despite large variation in dietary phosphorus intake across individuals with CKD, dietary phosphorus was not associated with FGF-23, PTH, or vitamin D metabolites.

**Funding:** Other NIH Support - NIH NIDDK T32 “Research Training in Renal Disease”

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**FR-PO1231**

**Combinational Usage of Vitamin D Status and the Earliest Marker Fibroblast Growth Factor 23 Improves Risk Stratification for Renal Outcome**

**Methods:** This prospective cohort consisted of 738 predialysis outpatient means estimated glomerular filtration rate (eGFR), 35 mL/min/1.73 m² in 2 nephrology units. The endpoint was doubling of serum creatinine or dialysis initiation. The endpoint was doubling of serum creatinine or dialysis initiation. The endpoint was doubling of serum creatinine or dialysis initiation. The endpoint was doubling of serum creatinine or dialysis initiation. The endpoint was doubling of serum creatinine or dialysis initiation. The endpoint was doubling of serum creatinine or dialysis initiation.

**Results:** At baseline, the increase in intact FGF23 levels with eGFR decline was especially pronounced changes in the other MBD-related factors. For a median duration of 4.4 years, 213 patients reached the endpoint. In Cox proportional hazards model, high FGF23 and high 25D levels predicted CKD progression (interaction P = 0.01), while 1,25-dihydroxyvitamin D, PTH, phosphate levels, or active vitamin D therapy did not. Adding FGF23 and 25D to the base model of age, sex, diabetes, predialysis FR, prior cardiovascular disease, systolic blood pressure, hemoglobin, and albumin led to a net reclassification improvement of 9.05% (P=0.001). Dividing patients into 4 groups by the median of 25D and FGF23, adjusted hazard ratios for the outcome of High FGF23-Low 25D, Medium 25D-Low FGF23, Low FGF23-High 25D, and Low FGF23-Low 25D were 2.52 and 2.91, respectively. A separate analysis of 8 patients with PTX and were divided into two types: diffuse type (D-type) and nodular type (N-type). The expression of Klotho and FGF1 were detected by Western blot. 3. Parathyroid glands from patients with SHPT were divided into four groups in vitro as follows: blank-control group, FGF23 stimulation group, FGF23 (0.1µg/ml) stimulation group, FGF23 antibody + FGF23 stimulation group. According to the recommended concentration of FGF23 0.3µg/ml and 0.1µg/ml FRG3 and FGF23 (0.1µg/ ml) stimulation for 0h, 2h, 6h, 12h, 24h, 36h.

**Results:** 1.FGF23 was significantly elevated in SHPT patients. In 21 PTX patients, postoperative FGF23 were significantly decreased compared with preoperative levels, and this was followed by a reduction in PTH levels. Calcium levels, phosphorus levels, and calcium-phosphorus product levels were significantly decreased after PTX, and this was followed by a reduction in plasma FGF-23 levels in time-course study. 2.Nodular type of parathyroid gland We measured lower Klotho and FGF1 expression than diffuse type of parathyroid tissue. 3.The administration of recombinant FGF23 0.1µg/ml and FGF3 antibody 1.0µg/ml together, the level of PTH was decreased at 2h.

**Conclusions:** Parathyroid glands regulate circulating FGF23 levels in SHPT. Nodular type of parathyroid tissue exhibited lower Klotho and FGF1 expression than diffuse type of parathyroid tissue. 3.The administration of recombinant FGF23 0.1µg/ml and FGF3 antibody 1.0µg/ml together, the level of PTH was decreased at 2h.

**Funding:** High serum phosphate, parathyroid hormone (PTH) and fibroblast growth factor 23 (FGF23) levels and vitamin D deficiency, when studied separately, were found to predict the progression of chronic kidney disease (CKD). However, not all mineral bone disease (MBD)-related factors have been measured simultaneously.

**Methods:** This endpoint was doubling of serum creatinine or dialysis initiation. Our findings provide a new rationale for aVD therapy in fibrotic kidney diseases even without renin up-regulation.

**Conclusion:** Despite large variation in dietary phosphorus intake across individuals with CKD, dietary phosphorus was not associated with FGF-23, PTH, or vitamin D metabolites.

**Funding:** Other NIH Support - NIH NIDDK T32 “Research Training in Renal Disease”

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

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**397A**
The Effect of KAI-4169, a Novel Treatment for Chronic Kidney Disease-Mineral and Bone Disorder, on Serum Phosphorus Kinetics

**Methods:** Twenty-eight subjects on hemodialysis were given a single dose of KAI-4169 or placebo. The 5, 10 and 20 mg cohorts were studied in a 2-period cross-over design while the 40 and 60 mg cohorts were randomized to KAI-4169 or placebo with 8 subjects per cohort. Immediately following hemodialysis, subjects were admitted to a Phase 1 unit and observed for 3 days. Baseline laboratory testing was performed 2 hours post hemodialysis.

**Results:** Following injection of KAI-4169 post dialysis, there is a rapid 60-80% decrease in the levels of intact PTH followed by a dose dependent return towards baseline over the remaining interdialytic interval (Figure A). Phosphorus values which were decreased by dialysis, rose rapidly over the first 8 hours to a plateau and then increased more slowly during the remaining interdialytic interval (Figure B).

Interestingly, the rate of return to the plateau level of phosphorus was markedly modified by KAI-4169. The 5 mg dose had minimal effect but higher doses markedly decreased the rise of serum phosphorus towards pre-dialysis values.

**Conclusions:** These observations suggest that the marked reduction in PTH sustained over 72 hours appears to alter serum phosphorus kinetics post dialysis such that phosphorus efflux, presumably from bone, is markedly attenuated. This would suggest that phosphorus efflux from bone is a significant contributor to the generation and maintenance of hyperphosphatemia in patients with secondary hyperparathyroidism on hemodialysis.

**Funding:** Pharmaceutical Company Support

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Vitamin D Deficiency in Acute Kidney Injury

**Methods:** We recruited 28 participants with AKI and 30 controls from the medical intensive care unit and general hospital wards of Columbia University Medical Center. The following serum values were measured at baseline and repeated 5 days later: 25-hydroxyvitamin D (25(OH)D), 1,25-dihydroxyvitamin D (1,25(OH)2D), 24R,25-dihydroxyvitamin D (25(OH)D3), Vitamin D Binding Protein (VDBP), Fibroblast Growth Factor 23 (FGF-23), and Parathyroid Hormone (PTH).

**Results:** Compared with controls, participants with AKI had lower baseline levels of 25(OH)D, 1,25(OH)2D, 24R,25(OH)2D, and VDBP, and higher levels of FGF-23 and PTH. Among participants with AKI, there was a significant inverse correlation between FGF-23 and 25(OH)D (p=0.018), however, no significant correlations were found between FGF-23 and 1,25(OH)2D or between FGF-23 and 24R,25(OH)2D.

Baseline serum values in control vs. AKI median (IQR)

<table>
<thead>
<tr>
<th>Control</th>
<th>AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D (ng/ml)</td>
<td>14 (8-21)</td>
</tr>
<tr>
<td>1,25(OH)2D (pg/ml)</td>
<td>25 (15-35)</td>
</tr>
<tr>
<td>24R,25(OH)2D (ng/ml)</td>
<td>3.5 (0.6-2.6)</td>
</tr>
<tr>
<td>VDBP (mg/dl)</td>
<td>29 (25-36)</td>
</tr>
<tr>
<td>FGF-23 (RU/ml)</td>
<td>263 (87-577)</td>
</tr>
<tr>
<td>PTH (pg/ml)</td>
<td>40 (30-80)</td>
</tr>
<tr>
<td>Ca (mg/dl)</td>
<td>9.6 (9.3-9.8)</td>
</tr>
<tr>
<td>PO4 (mg/dl)</td>
<td>3.4 (2.9-4.0)</td>
</tr>
</tbody>
</table>

**Conclusions:** AKI is associated with vitamin D deficiency and elevation of FGF-23. While elevated FGF-23 may contribute to vitamin D deficiency in AKI, the mechanism does not appear to be mediated by CY24 and enhanced catabolism of 25(OH)D, given our findings of a decreased 24R,25(OH)2D. The mechanism may be related to alterations in vitamin D distribution or to diminished substrate delivery, as the VDBP levels were also decreased. The significance of these preliminary findings is unclear and will require future investigation. Vitamin D deficiency in AKI may have important physiologic consequences such as impaired host immunity, and may be a marker of poor outcomes.

**Funding:** Private Foundation Support

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Safety and Efficacy of Cholecalciferol (D3) Treatment in Hemodialysis Patients: Effects on Bone and Mineral Metabolism

**Methods:** We studied 86 CKD (74% male; 21% diabetic; age 65±13 years; CKD stages 3: 48%, 4: 43%, 5: 9%) FGF23 (3-Terminel, Immunospe) was evaluated by ELISA. FEP (%) calculated using formula (UP/OP × PCT)/(PP/UC × C) x 100, Blood and urine samples were taken simultaneously in a fasting steady state, after discarding overnight urine. Glomerular filtration rate (eGFR) was estimated by the MDRD4 equation.

**Results:** Compared with controls, patients with CKD pts (p<0.001) and FGF23 was inversely correlated with eGFR (p=0.001) and albumin (p=0.022) and directly correlated with P levels (p=0.001), iPTH (p<0.001) and with FEP (p=0.001) was inversely correlated with eGFR (p<0.001) and directly with PTH (p<0.001). Patients with P<4.5 mg/dl showed higher FGF23 levels (p=0.001) and FEP values (p=0.010).

In multivariable analysis, adjusting for age, eGFR and PTH levels, FGF23 was directly correlated with several levels (p=0.008) and inversely with albumin levels (p=0.01); FEP was inversely correlated with eGFR (p=0.001) and P levels (p=0.001) and directly with logFGF23 (p=0.008).

**Conclusions:** In summary, in this group of CKD pts, P levels were increased only in 17% of pts; while FEP-20% was detected in 88% of pts. Fasting FEP was inversely associated with eGFR and positively associated with FGF23 levels. FEP evaluated by this simple method may be a useful tool in the assessment of the altered mineral metabolism of CKD pts.

**Funding:** Private Foundation Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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398A
Methods: We screened 97 patients for 25(OH)D deficiency. 79 patients with 25(OH)D levels <20 ng/ml were randomized to treatment with D3 (50,000 IU/week to goal >35 followed by 10,000 IU/week) or control (no therapy) in a 2:1 ratio. After 6 weeks, 3, and 6 months, patients were assessed for any side effects, medications and monthly parameters. Results: In the D3 treatment arm, baseline median 25OHID increased from 13.5±mg/L to 40.5 ± at 6 weeks (n=51; p<0.001), 43.4± at 3 months (n=38; p<0.001) and 38.6 ± at 6 months (n=20; p<0.001). Baseline median serum Ca was 9.3±g/dL and remained unchanged at 6 weeks (9.2±p<0.075), 3 months (9.4±p<0.064), and 6 months (9.4±p<0.052). Median PTH was 36±mg/mL at baseline, 381± at 6 weeks (p=0.212), 372± at 3 months (p=0.757), and 292± at 6 months (p=0.07).

At our dialysis center, therapy with activated Vit D is dose-adjusted to serum PTH level. Intriguingly, normalization of 25OHID with oral D3 resulted in a significant decrease in baseline serum PTH level. Vitamin D3 treatment in dialysis patients required to maintain optimal bone mineralization. Because oral D3 is inexpensive, these findings could have important economic implications.

Funding: Private Foundation Support

FR-PO1237

Cinacalcet Therapy & Mortality among Hemodialysis (HD) Patients: International Results from the Dialysis Outcomes & Practice Patterns Study (DOPPS) Francesco Tentori, Diane Steffick, Ronald L. Pisoni, Brenda W. Gillespie, Peter G. Kerr, Stefan H. Jacobson, Takashi Akiba, Bruce M. Robinson, ‘Arbor Research Collaborative for Health, Ann Arbor, MI; ‘Univ of Michigan, Ann Arbor; ‘Monash Medical Centre & Univ, Australia; ‘Danderyd Hospital, Stockholm, Sweden; ‘Tokyo Women’s Medical Univ, Japan.

Background: Elevated parathyroid hormone (PTH) is associated with poor outcomes in dialysis patients. By acting on parathyroid calcium-sensing receptors, calcimimetics (cinacalcet) may provide better PTH control than vitamin D (VitD) alone. Among VitD treated patients from a US dialysis provider, those also using cinacalcet had lower mortality. We studied cinacalcet use & mortality in a HD cohort from 12 countries with wide variation in clinical practices.

Methods: 22,129 patients in DOPPS3 & 4 (2005-2010) were analyzed; 4,534 (20%) used cinacalcet. Cox models were used to predict survival with a time-varying cinacalcet indicator set at first reported prescription.

Results: Cinacalcet use rose over time from 8% (2005) to 19% (2010) & varied across facilities in each country (e.g. 0 to 90% in US). Patients on cinacalcet were younger (56.4±17.9 yrs, p<0.001) and had higher serum creatinine levels (5.3± ±2.6 mg/dL, p<0.001). In contrast, the control arm (n=28), we did not observe changes in median 25OHID (13.1±ng/mL at baseline vs. 10.4 at 6 months, n=13, p<0.04). There were no changes in Ca (9.1±mg/dL at baseline vs. 9.8 at 6 months, p=0.158) or PTH (293±mg/mL at baseline vs. 329± at 6 months, p=0.158). Moreover, activated Vit D requirements were unchanged.

Conclusions: The early results of this randomized trial indicate that cinacalcet is safe and effective in hemodialysis patients and suggest that repletion lowers dose of activated Vit D required to maintain optimal bone mineralization. Because oral D3 is inexpensive, these findings could have important economic implications.

Funding: Private Foundation Support

FR-PO1238

Characterization of KAI-4169, a Novel Peptide for the Treatment of Chronic Kidney Disease – Mineral and Bone Disorder, in a Phase I Study in Healthy Males Kevin J. Martin, Gregory Bell, Karen Pickthorn, Saling Huang, Peter Hodson, Munro Peacock, Saint Louis University School of Medicine; ‘KAI Pharmaceuticals; ‘Nuclear Network; ‘Indiana University.

Background: KAI-4169 is a novel peptide agonist of the CaSR that is being developed as a therapy for patients with chronic kidney disease-mineral and bone disorder (CKD-MBD). This first-in-man study was conducted to evaluate safety, tolerability, pharmacokinetics and pharmacodynamics of KAI-4169 in healthy males.

Methods: KAI-4169, administered as an IV bolus, was evaluated in a single-center, randomized, double-blind, placebo-controlled, single-dose, dose escalation Phase I study. Eight subjects were enrolled and ends of 4 cohorts (0.5, 2, 10, 100 mg) and were randomized 6:2 to KAI-4169 or placebo. Serum for routine chemistries and PTH were obtained prior to dosing and every 2-6 hours following dosing for 48 hours. Plasma for KAI-4169 analysis were collected at regular intervals following dosing. Urine was collected 24 hours prior to dosing and for 48 hours following dosing.

Results: The plasma terminal half-life of KAI-4169 was ~20 hours and KAI-4169 exposure was dose-dependent. KAI-4169 resulted in dose-dependent reductions in iPTH with a mean percent change from baseline at 30 minutes following dosing of -3.5, -21.7, -55.4, -69.0 and -72.6% for placebo, 0.5, 2, 10 mg and 10 mg groups, respectively. Dose-dependent reductions in serum calcium were observed with a mean maximal reduction of -16% at the highest dose. There was no significant change in the urine Ca/Cr ratio but tubular reabsorption of Ca tended to decrease with dose in the period immediately following dosing. Mean serum phosphorus (Pi) changes were similar to placebo except in the 10 mg cohort where it increased; however, apparent dose-dependent reductions in the Pi/Cr ratio and dose-dependent increases in tubular reabsorption of Pi were observed.

Conclusions: KAI-4169, a novel peptide agonist of the CaSR, was well tolerated at single doses up to 10 mg in healthy men and resulted in sustained dose-dependent reductions in PTH and calcium. KAI-4169 may represent a novel therapeutic approach for the treatment of CKD-MBD.

Funding: Pharmaceutical Company Support

FR-PO1239

Evidence for Increased Catabolism of 25(OH)D in CKD Hala Alshayeb, Barry M. Wall, Arif Showkat, Geeta G. Gyanamli, Valentin David, Bing Dai, Leigh Darryl Quarles.

1 Nephrology, UTHSC, Memphis, TN; 2 Nephrology, VAMC, Memphis, TN.

Background: Low circulating 25(OH)D, a marker of vitamin D deficiency, is prevalent in CKD. 1,25(OH)D is synthesized from 25(OH)D by CYP27b1 and both D metabolites and 25(OH)D levels are increased in CKD. Serum 25(OH)D levels in Col4a3-/- mice (Pearson r=-0.65, p<0.00001). CKD and non-CKD (n=14) patients with 25(OH)D levels ≤20 ng/ml. We assessed the effects of coinstantaneous treatment with active vitamin D analogues on cholecalciferol-induced changes in serum 25(OH)D in patients with ESRD (n=14).

Methods: We examined serum 25(OH)D and 25(OH)D levels and kidney CyP24 mRNA expression in a nutritionally replete Col4a3-/- mouse CKD model. In addition, we assessed the ability of cholecalciferol po, 10,000 IU/week for 8 weeks to increase serum 25OHID level in CKD (n=14) and non CKD (n=14) patients with 25(OH)D levels ≤ 20 mg/ml. We assessed the effects of concomitant treatment with active vitamin D analogues on cholecalciferol-induced changes in serum 25(OH)D in patients with ESRD (n=14).

Results: The Co343/- mice exhibited reductions in renal function that were associated with progressive increases in serum FGF23 levels and elevations of CyP24 message expression in the kidney. Serum FGF23 concentrations were negatively correlated with serum 25(OH)D levels in Co343/- mice (Pearson r=-0.65, p<0.0001). CKD and non-CKD patients had similar baseline 25(OH)D and 1,25(OH)D2 concentrations, but the CKD group had significantly higher baseline PTH levels (167±117 vs 60±27). The change in 25OHID and 1,25(OH)D2 levels were lower in CKD vs non-CKD (12±9 vs 19±8, p=0.05) and (4±24 vs 16±32, p=0.1). Serum iPTH levels decreased significantly after treatment in CKD (-42±68, p<0.04) but not in non-CKD (-10±25, p=0.16). In the ERSD group co-treatment with doxercalciferol resulted in significantly lower cholecalciferol induced increments in 25(OH)D (11.66 compared to the non-doxercalciferol treated group 22±12 (p=0.04).

Conclusions: Experimental CKD in mice have FGF23-associated reductions in 25(OH)D D and patients with impaired renal function are resistant to cholecalciferol treatment possibly due to FGF23 and or doxercalciferol-mediated CyP242 catabolism of 25(OH)D.

Funding: NIDDK Support

FR-PO1240

Vitamin D Deficiency in Fabry Disease Christiane Drechsler, Stefan Pilz, Christoph Wanner.

1 Dept of Medicine 1, Div of Nephrology, University of Wuerzburg, Germany; 2 Dept of Endocrinology, University of Graz, Austria.

Background: Patients with Fabry disease frequently develop left ventricular (LV) hypertrophy and renal fibrosis. Due to heat intolerance and inability to sweat, patients tend to be vitamin D deficient. We evaluated the effects of vitamin D supplementation on LV mass and adverse symptoms in patients with Fabry disease.

Methods: 25-hydroxyvitamin D (25(OH)D) was measured in 111 patients with genetically proven Fabry disease. LV mass and HCM were assessed by echocardiography and magnetic resonance imaging. In cross-sectional analyses, associations with adverse

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clinical outcomes were determined by linear and binary logistic regression analyses, respectively, and adjusted for age and sex.

Results: Patients had a mean age of 40.1±13 years (42% male), and mean 25(OH)D of 23.5±11.4 ng/ml. Those with severe vitamin D deficiency (25(OH)D ≤ 10 ng/ml) had an adjusted odds ratio of 2.0 (95% CI: 1.1–3.6) compared to those with sufficient 25(OH)D levels (p=0.005). The mean LV mass was meaningfully different with 170.7±75.7 in severely deficient, 154.6±50 in deficient and 128.5±48 in vitamin D sufficient patients, respectively (p=0.013). With the severity of vitamin D deficiency, the median levels of proteinuria increased, as did the prevalences of depression, edema, corona verticillata and the left ventricular hypertrophy.

Conclusions: Vitamin D deficiency was strongly associated with HCM and higher LV mass in patients with Fabry disease as well as adverse clinical symptoms. Whether vitamin D supplementation improves complications of Fabry disease remains to be determined.

FR-PO1241
Regulators of Bone Mineral Disorder and Vascular Function in Patients with Chronic Kidney Disease

Methods: In this cross-sectional study of patients with stage 3-5 CKD (n=125) and 40 healthy controls (HC), regulators and markers of BMD [FGF-23, fetuin-A, 25(OH)D, calcium, phosphate (PO4), intact parathyroid hormone (iPTH)] and measures of vascular function [endothelial dysfunction, flow mediated dilatation (FMD) and systemic arterial compliance (SAC)] were evaluated.

Results: Compared with HC, CKD subjects had lower 25(OH)D, higher PO4, fetuin-A and FGF-23 levels (all p<0.05) but had no difference in serum calcium levels. CKD subjects with lower FMD (median cut-off <4.19%) had higher FGF-23 (p=0.05) and fetuin (p=0.02) levels while those with impaired large artery compliance (median cut-off <14.58 ml/mmHg) had lower 25OHLD (p=0.05) but with no difference in serum calcium, PO4 and iPTH. In a stepwise multiple regression analysis, higher FGF-23 was independently associated with reduced FMD (β=-0.28, p<0.001) while lower 25OHLD was independently associated with increased large artery compliance (β=6.19, p=0.01).

Conclusions: Increased serum FGF-23 level in CKD is independently associated with endothelial dysfunction, whereas low 25OHLD was independently associated with impaired large artery compliance. These data support a link between regulators of BMD and vascular dysfunction in CKD.

Funding: Pharmaceutical Company Support, Private Foundation Support

FR-PO1242
Comparison of Alfacalcidol and Paricalcitol for Treatment of Secondary Hyperparathyroidism in Hemodialysis Patients: A Randomised Cross-Over Study

Methods: A randomized clinical trial with a 16-week cross-over study, intravenous alfacalcidol and paricalcitol were compared in chronic hemodialysis patients with secondary hyperparathyroidism. Doses were increased every second week until parathyroid hormone were sufficiently suppressed or phosphate or ionised calcium were increased above accepted levels.

Results: A total of 86 hemodialysis patients were randomised. Due to the presence of a period effect only data from the first treatment period (n=80) were available for statistical analysis. The proportion of patients reaching a 30% decrease in parathyroid hormone during the last four weeks of the treatment period were similar in the two groups (alfacalcidol 82% and paricalcitol 93% (P=0.180). There was no difference in the incidence of hypercalcemia or hyperphosphatemia between the treatment groups. A significant interaction effect between baseline parathyroid hormone and treatment was found (P=0.012), suggesting that alfacalcidol suppressed parathyroid hormone irrespective of baseline level, whereas suppression by paricalcitol depended on baseline level.

Conclusions: We found no over-all difference between alfacalcidol and paricalcitol with respect to suppression of secondary hyperparathyroidism and induction of hypercalcemia and hyperphosphatemia in hemodialysis patients.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1243
Lack of Signalling through Vitamin D Receptor Leads to Stress-Induced Premature Senescence in Vascular Smooth Muscle Cells

Methods: FVSMC were cultured in the presence or absence of 1α,25(OH)2D3 (1α,25(OH)2D3 was added at 50 nM at day 5 and maintained up to day 10). Cell proliferation was assessed by cell counting, and cell morphology was observed using phase-contrast microscopy.

Results: There was a significant decrease in cell proliferation in the absence of 1α,25(OH)2D3 compared to the control group. The cells showed a flattened morphology and an increased number of nuclei, indicative of senescence.

Conclusions: The lack of signalling through the vitamin D receptor leads to stress-induced premature senescence in VSMC.

Funding: Government Support - Non-U.S.

FR-PO1244
Effects of Cinacalcet Treatment on Serum Soluble Klotho Levels in Hemodialysis Patients with Secondary Hyperparathyroidism

Methods: A total of 86 hemodialysis patients were randomised. Due to the presence of a period effect only data from the first treatment period (n=80) were available for statistical analysis. The proportion of patients reaching a 30% decrease in parathyroid hormone during the last four weeks of the treatment period were similar in the two groups (alfacalcidol 82% and paricalcitol 93% (P=0.180). There was no difference in the incidence of hypercalcemia or hyperphosphatemia between the treatment groups. A significant interaction effect between baseline parathyroid hormone and treatment was found (P=0.012), suggesting that alfacalcidol suppressed parathyroid hormone irrespective of baseline level, whereas suppression by paricalcitol depended on baseline level.

Conclusions: We found no over-all difference between alfacalcidol and paricalcitol with respect to suppression of secondary hyperparathyroidism and induction of hypercalcemia and hyperphosphatemia in hemodialysis patients.

Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1245
PK/PD Modeling of Transdermal Delivery of a Novel Peptide, KAI-4169, for the Treatment of Chronic Kidney Disease-Mineral and Bone Disorder

Methods: KAI-4169 disposition parameters were directly extracted from Phase 1 data. From preclinical data, bioavailability was estimated and a transit absorption model was constructed to estimate the number of compartments and mean transit time for drug to
pass through these compartments to reach the central circulation. Using these parameters, a compartmental description of KAI-4169 and transit absorption model was constructed to simulate plasma KAI-4169 after daily transdermal administration.

Phase 1 PK/PD data were then leveraged to simulate PTH response with KAI-4169 patch administration in Stage 4 CKD patients.

**Results:** The novel peptide KAI-4169 can be delivered systemically by patch technologies. In HV and HD subjects, KAI-4169 demonstrated low PK variability and a predictable exposure-response relationship. Simulations performed with the integrated PK/PD model indicated that daily use of KAI-4169 will result in dose-dependent sustained exposure to KAI-4169 with corresponding reductions in PTH.

**Conclusions:** Daily transdermal patch administration of KAI-4169 is predicted to provide sustained PTH control in Stage 4 patients with CKD-MBD.

**Funding:** Pharmaceutical Company Support

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**FR-PO1246**

**Calcidiol Is Less Effective Than Calcitriol in Regulating VDR Target Gene Expression in Human Coronary Artery Smooth Muscle Cells**

**Background:** Vitamin D receptor (VDR) activation by agonists such as calcitriol, paricalcitol and doxercalciferol is associated with cardiovascular benefits in chronic kidney disease patients, but whether VDR’s hormone, calcitriol, and prehormone, calcidiol, exhibit similar effects requires more studies.

**Methods:** Primary culture of human coronary artery smooth muscle cells (HCASMCs) were treated with the VDR agonist calcitriol or the prehormone calcidiol in the presence of normal (0.9 mM) or elevated (2.06 mM) Pi. The expression of VDR target genes were determined by real-time PCR and proteins detected by Western blotting.

The expression and activity of CYP27B1 (the enzyme responsible for converting calcidiol to calcitriol) was also measured.

**Results:** Treating HCASMCs with 2.06 mM Pi for 24−48 hr significantly elevated the VDR mRNA (233%) and protein levels (181%). Calcitriol and calcidiol induced CYP24A1 expression with EC50 values at 70 and 662 nM, respectively. The effects of calcitriol (CYP24A1) was also measured.

**Conclusions:** Calcidiol is less effective than calcitriol in regulating VDR target gene expression.

**Funding:** Pharmaceutical Company Support

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**FR-PO1247**

**Relationship between Parathyroid Hormone (PTH) and Serum Phosphorus (P) Levels before and during Treatment with Cinacalcet among Hemodialysis Patients with Secondary Hyperparathyroidism (SHPT)**


**Background:** Efforts to control hyperphosphatemia in patients on HD focus mainly on controlling PTH and P levels. 

**Methods:** We studied 223 HD patients with mean age (± SD) of 62.7±15.3 years, 48% female, 27% diabetics, with mean HD time of 42.9±39.3 months. Univariate and multivariate analysis were performed and a p<0.05 was considered significant.

**Results:** During the study period, patients were hospitalized at least once and 29% of the patients died (mainly from CV causes). [25(OH)D3] levels were significantly lower in patients that died from CV causes (16.4±7.8 vs. 22.6±12.6 ng/mL, p<0.006). [25(OH)D3] levels were also lower in patients hospitalized during the study period.

**Conclusions:** In conclusion, [25(OH)D3] serum levels seem to be a good marker of morbidity and mortality (according to hospitalizations) and mortality (overall and CV) in HD patients.

**Funding:** Pharmaceutical Company Support

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**FR-PO1248**

**25-Hydroxyvitamin D3 Levels Are Predictors of Mortality and Hemodilysis Patients**

**Patrick Mattias,1,2 Cristina Jorge,1,2,3 Carina Ferreiro,1,2 Marília Borges,1,2 Tiago Amaral,1,2 Marco Mendes,1,2,3 Célia Gil,1,2 José Cortez,4 Aníbal Ferreira,2,3,5 *Dialysis Clinic, Hemodial, Vila Franca de Xira, Portugal; 1Dialysis Clinic, Dialvera, Alverca, Portugal; 2NDAN, Lisboa, Portugal; 3Laboratório Dr. Fernando Teixeira, Lisboa, Portugal.*

**Background:** Vitamin D deficiency has been associated with the development of cardiovascular (CV) disease and mortality in the general population.

**Methods:** We enrolled 223 HD patients with mean age (± SD) of 62.7±15.3 years, 48% female, 27% diabetics, with mean HD time of 42.9±39.3 months. Univariate and multivariate analysis were performed and a p<0.05 was considered significant.

**Results:** During the study period, patients were hospitalized at least once and 29% of the patients died (mainly from CV causes). [25(OH)D3] levels were significantly lower in patients that died from CV causes (16.4±7.8 vs. 22.6±12.6 ng/mL, p<0.006). [25(OH)D3] levels were also lower in patients hospitalized during the study period.

**Conclusions:** In conclusion, [25(OH)D3] serum levels seem to be a good marker of morbidity (according to hospitalizations) and mortality (overall and CV) in HD patients.

**Funding:** Pharmaceutical Company Support

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**FR-PO1249**

**Serum Phosphorous Variability Is Associated with Elevated Mortality Risk in Hemodialysis and Peritoneal Dialysis Patients**

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**Background:** To determine the association between serum phosphorous levels and variability, and mortality in ESRD patients.

**Methods:** Data on all ESRD patients from a major dialysis chain from 1/1/2006 to 12/1/2009 were divided into six-month intervals generating a total of 119,191 patients and 852,239 six-month windows. For each patient-interval we obtained treatment modality (PD vs HD), average and standard deviation of serum phosphorous levels, age, gender and race, prevalent diabetes, hypertension, dialysis vintage, and K/DOQI compliance. As a result, we were left with a total of 11,992 patients. For each patient-interval we performed time dependent proportional hazards regression for time to death while adjusting for prevalent diabetes, hypertension, dialysis vintage, and mortality in ESRD patients.

**Results:** Characteristics of the study population are shown in Table 1. Median serum phosphorous was 4.0 mg/dl (Q1:2.2, Q3:6.0). Median serum phosphorous levels were incrementally higher by disease category of PTH levels: 300-500 pg/mL; 500-800 pg/mL; and >800 pg/mL. Among compliant patients, the median serum phosphorous was below the all-patient average of the standard deviations, were classified: compliant when average serum phosphorous was below the K/DOQI guidelines standard of 5.5 mg/dl, non-compliant otherwise, consistent when standard deviation of serum phosphorous was below the all-patient average of the standard deviations, inconsistent otherwise. Time dependent proportional hazards regression for time to death was performed seperately for HD and for PD.

**Conclusions:** Percent of patients were compliant, 56% were consistent, 40% were compliant and consistent. After adjusting for comorbidities, other lab tests, and medication dosing and compliance, higher serum phosphorous levels and variability were associated with higher mortality in both HD and PD patients. The Figure presents relative mortality risk (compliant/consistent is the baseline) - all values were significantly higher than 1 (p-value < 0.001)
**FR-PO1250**

**Vitamin D Catabolism in Chronic Kidney Disease**


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**Background:** The current paradigm of decreased 1,25-dihydroxyvitamin D \([1,25(OH)2D]\) activation in chronic kidney disease (CKD) is incomplete because it overlooks the role of vitamin D catabolism. 24,25-dihydroxyvitamin D\([24,25(OH)2D]\), the first product in the metabolism of 25-hydroxyvitamin D\([25(OH)D]\) by CYP24A1, is a biomarker of vitamin D catabolism. We developed a novel assay to quantify serur 24,25(OH)\(_2\)D and studied its determinants, functional significance, and health outcomes in CKD.

**Methods:** We performed a case-cohort study in the Nephrology clinic-based Seattle Kidney Study. We randomly sampled 277 of 331 participants for inclusion in descriptive analyses and added 34 cases for mortality analyses (total N=90 cases of death). Vitamin D metabolites were measured from frozen baseline serum using HPLC-tandem mass analyses and added 34 cases for mortality analyses (total N=90 cases of death). Vitamin D metabolites were measured from frozen baseline serum using HPLC-tandem mass spectrometry. The Barlow method was used for assigning case-cohort sampling weights in survival analyses.

**Results:** At baseline, participants had a mean eGFR of 46ml/min/1.73m\(^2\) and a mean age of 61 years. 83% were men and 69% were white. 24,25(OH)\(_2\)D concentration declined in survival analyses.

**Conclusions:** Serum phosphorous variability was found to be an independent predictor of mortality in both PD and HD patients. Optimal medical management should focus on managing both serum phosphorous levels and their variability. Unmeasured variables may account for patients with higher and more variable phosphorous levels having higher mortality.

**Funding:** Pharmaceutical Company Support

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**FR-PO1252**

**Vitamin D Suppresses High Glucose-Induced GLUT1 Expression in Mesangial Cells by Targeting NF-\(\kappa\)B**

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**Background:** Glucose transporter 1 (GLUT1) is a facilitative glucose transporter that is up-regulated by high glucose (HG). In diabetes GLUT1 overexpression in mesangial cells stimulates glucose uptake and the synthesis of extracellular matrix, leading to glomerular injury. Glucosylated GLUT1 overexpression in transgenic mice causes glomerulosclerosis even in non-diabetic conditions. Our previous studies showed that vitamin D reduces glomerulosclerosis in diabetic nephropathy.

**Methods:** We used mesangial cell cultures to explore the molecular mechanism underlying vitamin D inhibition of glomerulosclerosis.

**Results:** Exposure of primary mesangial cells to HG media (30 mM) markedly stimulated GLUT1 expression at mRNA and protein levels, and the stimulation was blocked by 1,25-dihydroxyvitamin D \([1,25-(OH)2D]\). Consistently, 1,25-VD also inhibited HG-induced glucose uptake in mesangial cells. The HG-stimulation was blocked by NF-\(\kappa\)B inhibitor BAY 11-7082, suggesting the involvement of NF-\(\kappa\)B in HG induction of GLUT1. A putative cis-\(\kappa\)B site was identified in the GLUT1 enhancer-2 at +17282 bp in silico analysis. EMSA showed that this \(\kappa\)B site could be bound by p65. CHIP assays with anti-p65 antibodies confirmed the interaction of p65 with this \(\kappa\)B site in mesangial cells. HG increased p65 binding to the \(\kappa\)B site, which was blocked by 1,25-VD. Luciferase reporter assays using pEn-GLUT1-Luc plasmid showed that HG induced luciferase activity in the wild-type plasmid, but not in a mutant plasmid in which this \(\kappa\)B site was mutated. The HG-induced luciferase activity was inhibited by 1,25-VD treatment.

**Conclusions:** Taken together these data suggest that 1,25-VD suppresses HG-induced GLUT1 expression by targeting the \(\kappa\)B-mediated pathway. Suppression of GLUT1 may contribute to the renoprotective activity of vitamin D against glomerulosclerosis in diabetes.

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**FR-PO1253**

**Serum 25-Hydroxyvitamin D Levels and Vascular Calcification in Predialysis and Dialysis Patients with Chronic Kidney Disease**

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**Background:** The role of vitamin D in the process of vascular calcification is controversial in patients with chronic kidney disease. We investigated whether serum 25-hydroxyvitamin D associates with vascular calcification in predialysis and dialysis patients.

**Methods:** We included 209 patients. Vascular calcification was evaluated by examining plain X-rays of pelvis and hands as previously described. The augmentation index (AIx) was assessed with a commercially available device (VP-2000, Colin Corporation).

**Results:** We found a high prevalence of vitamin D deficiency in our population (77.0%). Vascular calcification was present in 36.4% of all patients. The presence of vascular calcifications was significantly associated with lower 25(OH)D levels in predialysis, dialysis and all patients. Multivariate analysis showed that 25(OH)D levels were inversely associated with simple vascular calcification score \(\geq 1\) (OR; 0.037, 95% CI; 0.86 - 0.99, \(P = 0.037\)). Lower 25(OH)D levels were associated with higher AIx in predialysis and all patients, but this inverse relationship was abolished in multivariate analysis.

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**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

*Underline represents presenting author.*

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*Pit-2 (SLC20A2) were not increased. In normal rats, edecalcitol increased NaPi-IIb 8- and 3-fold in the duodenum and jejunum, respectively, but had no effect in ileum or lung, despite the presence of the VDR and induction of 24-hydroxylase mRNA. Calcitriol had no effects in any of the tissues. Renal NaPi-Ia and NaPi-IIc were not regulated by either vitamin D compound under these conditions.

**Conclusions:** These studies indicated that the vitamin D analog edecalcitol is a unique potent stimulator of intestinal Pi absorption via induction of NaPi-IIb. The mechanisms responsible are under investigation.

**Funding:** Pharmaceutical Company Support
FR-PO1254

Long-Term Effect of Cinacalcet Hydrochloride Treatment on the Parathyroid Gland Volume in Patients with Advanced Secondary Hyperparathyroidism

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Background: Cinacalcet hydrochloride has been shown to lower serum PTH and to improve achievement rate of the guidelines for secondary hyperparathyroidism (SHPT). However, long-term effect of cinacalcet hydrochloride treatment with intravenous vitamin D therapy on the parathyroid gland (PTG) volume has not been fully elucidated.

Methods: The present study comprised of 60 hemodialysis patients from 10 dialysis centers in Japan, who received more than two years of cinacalcet treatment for advanced SHPT, resistant to intravenous vitamin D therapy (intact PTH, 568 ± 268 pg/ml). The serial changes in the biochemical parameters and maximum PTG volume were determined. We also investigated the factors that determined “marked PTG reduction”, and the achievement rate of the guideline recommended by the Japanese Society of Dialysis Therapy (JSDT); 8.4 ≤ calcium ≤ 10.0 mg/dL, 3.5 ≤ phosphorus (P) ≤ 6.0 mg/dL, 60 ≤ intact PTH ≤ 180 pg/ml.

“Marked PTG reduction” was defined as more than 30% reduction in maximum PTG volume after two years of treatment, and was used as the endpoint in the following analysis.

Results: Cinacalcet treatment with a mean dose of 47.5 mg/day for two years achieved approximately 25% reduction in PTG volume overall. A total of 33 patients showed “marked PTG reduction”. The achievement rate of the JSDT guideline increased from 5% to 48%.

Conclusions: Cinacalcet treatment with intravenous vitamin D therapy for two years effectively reduced maximum PTG volume even in patients with advanced SHPT, leading to the increased achievement rate of the JSDT guideline.

Funding: Private Foundation Support

FR-PO1255

Serum 25-Vitamin D Level in Children: Do We Need To Change the Reference Range Based on 2011 Institute of Medicine (IOM) Report?

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Background: The 2011 IOM Report recommends serum 25-Vitamin D (S-VitD) of ≥20 ng/ml as normal value. The traditional reference range (RR) of S-VitD for children in our clinical lab has been ≥30 ng/ml. Characteristically, in subjects with normal kidney function the onset of Vit D insufficiency is indicated by a rise in serum PTH (S-PTH). Considering the new IOM report, the aim of this study was to re-examine our RR for S-VitD based on S-PTH.

Methods: The database of clinical lab of our tertiary children’s hospital from Jul 09 to Jan 11 was analyzed for S-VitD, S-PTH and serum creatinine (S-Cr). Data were included only of the child’s initial sample and if all measurements done on same blood sample. S-VitD was measured by Tandem Mass Spectrometry on AB4000 QTrap, S-PTH by immunoassay (ImmuliteTM) and S-Cr on Vitros autoanalyzer. To exclude patients with kidney failure, primary hyperpara and pseudohypopara we included only children with S-Cr 50.6 mg/dL and S-PTH ≤200 pg/ml.

Results: There were 304 samples with S-Cr ≤0.6 mg/dL and S-PTH ≤200 pg/ml. Using S-VitD <30, 39% were insufficient/deficient compared to 10% for S-VitD ≥20 mg/ml. The inflection point by Gaussian-Newton method for S-VitD (Figure) was at 31.7 mg/ml (95%CI 26.4-37.0). S-PTH ≥75 pg/ml was recorded in 23 children (19.3%) with S-VitD <30 mg/ml compared with only 12 (6.5%) with S-VitD ≥30 mg/ml (Z2-test p=0.0006). OR for S-PTH ≥75 for S-VitD ≥30 was 0.29 (95%CI 0.14-0.61). Similar analysis in 553 children with S-Cr ≤10 mg/dL yielded identical results with inflection at 30.1 ng/ml (95%CI 24.1-36.1).

Conclusions: Based on both the inflection point for S-VitD and incidence of elevated S-PTH, and in contrast to the IOM recommendation, we suggest to maintain in children a cut-off value of ≥20 ng/ml.

FR-PO1256

Characterization of KAI-4169, a Novel Peptide for the Treatment of Chronic Kidney Disease – Mineral and Bone Disorder, in a Single-Dose Study in Hemodialysis Subjects

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Background: KAI-4169, a novel peptide agonist of the calcium sensing receptor (CaSR), is being evaluated in hemodialysis subjects as a treatment for CKD-MBD. This randomized, double-blind, placebo-controlled, single-dose, dose-escalation study was designed to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of KAI-4169 administered as an IV bolus.

Methods: Twenty-eight subjects were enrolled in one of five cohorts (5, 10, 20, 40, 60mg). Cohorts 1-3 were treated in a 2-period crossover design with 4 subjects/cohort. In Cohorts 4 and 5, 8 subjects were randomized 1:1 to KAI-4169 or placebo. Major inclusion criteria included hemodialysis for at least 3 months, serum iPTH >300 pg/mL, serum cCa ≥9.0 mg/dL and the dose of vitamin D or analogs had to be stable for at least 3 weeks prior to screening. Subjects were admitted to a clinical research unit following hemodialysis and were observed for 3 days prior to discharge for hemodialysis.

Results: Treatment with KAI-4169 resulted in rapid suppression of iPTH with mean maximal reduction from baseline of 64, 73, 75, 84 and 86 % at the 5, 10, 20, 40 and 60 mg dose levels, respectively.

Conclusions: KAI-4169, a novel peptide agonist of the CaSR, administered as an IV bolus resulted in sustained, dose-dependent reductions in serum iPTH and was well tolerated at single doses up to 60 mg. KAI-4169 represents a novel therapeutic approach for the treatment of CKD-MBD.

Funding: Pharmaceutical Company Support

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Underline represents presenting author.
FR-PO1257
Are Current KDOQI/JSDT Guidelines Sufficient for Preventing the Progression of Secondary Hyperparathyroidism? Sato Megumi,1 Masanori Jotoku,2 Yuzuuro Sato,2 Ryota Ikece,3 Masataka Tsunoda,3 Naomi Sasaki,3 Nobuo Hashimoto.1 1Department of Nephrology, Sato Juntokkakanai, Matsuyama, Ehime, Japan; 2Department of Internal Medicine, Sato Junktakkanai, Matsuyama, Ehime, Japan; 3Department of Nephrology and Dialysis, H.N.Medic Kitahiroshima, Kitahiroshima, Hokkaido, Japan; 4Department of Nephrology and Dialysis, H.N.Medic Sapporo-Higashi, Sapporo, Hokkaido, Japan; 5Department of Nephrology and Dialysis, H.N.Medic Sapporo, Hokkaido, Japan.

Background: There are several guidelines for secondary hyperparathyroidism(2°HPT) in the world, and each guideline has each standard values for Ca, P and PTH. In this study we compared the incidence of refractory 2°HPT between the dialysis patients who had been categorized by the current guidelines from the Kidney Disease Outcomes Quality Initiative KDOQI and Japanese Society for Dialysis Therapy (JSDT).

Methods: 180 chronic hemodialysis patients were enrolled into the study protocol. The patients were divided into the next 3 groups based on the averaged cCa and P: Group A: ≤cCa ≤P met KDOQI guideline (n=107), Group B: gap area between JSDT and KDOQI (n=47), Group C: the rest patients (n=26). Refractory 2°HPT was identified as the association of Cinacalcet hydrochloride(CH) or receiving of parathyroidectomy (PTx). The incident rates of refractory 2°HPT in each group were evaluated after 3-year-followup period.

Results: The mean values in cCa, P, i-PTH were shown in table 1. The incident rate in each group was significantly different from each other; 1/107 (0.9%) in Group A, 12/47 (25.5%) in Group B and 14/26 (53.9%) in Group C (Table 1).

Conclusions: The JSDT guideline is not sufficient to prevent the onset of refractory 2°HPT because the incidence in Group B was significantly higher than that in Group A. In conclusion, ideal standard values in cCa and P should be stricter than the current values in both JSDT and KDOQI guidelines for preventing refractory 2°HPT.

FR-PO1258
Proteinuria Modifies Response to Cholecalciferol in CKD Amay Parikh,1 Herbert S. Chase,2 Linda Vernocchi,1 Leonard Stern.1 1Columbia University, New York, NY; 2Biomedical Informatics, Columbia University, New York, NY.

Background: We previously showed that the response to cholecalciferol replacement therapy in CKD, assessed by the 25-OH Vitamin D (25VitD) level, was associated with eGFR. Non-responders displayed a worsening eGFR over time compared to Responders. The effect on eGFR by the cholecalciferol response was modified by proteinuria.

Methods: 180 chronic hemodialysis patients were categorized by the current guidelines from the Kidney Disease Outcomes Quality Initiative KDOQI and Japanese Society for Dialysis Therapy (JSDT).

Results: No difference was observed in microalbuminuria between the two groups. With similar proteinuria, the eGFR declined faster in the NON-RESPONDERS (1.47 g/d) than RESPONDER (0.89 g/d) (p<0.004). During 50 months of follow-up, proteinuria 2°HPT was higher than RESPONDER (0.57) was independently associated with selected physical performance measures.

FR-PO1260
Vitamin D Deficiency (VDD) Is Associated with Increased Frequency of Vascular Access Dysfunction (VAD) in Chronic Hemodialysis Patients (HDPs) Reuben Valentin,1 Jose A. Velaz,2 Khalidoune Souda,3 Christine L. Gear,1 Jonathan A. Geffond,3 Wajeeh Y. Qunibi.1 1Division of Nephrology, University of Texas Health Science Center at San Antonio, TX; 2Epidemiology and Biostatistics, University of Texas Health Science Center at San Antonio, TX.

Background: VDD is prevalent worldwide and is associated with obesity, diabetes, hypertension and other cardiovascular risks. Vitamin D receptors are present in vascular smooth muscle and endothelial cells. Vascular access complications in HDPs are a major source of morbidity and increased health costs. In this study we sought to examine the relationship between 25OH D levels and dialysis access interventions for thrombosis, failure to mature, pseudoaneurysm, steal syndrome, stenosis within 1 year of initial 25OH D level.

Methods: We reviewed medical records of 256 HDPs who had 25OH D level and arteriovenous fistula or arteriovenous graft. We performed univariate Poisson regression with the number of access interventions regressed onto pre-specified demographic and laboratory variables and then included significant variables as covariates in a multiple logistic regression model of the odds of having one or more intervention.
Results: Mean age was 55±12.5 years. 48% were female. Mean 25OHID level was 19.1±11.9 ng/ml. Serum calcium, iPTH, homoglobin, platelets, albumin were significantly associated with access interventions (p<0.05). The per-unit change in intervention risk associated with 25OHID level remained significant (p=0.0246; 95%CI [0.94, 1.01]) after adjusting for these variables.

Methods: Logistic Regression analysis of Risk of Access Intervention

Variable         Log Odds Std Err OR 95% CI P-Value
Calcium         -0.033  0.018  -0.97  (0.94, 1)  0.0246
Homoglobin      -0.004  0.005  -1.00  (0.85, 1.2) 0.39
Platelet         2.632  1.76   13.9  (0.44, 491.8) 0.1359
Albumin         -0.051  0.038   -0.99  (0.82, 1.25) 0.1659

The adjusted OR of the risk of access intervention for patients with 25OHID deficiency (<15 ng/ml) was 1.98 (p=0.042; 95%CI [1.15, 3.41]) compared to those with 25OHID ≥15 ng/ml.

Conclusions: VDD is associated with the development of VAD. Treatment of VDD for the purpose of preventing VAD must await randomized, controlled studies.

FR-PO1261
Parathyroid Hormone, Calcium, and Phosphorus Metabolism in Children Undergoing Chronic Peritoneal Dialysis
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Background: Chronic kidney disease-mineral and bone disorder (CKD-MBD) remains a significant problem in children on dialysis. Parathyroid hormone (PTH), calcium and phosphorus metabolism is a key component of CKD-MBD and PTH is at the center of the mineral imbalance, consequent skeletal disease and growth failure. However, the optimal PTH target in CKD patients is a matter of long-standing controversy.

Methods: We assessed the status of CKD-MBD in children on peritoneal dialysis for more than one year at Asan Medical Center between May 2001 and July 2010 and compared biochemical profile with recommendations of KDOQI guidelines. We also evaluated the relationship between PTH and growth.

Results: Twenty-five boys and 16 girls with median age of 16 years (3-22 years) were included. The median age at initiation of peritoneal dialysis was 10 years (0-17 years) and mean±SD duration of peritoneal dialysis was 39.9±22.0 months. Mean±SD values of serum PTH, Ca, P, Ca×P and PTH above the target ranges recommended in the KDOQI guideline. Patients were followed for 16±9 months. There was no significant correlation between PTH and bone markers in this study.

Conclusions: Biochemical profile was outside the guideline targets in more than 50% percent of patients despite effort to control Ca, P and Ca×P. Signs and symptoms of CKD-MBD were observed in 15 percent of patients whose PTH level was significantly higher. There were no relationships between growth and time-averaged PTH concentrations regardless of growth hormone therapy.

FR-PO1262
Cinacalcet Treatment Decreases Parathyroid Gland Volume in Hemodialysis Patients with Secondary Hyperparathyroidism – A Long-Term Follow-Up Study
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Background: We and others have recently reported that cinacalcet treatment significantly decreases parathyroid gland volume (PTV) in hemodialysis patients with secondary hyperparathyroidism (SHPT) in 6 months (Ichii, et al. Nephron Clin Pract;115:c195, 2010) and in 12 months (Komaba, et al. Clin J Amer Soc Nephrol 5:2305, 2010). However, further long-term effect of cinacalcet on PTV is unknown. In the present study, we examined changes in PTV after cinacalcet treatment for longer term.

Methods: Cinacalcet was administered in patients with SHPT in whom active vitamin D and phosphate binders could not sufficiently reduce PTH levels. Dosage of cinacalcet was adjusted according to the Japanese guideline for the treatment of SHPT (Ther Apher Dial 12:514, 2008). PTV was measured four times by ultrasonography before (-12.3±3.8 months), at the start of (-7.0±2.9 months and at 18.6±2.8 months after cinacalcet treatment in 49 patients with SHPT (61±19 years, hemodialysis duration 166±85 months).

Results: Intent-PTV was significantly increased from 500±279 pg/ml 12 months before, to 607±288 pg/ml at the start of cinacalcet treatment (p<0.01). It decreased to 244±152 pg/ml at 7.0 months (p=0.0001), and to 202±161pg/ml at 18.6 months (p<0.0001), PTV significantly increased from 681±638 mm3 12 months before, to 994±754 mm3 at the start (p<0.0001). PTV was significantly decreased to 737±553 mm3 (p<0.001) at 7.0 months, and was continuously decreased to 643±552 mm3 (p<0.001) at 18.6 months. Although, out of 49 patients, there were 12 patients in whom PTV was decreased during the period, intent-PTV in these patients was significantly decreased (p<0.0001).

Conclusions: In the present study, cinacalcet treatment was demonstrated to decrease PTV, along with decreasing intact PTH, for longer periods. This study suggests that cinacalcet treatment may postpone parathyroidectomy and/or reduce its cases.

FR-PO1263
Serum 25-Hydroxyvitamin D Concentration and Risk of Clinical Disease Events in a Community-Based Population of Older Adults
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Background: Circulating concentrations of 25-hydroxyvitamin D [25(OH)D] are used to define vitamin D deficiency. While not optimal, threshold 25(OH)D concentrations for populations with chronic kidney disease have largely been extrapolated from the general population. Current clinical 25(OH)D targets are based on associations with markers of bone and mineral metabolism. These targets may not reflect optimal levels for other chronic diseases that are potentially affected by vitamin D and do not account for seasonal 25(OH)D variation. The goal of this study was to evaluate the relationship of serum 25(OH)D concentration with the incidence of major disease outcomes that are pathophysiologically relevant to vitamin D.

Methods: We studied 1,662 Caucasian older adults from the community-based Cardiovascular Health Study. We measured baseline serum 25(OH)D concentration using a high performance liquid chromatography-tandem mass spectrometry assay that conforms to National Institute of Standards and Technology reference standards. We defined the primary outcome measure as the time to a composite outcome of incident hip fracture, myocardial infarction, cancer, or death from any cause.

Results: Over 11 years median follow-up, the composite outcome occurred in 1,041 participants (63%). The association between 25OHID < 20 ng/ml with the composite clinical outcome varied by season (p=0.03). Serum 25(OH)D concentration below the lowest season-specific 22nd percentile (< 14 ng/mL in winter, < 18 ng/mL in spring, < 22 ng/mL in summer, and < 19 ng/mL in fall) was associated with an 18% greater adjusted risk of the composite outcome (95% confidence interval 1%, 38%). Compared with a static 25(OH)D threshold of 20 ng/ml, season-specific thresholds reclassified 8% of participants and improved risk prediction (net reclassification index 2.8%, p=0.047).

Conclusions: These findings suggest that threshold concentrations of 25(OH)D associated with increased risk of relevant clinical health events center near 20 ng/ml. In addition, season-specific targets for 25(OH)D concentration may be more appropriate than static targets when evaluating health risk.

FR-PO1264
VS-110: A Novel Vitamin D Receptor Modulator with Cardiovascular Protective Effects in 5/6 Nephrectomized Uremic Rats
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Background: Vitamin D receptor modulators (VDRMs) such as calcitriol, paricalcitol and doxercalciferol are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). A majority of CKD patients die from cardiovascular complications. Clinical observations demonstrate that VDRM therapy may provide cardiovascular and survival benefit for CKD patients. However, current on-market VDRMs have a narrow therapeutic index (TI) at 1-4-fold (estimated from the hypercalcemic toxicity and PTH suppressing efficacy). Hypercalcemia remains a serious concern, which leads to the need for frequent drug dose titration and serum calcium monitoring. Significant clinical benefit can be derived from a VDRM with expanded TI and cardiovascular protective effects.

Methods: The 5/6 nephrectomized (NX) male Sprague-Dawley rats were 6 week after the surgery exhibited established uremia, elevated parathyroid hormone (PTH), endothelial dysfunction and left ventricular hypertrophy.

Results: Treatment of 5/6 NX rats by VS-110, a novel VDRM, at 0.01 - 1.0 µg/kg (oral gavage, once or twice, for two weeks) suppressed serum PTH effectively without raising serum calcium, demonstrating a >50-fold TI. Similar results were obtained when VS-110 was given to 5/6 NX rats by i.p., 3x/week for two weeks. When the 5/6 NX uremic rats were treated with VS-110 (0.01 - 1 µg/kg) for two weeks, VS-110 improved endothelium-dependent aortic relaxation, reduced left ventricular (LV) fibrosis and attenuated LV hypertrophy in a dose-dependent manner without affecting serum calcium. Real-Time PCR showed that VS-110 induced CYP24A1 and CD14 expression in HL-60 cells with EC50 values at 6.8 and 0.4 nM, respectively. VS-110 induced HL-60 differentiation with an EC50 value at 1.7 nM (vs. calcitriol at 13.9 nM) and inhibited the proliferation of primary human keratinocytes with an IC50 value at 1.4 nM (vs. calcitriol at 10 nM).

Conclusions: These studies demonstrate that VS-110 is a novel VDRM with greatly expanded TI and an overall therapeutic product profile that supports clinical development for expanded use in pre-dialysis CKD patients to realize the cardiovascular protective effects of VDR activation.

Funding: Pharmaceutical Company Support
FR-PO1265

Rise in Uncontrolled Secondary Hyperparathyroidism (SHPT) among Blacks after Implementation of the US Prospective Payment System (PPS): Results from the DOPPS Practice Monitor (DPM)
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Background: Increased financial constraints may lead to lower utilization of intravenous (IV) vitamin D analogs under the new bundled prospective payment systems (PPS), leading to poorer control of secondary hyperparathyroidism (SHPT). Because Black patients require higher vitamin D doses (Wolf JASN 2008) on average, they may be particularly susceptible to this change.

Methods: The DOPPS Practice Monitor (DPM) provides timely, public reporting of trends in dialysis care as the new PPS and QIP are implemented (www.dopps.org/dpm). The DPM follows a nationally representative sample of ∼140 US dialysis units with ≥20 chronic hemodialysis patients. We studied trends in parathyroid hormone (PTH) values and SHPT therapies from July 2010 to February 2011.

Results: The % of patients with PTH measured over 3 months did not change (93-96%). The median PTH value rose among Blacks from 296 (interquartile range [IR]: 214-469) to 379 pg/ml (IR: 236-606), and among non-Blacks from 244 (IR: 173-354) to 283 pg/ml (IR: 192-435). The prevalence of severe uncontrolled SHPT (defined as PTH >600 pg/ml) rose sharply among Blacks (from 16 to 26%) and slightly among non-Blacks (from 9 to 11%). Preliminarily, these changes do not appear to be due to decreased overall use of SHPT treatments (% prescribed IV vitamin D rose slightly in both race groups, cinacalcet to 11%).

Conclusions: All individual-level and facility-level assessments support the conclusion that doxercalciferol is non-inferior to paricalcitol on bone and mineral outcomes in HD patients. The small increase in PTH coincides with the increase in the KDIGO upper limit from 300 to 600 pg/ml. Funding: Pharmaceutical Company Support.

FR-PO1267

Cinacalcet Hydrochloride Induces Apoptosis of Parathyroid Cells in Humans and In Vitro: Histological and Cytological Analyses
Ryoko Tatsumi, Takatoshi Kakuta, Kaichiro Sawada, Genta Kanai, Hirotaka Kobama, Masafumi Fukagawa. Division of Nephrology, Endocrinology and Metabolism, Tokai University School of Medicine, Isehara, Japan.

Background: Secondary hyperparathyroidism develops during the long course of chronic kidney disease. Recently, cinacalcet hydrochloride has been available as one of the treatment options for secondary hyperparathyroidism. There have been a number of reports on the effects of cinacalcet on biochemical parameters of secondary hyperparathyroidism. In this study, we examined histological and cytological changes of parathyroid glands removed from patients with severe secondary hyperparathyroidism, particularly focusing on apoptosis in parathyroid cells.

Methods: The study subjects were 16 dialysis patients who underwent parathyroidectomy for severe secondary hyperparathyroidism in our hospital from April 1, 2007 to March 31, 2010, in whom 8 patients had been treated with cinacalcet hydrochloride and the others had not. We compared the number of TUNEL-positive cells between cinacalcet group and non-cinacalcet group. We also examined the effects of cinacalcet on parathyroid cell death in vitro cell culture with the TUNEL staining, using parathyroid cells from patients with severe secondary hyperparathyroidism.

Results: The number of TUNEL-positive cells in the cinacalcet group was significantly higher than that of non-cinacalcet group (12.7 ± 4.3 versus 3.2 ± 2.4 per 1000 cells, P <0.001). In vitro examination also showed significant increases of apoptotic cells with the addition of 10 μM cinacalcet hydrochloride into the culture medium, further supporting the apoptotic effect of cinacalcet on parathyroid gland cells.

Conclusions: These results suggest that treatment with cinacalcet induces apoptosis in hyperplastic parathyroid cells in patients with secondary hyperparathyroidism.

FR-PO1266

Effects of Switching from Intravenous Paricalcitol to Doxercalciferol on Dialysis Patient Bone and Mineral Outcomes
T. Christopher Bond, Steven M. Wilson, Mahesh Krishnan, Tracy Jack Mayne. DaVita Clinical Research, Minneapolis, MN.

Background: The relative effectiveness of various forms of intravenous vitamin D among hemodialysis (HD) patients is still in question. We assessed outcomes before and after a planned switch of dialysis patients from paricalcitol to doxercalciferol.

Methods: This single-arm, prospective study measured pre/post levels of phosphorus, corrected calcium and parathyroid hormone (PTH) to assess non-inferiority of clinical endpoint. Patient-time within range for all 3 laboratory measures was constant or increased across the time frame of the study. The facility-level analysis of laboratory values showed results similar to those seen in the individual-level analysis. There was no change in hospitalizations or hospitalized days per patient-year.

Results: The % of patients with PTH measured over 3 months did not change (93-96%). The median PTH value rose among Blacks from 296 (interquartile range [IR]: 214-469) to 379 pg/ml (IR: 236-606), and among non-Blacks from 244 (IR: 173-354) to 283 pg/ml (IR: 192-435). The prevalence of severe uncontrolled SHPT (defined as PTH >600 pg/ml) rose sharply among Blacks (from 16 to 26%) and slightly among non-Blacks (from 9 to 11%). Preliminarily, these changes do not appear to be due to decreased overall use of SHPT treatments (% prescribed IV vitamin D rose slightly in both race groups, cinacalcet to 11%).

Conclusions: Our results indicate a notable increase in PTH levels overall, and in severe uncontrolled SHPT (>600 pg/ml) among Blacks, over the early PPS transition period. Additional evaluation of the causes of this trend and its potential consequences are warranted. Funding: Pharmaceutical Company Support.

FR-PO1268

Differential Effects of Active Vitamin D Compounds on Secondary Hyperparathyroidism and Vascular Calcification
W. Charles O’Neill1, Xiaonan H. Wang,1 Hartmut H. Malluche.2 1Emory University; 2University of Kentucky; 1Amgen, Inc.

Background: Active vitamin D compounds suppress secondary hyperparathyroidism but can also produce vascular calcification. Whether PTH can be suppressed at doses that do not augment vascular calcification is unclear. Since phosphate also affects PTH secretion and vascular calcification, we reasoned that the differential effects on PTH and vascular calcification depend on phosphate intake.

Methods: Renal failure was produced in rats by feeding adenine, and calcitriol or paricalcitol was given 3 times per week at varying doses. After 28 days, aortic calcium was measured by the cresolphthalein method after extraction in HCl. PTH was measured by immunoussay using an antibody against rat PTH 1-34.

Results: Plasma calcium, measured by radioimmunoassay 48 hours after dosing, was 6.3 ± 1.8, 7.5 ± 1.0, 24.6 ± 4.1, and 79.6 ± 6.1 pg/ml at 10, 40, and 100 ng/kg calcitriol, indicating that the higher doses resulted in persistent elevations. With 1.06% dietary phosphorus, suppression of PTH (348 ± 66 vs. 719 ± 85 pg/ml, p<0.001) required 100 ng/kg calcitriol, while 40 ng/kg increased aortic calcium (3695 ± 733 vs. 1077 ± 865 nmol/mg, p<0.005, normal: <25). Similarly, with 0.73 % phosphorus intake, 40 mg/kg calcitriol increased aortic calcium (5091 ± 1055 vs. 367 ± 217 nmol/mg, p<0.005) without suppression of PTH (304 ± 138 vs. 276 ± 55 pg/ml). Paricalcitol increased aortic calcification at 160 ng/kg (2623 ± 562 vs. 1586 ± 388 nmol/mg, p<0.007) while 320 ng/kg was required to suppress PTH (197 ± 77 vs. 636 ± 56 pg/ml, p<0.001). However, with a 0.4% phosphorus diet (normal rat chow), PTH was suppressed at 40 mg/kg calcitriol (29 ± 16 vs. 570 ± 109 pg/ml, p<0.002) without significantly increasing aortic calcification (558 ± 551 vs. 88 ± 20 pg/ml).

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Underline represents presenting author.
406A
47 mmol/l). Aortic calcium content correlated weakly with plasma phosphate (r=0.28, p <0.03) but not with FTH or calcium. A significant increase in plasma calcium occurred only with paricalcitol.

Conclusions: Suppression of secondary hyperparathyroidism by calcitriol without promoting vascular calcification is dependent on phosphate intake. There was no advantage of paricalcitol over calcitriol.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-PO1269

Chronic Exposure to Laminar Shear Stress Induces an Anti-Coagulant, Anti-Inflammatory Phenotype in Glomerular Endothelial Cells and Results in Communication with Podocytes

Sadie Slater, Gavin Iain Welsh, Moin Saleem, Peter W. Mathieson, Simon C. Satchell. Academic Renal Unit, University of Bristol, Bristol, United Kingdom.

Background: The importance of podocyte to glomerular endothelial cell (GEnC) communication in the glomerulus is widely recognised. In systemic circulations chronic laminar shear stress (LSS) plays a crucial role in determining EnC behavior and regulating EnC communication with smooth muscle cells. Here we investigate signaling pathways activated by LSS in GEnC and the effect on co-cultured podocytes.

Methods: Conditionally immortalised human GEnC and podocytes were utilised. GEnC were cultured under static conditions, or on an orbital shaker set to generate 10dynes/cm² LSS for 24, 48, 72, or 96h. Western blotting examined changes in protein expression. A nitrate assay measured nitric oxide (NO) production. Changes in GEnC barrier properties in response to LSS were measured using an ECIS (Electric Cell Impedance Sensing) system. Changes in podocyte protein expression and barrier properties in response to GEnC LSS were investigated using a GEnC-podocyte co-culture model, or by placing conditioned media from static or LSS GEnC on podocytes.

Results: LSS in GEnC increased expression of KL2 (p<0.0001), thrombomodulin (p=0.003), eNOS (p=0.003), and NO (p=0.005). These changes are associated with ERK phosphorylation and were prevented by addition of a MAPK kinase inhibitor, OU126. ECIS demonstrated GEnC monolayer resistance decreased in response to LSS. Podocytes exposed to spermine, a nitric oxide donor, showed decreased monolayer resistance and increased phosphorylation of a key protein in actin regulation, VASP. Similarly, GEnC LSS exposed to spermine, a nitric oxide donor, showed decreased monolayer resistance and increased VASP phosphorylation compared to static media.

Conclusions: We have demonstrated chronic LSS in GEnC results in an anti-inflammatory, anti-coagulant phenotype, mediated via the MEK5 pathway, leading to increased phosphorylation of a key protein in actin regulation, VASP. Similarly, GEnC LSS exposed to spermine, a nitric oxide donor, showed decreased monolayer resistance and increased VASP phosphorylation compared to static media.

FR-PO1270

Interferon Beta Modulates Glomerular Endothelial Cell (GEnC) Barrier Properties through Activation of the Small GTPase Rap1

Georgina Cope, Simon C. Satchell, Candida Tasman, Peter W. Mathieson, Gavin Iain Welsh, Simon C. Satchell. Academic Renal Unit, University of Bristol, Bristol, United Kingdom; Microvascular Research Laboratories, University of Bristol, Bristol, United Kingdom; Dementia Research Group, University of Bristol, Bristol, United Kingdom.

Background: Interferon beta (IFNβ) mediates tightening of GEnC monolayers is partially dependent on IFNβ mediated tightening of GEnC monolayers. However, the pathway through which IFNβ mediates this effect remains unknown. In order to elucidate novel pathways, we investigated the role of the small GTPase Rap1.

Methods: We have previously shown that IFNβ mediates barrier properties in GEnC. In vitro exposure of glomerular endothelial cells (GEnC) to IFNβ at 1000 units/ml of IFNβ reduced their permeability, suggesting that IFNβ may act directly on cells of the glomerular filtration barrier to maintain its permeability. Others have shown that endothelial barrier properties are enhanced by activation of the small GTPase Rap1. We therefore tested the hypothesis that IFNβ acts via Rap1.

Results: IFNβ induced Rap1 activation at a level comparable to 8′me cAMP, an activator of Rap1. Co-incubation with GGTTI-298 significantly reduced the tightening of the GEnC barrier provoked via IFNβ. To determine the isoform of Rap1 responsible we used targeted siRNA to Rap1. This resulted in Rap1 knockdown and attenuated the increase in resistance caused by IFNβ for 6 hours, while GGTTI-298 mediated a sustained inhibition. Activation of Rap1 in GEnC via IFNβ induced its association with actin and RIAM. This interaction was reduced by co-incubation of GGTTI-298 and IFNβ. IFNβ induction of the Rap1α activator of Rap1α. Co-incubation with GGTI-298 significantly reduced the tightening of GEnC monolayers in response to IFNβ.

Conclusions: We have demonstrated that IFNβ mediates barrier properties through activation of the small GTPase Rap1. Activation of Rap1 in GEnC reduces the permeability of GEnC monolayers by reducing the association of Rap1 with actin and RIAM. This interaction is reduced by co-incubation of GGTTI-298 and IFNβ.

Funding: NIDDK Support

FR-PO1277

Endocytic Proteins in Podocytes Affect the Stability of Foot Processes in Kidney Glomeruli

Keita Soda, Xuefei Tian, Renia Zheng, Shuta Ishibe. Internal Medicine, Section of Nephrology, Yale University, School of Medicine, New Haven, CT.

Background: Membrane trafficking linking actin regulation plays a pivotal role in maintaining cell homeostasis. However, membrane dynamics in podocytes remain unclear. We show that endocytic proteins, Dynamin (Dyn1 and Dyn2) and Syndapin 1 (Sy1) affect the stability of glomerular filtration barrier in the kidney.

Methods: Podocyte specific double knockouts of Dyn1 and Dyn2 were generated using the Cre-LoxP system (Dyn dKO). Kidney sections were analyzed with H and E, PAS, trichrome staining and electron microscopy (EM). Albuminuria and urine creatinine were measured by ELISA. Endocytosis assays were performed on primary wild type (WT) and mutant podocytes.

Results: Dyn dKO mice were born at the expected Mendelian frequency. However, these mice failed to gain weight by 4 weeks (1.3±0.1 vs. 1.4±0.1, 10.5±0.4 vs. 8.5±0.3, 21.8±1.8 vs. 13.3±0.8 (wild vs. Dyn dKO; 4 and 8 weeksi)); developed severe albuminuria (16.3±0.9 vs. 716.7±37.0, 15.4±0.8 vs. 21.8±1.8 vs. 13.3±0.8 (wild vs. Dyn dKO;p1, 4 and 8weeks)) and progressive renal failure (0.35±0.06 vs. 0.78±0.09, 0.34±0.06 vs. 1.3±0.04, 0.32±0.05 vs. 1.47±0.06 (wild vs. Dyn dKO;4 and 8 weeks) (µg/mg)); and creatinine measurements (0.32±0.05 vs. 1.47±0.06 (wild vs. Dyn dKO;4 and 8 weeks)) (µg/mg)).

Conclusions: These findings provide insight into Dynamins and Syndapin 1 as fundamental role regulating endocytosis and actin dynamics within the glomerular filtration barrier.

FR-PO1273

Calprotectin, an Endogenous Toll-Like Receptor 4 Ligand (TLR4), Is Critical for Induction of Glomerulonephritis


Background: Calprotectin is an endogenous TLR4 agonist, expressed in neutrophils, monocytes and infiltrating macrophages. We investigate disease induction using the murine nephrotoxic nephritis (NTN) model in calprotectin deficient mice, as well as potential amplification of human monocytic calprotectin expression by MPO-ANCA and PR3-ANCA, investigation of serum levels and cell surface expression in patients.

Methods: Accelerated NTN experiments performed on wild-type (WT) and calprotectin deficient mice (Cal–/–), with day 7 sacrifice. Renal histology was scored for thrombosis and interstitial fibrosis, while EM demonstrated severe foot process effacement. To further explore the role of the endocytic pathway, Dyn binding partner S1 was examined. S1 null mice had severe albuminuria at birth (25±5 vs 890±67 (wild vs. S1 KO)(μg/mg)) and robust process effacement. Compared to wild type podocytes, Dyn dKO or S1 null podocytes had robust Amp2 activation at clathrin coated pits as well as endocytic defects characterized by significantly decreased Nephrin uptake compared to wild type (Dyn dKO; <4%, S1 KO; <70%, p<0.05 respectively).

Conclusions: These findings provide insight into Dynamins and Syndapin 1 as fundamental role regulating endocytosis and actin dynamics within the glomerular filtration barrier.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Indomethacin Blocks the Fluid Flow Shear Stress (FFSS)-Induced Increase in Glomerular Albumin Permeability (P<sub>alb</sub>)

In Vitro

Tarak Srivastava,1 Ellen T. McCarthy,2 Ram Sharma,1 Mukut Sharma,1 Neel Hospody,3 Nephrology, CMH, UMBC, Kansas City, MO; 2Nephrology, KUMC, Kansas City, KS; 3Nephrology, KU Med Ctr; Kansas City, MO.

Background: FFSS, the force tangential to the podocyte surface that results from flow of ultrafiltrate, increases with SNGFR. Increased SNGFR occurs in congenital or acquired sodium reabsorption defects and in early diabetic nephropathy, conditions that result in microalbuminuria and CKD. We have shown that FFSS alters the actin cytoskeleton in podocyte monolayers and increases COX-2 expression and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels. We have also shown that PGE<sub>2</sub> increases albumin permeability (P<sub>alb</sub>) in isolated glomeruli and that increased P<sub>alb</sub> precedes proteinuria in models of diabetes and hypertension. We hypothesized that increased FFSS alters filtration barrier through COX2 metabolites.

Methods: Glomeruli from Sprague-Dawley rats (200-225 g) were subjected to 0.3 dynes/cm² FFSS for 30, 60 or 120 mins followed by a recovery for 120 mins at 37°C. Indomethacin (5μM) was included in the medium in some experiments. In glomerular P<sub>alb</sub> were determined using an in vitro assay. Untreated baseline and time matched glomeruli were used as controls. Murine podocytes were subjected to FFSS for 120 minutes and analyzed for changes in gene expression using Affymetrix GeneChipMouse Exon 1.0ST Array.

Results: Results show that FFSS increased Palb at 30, 60 and 120 minutes. The effect of FFSS on Palb persisted after 120 minutes of recovery. Partial results presented in the Table below show that indomethacin significantly blocked the effect of FFSS on Palb.

<table>
<thead>
<tr>
<th>Group</th>
<th>P&lt;sub&gt;alb&lt;/sub&gt; 0.16±0.11</th>
<th>P&lt;sub&gt;alb&lt;/sub&gt; 0.16±0.00</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.53±0.04</td>
<td>0.75±0.06</td>
</tr>
<tr>
<td>FFSS (120 min)</td>
<td>0.62±0.06</td>
<td>0.62±0.06</td>
</tr>
<tr>
<td>Indomethacin+FFSS-Recovery</td>
<td>0.16±0.11</td>
<td>0.00±0.00</td>
</tr>
</tbody>
</table>

n=20 glomeruli/group

Initial analysis of the gene array results has shown a 3-fold upregulation of COX-2 expression in FFSS-treated compared to control podocytes (data not shown here).

Conclusions: These results suggest that fluid-induced increase in FFSS increases P<sub>alb</sub> through PGE, an arachidonic acid metabolite of the COX pathway. We postulate that changes in the glomerular filtration barrier secondary to FFSS may explain the microalbuminuria in CKD.

Funding: NIDDK Support, Private Foundation Support

The Podocyte Slit-Diaphragm Molecule MAGI-1 Is Required for Nephrin Localization and Function

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Background: In podocytes, MAGUK with inverted domain structure-1 (MAGI-1) is specifically expressed at the slit-diaphragm and functions as a linker protein that directly interacts with several other critical proteins including nephrin, α-actinin-4, and synaptopodin. Previously, the function of MAGI-1 and its roles in podocyte dynamics in proteinuric diseases were entirely unknown.

Methods: To study the mechanisms of MAGI-1 function in greater detail, we generated stable MAGI-1 deficient podocytes using targeted shRNA lentiviral infections of an established human cell line.

Results: MAGI-1 deficient podocytes have significant abnormalities in cellular morphology characterized by a simplified and overall smaller cell size, less intricate cytoskeletal architecture, and less complex projections and lamellipodium. Podocytes lacking MAGI-1 also divide at a dramatically lower rate and adhere abnormally to many components of the glomerular basement membrane including collagen type IV and laminin.

To begin to investigate MAGI-1 function, we examined the impact of MAGI-1 deficiency on the localization of its known interacting partners. In MAGI-1 deficient podocytes transfected with a FLAG-nephrin expression construct, membrane expression of nephrin was almost completely lost with significant accumulation in the cytoplasm. These results were confirmed when analyzing endogenous nephrin in fully differentiated podocytes. Ongoing work is focused on dissecting involved mechanisms.

Conclusions: Our data suggests that MAGI-1 deficiency has a significant impact on podocyte architecture in culture. These effects may be mediated by the loss of nephrin surface expression in the setting of MAGI-1 deficiency. We speculate that loss of MAGI-1 results in destabilization of the protein complex associated with nephrin’s cytoplasmic domain resulting in increased nephrin endocytosis.

Funding: NIDDK Support

Nephrin Regulates Lamellipodia Formation by Assembling a Protein Complex That Includes Sh1p2, Filamin and Lamellipodin

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Substrate Elasticity and Geometry Affect Podocyte Cell Mechanics

Eric Schordan,1 Sandra Schordan,1 Rudolf Merkel,2 Bernd Hoffmann,2 Nicole Endlich,2 Karlhans Endlich.1 Anatomy and Cell Biology, University Medicine Greifswald, Greifswald, Germany; 1Institute of Complex Systems (IC5-7), Forschungszentrum Juelich GmbH, Juelich, Germany.

Background: Podocyte foot processes cover the outer aspect of the capillaries in the renal glomerulus. They are the important determinants of the glomerular filtration barrier. In addition, podocytes stabilize the capillary wall with a contractile actin cytoskeleton counteracting the high hydrostatic capillary pressure. Chronic kidney disease is predominantly caused by damage and failure of podocytes, e.g. by increased capillary pressure.

Methods: In the present study we investigate the cytoskeleton and cell mechanics of podocytes in response to different elasticities and geometries of the substrate.

Results: We find that soft substrates (8 to 56 kPa mimicking basement membrane elasticity) as compared to stiff substrates (490 kPa or rigid plastic) modify cell morphology by inducing cell orientation, actin cytoskeleton reorganization and formation of longer protrusions. Additionally, submerged displacement markers, increase with increasing substrate stiffness. Recently, we demonstrated that stiff substrates (8 to 56 kPa) prevent cell migration demonstrating that the presence of a substrate stiff enough to evoke cell spreading is required for migration. To test this hypothesis we cultured podocytes on substrates with varying stiffness but no effective substrate stiffness sufficient to prevent cell spreading was found. These results indicate that the stiffness of the substratum is determined by a balance of cell biomechanics and substrate stiffness. Further, we find that cell mechanics are an adaptive response to substrate stiffness and that substrate stiffness influences cell mechanics.

Conclusions: Our studies demonstrate that elasticity and geometry affect podocyte cell mechanics. Moreover, there is a coupling between substrate elasticity, traction force and stress fiber formation.

Funding: Government Support - Non-U.S.
ubiquitination of the same site thus lead to stabilization. The aim of our studies was to analyze the role of Sumoylation in regulating localization and stability of nephrin.

Methods: First we analyzed sumoylation of nephrin by in vitro Sumoylation. For mapping the potential sites that can be modified by Sumoylation we used Sumos 2,0. We found one predicted Sumoylation sequence at the cytoplasmic tail of nephrin that is conserved in mouse and human. To explore if this predicted site is modified by Sumoylation we created nephrin-mutants in which lysine was converted to arginine. To show that Sumoylation may regulate stability of nephrin we examined the steady-state level by treatment with cycloheximide. Furthermore we performed endocytosis assays, subcellular fractionation, and confocal microscopy.

Results: We could show that nephrin is sumoylated by Sumo-1, -2 and -3. By immunoprecipitation and in vitro Sumoylation we could verify that the lysine-mutant cannot be sumoylated. Cycloheximide-experiments showed that the lysine-mutant has a lower steady state level compared to WT-nephrin. Furthermore endocytosis of the lysine-mutant is enhanced and subcellular fractionation and confocal microscopy showed that the lysine mutant accumulated at the pericellular region and cytosol.

Conclusions: These data suggest that Sumoylation determines localization and enhances stability of nephrin. Next to glycosylation and phosphorylation, Sumoylation is a posttranslational modification that may play a role in the tight regulation of nephrin trafficking and turnover at the slit diaphragm.

Funding: Government Support - Non-U.S.

FR-PO1279
Role of Guanine Nucleotide Exchange Factor-H1 in Complement-Mediated RhoA Activation in Glomerular Epithelial Cells Flaviana Mouawad, Lamine Aoudjit, Ruihua Jiang, Tomoko Takano. Medicine, McGill University, Montreal, QC, Canada.

Background: In the rat model of membranous nephropathy (passive Heymann nephritis), complement C5-9 causes visceral glomerular epithelial cell (GEC) injury. We reported previously that complement activates the small GTPase, RhoA, in GEC in vitro and in vivo. The present study addresses the role of guanine nucleotide exchange factor (GEF)-H1, an activator of RhoA, in complement-mediated RhoA activation in GEC.

Methods: Rat GEC and immortalized mouse podocytes were used. Complement stimulation was done by serially exposing cells to antibody and normal human serum (NS) or de-complemented serum (control). Affinity precipitation by RhoG17A, which binds to active GEF, followed by immunoblotting was used to quantify GEF-H1 activity. RhoA activity in live cells was visualized by fluorescence resonance energy transfer in live cells. Nuclear extracts from glomeruli were used to study NFAT activation in vivo by electromobility shift assay (EMSA).

Results: Constitutively active (CA)-RhoA activated the fibronectin promoter, which was inhibited by BAPTA (Ca2+ chelator), W7 (calmodulin inhibitor), FK506 (calcineurin inhibitor), and VIVIT (NFAT inhibitor). Angiotensin II, which is known to activate RhoA in podocytes, also transactivated the fibronectin promoter, which was inhibited by the same set of inhibitors. CA-RhoA activated the NFAT-responsive promoter and CA-NFAT in turn activated the fibronectin promoter, which was further augmented by an activator of protein kinase C, phospholipid (nisistatin) as well as VIVIT (NFAT inhibitor).

Funding: Government Support - Non-U.S.

FR-PO1280
The Novel Podocyte Protein 4.1O Interacts with Slit Diaphragm Proteins Nephrin, and Ameliorates Nephrin Endocytosis Eve Koenen-Heinze,1 Sunja Obst,1 Marcus G. Porezzos2, Magdalena Wulczyn2, Ivo Quack,2 Sebastian Alexander Potthoff,1 Andrzej S. Krolewski,2 Lars C. Rump,1 Lorenz Sellin.1 1Nephrology, Heinrich Heine University, Duesseldorf, Germany; 2Section on Genetics and Epidemiology, Joslin Diabetes Center, Aurora, CO; 3National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD.

Background: Microalbuminuria is an early marker for diabetic nephropathy. A GWAS for diabetic nephropathy revealed FRMD3 as a candidate gene in type 1 diabetics. FRMD3 encodes for the protein 4.1O. 4.1 proteins serve as adaptors between plasma membrane proteins and the actin cytoskeleton. The 4.1O orthologue in zebrafish is required for prevention of proteinuria. Nephrin is endocytosed upon binding to the adaptor protein β-arestin2. The expression and molecular function of 4.1O in human podocytes is unknown so far.

Methods: Human podocytes were differentiated over 14 days. RNA and protein were isolated and followed by RT-PCR or western blot respectively for 4.1 family members. Cells expressed 4.1O and nephrin or GLEPP1 or truncations of the indicated proteins. After cell lysis co-immunoprecipitation was performed. Using monoclonal and polyclonal antisera immunofluorescence for 4.1O was done.

Results: FRMD3 (4.1O) has been shown to be a candidate gene for diabetic nephropathy in type 1 diabetics. In zebrafish, its deletion leads to proteinuria. 4.1O is a novel human podocyte protein that interacts with nephrin and GLEPP1. Binding of 4.1O to nephrin prevents β-arestin2 binding to nephrin. It is therefore conceivable that 4.1O is a relevant adaptor to slit diaphragm proteins and the actin cytoskeleton. Furthermore it is postulated that 4.1O plays an important role in the protection for progression of proteinuric kidney disease by inhibition of nephrin endocytosis.

Funding: NIDDK Support

FR-PO1281
RhoA Regulates the Expression of Fibronectin Via Nuclear Factor of Activated-T Cells in Podocytes Lei Zhu, Ruihua Jiang, Lamine Aoudjit, Tomoko Takano. Medicine, McGill University, Montreal, QC, Canada.

Background: 4.1O is a small GTPase which regulates the actin cytoskeleton. We reported previously that high level expression of active RhoA in podocytes in mice induces glomerulosclerosis, accompanied by fibronectin upregulation. The aim of this study was to investigate the mechanism by which RhoA upregulates fibronectin expression in podocytes.

Methods: Differentiated mouse podocytes or cultured rat podocytes were used for studies with cultured cells. Double luciferase assay was used to study the activation of the fibronectin promoter and the NFAT responsive promoter. CA-RhoA activity was monitored by fluorescence resonance energy transfer in live cells. Nuclear extracts from glomeruli were used to study NFAT activation in vivo by electromobility shift assay (EMSA).

Results: Constitutively active (CA)-RhoA activated the fibronectin promoter, which was inhibited by BAPTA (Ca2+ chelator), W7 (calmodulin inhibitor), FK506 (calcineurin inhibitor), and VIVIT (NFAT inhibitor). Angiotensin II, which is known to activate RhoA in podocytes, also transactivated the fibronectin promoter, which was inhibited by the same set of inhibitors. CA-RhoA activated the NFAT-responsive promoter and CA-NFAT in turn activated the fibronectin promoter, which was further augmented by an activator of protein kinase C, phospholipid (nisistatin) as well as VIVIT (NFAT inhibitor).

Funding: Government Support - Non-U.S.

FR-PO1282
Albumin Exposure Induces an Inflammatory Response and Reduces VEGF Expression in Cultured Podocytes Kavo Okamura,1 Patrick Daniel Dummer,2 Jeffrey B. Kopp,2 Judith Blaine.1 Medicine, University of Colorado Denver, Aurora, CO; 2National Institute of Diabetes and Digestive and Kidney Disease, Bethesda, MD.

Background: Albuminuria exposure induces an injury response in cultured proximal tubular cells, but the effects of albumin on cultured podocytes have not been as well investigated. In vivo, podocytes express multi-ligand receptors that facilitate albumin uptake, including low-density lipoprotein-related protein 2 or megalin and neonatal Fc receptor.

Methods: We studied human urine-derived podocyte-like cells, transformed with human telomerase reverse transcriptase and temperature-sensitive SV40 T antigen (Sakairi, Am J Physiol 2010). Podocytes were cultured under non-permissive conditions (37°C) at which they express differentiation markers including WT1, synaptopodin, and nestin. Podocytes were exposed to medium supplemented with 10% heat-inactivated fetal bovine serum (fetal albumin) or 2 mg/ml, supplemented with either 5% murine serum-free recombinant human serum albumin or dextran of a similar molecular mass as an oncotic control. Cytokine mRNA levels were assessed by quantitative RT-PCR over an 8 hour time course and cytokine medium levels were assessed over a 48 hour time course. Results are expressed as fold-change compared to oncotic control.

Results: Albumin exposure increased expression of interleukin-lbeta (IL-1b) expression (peak mRNA increase + fold at 3 hours ), increased tumor necrosis factor alpha (TNFa) expression (peak mRNA increase + fold at 3 hours, peak protein increase + fold at 6 hours), increased interleukin-10 expression + fold at 6 hours), increased interleukin-10 expression + fold at 48 hours). In addition, vascular endothelial growth factor (VEGF) mRNA expression was reduced by + fold at 6 hours compared to dextran treated controls.

Conclusions: These results suggest that exposure of cultured podocytes to albumin concentrations similar to those present in nephrotic urine induce an inflammatory factor cascade, with increased expression of IL-1b and TNFα followed by IL-6, and reduce VEGF mRNA expression. Together these may contribute to dysfunction of podocytes and glomerular endothelial cells in nephrotic syndrome.

Funding: NIDDK Support

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409A
FR-PO1283

Podocyte Failure Caused by Hypertrophic Stress in Rats Expressing a Podocyte-Specific Dominant Negative 4E-BP1 Transgene of the mTOR Pathway. Acceleration of Proteinuria, FSGS and Progression to ESKD by Conditions Causing Glomerular Growth, and Prevention by Calorie Restriction

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Background: Progressive podocyte depletion drives progressive glomerulosclerosis. Podocyte depletion could result from reduction in podocyte number, size and/or function.

Methods: To test the hypothesis that reduced capacity of podocytes to respond to hypertrophic stress would lead to glomerulosclerosis we developed Fischer 344 rats expressing a human AA-4E-BP1 dominant negative transgene driven by the podocyte-specific podocin promoter. 4E-BP1 (in the mTOR pathway) controls CAP-dependent translation and plays a role in determining cell size.

Results: Tg rat podocytes uniformly expressed the transgene, were born with the expected ratios without increased proteinuria and normal kidney histology. At 100g homozygous (but not heterozygous) rats had reduced podocyte and glomerular tuft volume. Both hetero and homozygous tg rats developed proteinuria, FSGS and reached ESKD by 12 months. Accelerated glomerular hypertrophy was induced by uninephrectomy (NX). Wi rats with NX develop minor proteinuria and few FSGS lesions by 14 weeks. In contrast NX of heterozygous tg rats caused proteinuria after 3 weeks, FSGS by 8 weeks and ESKD by 14 weeks. NX of homozygous tg rats caused rapid development of high level proteinuria, glomerulosclerosis and ESKD by 9 weeks. Proteinuria occurred in direct proportion to body weight gain. Morphometry showed development of a mismatch between glomerular size and total podocyte volume in hetero and homozygous rats. Both proteinuria and FSGS were completely prevented by calorie restriction.

Conclusions: Reduced capacity of podocytes to respond to hypertrophic stress in response to growth signaling through the mTOR pathway can trigger proteinuria, FSGS and progression to ESKD. Calorie restriction could be a useful adjunctive therapy to slow and/or prevent progression to ESKD.

Funding: NIDDK Support

FR-PO1284

Motor Protein Myo1c Is a Critical Component of the Glomerular Filtration System

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Background: Podocyte cells along with their specialized junctions “slit diaphragm” form the key components of glomerular filtration assembly. These structures are critical for the glomerular filtration function including the selective passage of low molecular weight waste products and the retention of blood plasma proteins. Our recent study identifies Myo1c as a novel Nephrin and Neph1 interacting motor protein that transports these proteins from cytoplasm to podocyte cell membrane.

Methods: This study investigates the role of Myo1c in maintaining the glomerular filtration function using zebrafish as a model system.

Results: Knockdown of Myo1c gene in zebrafish using antisense morpholino resulted in abnormal developmental phenotype.

Conclusions: In conclusion, Myo1c is a component of Nephrin and Neph1 signaling pathway and plays a role in maintaining glomerular function.

Funding: NIDDK Support, Other NIH Support - KO1

FR-PO1285

Septin-7, a Novel Interaction Partner of CD2AP, Negatively Regulates Glucose Uptake

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Background: Podocytes are insulin sensitive and take up glucose in response to insulin using glucose transporters GLUT4 and GLUT1. Glucose uptake depends on nephrin, which interacts with VAMP2 residing on GLUT4 storage vesicles (GSVs) and facilitates the fusion of the vesicles with the plasma membrane. CD2AP, an adaptor protein essential for the glomerular filtration barrier, interacts with nephrin and a number of proteins involved in various signaling and vesicular trafficking pathways.

Methods: Here we describe the identification of the small GTPase septin-7 as a novel interaction partner of CD2AP by pull-down assay and mass spectrometry and show by 2-deoxy-D-glucose uptake assay that septin-7 regulates glucose uptake.

Results: Septin-7 partially co-localizes with CD2AP in rat glomeruli and pull-down assay on rat glomerular lysate indicates that their interaction is mediated by the 3rd SH3 domain of CD2AP and the C-terminus of septin-7. Further, co-immunoprecipitation assays show that both CD2AP and nephrin interact with septin-7. Septins are filament-forming proteins and also septin-7 is expressed in cultured human podocytes in a filamentous pattern that depends on the intact actin cytoskeleton. In addition to regulating cytokinesis, mammalian septins function in exocytosis. We found that septin-7 interacts with regulators of vesicular trafficking both on GSVs and plasma membrane suggesting that septin-7 could regulate the exocytic transport of GSVs. In line with this, knockdown of septin-7 in HIRe cells, rat fibroblasts that stably express human insulin receptor, increased the glucose uptake activity of the cells. Further, the interaction of VAMP2 and ectopically expressed nephrin increased in septin-7 depleted HIRe cells.

In addition, the knockdown also induced pericardial edema and whole body edema at 72hpf (arrows) which suggests impairment of pronephric osmoregulatory function. Histological analysis of mutant fish revealed dilated nephric tubules consistent with loss of slit diaphragm proteins Nephrin and Neph1 in zebrafish. Further investigation to determine changes in the podocyte foot processes using electron microscopy and loss of filtration barrier using transgenic fish is under progress.

Conclusions: Reduced capacity of podocytes to respond to hypertrophic stress in response to growth signaling through the mTOR pathway can trigger proteinuria, FSGS and progression to ESKD. Calorie restriction could be a useful adjunctive therapy to slow and/or prevent progression to ESKD.

Funding: NIDDK Support
Conclusions: The data indicate that septin-7 may form a filamentous barrier and thus hinder vesicle trafficking, and consequently, depletion of septin-7 facilitates the fusion of the vesicles with the plasma membrane. Involvement of septin suggests that septin-7 may participate in the regulation of glucose transport also in podocytes.

Funding: Private Foundation Support

FR-PO1286

Ang II Induces Nephrin Dephosphorylation and Podocyte Injury through Caveolin 1-Dependent Mechanism Zhihong Ren,1 Wei Liang,1 Cheng Chen,2 Pravin C. Singhal,2 Guohua Ding,1,2 Division of Nephrology, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; 3Medicine, North Shore-Long Island Jewish Health System, Manhasset, NY.

Background: It has been suggested that nephrin contributes to the mechanism of Ang II-induced podocyte injury. Caveolin-1 has been demonstrated to play a crucial role in signal transduction. In the present study, we investigated the effect of Ang II on nephrin phosphorylation in podocytes and whether caveolin-1 was involved in this process.

Methods: Cultured podocytes were exposed to Ang II (10–6 mol/L) and pretreated with or without losartan (10–6 mol/L) for variable time periods. Nephrin and caveolin-1 expression and their phosphorylation were evaluated by Western-blotting and immunofluorescence. Caveolar membrane fractions were isolated by sucrose density gradient centrifugation, then the distribution and interactions between ATP, nephrin, Csk and caveolin-1 were analyzed using Western-blotting and co-immunoprecipitation. Podocyte apoptosis was evaluated by cell nucleus staining with Hoechst-33342.

Results: Caveolin-1 was expressed in podocytes and had a low level of phosphorylation in control condition. After Ang II stimulation, caveolin-1 phosphorylation was increased, but its total protein expression had no significant change. Nephrin and caveolin-1 were found to be co-localized in caveolar fractions. AT1 and Csk moved in caveolar fractions and had an interaction with caveolin-1 after the stimulation of AngII for 3 hour. Transfection of caveolin-1 plasmid(pEGFP-C3-cav-1) significantly increased AngII-induced nephrin dephosphorylation and podocyte apoptosis. Furthermore, knockdown caveolin-1 protein using siRNA inhibited nephrin dephosphorylation and ameliorated Ang II-induced podocyte apoptosis.

Conclusions: In conclusion, these data indicate that AngII induces nephrin dephosphorylation and podocyte injury through a caveolin-1-dependent mechanism.

Funding: Government Support - Non-U.S.

FR-PO1287

Role of Scribble for Podocyte Function and Cell Polarity Eugen Widmeyer, Bjorn Harlében,1 Nicola Wanner,1 Sung Tae Kim,2 Jeffrey H. Minter,2 Dontcho Kochev,1 Gerold Huber,1 Kerstin Kerjaschki,3 Gerd Walz,1 Tobias B. Huber,1

Background: The kidney filter represents a unique assembly of podocyte epithelial cells that tightly envelop the glomerular capillaries with their foot processes and the interposed slit diaphragm. So far, very little is known about the guidance cues and polarity signals that tightly enwrap the glomerular capillaries with their foot processes and the interposed slit diaphragm. So far, very little is known about the guidance cues and polarity signals that tightly enwrap the glomerular capillaries with their foot processes and the interposed slit diaphragm.

Results: We report that Scribble translocates from the lateral side of polygonal shaped immature podocytes to the basal cell membrane during podocyte differentiation. Immunogold electron microscopy reveals membrane associated localization of Scribble predominantly basal of the slit diaphragm and partially at the slit diaphragm.

Funding: Government Support - Non-U.S.

FR-PO1288

Functional and Spatial Analysis of C. elegans SYG-1 and SYG-2, Orthologs of the Nephrin/Neph Cell Adhesion Module Directing Selective Synaptogenesis Elke Neumann-Haefelin,1 Nicola Wanner,1,2 Gerd Walz,1,2 Tobias B. Huber,1,2,3 Renal Division, University Hospital, Freiburg, Germany; 2Spemann Graduate School of Biology and Medicine, Albert-Ludwigs-Universität, Freiburg, Germany; 3Centre for Biological Signalling Studies (bioss), Albert-Ludwigs-Universität, Freiburg, Germany.

Background: The assembly of specific synaptic connections represents a prime example of cellular recognition. Members of the immunoglobulin superfamily are ancient protein families with diverse functions, which are involved in the organism's signal transduction system. The correct connectivity patterns of the IgSF proteins nephrin and Neph 1 are crucial for the assembly of functional neuronal circuits and the formation of the kidney slit diaphragm, specifying the filtration barrier.

Methods: Here, we utilize the nematode C. elegans model for studying the requirements of synaptic specificity mediated by nephrin-Neph proteins.

Results: In C. elegans, the nephrin/Neph 1 orthologs SYG-2 and SYG-1 form intracellular contacts strictly in trans between epithelial guidepost cells and neurons specifying the localization of synapses. We demonstrate a functional conservation between mammalian nephrin and SYG-2. Expression of nephrin effectively compensated loss of syg-2 function in C. elegans and restored defective synaptic connectivity further establishing the Cerberry system as a valuable model for slit diaphragm function. Next, we investigated the effect of SYG-1 and SYG-2 trans homodimerization respectively. Strikingly, synaptic assembly could be induced by homophilic SYG-1 but not SYG-2 binding indicating a critical role of SYG-1 intracellular signalling for morphogenetic events and pointing towards the dynamic and stochastic nature of extra- and intracellular nephrin-Neph interactions to generate reproducible patterns of synaptic connectivity.

Conclusions: In summary, our findings corroborate that C. elegans is a useful tool for investigating fundamental nephrin/Neph protein functions. Furthermore, we present novel insights into the mechanisms of SYG-1 and SYG-2 homotypic adhesion properties and intracellular functions.

Funding: Government Support - Non-U.S.

FR-PO1289

Effect of Amyloidogenic Immunoglobulin Light Chains on the Contractile Cytoskeleton of Human Podocytes In Vitro Laura Econimo,1 Julie A. Tomolonis,1 Laura M. Denber,2 David C. Seldin,2 Lawreen H. Connors,3 Joel M. Henderson,1,3 Pathology and Laboratory Medicine, Boston University Medical Center, Boston, MA; 4Amyloid Treatment & Research Center, Boston University Medical Center, Boston, MA.

Background: Podocyte injury and proteinuria are hallmarks of renal involvement in light-chain (AL) amyloidosis, but the mechanisms underlying these effects are not well established. Recent work suggests that amyloidogenic light chains (LC) may have direct toxic effects on cells. Given the importance of the cytoskeleton to podocyte barrier function, we hypothesized that amyloidogenic LC may elicit a toxic effect on podocytes, characterized by derangement of cytoskeletal structure and function. In this study, we investigated the effect of amyloidogenic LC on human podocyte cytoskeletal structure and contractile function in vitro.

Methods: Human LC were isolated from urine of patients with specific LC type and soluble protein endocytosis, and traction force microscopy was used to measure cell traction.

Results: All of the proteins to which podocytes were exposed (LC or TTR) were confirmed to be endocytosed by the cells. Cells exposed to amyloidogenic LC appeared small and exhibited cytoskeletal disorganization, as compared to cells receiving other treatments. Cells exposed to amyloidogenic LC showed a significant decrease (up to 62%) in traction force magnitude compared to vehicle control; cells exposed to other LC or TTR exhibited less marked decreases (20 to 31%) in traction force relative to control.

Conclusions: The results suggest that amyloidogenic LC have a direct toxic effect on podocytes, manifest as contractile cytoskeleton dysfunction.

Funding: NIDDK Support

FR-PO1290

Complement Modulates the Ubiquitin-Proteasome System and Endoplasmic Reticulum-Associated Degradation in Glomerular Epithelial Cells Thomas M. Kitzler,1 Joan Papillon, Julie Guillemette, Simon S. Wing, Andrey V. Cybulsky,2 Department of Medicine, McGill University, Montreal, QC, Canada.

Background: In experimental membranous nephropathy, complement C5b-9 induces sublethal glomerular epithelial cell (GEC) injury and proteinuria. C5b-9 also activates mechanisms that restrict injury or facilitate recovery. The actions of C5b-9 are mediated by pathways, including mitogen-activated protein kinases and protein kinase C (PKC). Recently, we showed that in GECs, C5b-9 augmented the function of the ubiquitin-proteasome system (UPS); moreover, protein catabolism-mediated cytotoxicity. This study addresses mechanisms by which complement modulates the UPS.

Methods: We monitored UPS function by transfection of a UPS reporter, GFP (CL1 degreen fused with green fluorescent protein). By analogy, CD3δ-yellow fluorescent protein (YFP) was employed as a reporter of endoplasmic reticulum-associated degradation (ERAD). Ubiquitin mRNA was quantified by RTq-PCR.

Results: In GECs, sublytic C5b-9 decreased GFPu due to proteosomal degradation. This reduction in GFPu was attenuated by the c-jun N-terminal kinase (JNK)-directed inhibitor, SP600125, or by expression of negative JNK antisense, whereas inhibition of the extracellular signal-regulated kinase and p38 pathways were ineffective. Complement-induced GFPu degradation was also blocked by the PKC-directed inhibitor, GF109203X, or by depletion of PKC (prolonged pretreatment with phenol myristate acetate). Complement increased the level of CD3δ-YFP in GECs. The overall ubiquitination of proteins was increased the level of CD3δ-YFP in GECs. The overall ubiquitination of proteins was enhanced in complement-treated GECs and in glomeruli of rats with experimental membranous nephropathy, although ubiquitin mRNA was unchanged in GECs.

Conclusions: In GECs, complement increased ubiquitination of proteins, consistent with a state of cellular protein misfolding, and augmented overall UPS function, which was at least in part dependent on JNK and PKC. In parallel, ERAD was impaired, perhaps due to the induction of protein degradation by activated UPS.

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due to an overabundance of misfolded proteins in the ER, and/or reduced capacity for refolding of the protein. This perspective and investigation of UPS function may be a new strategy to limit complement-mediated GEC injury.

**FR-POI291**

**Transforming Growth Factor (TGF)-β1 Induced DNA Methylation of Wilms’ Tumor Suppressor Gene (WT1) Promoter in Human Podocytes**

**Hirokatsu Hirota, Keiji Hirohata, Toru Sukaiki, Satoshi Takahashi, Hidekazu Ikeuchi, Akito Maeshima, Yoshihisa Nojima. Department of Medicine and Clinical Science, Gunma University Graduate School of Medicine, Maebashi, Gunma, Japan.**

**Background:** WT1, firstly identified as a tumor suppressor gene, is essential for normal podocyte function. Previous reports have shown that WT1 promoter is often methylated in cancers, leading to transcriptional silencing. Recently, it was reported that TGF-β1 downregulates WT1 expression in podocytes. We investigated the possibility that the reduction of WT1 expression is caused by promoter methylation.

**Methods:** We used conditionally immortalised human podocytes in vitro and murine podocytes. The reduction of WT1 expression in podocytes was dependent on CXCR2 and ENA-78. The ability of ANCA to enhance neutrophil recruitment following podocyte injury by PAN. In vivo-VEGF-A was studied both pre and post the development of albuminuria (which occurs at 5 weeks in the podIRKO model). At 4 weeks the production of VEGF-A was reduced by 40% by measuring quantitative PCR of a single cell podocyte suspension and this continued and was reflected by decreased in-situ VEGF-A hybridisation signal after albuminuria has developed in podIRKO animals, when measured at 8 weeks.

**Conclusions:** WT1 is inducible in the podocyte. Our results suggest that when podocyte insulin resistance is present then VEGF-A production is reduced. This occurs prior to the development of albuminuria. The reduced insulin stimulated VEGF-A expression could explain the development of podocyte induced apoptosis observed in our transgenic models and also that commonly observed in human diabetic nephropathy.

**Funding:** Government Support - Non-U.S.

**FR-POI295**

**Podocyte Membrane Traffics HIV Accumulation Via DC-SIGN**

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**Background:** HIV infection of podocytes has been considered to play a critical role in development of HIV-associated nephropathy. Podocytes display a dysregulated growth and proliferative phenotype. We recently reported that DC-SIGN facilitates HIV-1 entry and thus, showed the role of actin and microtubules in the podocyte viral trafficking. Since Brefeldin A also enhanced podocyte HIV-1 accumulation, it seemed that

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Golgi-ER pathway might be linked to podocyte viral sorting. Interestingly, CHPs treated with fluoxetine, cytochalasin B, and Brefeldin A showed a two-fold increase in DC-SIGN expression. Thus, it appears that in addition to the blockade of the membrane traffic and enhanced expression of DC-SIGN has contributed to HIV entry into CHPs.

**Conclusions:** These findings indicated that alteration of membrane traffic promoted podocyte HIV-1 accumulation by enhancing podocyte expression of DC-SIGN.

**Funding:** NIDDK Support

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**FR-PO1296**

aPKC lambda/ iota and zeta Are Required for Podocyte Process Development

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**Background:** The kidney filter represents a unique assembly of podocyte epithelial cells that tightly enwrap the glomerular capillaries with their foot processes and the interconnected slit diaphragm. Previously, we identified the Par3, Par6, aPKC polarity complex as novel interactors of Nephrin and Nef at the slit diaphragm. Podocytes express both isoforms of aPKC, lambda/ iota and zeta. Genetic deletion of aPKC lambda/ iota in podocytes results in severe proteinuria, nephrotic syndrome, progressive glomerulosclerosis and death at 3-4 weeks after birth. Interestingly, while podocyte-specific aPKC lambda/ iota KO mice develop such a severe glomerulopathy phenotype they still born with a podocyte process network. We speculated that the aPKC zeta isoform might act as functional backup compensating for the loss of aPKC lambda/ iota during glomerular development. Here we report that podocyte-specific double knockout of both aPKC isoforms lambda/ iota and zeta results in a dramatic phenotype of podocytes that fail to form correct primary and secondary processes. This severe phenotype leads to glomerular developmental arrest, mesangiolysis, incomplete capillary network and perinatal death.

These results suggest that aPKC signaling is the essential step in formation of the podocyte process network identifying a fundamental signaling program for the regulation of the complex three-dimensional podocyte network.

**Funding:** Government Support - Non-U.S.

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**FR-PO1297**

The Reno-Protective Effect of Ethyl Pyruvate in Streptozotocin-Induced Diabetic Nephropathy Model

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**Background:** Pyruvate is an endogenous anti-oxidant and anti-inflammatory substance. The present study was implemented to investigate the protective effect of pyruvate against the development and progression of diabetic nephropathy in an in-vitro and in-vivo model.

**Methods:** Diabetic condition was induced by injecting streptozotocin(STZ, 65mg/kg) intraperitoneally from the S-D rats. Those that developed diabetes after 48 were treated with ethyl pyruvate (40mg/kg) intraperitoneally every other day. Rat mesangial cells cultured primarily from the S-D rat were treated in high glucose (HG, 50mM) or normal glucose (NG, 5 mM) condition. Results: HG increased mRNA and protein expression levels of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in a dose dependent manner. And NADPH oxidase-dependent reactive oxygen species (ROS) generation was increased in HG-stimulated cultured mesangial cells. Ethyl pyruvate (EP) decreased NADPH-dependent ROS generation and reduced mRNA and protein expression levels of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in a dose dependent manner. Diabetic rats without pyruvate treatment and nondiabetic rats were used for control. Pyruvate-treated diabetic rats exhibited decreased albuminuria, and NADPH-dependent ROS generation. Immuno histochemistry study showed that ethyl pyruvate decreased protein expression of collagen type IV and fibronectin compared to non-treated diabetic rat. Parallel changes were shown in the tissue mRNA and protein expression level of MCP-1, TGF-b1, laminin, fibronectin and type IV collagen in the kidney.

**Conclusions:** These findings suggest that pyruvate protects against kidney injury via NADPH oxidase inhibition.

**Funding:** Private Foundation Support

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**FR-PO1298**

Distribution of the Hindiii Restriction Fragment Length Polymorphism and CR1 Expression on Blood Leukocytes of Patients with Primary Glomerulonephritides and Lupus Nephritis

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**Background:** CR1 is a membrane receptor for C3b and C4b expressed on blood cells. Being involved in the processing and clearance of immune complexes (IC) and regulation of B-cell function, it protects from the development of IC diseases. CR1 expression on erythrocytes was supposed to be linked to high (H) and low (L) expression alleles identified by HindIII restriction fragment length polymorphism (RFLP). Intriguingly, there are no studies that would have examined the influence of this polymorphism on the level of CR1 expression on blood leukocytes (BL) in patients with primary and secondary glomerulonephritides (GN).

**Methods:** The surface expression of CD35 was determined by flow cytometry analysis of whole blood samples from patients with primary and secondary GN. Expression of CR1 on the lymphocytes (LN) and 44 healthy controls (C), with back gating on monocytes (M,C,D14), neutrophils (N, CD15) and B-lymphocytes (CD19). DNA was isolated from BL, amplified using primers specific for intron 27 of CR1 gene and HINDIII RFLP analysis was performed thereafter.

**Results:** Among 140 examined individuals, the HI allele was found in 65%, HL in 29.9% and LL in 5.71% of them. Unexpectedly, the highest expression of CR1 on all cell types was associated with the LL allele. In this respect, significant differences were found between individuals having the IL vs. HI alleles (p<0.001) and LL vs. HI alleles (p=0.005). The comparison of subjects having the HL and HI alleles revealed that those with the HI allele displayed significantly higher CR1 expression on B-cells (p<0.01) and N (p<0.05), but this could not be demonstrated on M. The prevalence of the HI allele was the highest in examined groups (PGN, 76.5%, CD14, 48.7%, C, TGN, 60%, LN and BL 83.3%, 32.5% and 25%) and LL allele (9.2%, 3.2% and 2.3%, respectively).

**Conclusions:** We found a genetic link between the HINDIII RFLP and CR1 expression on BL in Polish population. However, no impact of this polymorphism on the development of IC diseases could be confirmed.

**Funding:** Government Support - Non-U.S.

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**FR-PO1299**

Toward the Practical Use of Nephrin Peptides as a TRPC6 Inhibitor

Shoichiro Kanda,1 Yukata Harita,2 Takashi Sekine,1 Takashi Igarashi,2 Tatiana Drozdova, Andrey V. Cybulsky. 1 Medicine, McGill University, Montreal, Canada.

**Background:** Nephrin, a key component of the filtration slit diaphragm, undergoes post-translational modifications in the endosomal reticulum (ER). Mutations in nephrin lead to proteinuria. We examined the effects of missense mutations in nephrin on protein folding in the ER, cellular trafficking, and induction of the unfolded protein response (UPR).

**Methods:** Wild-type (WT) nephrin, and the 1171N, 2720C, S366R, S724C and R743C mutants were analyzed in 293T cells or glomerular epithelial cells (GECs) by transient transfection. Association of nephrin with the ER chaperone, calnexin, was studied by co-immunoprecipitation. Activation of the UPR was assessed by monitoring expression of the ER chaperone, Grp94, phosphorylation of eukaryotic translation initiation factor 2a (eIF2a), and induction of C/EBP homologous protein-10 (CHOP), as well as activity of transcription factor 6 (ATF6)-luciferase activity.

**Results:** All nephrin mutants showed increased association with calnexin, compared with WT nephrin. The 1171N and G270C mutants increased expression of Grp94 in 293T cells, and stimulated ATF6-luciferase activity in both 293T cells and GECs. Nephrin S366R and S724C tended to increase the expression of Grp94 and ATF6-luciferase activity were less consistent. The R743C mutant did not enhance Grp94 expression, nor ATF6-luciferase activity. All nephrin mutants did not increase eIF2a phosphorylation, nor CHOP expression. Immunofluorescence microscopy showed WT nephrin at the plasma membrane, while the 1171N and S366R mutants were perinuclear, colocalized with calnexin. Moreover, the two nephrin mutants induced aggregation of the ER chaperone, calreticulin, compared with WT. Treatment of cells with castanospermine (which reduces the interaction between the ER and calnexin) resulted in a portion of nephrin 1171N and S366R appearing at the plasma membrane.

**Conclusions:** Nephrin peptides inhibit phospho-dependent TRPC6 activity, and have a potential as a therapeutic reagent with appropriate delivery in vivo.

**Funding:** NIDDK Support
Conclusions: Certain nephrin mutants show impaired folding in the ER, and activate the ATF6 branch of the UPR. Induction of ER chaperones may represent a cytoprotective response, allowing cells to withstand proteotoxic injury. Blocking the interaction of nephrin with calnexin results in a partial rescue of certain nephrin mutants to the plasma membrane.

Funding: Government Support - Non-U.S.

FR-PO1301
Cyclophilin A Inhibits Glomerular Endothelial Cell Proliferation

Background: The glomerular endothelial cells (ECs) are strategically situated at the interface between blood and renal tubular epithelial cells, and their proliferation in response to injurious stimuli is closely associated with glomerular filtration barrier dysfunction. We have previously reported the cytoprotective roles of cyclophilin A (CypA) in podocyte injury induced by puromycin aminonucleoside (PAN) and angiotensin II (Ang II). Here, we investigated the role of CypA in glomerular ECs.

Methods: The human glomerular endothelial cells (HGECs) were isolated and cultured. The effects of CypA on HGEC proliferation were examined with MTT assay. The expression of cell cycle-related molecules was analyzed by western blotting. The localization of CypA was determined using immunofluorescence. The mechanism underlying CypA-mediated HGEC proliferation inhibition was investigated.

Results: CypA significantly inhibited HGEC proliferation. CypA treatment led to a decrease in the expression of cyclin D1 and cyclin A2, and an increase in the expression of p21cip1 and p27kip1. CypA also induced HGEC cell cycle arrest in G0/G1 phase. CypA reduced the nuclear translocation of NF-kB, and increased the expression of I-kBalpha. CypA inhibited the phosphorylation of Akt and its downstream target mTOR.

Conclusions: Our results indicate that CypA inhibits HGEC proliferation through regulating cell cycle-related molecules and suppressing NF-kB activity. CypA may be a potential therapeutic target for glomerular diseases.

Funding: National Natural Science Foundation of China (81970959, 81770886)
A Novel Function of the MYPT Family Member TIMAP in Endothelial Cells

**FR-PO1306**

**A Novel Function of the MYPT Family Member TIMAP in Endothelial Cells**

**EC** Marva Obeidat, Laaj Li, Barbara J. Ballermann. Medicine, University of Alberta, Edmonton, AB, Canada.

**Background:** TIMAP is a prenylated EC-specific protein phosphatase 1 (PP1c) regulatory subunit in the myosin phosphatase (MYPT) family, first identified by us in glomerular EC. MYPTs control P-MLC2 activity toward myosin light chains (MLC). Whether TIMAP regulates the MLCK-mediated phosphorylation of MLC2 bound to immobilized TIMAP(WT) was investigated. From our experiments we can hypothesize that leakage of albumin is caused by excessive entry of calcium through the NMDAR into endothelial cells and leads to endothelial dysfunction through activation of the MAPKinase pathway.

**Funding:** Private Foundation Support, Government Support - Non-U.S.

**FR-PO1307**

The Impact of Interplay between c-mip and WT1 Activity in the Pathophysiology of Podocyte Diseases

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**FR-PO1308**

EphB4 Forward Signaling Maintains Podocyte Homeostasis during Thy1.1 Nephritis

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**Background:** Glomerular diseases starting with mesangiolysis have a high potential for recovery, the molecular mechanisms remain however to be defined. Eph receptor tyrosine kinases and their ligands (ephins) play a pivotal role in the homeostasis of many adult organs and are also widely expressed in the kidney. The aim of our study was to assess their role in the glomerular recovery from Thy1.1 nephritis, a rat model of reversible mesangio proliferative glomerulonephritis (GN).

**Methods:** Western blotting and immunohistochemical methods were used to follow the expression and activation status of Eph receptors and Ephrin signaling during proteinuria, and nephritic glomerular and nephritic glomerular. Next, NV-BH7G12, a novel inhibitor of EphB4 phosphorylation, was applied to control or nephritic rats.

**Results:** EphB4 and ephrinB5 were expressed in healthy glomeruli and upregulated during Thy1.1 nephritis. EphB4 was mostly expressed at the apical membranes of podocytes, whereas ephrinB5 was mainly at the foot processes. Importantly, EphB4 was strongly phosphorylated around day 9 of nephritis. NPV-BH7G12 induced no glomerular changes in controls. Nephritic animals treated with vehicle showed neither morphological evidence of podocyte injury nor loss of podocytes. In contrast, NV-PBH7G12 application resulted in a reduction of glomerular injury and podocyte loss.

**Conclusions:** In aggregate, our results identify EphB4 signaling as a novel pathway allowing podocytes to adapt to transient capillary collapse and thus survive.

**Funding:** Government Support - Non-U.S.

**FR-PO1309**

Divergent Mechanisms of Proteinuria and Fibrogenesis in a Mouse Model of Adriamycin Nephropathy


**Background:** Transforming growth factor (TGF-β)-β is the most important mediator of renal fibrogenesis. We initially showed that P3K and TGF-β contribute to collagen I expression in podocytes. Using these findings, we developed a modified mouse model of adriamycin (ADR) nephropathy manifesting proteinuria and FSGS-like histology. Here, we evaluated the role of the p110γ isoform of PI3K in this model.

**Methods:** A single intravenous injection of ADR to 8 week-old 129SvJ mice induced glomerulopathy. Kidney tissue and cultured mouse podocytes were evaluated by standard immunohistochemistry and qPCR.

**Results:** In vivo administration of soluble TGF-β receptor II protected against glomerular disease, but did not change ADR-induced proteinuria or tubular injury. Akt phosphorylation was detected by immunostaining of ADR-treated mouse kidneys, suggesting PI3K activation. mRNA encoding p110γ, but not other PI3K isoforms, was selectively upregulated in ADR-kidneys. Though p110γ is highly enriched in leukocytes, we found that cultured podocytes express p110γ and that p110γ staining colocalizes with nephrin in cultured podocytes. Treatment of cultured podocytes with PI3K inhibitor 4E-BP1 and PI3K inhibitor 4E-BP1 prevented ADR-induced proteinuria and substantially decreased mesangial recovery. Additionally, NPV-BH7G12 inhibited cellular repair by intrinsically angiogenic, suggesting a previously unrecognized role of podocytes in regulating cytoskeletal and transcriptional expression.

**Conclusions:** In aggregate, our results identify PI3K signaling as a novel pathway allowing podocytes to adapt to transient capillary collapse and thus survive.

**Funding:** Government Support - Non-U.S.
FR-PO1310

Hypoxia-Inducible Factor 1α Expression Is Upregulated and Accelerates Fibrosis in Adriamycin-Induced Murine Glomerulonephropathy

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Background: Hypoxia-inducible factor (HIF)-1α has been associated with ischemic renal injury, but its fibrogenic role in normoxia is less characterized. We recently reported that transforming growth factor (TGF)-β upregulates HIF-1α expression in normoxic cultured human kidney cells. Transcriptional inhibition of HIF-1α blocked TGF-β1-induced type I collagen expression (Basu, JASN 2011). Here, we tested a possible role for HIF-1α in a mouse model of kidney fibrosis.

Methods: Glomerulonephropathy was induced in male 129x1/Svj mice by a single injection of adriamycin (ADR, 15 mg/kg, iv). Some mice were transferred to a hypoxia chamber (10% oxygen) the day after the ADR administration. On day 10, mice were sacrificed 1 hr after Hypoxprobe administration (60 mg/kg, iv), which accumulates under hypoxia (PO2 < 10 mmHg) and is detectable by immunoblot post mortem. Kidneys were harvested to evaluate histology and mRNA expression by real-time PCR.

Results: As described, ADR-treated mice in normoxia develop proteinuria and TGF-β-mediated histopathological changes consistent with FSGS, associated with increased ECM mRNA expression starting at day 7 and plateaus by day 14. (Finer and Hayashida, ASN2010). Hypoxprobe adducts were minimally detected in control mouse kidney cortex, whereas they strongly decorated proximal tubules of the ADR-treated mouse kidneys. HIF-1α mRNA expression, determined in whole kidney lysates, was increased 4.3x by ADR compared to control. Glomeruli of the ADR-treated mice showed mesangial expansion and fibrosis but no Hypoxprobe staining, suggesting a non-hypoxic mechanism for glomerular changes. The kidneys of ADR mice housed in hypoxia showed greater histopathological changes. COL1A2 mRNA expression in whole kidney lysates was not affected in control changes. The kidneys of ADR mice housed in hypoxia showed greater histopathological changes compared to control. Glomeruli of the ADR-treated mice showed mesangial expansion and fibrosis but no Hypoxprobe staining, suggesting a non-hypoxic mechanism for glomerular changes. Hypoxia did not increase whole-kidney HIF-1α expression greater than that seen with ADR alone.

Conclusions: These results suggest a synergistic role for HIF-1α in both hypoxic and normoxic kidney fibrosis. Funding: NIDDK Support

FR-PO1311

Renoprotective Effect and Mechanism of Bortezomib in Adriamycin-Induced Nephropathy Rats


Background: Many studies indicate that the proteasome inhibitor could exert potent anti-fibrotic inflammatory effects. Bortezomib demonstrates a potent antitumor activity against several human cancers and has been clinically used in patients with refractory multiple myeloma.

Methods: Adriamycin nephropathy was induced in Sprague-Dawley rat by a intravenous injection of adriamycin. 4 weeks after injection, bortezomib was given intraperitoneally (30mg/kg or 60mg/kg, twice in week) for 4 weeks. At the end of study, the biochemical indicators were measured and the pathological changes of the renal tissue were evaluated by light microscope. Transmission electron microscopy was used to observe the ultrastructure change of rat kidney. Immunohistochemistry and mRNA expression was applied to observe the expression levels of FSP-1, α-SMA, Coll III, TGF-β, Smad2, Smad3 and the macrophage infiltration in rat kidney.

Results: During the course of nephrotic syndrome, serum creatinine(Scr) and blood urea nitrogen (BUN) were significantly elevated (p<0.05). Histological examinations of kidney tissue demonstrated evident tubulo-interstitial inflammation and fibrosis (p<0.05). Compared with untreated adriamycin nephrotic group, bortezomib treatment could significantly reduce the level of urine protein, the Scr and BUN, which was accompanied by the attenuation of the macrophage infiltration, pathological changes and ultrastructure change in rat kidney. Immunohistochemistry results showed that the expression of FSP-1, α-SMA, Coll III, TGF-β and Smad3 significantly increased in adriamycin-induced nephritic group (p<0.05), but no significant difference in Smad2 (p>0.05) was found. Compared with the untreated adriamycin-induced nephrotic group, bortezomib treatment could significantly decreased the expression of FSP-1, α-SMA, Coll III, TGF-β and Smad3 (p<0.05), but Smad2 was increased (p<0.05).

Conclusions: These results demonstrated that bortezomib could significantly ameliorate nephrotic syndrome in adriamycin-induced nephrotic rats and progression of renal fibrosis through a TGF-β/Smad-dependent pathway.

FR-PO1312

Podocyte Damage in Fabry Disease

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Background: Fabry disease is an X-linked lysosomal storage disorder resulting from an inborn deficiency of lysosomal α-galactosidase A (α-gal A). This deficiency leads to accumulation of neutral glycosphingolipids, mostly globotriaosylceramide (Gb3), in various tissues including kidney, heart, vessels and brain. End-stage renal disease is very common in hemizygous males and limits life expectancy in these patients but renal involvement leading to proteinuria, hematuria, and renal insufficiency is not well understood. Histological studies suggest that the accumulation of Gb3 in podocytes plays an important role in the pathogenesis of glomerular damage. However, the pathophysiological role of Gb3 in podocytes is difficult to study due to the lack of an appropriate animal or cell culture model.

Methods: We have established a human cell culture model of podocyte damage in Fabry disease by using RNA interference technology in combination with lentiviral gene transfer. An established human podocyte cell line was transduced with various short hairpin RNA (shRNA) constructs against human α-gal A. Reduction of α-gal A mRNA levels and α-gal A activity were confirmed by qPCR and by a photometric assay.

Results: Lipid chromotography revealed Gb3 accumulation in α-gal A knockdown cells. We observed a decrease of AKT phosphorylation as well as reduced levels of phospho-mTOR in α-gal A knockdown cells, associated with an increase in autophagy.

Conclusions: Our data suggest that podocyte damage in Fabry patients is linked to a dysregulation of autophagy.

This novel cell line will serve as a promising tool for further studies on the pathophysiological mechanisms responsible for glomerular dysfunction in Fabry disease.

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FR-PO1313

Inverted Formin 2 Is Essential for Maintenance of Podocyte Morphology and Signaling

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Background: Podocytes are terminally differentiated cells whose morphology and function depend on the fine regulation of actin skeleton dynamics. Mutations in inverted formin 2 (INF2), an actin regulating protein, can cause familial focal segmental glomerulosclerosis (FSGS). INF2 serves as a potential regulator of actin dynamics through its dual polymerization/dem polymerization activity and by interference with diaphanous-related formin (mDia) mediated actin polymerization. Here we investigated the role of INF2 in the maintenance of actin-based morphology and signaling in podocytes to gain insight into the mechanism of this form of disease.

Methods: In cultured human podocytes, silt diaphragm (SD) signaling was initiated by neprhin clustering and phosphorylation. The distribution pattern of SD proteins and the neprhin signalosome was compared with/without INF2 knockdown by siRNA sequences targeting INF2. The translocation of SD proteins and signalosome components were observed by immunofluorescent stain.

Results: In cultured podocytes, neprhin and podocin localized along the ruffles of the cells, at the tip of actin filaments. The phosphorylated neprhin recruited adapter protein NCK/2 actin serving protein mDia to the signalosome, and induced local actin tail formation. With INF2 knockdown, the de novo expressed neprhin or podocin was stuck in the cytoplasmic endosomes rather than being transported to the ruffles of the cells. The recruitment of mDia and the formation of actin tails were disrupted.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Conclusions: INF2 is an essential protein in maintaining the actin cytoskeleton-dependent morphology of podocyte, distribution of protein 53D, and the response of actin-regulating signal transduction initiated by nephrin phosphorylation. The decomposition of foot process integrity may be associated with the deficiency of INF2 in the SD complex-cytoskeleton pathway.

FR-POI314
Role of Calcium-Indepet Phospholipase A2γ in Complement-Mediated Glomerular Epithelial Cell Injury Hanan Elmiram, Tomoko Takano, Joan Papillon, Andrey V. Cybulsky. Nephrology, McGill University, Montreal, QC, Canada.

Background: In experimental membranous nephropathy, complement C5b-9 induces glomerular epithelial cell (GEC) injury in urine. The effects of C5b-9 are mediated via signaling pathways, including calcium-independent phospholipase A2γ (iPLA2γ), protein kinase C (PKC), and mitogen-activated protein kinases (MAPKs), that is extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38. The iPLA2γ pathway is cytoprotective. The present study addresses the mechanisms of iPLA2γ activation.

Methods: Cultured GEC were stably transfected with iPLA2γ cDNA. GEC were incubated with normal and serum-free medium (to assemble complement C5b-9) or heat-inactivated serum (control). To study MAPKs in iPLA2γ activation, COS-1 cells were transfected with iPLA2γ and cycloxygenase-1 (COX-1). PLA2γ activity was monitored by quantifying prostaglandin E2 (PGE2) production. Mutations in two putative ERK phosphorylation sites in iPLA2γ (S168, S271A) were created by PCR-based mutagenesis.

Results: Complement-mediated production of PGE2 was amplified in GEC that overexpress iPLA2γ compared with control cells, and the effect of iPLA2γ was blocked by the iPLA2γ inhibitor, bromo-enol lactone (BEL). Activation of S168A, S271A and S168A/S271A mutants was comparable to the wild type enzyme.

Conclusions: Complement-mediated activation of iPLA2γ is mediated via PKC and ERK. PKC is necessary, but is insufficient for iPLA2γ activation. ERK is necessary and sufficient, but appears not to act directly on iPLA2γ. Defining the mechanisms by which complement contributes to GEC injury and proteinuria will provide opportunities for development of novel therapeutic approaches.

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FR-POI315
Serum Albumin-Induced Podocyte Cox-2 Expression Is Inhibited by Glucocorticoids and MAPK Inhibitors Shinya Agraewal,1 Adam J. Guess,1 Ruma Pall,1 Rainer Benndorf,1,2 William E. Smoyer,1,2 Center for Clinical and Translational Research, Research Institute at Nationwide Children’s Hospital, Columbus, OH; 1Department of Pediatrics, Ohio State University, Columbus, OH.

Background: Proteinuria is a hallmark of glomerular disease, the third leading cause of ESRD in the US. Proteinuria is also a known risk factor for progressive glomerular disease, and results in increased podocyte exposure to serum albumin (SA). Additionally, oxidized forms of SA have been found in patients with glomerular disease. Based on this, we analyzed the ability of SA and oxidized SA to induce Cox-2 in podocytes, and whether glucocorticoids (GC), thiazolidinediones (TZDs) or selected MAPK inhibitors could block this induction.

Methods: Cultured murine podocytes were treated with SA and oxidized SA at physiological concentrations and cell viability and Cox-2 expression were quantified. In addition, since GC, TZDs and MAPK inhibitors have all been reported to reduce both proteinuria and direct podocyte injury, we also analyzed their ability to regulate SA-induced Cox-2 expression.

Results: SA increased Cox-2 mRNA and protein expression in podocytes in a dose-dependent manner. Moreover, SA-induced Cox-2 expression was reduced by inhibitors of ERK1/2, p38 MAPK, and MK2, but not SAPK/JNK, although all of these were activated by SA exposure. In addition, GC, but not TZDs, inhibited SA-induced Cox-2 expression. Notably, treatment with oxidized SA led to a greater induction of Cox-2 protein than un-treated SA. Lastly, increasing doses of SA led to progressive reductions in podocyte viability.

Conclusions: Physiologic concentrations of SA induce podocyte Cox-2 expression, which can be inhibited by both GC and selected MAP inhibitors. Since GC and MAPK inhibitors are now known to directly protect podocytes against injury, our results suggest that Cox-2 may be a molecular mediator of SA-induced podocyte injury.

Funding: NIDDK Support

FR-POI316
15-Deoxy-Delta<sub>12</sub>,14-Prostaglandin J<sub>2</sub> Inhibits the Expression of Chemokines by Blocking NF-kB Nuclear Translocation Via PPAR-Independent Mechanism in Lipopolysaccharide-Stimulated Renal Tubular Epithelial Cells Ying Lu, Qiao Zhou, Fang Zhong, Xu Hao, Cong Li, Weiming Wang, Nan Chen. Nephrology, Ruijin Hospital, Shanghai, China.

Background: 15d-PGJ2 is a high-affinity ligand for peroxisome proliferator-activated receptor (PPARg), and has been suggested to exert anti-inflammatory effects in vivo. The aim of the study was to investigate the effect and mechanism of 15d-PGJ2 on the expression of chemokines in Lipopolysaccharide–stimulated renal tubular epithelial cells.

Methods: Conditionally immortalized murine podocytes were differentiated for at least 14 days. The cells were subsequently transfected with PPARγ reporter plasmid. To study the effect of 15d-PGJ2 on the expression of chemokines, cells were treated with 15d-PGJ2. Chemokines including interleukin-8 (IL-8) and chemotactic protein-1 (C5a) were determined by real-time PCR and ELISA. The location of nuclear factor-kB (NF-kB) was detected by immunofluorescence analysis.

Results: The results showed that, compared with the control group, IL-8 and MCP-1 were significantly increased at both transcription and post-transcription level in LPS-stimulated group. Accordingly, p-IκBα in cytoplasm and NF-kB in nucleus was significantly increased in LPS-stimulated HK-2 cells compared with the control group. Pre-treatment HK-2 cells with 15d-PGJ2 abolished LPS-induced IL-8 and MCP-1 overproduction. Knockdown of PPARγ by RNAi in HK-2 cells shows that 15d-PGJ2 also inhibited LPS-induced IL-8 and MCP-1 overexpression in these cells. Nuclear translocation of p65 and phosphorylation of IκBα induced by LPS were restored by 15d-PGJ2 in both HK-2 cells and PPARγ-knockdown HK-2 cells.

Conclusions: In the study, we demonstrated that 15d-PGJ2 could inhibit the expression of chemokines by blocking NF-kB translocation into nucleus via a PPAR-independent mechanism in LPS-stimulated renal tubular epithelial cells.

Funding: NIDDK Support

FR-POI317
Regulation of Fatty Acid Oxidation Profoundly Affects Palmitic Acid Induced Podocyte Cell Death Konrad van Kamps,1 Jonas Sieber,1 Peter H. Mundel,1 Andreas Werner Jehle.1,3 Department of Biomedicine, Molecular Nephrology; University Hospital Basel, Switzerland; 1Division of Nephrology, Massachusetts General Hospital, Boston, 1Department of Internal Medicine, Kantonsspital Bruderholz, University of Basel, Basel, Switzerland.

Background: Diabetes mellitus type 2 is associated with altered lipid metabolism leading to elevated levels of free fatty acids (FFAs). Recently, we have reported the antagonistic effects of palmitic acid versus monounsaturated FFAs (MUFA's) on podocyte survival (Am J Physiol Renal Physiol. 2010 Oct;299(4):F821-9). The objectives of this study were to elucidate whether inhibition or stimulation of fatty acid oxidation (FAO) may affect palmitic acid induced podocyte cell death.

Methods: Conditionally immortalized murine podocytes were differentiated for at least 11 days and palmitic acid or oleic acid were complexed to BSA. Apoptosis and necrosis

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were quantified by annexin V and propidium iodide labeling. We used etomoxir, a carnitine- palmitoyltransferase I inhibitor, to reduce FAO. Carnitine-5-oxo-4-carboxabide ribonucleoside (AICAR) was used to stimulate FAO. Levels of pAMPK, pACC, AMPK and ACC were measured by immunoblotting. We used tritated palmitic acid and measured tritiated water to assess oxidation of palmitic acid.

(AICAR) dose-dependently increases palmitic acid induced cell death up to 200%. Contrariwise, we show that AICAR significantly decreases palmitic acid induced apoptosis and necrosis by 49.5±1.5% (p=0.004), and 55.7±4.0% (p=0.01) respectively. AICAR upregulated the phosphorylation of both AMPK and ACC.

Conclusions: AICAR upregulated the phosphorylation of both AMPK and ACC. Furthermore, palmitic acid increases oxidation of palmitic acid by 124.0±4.9% (p=0.001) as assessed by the metabolism of tritiated palmitic acid. Similarly, oleic acid increases oxidation of palmitic acid by 51.3±6.5% (p=0.001).

Keywords: AICAR; palmitic acid; AMPK; ACC; palmitic acid induced cell death; oxidation; mitochondrial dysfunction.

FR-PO1318
DNaSe I Induces Other Endonucleases, DNA Damage and Apoptosis Pathways in Kidney Tubular Epithelial Cells by Its DNA Cleavage Activity
Tariq Fahmi, Xiaoying Wang, Ellen T. Gross.

Background: Every mammalian cell has cytotoxic endonucleases which degrade host DNA prior and after cell death. The endonucleases have similar mode of action and often simultaneously induced to cleave DNA after cell injury. The mechanism for the simultaneous induction of endonucleases is unknown. DNA degradation by DNase I, the most active kidney endonuclease, has been observed in kidney ischemia-reperfusion (IR), and its inactivation was protective against toxic or ischemic kidney injuries suggesting that DNase I is responsible for kidney cell death. We hypothesized that DNase I may be a universal regulator of cell death, both during renal ischemia and as well as some apoptosis and DNA damage pathways.

Methods: To test this, rat kidney tubular epithelial N825E were transfected with rat DNase I gene or its inactive mutant in pECFP expression vector for 6, 12 and 24 hrs, while control cells were transfected with “empty” pECFP. DNase I-CFP expression was monitored by fluorescence. RNA was analyzed for all known cytotoxic endonucleases and ~200 markers of DNA damage and apoptosis pathways using real-time RT-PCR.

Results: The measurements showed certain endonucleases, DNA damage and apoptosis pathways were induced by active DNase I, including: (1) several cytotoxic endonucleases except DNase II, with the maximal effect for DNase gamma and DNase X; (2) beta-actin, the only known endogenous inhibitor of DNase I; (3) Fox, previously described being transcriptionally regulated by DNase I, and its inhibitor FAIM; (4) caspases 3, 7, 8 and 12; (5) DNA polymerase beta (indicative of single-stranded DNA breaks); (6) p53 and Tps3bp2 (likely a result of DNA damage); and (7) Apaf 1 (likely a result of p53 activation).

Conclusions: These results suggest for the first time that DNase I activity may induce other endonucleases and several key regulators of DNA damage and apoptosis pathways during kidney injury. Funding: NIDDK Support, Veterans Administration Support

FR-PO1319
Ethanol Causes Oxidative Stress, Alters Actin Cytoskeleton and Decreases Expression of Cytotrome P450 Isoforms in Murine Podocytes
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Background: The contribution of ethanol (EtOH) to progression of chronic kidney disease is controversial, although EtOH is associated with hypertension. Effects of EtOH on podocytes are unknown. We have shown that EtOH alters podocyte expression of cytchrome P450 (CYP450) isoforms in cultured podocytes, the only known endogenous inhibitor of 20-HETE synthesis and that 20-HETE is protective in several models. We hypothesized that EtOH causes oxidative stress and alters podocyte structure and that 20-HETE is protective in several models. We hypothesized that ethanol causes oxidative stress and decreases synthesis of protective eicosanoids.

Methods: Immortalized podocytes were incubated with EtOH (1 to 20 µM) for 1 to 24 hr. These concentrations correspond to blood alcohol levels seen in humans. We examined podocyte superoxide generation using the fluorescent probe hydroethidine (HE), visualization podocyte actin cytoskeleton using confocal microscopy and measurement of CYP41a12 and CYP41a12b using quantitative RT-PCR. 20-HETE (100 nM) was included in the incubation medium in some groups.

Results: Incubation with EtOH increased superoxide levels, indicated by increased HE fluorescence. Furthermore, EtOH caused disruption of actin filaments. These effects were dose- and time-dependent. Incubation of 20-HETE abrogated the increase in superoxide and prevented actin cytoskeletal disruption. EtOH (20 µM) decreased expression of CYP41a12 and CYP41a12b at 24 hr (P<0.02 and P<0.001 vs control, respectively) while lower concentrations increased expression of some CYP41a12.

Conclusions: EtOH in meaningful concentrations increased oxidative stress and altered actin cytoskeleton in podocytes, effects reversed by 20-HETE. We posit that excessive EtOH consumption may alter glomerular structure and function by mechanisms that include increased oxidative stress and decreased synthesis of protective eicosanoids. Such changes may exacerbate underlying glomerular pathologic conditions and contribute to progressive renal injury. In contrast, moderate EtOH intake may provide protection by increased 20-HETE.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1320
Role of Podocytes’ Collagen Receprors in Renal Fibrosis of COL4A3 Knockout Mice
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Background: Podocytes sense the composition of glomerular basement membrane by collagen receptors such as DDr1 (discoidin domain receptor 1) and ITG2A (Integrin a2). Therefore, collagen receptors might play an important role in maintenance normal composition of the glomerular basement membrane and in type IV collagen diseases such as Alport syndrome. In the present study, we aimed to investigate the role of collagen type IV in COL4A3 knockout mice with wild-type controls.

Methods: Phosphorylation of Akt/PKB and STAT1 was analyzed by Western Blotting. Quantification of protein bands was carried out by ImageJ and the ratio of phosphorylated to unphosphorylated protein was calculated. Matrix accumulation and fibrosis was analyzed by immuno-histochemistry.

Results: Kidney lysates of wildtype mice showed an age-dependent (50d, 100d, 150d) increase of phosphorylated Akt/PKB. Even the lowest level of activated Akt in 50 d wildtype mice was higher than in COL4A3 knockout mice of any age. Mice without ITG2A and DDr1- or DDr2-treatment showed the same increased accumulation of matrix and fibrosis. Phosphorylated STAT1 also increases with age in wildtype mice. In contrast to Akt/PKB, activation of STAT1 is at its maximum at 4.5 weeks of age and slightly decreases until week 9 in COL4A3 knockout mice. The fraction of phosphorylated STAT1 in Alport mice is lower than in wildtype mice.

Conclusions: Akt is involved in cellular survival pathways. Therefore, decreased activation of STAT1 by DDR1 pathway in mutant mice indicates a role of DDR1 in the formation of matrix and fibrosis. Moreover, NIPP1 is important in spliceosome assembly and pre-mRNA splicing. By performing differential display analysis we have recently found out that NIP1 gene expression was differentially regulated in Co-BASA and AGE-BASA treated differentiated podocytes. The aim of this study was to address the NIPPI expression in podocytes.

Funding: The NIPPI expression was analyzed by Real Time PCR, the protein concentration in corresponding samples by Western Blot analysis and immunofluorescence staining. In addition, it was analyzed whether the reduced NIPPI expression correlates with changes in cell cycle or cell proliferation. Methods: NIPPI expression was analyzed by Real Time PCR, the protein concentration of Co-BASA and AGE-BASA treated differentiated podocytes compared with Co-BASA treated cells. Immunofluorescence showed a NIPPI translocation from the cytoplasm to the nucleus compared to the control cells. NIPPI sRNA transfection of podocytes reduced NIPPI expression in podocytes and induced p27Kip1 a cell-cycle inhibitor, previously shown to be induced by AGE-BSA in podocytes. Moreover, Podocytes revealed a lesser proliferation rate when NIPPI expression is reduced either by AGE-BASA or NIPPI sRNA.

Conclusions: AGE-BASA treated podocytes demonstrated a reduced NIPPI expression compared to control cells. This decreased NIPPI expression may explain several pathophysiological alterations of podocytes in diabetic nephropathy such as hypertrophy, cell-cycle arrest, and apoptosis.

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FR-PO1322
Role of Connective Tissue Growth Factor in High Glucose-Induced Epithelial-Mesenchymal Transition of Podocytes
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Background: Our previous studies demonstrated that connective tissue growth factor (CTGF) plays an important role in the early kidney hypertrophy and fibrosis of diabetic nephropathy, while the potential mechanism is still unclear. This study was to investigate the role of CTGF in high glucose-induced epithelial–mesenchymal transition in podocytes.

Methods: The differentiated podocytes cultured under different conditions for 24 hours at 37°C were divided into four groups as follows: Control (5 mM GS), 5 mM
FR-PO1323

Connective Tissue Growth Factor Binds to Epidermal Growth Factor Receptor To Modulate Renal Inflammation

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Background: Connective tissue growth factor (CTGF) has suggested as a risk marker for chronic kidney disease. CTGF inhibits fibrosis but its role in renal inflammation has not been described.

Methods: We created transgenic (TG) mice that expressed either V14Rho or N19Rho specifically in tubular cells with the Cre-loxP principle. Rho A activity was assessed by small GTPases belonging to the Rho GTPase family. The morphologic changes in the kidney were assessed by phase contrast microscopy. To study the relative marker of epithelial–mesenchymal transition (EMT), the mRNA and protein expression were analyzed by real-time RT-PCR and western blotting, respectively. In addition, the effect of inhibition CTGF with an antibody and RNA silencer of αV and β3 integrin. Moreover, erlotinib inhibited CTGF-induced proinflammatory mediators and ERK activation in tubular cells.

Results: In vitro, CTGF caused EGFR phosphorylation in the kidney, mainly located in tubulop epithelial cells. Treatment with Erlotinib inhibited CTGF-induced renal changes observed at 24 hours, including ERK activation (a downstream EGFR signalling), up-regulation of proinflammatory factors and interstitial inflammatory cell infiltration. In cultured tubulop epithelial cells, CTGF increased EGFR phosphorylation. This process was not due to EGFR transactivation, as shown by the lack of effect of matrix metalloproteinase inhibitors (GM6001 and TAPI-2). Direct binding was demonstrated by cross-linking and immunoprecipitation studies, that leads to CTGF-EGFR heterodimers formation. EGFR activation is regulated by integrins, as shown by different approaches: RGD peptides, neutralizing antibody and RNA silencer of αV and β3 integrin. Moreover, erlotinib inhibited CTGF-induced proinflammatory mediators and ERK activation in tubulop epithelial cells.

Conclusions: Our results suggest that CTGF directly binds to EGFR and activates its signalling pathway, leading to modulation of downstream mechanisms, such as ERK activation, and cellular responses, including renal inflammation.

Funding: Government Support - Non-U.S.

FR-PO1324

In Vivo Manipulation of Glutathione S-Transferase A4 Prevents 4-Hydroxynonenal-Induced Renal Tubular Cell Damage

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Background: Lipid peroxidation yields toxic products that can damage cell membranes, including aldehydes 4-hydroxynonenal (4-HNE). Since glutathione transference, GSTA4, can eliminate 4-HNE, we studied a mouse model of unilateral ureteral obstruction (UUO) to determine if changing GSTA4 expression influences UUO-induced fibrosis.

Methods: In vitro studies were accomplished using a PiggyBac transposon system. In these mice GSTA4 was expressed in a group of mice, its signalling pathway, leading to modulation of downstream mechanisms, such as ERK activation, and cellular responses, including renal inflammation. In vivo studies were done in tubulop epithelial cells.

Results: In vivo, CTGF caused EGFR phosphorylation in the kidney, mainly located in tubulop epithelial cells. Treatment with Erlotinib inhibited CTGF-induced renal changes observed at 24 hours, including ERK activation (a downstream EGFR signalling), up-regulation of proinflammatory factors and interstitial inflammatory cell infiltration. In cultured tubulop epithelial cells, CTGF increased EGFR phosphorylation. This process was not due to EGFR transactivation, as shown by the lack of effect of matrix metalloproteinase inhibitors (GM6001 and TAPI-2). Direct binding was demonstrated by cross-linking and immunoprecipitation studies, that leads to CTGF-EGFR heterodimers formation. EGFR activation is regulated by integrins, as shown by different approaches: RGD peptides, neutralizing antibody and RNA silencer of αV and β3 integrin. Moreover, erlotinib inhibited CTGF-induced proinflammatory mediators and ERK activation in tubulop epithelial cells.

Conclusions: Our results suggest that CTGF directly binds to EGFR and activates its signalling pathway, leading to modulation of downstream mechanisms, such as ERK activation, and cellular responses, including renal inflammation.

Funding: Government Support - Non-U.S.

FR-PO1325

Cdc42-Interacting-Protein-4 Promotes the Translocation of β-catenin to Nucleus in TGF-β1-Induced Renal Tubular Epithelial-To-Mesenchymal Transdifferentiation

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Background: Epithelial-to-mesenchymal transdifferentiation (EMT) in kidney is the transition of tubular epithelial cells into myofibroblasts, it is considered as one of the most important events underlying chronic renal diseases. During the process of EMT, epithelial cells lose the expression of E-cadherin. Snail family members are E-cadherin transcriptional repressors which have been implicated in promoting EMT. Evidence demonstrates that translocation and accumulation of β-catenin in the nucleus lead to enhanced binding to members of the T cell factor (TCF) family and lymphoid enhancer factor (LEF) family, in turn, β-catenin/TCF/LEF complexes activate target genes such as Snail family. Thus, it is essential to investigate what effects translocation of β-catenin to nucleus. Cdc42-interacting protein 4 (CIP4), a Cdc42 effector protein involved in cytoskeleton organization and actin polymerization, is suggested to associated with β-catenin and paly a role in EMT. We investigated this potential role of CIP4 in TGF-β1-induced renal tubular EMT.

Methods: Expression, interaction and colocalization of proteins were detected by western blot, immunoprecipitation and confocal imaging respectively.

Results: Expression of CIP4 was upregulated in rat proximal tubule cell line (NRK52E) stimulated by TGF-β1 accompanied by reduced expression of E-cadherin and increased expression of mesenchymal marker α-SMA. The interaction between CIP4 and β-catenin was detected by immunoprecipitation, CIP4 colocalized with β-catenin in cell membrane/ cell nucleus before/after TGF-β1 stimulation by confocal images. In normal condition, overexpression of CIP4 promoted translocation of β-catenin to nucleus accompanied by the reduced expression of E-cadherin. Furthermore, to knockdown CIP4 by using siRNA, we found that β-catenin translocated to nucleus was decreased, expression of E-cadherin was elevated.

Conclusions: In conclusion, CIP4 promotes translocation and accumulation of β-catenin to nucleus in TGF-β1-induced renal tubular EMT, which is accompanied by reduced expression of E-cadherin.

FR-PO1326

Mechanisms of Proteinuria Induced by Rho A GTPases

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Background: Podocytes play a pivotal role in maintaining the integrity of the glomerular filteration barrier. A growing literature suggests that this function is regulated by small GTPases belonging to the Rho GTPase family.

Methods: A constitutively active Rho A (V14Rho) or a dominant negative Rho A (N19Rho) were introduced into cultured podocytes using protein transduction by treating the proteins with the Tat human immunodeficiency virus (HIV) protein sequence [V14Rho(+)] or N19Rho(-)]. Cell permeable proteins lacking the Tat sequence were used as controls [V14Rho(−)] or [N19Rho(−)]. To investigate the role of Rho A in podocyte biology in vivo, we created transgenic (TG) mice that expressed either V14Rho or N19Rho specifically in podocytes using a doxycycline inducible strategy.

Results: V14Rho(+) enhanced both stress fiber formation and podocyte apoptosis, and the apoptotic effect was blocked by the Rho kinase inhibitor Y27632. In contrast, N19Rho(−) had no significant effect on podocyte apoptosis but decreased stress fiber formation and promoted the formation of monomeric actin (G actin). In TG mice, induction of either V14Rho or N19Rho caused a significant increase in albuminuria and foot process effacement. The mechanisms of these adverse effects, however, appeared to be different. Induction of V14Rho caused a reduction in expression of nephrin at both the mRNA and protein levels without affecting expression of the actin-associated, cytoskeletal protein synaptopodin. In contrast, induction of N19Rho had no effect on nephrin mRNA or protein levels but decreased synaptopodin expression.

Conclusions: These data suggest that basal Rho A activity also promote podocyte injury by mechanisms that appear to be different from the mechanisms that alter glomerular filtration barrier function following Rho A inhibition.

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FR-PO1379

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419A
Complete Differentiation of Rab3A-KO Podocytes Induced by GABAA-R Agonists  Silvia Armelloni, Min Li, Laura Giardino, Alessandro Corbelli, Masami Ikehata, Deborah Mattinzoli, Piergiorgio Messa, Maria Pia Rastaldi.
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Background: We have previously shown that Rab3A-KO mice have spontaneous macroalbuminuria with podocyte cytostatic changes and decreased expression of specific molecules. Aim of this study was the identification of intracellular pathways possibly linking Rab3A absence to podocyte damage.

Methods: RealTime-RTPCR-arrays were used to quantify differential gene expression. Cell proliferation was evaluated by FACS analysis of BrdU incorporation and by “Fucci assay”, based on cell transduction of GFP and geminin, fused to fluorescent markers. Activation of the MAPKinase pathway, and expression of GABA Receptors and cytoskeleton molecules were investigated by Western Blot and immunostaining.

Results: Rab3A-KO podocytes showed increased mRNA expression of Ntrk2, Fgfi2, Tsc2, Npyy and Npyy5 and a decreased expression of Ngn1 than WT cells. All overexpressed molecules are involved in proliferation and maturation of neuronal precursors and act by activating the MAPKinase pathway. Ngn1 instead regulates neuronal differentiation and synapse development.

FACS analysis showed increased BrdU incorporation in KO podocytes and the Fucci method detected more KO than WT cells in the G2 phase of the cell cycle.

KO cells also displayed higher p-MAPK/MAPK ratio than WT podocytes. Further, mRNA and protein of GABA-A Receptors (GABA-A-R) were in higher in KO than in WT cells, and expression of KO-cells with GABA-A-R agonists induced further p-MAPK increase, while incubation with a GABA-A-agonist, not with a GABAB-R agonist, caused p-MAPK reduction, increased the expression of the cytoskeleton molecules Arg and synaptopodin, and improved alpha-actinin-4 distribution.

Conclusions: Our results show that Rab3A null podocytes are less differentiated and, as it occurs in neuronal cells, a series of early genes induce proliferation through activation of the ERK/MAPK pathway.

Our data also show the potential involvement of GABA-A-Receptors in these processes; ionotropic GABA-A-R increases MAPK activity and can be modulated by specific agonists, which improve cell differentiation by cytoskeletal regulation.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1330

Endothelin(ET)-1, Via ET-R, Activates β-Arrestin-Mediated Signaling Pathways in Podocytes: Potential Implications for Proliferative Lesions in Chronic Kidney Disease Simona Buelli,1 Elena Gagliardi,1 Laura Rosano,2 Anna Pezzotta,1 Anna Bagnato,2 Giuseppe Remuzzi,3 Ariela Benigni.1
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We hereby study how this mechanism operates in proliferative kidney disorders, and whether ET-1 triggers podocyte migration via β-arrestin-mediated signaling.

Methods: Migration of differentiated podocytes and β-arrestin signaling pathway were assessed by wound healing assay and immunoprecipitation/immunoblotting.

Results: ET-1 (100nM) promotes podocyte migration via ETAR as selective antagonist BQ2312 significantly prevents cell motility. ET-1 upregulates podocyte constitutive expression of β-arrestin mRNA and protein and induces the formation of ETAR/β-arrestin/ Src signaling complex. By inducing Src activation, ET-1 promotes EGFR transactivation and Akt phosphorylation associated with active β-catenin accumulated in the cytoplasm, events preceding translocation of pro-migratory genes. Mice with adriamycin-induced nephropathy unexpectedly exhibit glomerular synchiae and few crescents at 4wk. Podocytes forming synchiae express high β-arrestin expression levels.

Conclusions: These results indicate that, as in cancer cells, ET-1 promotes podocyte migration and activates ET,R/β-arrestin-β-arrestin pathways providing new clues for therapies based on ET receptor antagonists in podocytopathies associated with crescentic lesions.

Funding: Private Foundation Support

FR-PO1329

Characterization of a β3 Adrenoceptor Pharmacophore That Predicts Mitochondrial Biogenesis Lauren P. Wills, Richard Trager, Christopher C. Lindsey, Yuri K. Peterson, Craig Cano Beeson, Rick G. Schnellmann. Pharmaceutical and Biomedical Sciences, Medical University of South Carolina, Charleston, SC.

Background: The renal tubular epithelia have capacity for repair and regeneration after insults, which can be improved by compounds that promote mitochondrial biogenesis.

Methods: To examine the impact of the β-adrenergic signaling pathway on mitochondrial biogenesis, primary renal proximal tubule cells (RPTC) and adult feline cardiomyocytes (AFC) were exposed for 24 hr to multiple β-adrenoceptor (β-AR) agonists: isoproterenol (non-selective β-AR agonist), BRL 37344 (selective β1-AR agonist), and formoterol (selective β2-AR agonist). The Seahorse Biosciences analyzer was used to quantify FCCP-uncoupled oxygen consumption rate (OCR), a marker of maximal electron transport chain activity and mitochondrial biogenesis.

Results: Isoproterenol and BRL 37244 did not alter mitochondrial respiration at any of the concentrations examined (10-1000 nM). Formoterol exposure (30 µM) resulted in increased FCCP-uncoupled OCR and mitochondrial DNA (mDNA) copy number. The effect of formoterol on OCR in RPTC was inhibited by the β2-AR antagonist propranolol. To examine the effects in vivo, C57BL/6 mice were exposed to 100 µg/kg of formoterol for 24 or 72 hr. Formoterol exposure increased kidney and heart mDNA copy number, increased mitochondrial cholesterol regulator PGC-1α (peroxisome proliferator-activated receptor gamma coactivator -1α), and induced multiple gene coding for electron transport chain

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420A
FR-PO1332

A Role for PI3-kinase in Regulating the EMT-Antagonist Protein, SARA
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Background: TGF-β1 promotes renal fibrosis, in part by promoting epithelial to mesenchymal transition (EMT). We have previously shown that the Snail anchor for receptor activation (SARA) helps maintain an epithelial cell phenotype, and that reduction of SARA by TGF-β1 or other means stimulates events associated with EMT. Investigation of signaling pathways that might regulate SARA expression demonstrated a role for PI3-kinase signaling, as evidenced by the fact that, like TGF-β1, the PI3-kinase inhibitor LY294002, caused a reduction in SARA expression and a concomitant increase in the expression of the EMT marker αSMA. However, our data suggested that the mechanism by which PI3-kinase inhibition depletes SARA differs from the mechanism that results from prolonged TGF-β1 treatment.

Methods: Standard protein, transfection, qPCR, and immunochemistry techniques were employed.

Results: To confirm the effects of chemical PI3-kinase inhibition, we established an HKC proximal tubule cell line that stably expresses an shRNA for the p85α-regulatory subunit of PI3-kinase. The activity of Akt, a downstream target of PI3-kinase, is inhibited by LY294002, but was enhanced in p85α knock-down cells. However, knock-down cells still have depleted SARA expression and increased αSMA expression. These data suggest that maintenance of SARA expression likely is mediated by PI3-kinase but independent of pAkt. It is known that SARA associates with early endosomal subcellular compartments. While, LY294002 treatment did not interfere with SARA localization, it did increase the intensity in endosome size and number. Potassium depletion, used to block internalization to the early endosome, inhibited the LY294002-induced SARA depletion. Further, co-immunoprecipitation studies demonstrated a SARA-p85α interaction that was reduced by LY294002 under control conditions, but was enhanced under conditions of inhibited internalization.

Conclusions: Our data therefore suggest that SARA is protected from degradation via its interaction with PI3-kinase at the cell membrane, but that the separation of this complex upon internalization to altered endosomal compartments leads to reduced SARA expression.

Funding: NIDDK Support

FR-PO1333

PI3K, but Not Smad3, Is Required for Normoxic Induction of HIF-1α expression by TGF-β1
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Background: The transforming growth factor (TGF)-β pathway, acting through the Smad signaling proteins, has been shown to interact with hypoxia-inducible factor (HIF) 1α in models of hypoxic kidney injury. Previously, we showed that TGF-β1 increases HIF-1α transcriptional activity and protein expression in a human proximal tubule cell (HKC) line under normoxia (21% O2) in a receptor-dependent manner. Blockade of the Smad3 signaling pathway with the dominant-negative Smad3A construct reduced normoxic TGF-β1-stimulated HIF-1α transcriptional activity. Interestingly, a dominant-negative HIF-1α construct reduced normoxic basal and TGF-β1-stimulated Smad3 transcriptional activity. Inactivation of tuberin and results in increased phosphorylation of S6Kinase, a major pathway, which increases protein translation and cell growth. Hyperglycemia increases cell matrix proteins but the pathogenic mechanisms are not fully understood.

Methods: In these studies if Smad3 was required for normoxic induction of HIF-1α expression by TGF-β1 or if phosphoinositide-3 kinase (PI3K) acts on specific residues result in its inactivation. Tuberin inactivation leads to activation of mTOR pathway, which increases protein translation and cell growth. Hyperglycemia increases cell matrix proteins but the pathogenic mechanisms are not fully understood.

Results: Our data show that inactivation of tuberin resulting activation of the mTOR pathway enhances tuberin phosphorylation and inactivation of tuberin and results in increased phosphorylation of S6 kinase, a major downstream target of mTOR. This is associated with increased fibroton and collagen IV protein expression in cultured PPT cells. Our data show that the increase in fibroton and collagen IV protein expression in tuberin-null cells is reversed upon the introduction of tuberin cDNA. In addition, our data show that blockade of mTOR with rapamycin prevents HIF-induced increase in fibroton promoter transcriptional activity in PPT cells.

Conclusions: In conclusions, we show for the first time a novel role of tuberin in the regulation of cell matrix proteins in proximal tubular cells. Our data provide an evidence that tuberin enhances tuberin phosphorylation/inactivation and results in an increase cell matrix proteins accumulation in kidney cortex.

Funding: NIDDK Support

FR-PO1334

Mammalian Diaphanos 1 (mDia1) Plays a Central Role in EMT Induced Redox Signaling 
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Background: RAGE (Receptor for Advanced Glycation end Products) is a multiligand receptor of the immunoglobulin superfamily involved in fundamental disease processes characterized by vascular pathology. Vascular stress leads to up-regulation of RAGE ligands in the vessel wall, thereby facilitating their engagement and activation of RAGE to induce pro-inflammatory and tissue-damaging responses. We previously reported that the gene expression domain of RAGE in liver with mDia-1, a member of the formin family involved in actin and microtubule reorganization. Methods: In the present study, we tested the role of mDia1 in RAGE induced oxidative stress generation and redox signaling pathways. We established primary cultures of murine aortic smooth muscle cells (SMCs) from wild-type, RAGE null and mDia-1 null mice. Recombinant Si00B was used as a prototypic RAGE ligand.

Results: We found that: 1/ RAGE activation leads to the translocation of cSRK kinase to the membrane, followed by Rac1 and Nox1 activation. Nox4 was not affected. 2/ RAGE induced superoxide production and consequent phosphorylation of GSK3β were required for WT SMCs migration. 3/ Presence of mDia1 was critical for RAGE induced cSRK translocation at the membrane, Rac1 and Nox1 activation, AKT/GSK3β phosphorylation and VSMC migration. Finally, in vivo, in a mouse model of guidewire induced femoral artery denudation, we found that mice devoid of mDia-1 had less neointimal expansion. Furthermore, NADPH oxidase activity, and phosphorylation of AKT/GSK3beta were lower in arteries of animals devoid of mDia1.

Conclusions: We conclude that mDia1 integrates oxidative and signal transduction pathways as a positive regulator of RAGE ligands, therefore acting as a regulator of pathological neointimal expansion.

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FR-PO1335

A Novel Role of Tuberin in the Regulation of Cell Matrix Proteins in Proximal Tubular Cell Lines
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Background: Matrix protein accumulation is major pathological features of diabetic nephropathy that eventually in renal failure. PI3-K/Akt pathway phosphorylates tuberin on specific residues result in its inactivation. Tuberin inactivation leads to activation of mTOR pathway, which increases protein translation and cell growth. Hyperglycemia increases cell matrix proteins but the pathogenic mechanisms are not fully understood.

Methods: Inactivation of tuberin and activation of downstream signal of mTOR, collagen IV and fibroton expression were measured in kidney cortical tissue of control and type 1 diabetic animals and in primary proximal tubular cells (PPT) incubated with normal or high glucose. In addition, promoter transcription activity of fibroton was measured in PPT cells treated with high glucose (HG) using luciferase assay.

Results: Our data show that inactivation of tuberin resulting activation of the mTOR pathway enhances matrix proteins accumulation in PPT cells exposed to HG and in kidney cortex of rats with type1 diabetes. We find also that high glucose enhances phosphorylation/translation of tuberin and results in increased phosphorylation of S6 kinase, a major downstream target of mTOR. This is associated with increased fibroton and collagen IV protein expression in cultured PPT cells. Our data show that the increase in fibroton and collagen IV protein expression in tuberin-null cells is reversed upon the introduction of tuberin cDNA. In addition, our data show that blockade of mTOR with rapamycin prevents HG-induced increase in fibroton promoter transcriptional activity in PPT cells.

Conclusions: In conclusions, we show for the first time a novel role of tuberin in the regulation of cell matrix proteins in proximal tubular cells. Our data provide an evidence that tuberin enhances tuberin phosphorylation/inactivation and results in an increase cell matrix proteins accumulation in kidney cortex.

FR-PO1336

Curecinin Blocks Ang II Induced TNFα Secretion by Inhibiting Phosphorylation of TNFα Converting Enzyme (TACE)
Siddhartha S. Ghosh, George Bassam Saffouri, Shobha Ghosh, Domenic A. Sia, Todd W. Gehr. Int Medicine/Nephrology, VCU, Richmond, VA.

Background: Angiotensin II (Ang II) by increasing TNFα secretion, can secondarily induce inflammation and aggravate renal failure. We showed that curecinin (CUR) ameliorates renal failure in rats by blocking TNFα secretion. However, the mechanism by which TNF secretion relates to Ang II/CUR is poorly elucidated. Phosphorylation of TNFα converting enzyme(TACE) regulates release of TNFα. Hence, we hypothesized that curecinin inhibits TACE signaling pathways including the MEK/ERK pathway mediate the phosphorylation of TACE. We theorized that Ang II, by activating the ERK pathway, can induce TACE

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phosphorylation and CUR, which is known to block ERK phosphorylation, can prevent TGF-β1-induced EMT and induce specific sRNA expression.

Methods: Thiglycolate elicited peritoneal macrophages (MO) from Sprague Dawley rats were plated for cell culture. The MO were treated with Ang II in the presence and absence of 10 μM CUR and/or 10 μM of ERK inhibitor PD 98059 (PD) for 24 hours; control MO were exposed to ethanol. TNFα was measured by ELISA. MO lysates were made with phosphatase and protease inhibitors (n=4–6).

Results: Ang II treated MO had a 65% increase in TNFα secretion (p<0.01), CUR and PD blunted it by 40.3% (p<0.01) and 17.4% (p>0.05) respectively. Western blot analyses showed that Ang II increased EMT markers such as α-SMA; RT-PCR for α-SMA and fibronectin were significantly increased (P<0.05). The mRNA expression of EMT markers decreased expression of E-cadherin mRNA (P<0.05) and protein (P<0.05), whereas increased expression of TNF-α (P<0.01) and IL-12 (P<0.01), with the peak level up to 15 fold increased expression of TNF-α (P<0.01) and 34 – 50.0% (p>0.05) respectively, with CUR and PD pre-treatment (CUR vs PD p<0.05).

Conclusions: Ang II increases TNFα secretion by phosphorylating TACE, a process effectively blocked by CUR. Although CUR was less effective than PD in blocking ERK phosphorylation it was more effective in blocking TNFα secretion and TACE phosphorylation. The superiority of CUR to PD may be a function of Ang II activating other phosphorylation pathways such as PKC, inhibitable by CUR but not by the MEK inhibitor PD.

FR-PO1337

High Glucose Induced Classically Activated Macrophage Facilitates Process of Epithelial Mesenchymal Transition in Renal Proximal Tubular Epithelial Cells Yansheng Jin, Xiaoliang Zhang, Kun Ling Ma, Linli Lv, Bi-Cheng Liu.

Institute of Nephrology, Tongji Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.

Background: It is the status of macrophage activation rather than infiltrated number in kidney tissues plays the key role in determining the outcome of kidney diseases. There are two types of macrophage activation including classical (M1) and alternative (M2) types. M1 induces inflammation and tissue injury while M2 may provide anti-inflammatory effect and promote tissue repair. Our previous study had demonstrated that macrophage-M1 induces inflammation and tissue injury while M2 may provide anti-inflammatory effect.

Methods: U937 and HK-2 cells were used in all experiments. High glucose (30mM) was used to stimulate U937 and M1 marker iNOS, TNF-α and IL-12 were detected by ELISA. Stimulated U937 were co-cultured with HK2 cells followed by measurement of EMT marker α-SMA,fibronectin and E-cadherin by western immunoblotting.

Results: Treatment of U937 cells with HG significantly increased the expression of iNOS mRNA (P<0.05) and the activity of iNOS (P<0.05), with the maximal iNOS activity achieved at hour 9 (P<0.01). Incubation of 30mM of HG with U937 cells for 24 hours increased expression of TNFα (P<0.01) and IL-12 (P<0.01), with the peak level up to 15 times higher than control. In addition, co-culture of HG treated U937 with HK-2 cells for 24 hours decreased expression of E-cadherin mRNA (P<0.05) and protein (P<0.05), whereas α-SMA and fibronectin were significantly increased (P<0.05). The mRNA expression of α-SMA and fibronectin in HK2 cells were also increased after incubation with HG treated U937. This change was similar with the positive control with IFNγ/LPS treatment compared to U937, which represents M1 activation, while it was not observed in the osmotic control (treatment with mannitol).

Conclusions: High glucose shifts macrophage differentiation into classically activated (M1) macrophage, which can facilitate the process of EMT in HK2 cells.

FR-PO1338

Erbn Inhibits TGF-β1-Induced Epithelial-to-Mesenchymal Transition in Renal Tubular Epithelial Cells through an ERK-Dependent Pathway Qiaodan Zhou, Rui Zeng, Chou Xu, Lilly Liu, Min Chen, Han, Gang Xu.

Division of Nephrology, Department of Internal Medicine, Tongji Hospital, Tongji Medical College, HUST, Wuhan, Hubei, China; Department of Urology, Tongji Hospital, Tongji Medical College, HUST, Wuhan, Hubei, China.

Background: Epithelial-to-mesenchymal transition (EMT) plays a crucial role in the progression of renal interstitial fibrosis, which ultimately leads to end-stage renal failure. Erbin, a member of LAP family, is recently reported to inhibit Smads and ERK pathway which are two important intracellular signaling involving in TGF-β1-induced EMT. However, the role of Erbin in the regulation of EMT and the underlying mechanisms remain to be fully understood. To that end, we aimed to evaluate the expression of Erbin in renal interstitial fibrosis nephropathy (RIF) and the potential role of Erbin in tubular EMT stimulated by TGF-β1.

Methods: Western blotting analysis for Erbin, E-cadherin and α-SMA; RT-PCR for Erbin; Immunohistochemistry for Erbin expression in Rat renal tissue; Masson staining for rat renal tissue; Immunofluorescence test for E-cadherin and α-SMA.

Results: In this study we demonstrated that the expression of Erbin was up-regulated in the tubular epithelia of 5/6-nephrectomized rats. We also showed here that TGF-β1 induced Erbin expression both in mRNA and protein level in NRK52E cells during their acquisition of an EMT phenotype. Importantly, elevated expression of Erbin inhibited EMT signaling and partial reversed EMT stimulated by TGF-β1. In the mean time, reducing Erbin expression by specific TGF-β1 phosphorylation with in vitro transcribed CTGF mRNA also increases the anti-EMT phenotype.

Conclusions: These results demonstrate that Erbin is a negative feedback molecule induced by TGF-β1 and inhibits TGF-β1-induced EMT via ERK signaling pathway.
HIF1 Modulates Chromatin Conformational Change by Recruiting KDM3A and Enhances Regulation of Glucose Transporter 3 in Endothelial Cells

Background: Hypoxia plays a crucial role in both acute and chronic kidney injury. Hypoxia inducible factor 1 (HIF1) is a master regulator of the gene expressions especially to organize glycolysis, cell proliferation and cell survival under hypoxia. Recently HIF1 was reported to regulate subsets of histone demethylases, which modify the chromatin structures. However, its role in epigenetic regulation remains unknown. We aimed at clarifying a new epigenetic mechanism via HIF1, which serve as the first-line defense against hypoxic milieu in various organs including the kidney.

Methods: We performed chromatin immunoprecipitation with deep sequencing (ChIP-Seq) to identify the genome-wide binding sites of HIF1 and clarified the histone modifications in human umbilical venous endothelial cells. We also examined chromatin conformation capture assay to identify the chromatin structural change under hypoxia.

Results: We identified that HIF1 binds to the distal regions from the transcriptional starting sites and plays an important role as an enhancer of glucose transporter 3 (SLC2A3). The reporter assay confirmed that the distal regions of SLC2A3 up-regulate the luc activity under hypoxia and overexpression of HIF1. We clarified the distal region enhances SLC2A3 expression by changing chromatin conformational structure via HIF1. Furthermore KDM3A (JMJD1a; jumonji-domain containing 1a) is a member of histone demethylases and up-regulated by HIF1 under hypoxia. We demonstrated that KDM3A is recruited to the enhancer regions of SLC2A3 by HIF1 and demethylates histone 3K4 to up-regulate the expression under hypoxia, while KDM3A is not recruited to those enhancer regions when HIF1 is knocked down. These results demonstrate that HIF1 is essential to change the chromatin conformation and recruit KDM3A under hypoxia.

Conclusions: Our findings provide new insights into the regulatory mechanism of HIF1 and epigenetic modifications of KDM3A in HUVEC under hypoxia.

Funding: Government Support - Non-U.S.

Interferon Gamma Promotes Starvation-Induced Autophagy by Activating the GCN2-ATF4 Pathway

Background: The aim of this study is to characterize the mechanisms and the consequences of IFN-γ-induced autophagy in human endothelial cells.

Methods: Human Renal Cortical Cells (HRCCs) were exposed to 10 ng/ml IFN-γ for 24 to 72 hours. Mechanisms and consequences of autophagy were evaluated by western blotting, real time PCR, immunofluorescence and small interfering RNA. An IFN-γ-induced autophagy in human epithelial cells. Conclusions: Our results suggest that the pathophysiological role of IFN-γ in human renal epithelial cells.

Funding: Government Support - Non-U.S.

A Circadian Oscillator Kid-1, Whose Transcription Is Directly Regulated by c-Myc, Participates in the Transcriptional Mechanism of Per2 through Intervening in the Network of c-Myc and Tif1β

Background: Discerning the role of the intrinsic kidney clock has important implications for cell cycle of renal tubular epithelial cells and renal function. We reported that a Zinc-finger transcriptional repressor Kid-1 is controlled by the kidney clock, but the mechanism of rhythmical Kid-1 mRNA expression remained to be determined.

Methods: There is no canonical E-box element in the promoter of Kid-1 gene. To determine whether Kid-1 is directly regulated by CLOCK, we knocked down CLOCK in NIH3T3 cells by siRNA. Predictably, Kid-1 mRNA expression level unchanged. Intriguingly, when the expression level of c-Myc was knocked down by siRNA, the level of Kid-1 mRNA was upregulated. c-Myc is a critical repressor for circadian Cyclin D1 transcription through its initiator element. Therefore, we searched Kid-1 gene for analogous sequences.

Results: We found a putative initiator element ACATTTC in Kid-1 promoter region, whose sequence is conserved among mice, rats and human. Luciferase assay and Chromatin immunoprecipitation (ChIP) assay in NIH3T3 cells confirmed that c-Myc negatively regulated Kid-1 transcription through its initiator element. Western blot analysis confirmed that transient overexpression of Kid-1 in NMRK2E cells resulted in downregulation of c-Myc and silencing of Kid-1 by siRNA in NMRK2E cells resulted in upregulation of c-Myc. Moreover, when Kid-1 was knocked down, the expression levels of Per2 mRNA and its protein product markedly decreased. ChIP assay confirmed that the more amount of lysine9 trimethylated histone H3 interacted with Per2 promoter region when Kid-1 was knocked down. Moreover, the association of c-Myc with Tif1β which is a transcriptional repressor appeared in Per2 promoter region when Kid-1 was knocked down.

Conclusions: These results suggest that Kid-1, whose transcription is negatively regulated by c-Myc, participates in the transcriptional mechanism of Per2 through intervening in the network of c-Myc and Tif1β.

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FR-PO1345

Hypercritical Role of mRNA Regulation for Hypoxia Inducible Factor 1 alpha (HIF-1alpha) Expression

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Background: Genes are regulated at multiple levels such as transcriptional as well post-transcriptional level. The expression of hypoxia-inducible factor 1 (HIF-1) is critical for many processes such as erythropoiesis, vascular growth, anaerobic metabolism, and iron transport mechanisms. Beside the oxygen-dependent hydrolylation of the regulative HIF-1 alpha subunit, there is increasing evidence for non-oxygen dependent pathways affecting HIF-1alpha expression.

In this study we addressed the question as to whether the HIF-1alpha mRNA is important for the regulation of the transcription factor complex and whether there are trans-acting factors that might act as regulators for HIF-1 activity.

Methods: We quantify the impact of transcriptional as well post-transcriptional gene regulation. Furthermore, we present a computational and experimental approach to identify RNA-binding proteins (RNA-BPs) that modulate mRNA stability of selected genes. For this purpose, we used gene expression data of a large set of microarray experiments available from the Stanford microarray database. We also applied modelling techniques to gain insight into the signalling dynamics of HIF-1.

Results: Based on large-scale expression data, we show that HIF-1alpha mRNA turnover is crucial for the activity of HIF-1 and expression of its downstream targets. We identify a hypercritical requirement in the HIF-1alpha mRNA level which highly impacts the activity of HIF-1 as a transcription factor. Further, we show, both computationally and experimentally, that the gene expression levels of a number of RNA-binding proteins is directly correlated with that of HIF-1 target genes.

Conclusions: Our integrated modelling and experimental approach highlights the importance of mRNA regulation especially under conditions that affect HIF-1alpha protein levels. We conclude that a number of RNA-binding proteins that modulate HIF-1alpha expression level and subsequently HIF-1 function.

Funding: Government Support - Non-U.S.

FR-PO1346

Regulation of HuR Expression in Renal Ischemia

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Background: HuR is a ubiquitously expressed RNA-binding protein that is increasingly shown to be a master regulator of genes associated with growth, cell division and stress response. We have demonstrated that renal HuR is specifically upregulated in proximal tubule (PT) cells following an ischemic event, where it protects these cells from subsequent apoptosis. The purpose of this study is to evaluate the transcriptional control of HuR in PT cells.

Methods: Cultured proximal tubule (PT) cell lines were grown under normal conditions or under conditions mimicking ischemic stress. Levels of HuR mRNA transcripts were determined by competitive RT-PCR and ribonuclease protection assay. Levels of HuR protein were determined by Western blotting. Gel mobility shift assays were used to detect binding of transcription factors to the 5' UTR of the HuR gene.

Results: We demonstrate that HuR mRNA is expressed in two forms with alternate 5' untranslated regions that are differentially expressed during normal growth and stress. We are exploring why PT cells express these forms under different cellular conditions, as insights into regulation of HuR will elucidate mechanisms controlling expression of genes promoting growth and cell survival. We are currently characterizing the mechanisms that control the expression of the alternate transcript, which is efficiently translated during normal growth, but down-regulated following cellular stress. Studies so far reveal that transcription factors Sp1 and NF-xB play a critical role in regulating this form of HuR mRNA. Further, we found that NF-xB stimulation of HuR expression results in its participation in a positive feedback loop that promotes expression of Akt, a protein kinase well-established as a mediator of cell survival.

Conclusions: HuR mRNA exists in two alternate forms which differ in the length of their 5'UTR and are differentially expressed during normal growth and cell stress. Elucidating these mechanisms will help us understand its protective role in ischemia-reperfusion injury, ischemic preconditioning and other models of renal cellular stress.

Funding: NIDDK Support

FR-PO1347

5-Hydroxytryptamine-Class 2 Receptor-Mediated Mitochondrial Biogenesis as a Potential Therapeutic Strategy for Treatment of Acute Kidney Injury

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Background: Renal proximal tubule cells (RPTC) exposed to acute oxidant injury exhibit mitochondrial dysfunction; recovery of cellular function in these cells is temporally related to recovery of mitochondrial function. Indicating that the mitochondria are a potential novel target for the treatment of AKI. Previous work in our laboratory demonstrated that DOI, a 5-hydroxytryptamine 2 receptor (5HT2) pan-agonist, stimulates mitochondrial biogenesis in vitro by increasing the expression of peroxisome-proliferator-receptor-y co-activator 1 (PGC-1alpha), the “master regulator” of mitochondrial biogenesis. Additionally, it was demonstrated that DOI accelerates the recovery of mitochondrial function after exposure of RPTC to acute oxidant injury, suggesting that 5-HT receptor agonists may represent a novel therapeutic strategy for the treatment of mitochondrial and cell injury.

Methods: The goal of these studies was to further explore the role of 5HT2 receptors as targets for induction of mitochondrial biogenesis using the Seahorse Biosciences analyzer as a respirometric screen in primary cultures of rabbit RPTC. Concentration-response experiments were conducted using six 5HT2 receptor agonists and six 5HT2 receptor antagonists.

Results: Four compounds increased maximal FCCP-uncoupled respiration, a test for mitochondrial biogenesis. CP-809101, a highly selective 5HT2c agonist, yielded a maximal biogenic response at 100 nM whereas three 5HT2a antagonists, SB-242084, ketanserin and MDL-100907, yielded a maximal biogenic response at 100 nM, 100 nM and 1 nM, respectively.

Conclusions: Based on these results, we postulate that 5HT2c agonism is responsible for the mitochondrial biogenic effect of DOI on cells exposed to oxidant injury. Furthermore, we suggest that 5HT2c receptor agonism signals mitochondrial biogenesis through a novel pathway.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1348

FoxO1 Is the Dominant Mediator of the Skeletal Muscle Atrophy Due to CKD and FoxO1 Can Be Inhibited In vivo by miR-486

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Background: The loss of muscle protein induced by chronic kidney disease (CKD) occurs via the ubiquitin-proteasome system (UPS). A pathway that activates the UPS in response to cell stress includes an upregulation of the expression of atrophy-related E3 ligases, Atrogin-1 and MuRF-1. It is generally accepted that forkhead transcription factors (FoxOs) can stimulate expression these E3 ligases, it is not known which isoform (Foxo1, Foxo3 or Foxo4) activates muscle proteolysis in vivo. Identifying which FoxO is required could lead to therapeutic strategies.

Methods: We studied mice with muscle-specific deletion of Foxo1 (Mfko) and examined how CKD (subtusal nephrectomy) affects muscle protein degradation. We also investigated if blocking Foxo1 expression by a micro RNA strategy changes muscle wasting.

Results: Mfko did not alter the expression of Foxo3a or Foxo4 in muscle and CKD did not cause significant muscle atrophy in Mfko mice. The expression of Atrogin-1/ MuRF-1 in muscle and the rate of muscle protein degradation were blocked by ~70% (p<0.05) relative to miR486 can damp Foxo1 translation. We examined if miR-486 influences protein metabolism in muscle cells. After transfecting a miR-486 mimic into primary cultures of mouse myotubes, the Foxo1 protein level decreased and its phosphorylation increased. Control myotubes treated with dexamethasone (Dex) had increased Atrogin-1/ MuRF-1 expression and protein degradation and these responses were largely blocked by expression of miR-486. In mice, we electroporated the miR-486 mimic into the mixed fiber, tibialis anterior (TA) muscles of CKD or Dex-treated mice. Muscle mass (the ratio of TA muscle weight to tibia length) in CKD mice significantly improved after electroporation of the miR-486 mimic and there was significant depression of Foxo1 protein and Atrogin-1/ MuRF-1 expression.

Conclusions: Our results demonstrate that Foxo1 is a dominant mediator of the CKD-induced activation of Atrogin-1/MuRF-1, and their contribution to muscle protein degradation. Manipulation of miR-486 could potentially blunt catabolic responses in muscle.

Funding: NIDDK Support

FR-PO1349

FGF23 Is Independently Associated with Vascular Calcification but Not Bone Mineral Density in Patients at Various CKD Stages

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Background: The hormone fibroblast growth factor 23 (FGF23) is involved in mineral homeostasis but may also have a role in vascular calcification and bone mineralization. Previous studies related to FGF23 and vascular and bone outcomes have been restricted to dialysis patients. The aim of the present study was to establish whether or not plasma FGF23 levels are associated with aortic and coronary calcification, arterial stiffness and bone mineral density in patients with early as well as late stages of CKD.

Methods: One hundred and fifty-three patients with CKD stages 2-5D were included in a cross-sectional study. In addition to routine biochemistry and intact FGF23 levels, aortic and coronary calcification and stiffness and bone mineral density (BMD) were assessed by multislice spiral computed tomography and automated pulse wave velocity (PWV).

Results: Plasma intact FGF23 levels were elevated in CKD patients; the elevation preceded that of serum phosphate in early-stage CKD. Patients with elevated FGF23 levels had higher aortic and coronary calcification scores than patients with lower FGF23 levels.
levels. Multivariate linear regression analysis indicated that only age (p<0.001) and FGF23 (p=0.008) were independently associated with aortic calcification score. Plasma FGF23 was neither associated with PWV nor with BMD.

Conclusions: Our data suggest that plasma FGF23 is an independent biomarker of vascular calcification in patients with various CKD stages including early stages. The associations between vascular calcification and FGF23 levels appear to be independent of BMD. It remains to be seen whether this association is independent of bone turnover and bone mass.

Funding: Government Support - Non-U.S.

FR-PO1350

microRNA-29 Is a Regulator of TGFβ-Dependent Fibrogenesis Phillip Kantharidis, Bo Wang, Rosemarie Carew, Chris Tikellis, Merlin C. Thomas, Mark E. Cooper. JDRF Danielle Alberti Memorial Centre for Diabetes Complications, Baker IDI Heart & Diabetes Institute, Melbourne, Victoria, Australia.

Background: Glomerular and interstitial fibrosis is a common pathogenic pathway for progressive kidney disease. The renal accumulation of extracellular matrix (ECM) is primarily driven by increased levels of pro-fibrotic mediators, including TGF-β1. Recent data have suggested an important role for specific microRNAs in enhancing fibrogenic signalling and sustaining pro-fibrotic phenotypes. In particular, the 3′UTR of most collagen contain target sites for miR-29 mediated translational repression. This study examines the potential functions of the microRNA-29 in TGF-dependent fibrogenesis.

Methods: The ectopic expression of pre-miR-29a/b/c or pre-miR-Control (miR-C) was induced in rat proximal tubular cells, primary messangial cells and immortalised human podocytes using Oligofectamine. Cells were then exposed to TGF-β1 (1-10ng/mL) for 3-10 days and the expression of collagen assessed using RT-PCR and immunoblotting.

Results: Treatment of renal cells with TGF-β1 reduced the expression of the miR-29a/b/c and increased fibrogenesis. By contrast, ectopic expression of miR-29 repressed the expression of collagen I and IV, at an mRNA and protein level, and attenuated TGF-dependent fibrogenesis in both renal cell lines. Luciferase-reporter constructs incorporating the 3′UTR of collagen I and IV demonstrated that TGF-β1 increased luciferase activity (collagen expression) and that miR-29 was able to prevent this increase.

Conclusions: miR-29 potentially plays an important role in TGF-β1-mediated collagen synthesis in renal cells. These miRNAs potentially represent a new target for the development of anti-fibrotic therapies for the treatment of chronic kidney disease.

Funding: Government Support - Non-U.S.

FR-PO1351

Increased Zinc-α2-glycoprotein White Adipose Tissue Synthesis in Chronic Kidney Disease: Nutritional and Metabolic Impacts Caroline Pelletier,1 2 Laetitia Koppe,1 2 3 Emilie M. Kalbacher,2 4 Denis Fouque,2 4 Christophe O. Soulage,1 4 CarMen u1000, INSERM, France; 2 Univ Lyon 1, France; 3 INSA, Lyon, France; 4 HCL, Lyon, France.

Background: Chronic kidney disease (CKD) is frequently associated with protein energy wasting which has been recognized as a predictive factor of mortality. Zinc-α2-glycoprotein (ZAG) has been proposed as a new adipokine involved in body weight control through its lipid mobilizing activity. We hypothesized that the urieemic environment in CKD may interact with the adipose tissue, resulting in an over-production of ZAG and therefore contributing to metabolic disturbances observed in CKD patients.

Methods: ZAG level was quantified in mouse 3T3-L1 adipocytes after incubation in culture medium containing either urea (30mM), plasma from healthy volunteers, CKD or haemodialysis (HD) patients (20%,v/v). ZAG was also measured in white adipose tissue (WAT) from 5/6 nephrectomized or controls rats. Plasmas from healthy volunteers, 8 CKD and 8 HD patients and subcutaneous adipose tissue (SAT) biopsies from 5 CKD patients and 9 non uremic individuals were collected. ZAG protein content was quantified in plasma, white adipose tissue or 3T3-L1 adipose cells by Western blotting.

Results: Uremic plasma but not urea or control sera, induced a significant increase in ZAG protein content (+224%,p<0.001) in 3T3-L1 adipocyte associated with an increase in basal lipolysis (+153%,p<0.005). 5/6 nephrectomized rats exhibited a significant decrease in WAT accretion (+44%,p<0.006) and a higher content of ZAG in WAT (+58%,p<0.02). ZAG protein level in WAT was negatively correlated with adipose tissue mass (p=0.006). Human plasma concentration of ZAG was increased in CKD patients as compared with healthy volunteers (+279%,p<0.001). Human SAT from CKD patients showed a higher content of ZAG protein level (+234%,p=0.042).

Conclusions: Undefined circulating factors in CKD, but not urea itself, increase ZAG production in adipocytes. These results suggest that the increase of ZAG serum levels reported in CKD patients could be due to an overproduction of ZAG by adipose tissue. The increase in ZAG could be a major contributor of fat mass loss and PEW in CKD patients.

Funding: Government Support - Non-U.S.

FR-PO1352

The Relationship between Vitamin D and Parathyroid Hormone in Chronic Kidney Disease: Is There a Threshold? Marie Metzger,1 Pascal Houllier,2 Martin Flamant,1 Jean-Philippe Haymann,3 Marc Froissart,3 Benedicte Stengel,2 Pablo A. Urena.1 2 3Inserm U1018, Villejuif, France; 2Paris Descartes University, Paris, France; 3Bichat Hospital, Paris, France; 4Tenon Hospital, Paris, France; 5Clinique du Landy, St Ouen, France.

Background: Vitamin D is essential for the regulation of parathyroid hormone (PTH) synthesis. According to the last KDIGO guidelines, serum 25(OH)D concentration might be measured in CKD patients and vitamin D deficiency corrected using treatment strategies recommended for the general population. However, there is no consensus on what define the adequate 25(OH)D values in CKD patients regarding to the level of PTH.

Methods: We used data from the NephroTest cohort including 916 adult patients with ND-CKD stages 1 to 5 and free from vitamin D supplementation to assess the relationship between PTH and 25(OH)D, and to test the existence of a threshold.

Results: Median values (IQR): measured GFR by 51Cr-EDTA: 38(28-52) ml/min/1.73m²; 25(OH)D: 17(13-27) ng/ml; PTH: 64(40-107) pg/ml. Measured ionized calcium (sd): 1.21 (0.07) mmol/L. PTH values were better predicted by a linear piecewise regression model of log(PTH) on 25(OH)D with a threshold of 9 ng/ml than by a linear regression model (p=0.01). Adjusting for ionized calcium and measured GFR significantly improved model predictions (R²=0.38 vs 0.10 for the model without adjustment) and reduced the 95%CI around the 9 ng/mL threshold = [6-21 ng/mL]. Estimated slopes were significantly negative before and after the threshold.

Funding: Government Support - Non-U.S.
Results: 1) HGF/FGF mRNA increased linearly in mDCT cells incubated with Glu at 10-3, 10-4, and 10-5 M (p<0.001 vs. 5.5 mM), but declined by 30% of 30 mM HGF/FGF protein increased linearly at 10-4 and 20 nM Glu (p<0.005 vs. 5.5 mM) but declined by 30 and 20 mM. 2) In a subset of clinical trial patients, with the randomization blind intact, MACR and uHGFIN/cr decrements were strongly associated; MACR stability/worsening was associated with higher MACR/urinary excretion.

Conclusions: 1) HGF/FGF mRNA/protein levels in mDCT cells are regulated at least in part by medium Glu content. Moderately high Glu increased, but extremely high Glu attenuated, HGF/FGF expression. 2) HGF/FGF correlated with treatment responses in DN. Taken together, these data suggest that HGF/FGF, which influences autophagy by regulating phagosomal-lysosomal trafficking, is regulated at least in part by Glu exposure; reflects tubular injury in vivo, is modifiable in DN, and may be a target for novel DN therapies and a measure of therapeutic success or failure.

**FR-PO1354**

**Osteoporogen Is Associated with Inflammation, Atherosclerosis and Mortality in CKD Patients Stage 3-5**

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**Background:** Osteoporogen (OPG) regulates bone mass by inhibiting osteoclast differentiation and activation, and also plays a role in vascular calcification. Recent research has implicated OPG in atherosclerosis, but epidemiological confirmation of this is sparse. In this study we evaluated the relationships between OPG levels and inflammatory and occult markers (OS) markers, atherosclerosis and mortality in CKD patients stage 3-5.

**Methods:** 162 patients (median age 61 years, 62% males; 50 hemodialysis (HD), 57 peritoneal dialysis (PD) and 50 CKD stages 3-4) were studied. All survivors completed 36 months of follow-up. Clinical characteristics were documented, and markers of mineral metabolism, inflammation, atherogenesis, bone metabolism and mortality were assessed similar to the mini-mental state exam. We used linear regression analyses to examine the association between abnormalities of mineral metabolism levels with outcomes in patients with advanced CKD not requiring dialysis (n=247) and ESRD (n=358), aged, 67± 12 years of follow-up. In multivariate analyses including all predictor variables, age, gender, race, diabetes, estimated glomerular filtration rate (eGFR), estimated glomerular filtration rate, iPTH, and log FGF23 were no longer significant (p>0.05 for each). Increasing log 1,25D levels were protective of cognitive impairment (β= -1.36±0.24; p<0.001). After adjustment for age, race, HIV and hepatitis C serostatus, and eGFR, estimated glomerular filtration rate was included in the Cox model for analysis, vitamin D was not associated with proteinuria (OR=1.36 per log-ing/liter lower, p<0.40). After adjustment for age, race, HIV and hepatitis C serostatus, diabetes, hypertension, and CKD, lower vitamin D levels were associated with over a 2-fold higher risk of proteinuria (OR=2.77 per log-ing/liter lower, p<0.04).

**Conclusions:** Vitamin D deficiency is prevalent in dialysis users, though fewer HIV-infected patients have low vitamin D levels. After accounting for CKD and CKD risk factors, lower vitamin D levels are associated with a higher risk of proteinuria. Studies are needed to determine predictors of vitamin D deficiency and whether vitamin D repletion ameliorates proteinuria in this setting.

**Funding:** NIDDK Support, Other NIH Support - NIDA

**FR-PO1355**

**Protein-Energy Wasting Abolishes the Association between Fat Mass and Bone Mineral Density in End-Stage Renal Disease Patients**

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**Background:** Low bone mineral density (BMD) is common in end-stage renal disease (ESRD) patients and leads to worse outcome. Many studies have demonstrated a positive association between fat mass and BMD. However, it is not known if protein-energy wasting (PEW) affects this association in ESRD.

**Methods:** 332 ESRD patients from an ongoing prospective cohort study including incident patients who were close to beginning dialysis replacement therapy were included. Total BMD was measured by dual energy X-ray absorptiometry (DXA) and expressed as T-score, indicating the number of standard deviations from the mean scores for 30-year old normal men and women separately. Fat mass was measured by dual energy X-ray absorptiometry. Truncal fat mass distribution was measured by DXA also. Subjective global assessment (SGA) was used as a surrogate of PEW. Spearman rank correlation analysis was used to determine the association between T-score and selected parameters.

**Results:** 100 patients had signs of PEW (SGA ≥2), and these patients had lower BMD t-score compared to the non-wasting group (1.67±0.3 vs 1.36±0.1; p<0.001). BMD t-score positively correlated with truncal fat mass (r=0.2775, p=0.001), non-truncal fat mass (r=0.2381, p=0.001), and total fat mass (r=0.3017, p=0.001) in non-wasted ESRD patients. However, in wasted patients these associations did not attain statistical significance (truncal fat mass, r=0.1604, ns; non-truncal fat mass, r=0.1538, ns; total fat mass, r=0.1564, ns).

**Conclusions:** Whereas BMD t-score positively correlated with truncal fat mass, non-truncal fat mass and total fat mass in non-wasted ESRD patients, no such associations were found in wasted ESRD patients. Our findings suggest that PEW abolishes the normal and expected association between fat mass and BMD.

**Funding:** Pharmaceutical Company Support

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

**426A**

Chronic Kidney Disease and Its Complications - II

FR-PO1358
Mineral Metabolism and Physical Function in Patients Undergoing
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2
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Background: Chronic kidney disease patients have a phenotype of premature aging,
inclusive of poor physical function. Declining eGFR is related to poor physical performance
using both objective and subjective measures. Abnormalities of parathyroid hormone (PTH),
Phosphorus (Pi), Calcium (Ca) as well as 25-hydroxy Vitamin D (25OHD) deficiency are
widespread in dialysis patients and may play a role in poor physical / muscle function in
these patients. In this analysis, we explore this potential relationship.
Methods: We examined the cross-sectional relationship of 25(OH)D, PTH, Pi, Ca
and physical performance in the baseline population of a published RCT of low intensity
exercise in 44 hemodialysis patients, using stored serum samples. Outcomes of interest
were the Short Physical Performance Battery (SPPB), which includes measures of strength,
endurance and balance and measures of lean body mass by Dual-energy X-ray absorpiometry
(DXA). Leisure time physical activity was assessed by self report using the Physical Activity
Scale for the Elderly (PASE).
Results: Serum samples were analyzed in 41 patients with median dialysis vintage of
2 years. 25OHD levels were less than 10 ng/ml in 38% of patients. A 10 pg/ml increase
in PTH was associated with a 0.05 kg decrease in whole body lean mass (p=0.02) after
adjusting for age, gender, randomization group, 25OHD, Ca and Pi. No relationship was
noted between 25OHD, Ca or Pi and lean muscle mass. Also, no baseline mineral metabolism
parameters were associated with SPPB or PASE scores.
Conclusions: We found a relationship between high PTH and low muscle mass by
DXA. High PTH increases intracellular Ca in muscle, and therefore likely plays a role in
muscle metabolism. Loss of muscle mass can be debilitating for CKD patients. We found
no associations between 25OHD levels, Ca or Pi and muscle mass/ function or on measures
of self report. This analysis provides further grounds for conducting interventional studies
of agents to lower PTH in CKD patients to prevent loss of muscle mass.
Funding: NIDDK Support, Other NIH Support - NIH General Clinical Research
Center M01 RR000054, Other U.S. Government Support, Private Foundation Support

FR-PO1359
Cholecalciferol Supplementation Does Not Affect Insulin Sensitivity in
Non-Diabetic Patients with Moderate Impairment of Renal Function
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Background: Reduced insulin sensitivity is a well-known phenomenon in end-stage
renal disease (ESRD), but is also present at other manifestations of kidney diseases such
as micro albuminuria, nephrotic syndrome and mild-moderate reduced renal function.
Alterations in vitamin D metabolism have been suggested to influence the insulin
resistance.
The aim of this study was to evaluate the potentially effect of 10 weeks high dosage
vitamin D3 (cholecalciferol) supplementation in non-diabetic patients with moderate
chronic kidney disease (CKD).
Methods: 24 patients with non-diabetic CKD stages 3-4 (Glomerular filtration rate
(GFR) measured using Iohexol® clearance 15-60 ml/min/1.73m2), low serum 25-OHvitamin D levels (< 75 nmol/L) and elevated fasting serum insulin levels (>10 mU/L)
were included in a randomized, placebo-controlled, two-way cross-over study to receive
daily either 3200 IU (80 µg) vitamin D3 (cholecalciferol; TillVal D®) or placebo in 10
weeks. Insulin sensitivity was assessed at the end of each treatment period as M-value, i.e.
glucose infusion rate divided by lean body mass (estimated with bio impedance) during the
assumed steady state (60-120 min) using a hyperinsulinemic (40 mU/m2/min) euglycemic
(5.6 mmol/L) clamp.
Results: 19 (79%) patients (M/F 13/6, age 65.8±13.7 years, BMI 28.8±4.7 kg/m2,
GFR 35±10.7 ml/min/1.73m2 , serum 25-vitamin D 51±15 nmol/L and fasting insulin 15±7
mU/L; mean±sd) completed both treatment and placebo periods. No significant difference
in insulin sensitivity (mean M-values) was found between cholecalciferol supplementation
and placebo (8.0±3.5 vs. 8.1±3.3 mg/kg lean body mass/min, p=0.89) despite significantly
difference in mean serum 25-OH-vitamin D between the placebo and vitamin D3 periods
(52±14 vs. 85±17 nmol/L, p <0001).
Conclusions: Preliminary analyses reveal that supplementation with high dosage
vitamin D3 (cholecalciferol) does not alter insulin sensitivity (M-value) in patients with
non-diabetic moderate impaired kidney function (CKD 3-4).

FR-PO1360
Hypomagnesemia and Glomerular Hyperfiltration in Diabetes Mellitus
Type 2 P.C. T. Pham,1 P.M. T. Pham,2 P.T. T. Pham.3 1Nephrology, UCLA-OVMC,
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Background: Glomerular hyperfiltration (GlomHyper) and hypomagnesemia are
commonly observed in diabetes mellitus type 2 (DM2). We examine the relationship
between DM2 GlomHyper and hypomagnesemia.
Methods: DM2 patients without known kidney disease evaluated at UCLA-OVMC
during January-March 2001 were included. Data retrieved include serum creatinine,
hemoglobin, hemoglobin A1C (HbA1C), routine electrolytes, lipid profiles, urinalyses,
history of hypertension, and pharmacy profiles. Estimations of the presenting glomerular

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filtration rate (eGFR) were determined by the CKD-epi formula. Multivariate analyses were
performed to determine if any clinical factors were associated with GlomHyper, defined
as eGFR greater than 120 mL/min/1.73 m2.
Results: There were 550 patients (54% females); mean age 57.5±11.0 years; eGFR
95.7±14.8 mL/min/1.73 m2. Twenty-nine patients had GlomHyper. GlomHyper had
significant negative correlations with age, hypertension, serum calcium, and the use of
aspirin, RAS inhibitors, and diuretics, and a positive correlation with HbA1C. Although
the correlation between serum magnesium and GlomHyper did not reach statistical
significance, it had a significant negative correlation with eGFR. Analysis of the interaction
between magnesium and calcium levels (calcium x magnesium) revealed a more significant
correlation with GlomHyper than either calcium or magnesium level alone, Pearson
coefficients: -0.13 (p=0.008),-0.11 (p=0.03), and -0.08 (p=0.07), respectively. Another
multivariate analysis revealed a significant correlation with lower magnesium levels and
GlomHyper in the stratum with calcium levels below median, but not in the higher calcium
stratum (coefficient: -0.27, p=0.0001).
Conclusions: The interaction factor (magnesium x calcium) revealed the strongest
correlation with GlomHyper compared to either factor alone. We speculate that the DM2
GlomHyper induces urinary loss of both cations, but while hypocalcemia directly exacerbates
GlomHyper via hypocalcemia-associated afferent arteriolar vasodilation, hypomagnesemia
exerts an indirect effect via hypoparathryoidism-induced hypocalcemia.

FR-PO1361
Fibroblast Growth Factor 23 and Chronic Kidney Disease Progression
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3
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Background: Recent reports have suggested that fibroblast growth factor 23 (FGF-23)
may be a risk factor for CKD progression. We assessed the relationship between circulating
levels of intact FGF-23 and other mineral metabolism related factors (parathyroid hormone
(PTH), serum calcium (Ca) and phosphate (P)) and their relationship to progression to end
stage renal disease (ESRD).
Methods: We followed 196 clinically stable adult CKD (eGFR>15 ml/min/1.73m2)
patients prospectively for a median of 35 months. Plasma levels of intact FGF-23 were
measured by ELISA (Immutopics, San Clemente, CA).
Results: FGF-23 levels were above reference ranges in 6% with median (P25-P75) of
17 (11-29) pg/mL. The mean±SD age of the study population was 61±15 years, 57% were
male, 19% African American and 32% diabetic; 19% of subjects were CKD KDOQI stage
1 and 2, 52% stage 3 and 29% stage 4. Hyperphosphatemia (P>4.5 mg/dL) was present
in 9% and elevated PTH (PTH>65 pg/mL) in 58%. FGF-23 and PTH levels correlated
inversely with eGFR and were positively correlated with each other (p<0.001). PTH levels
were inversely related to serum Ca (r=-0.24, p=0.002) and FGF-23 levels to serum P levels
(r=0.17, p=0.02). ESRD occurred in 35 participants, who had significantly lower eGFR
and higher PTH and FGF-23 levels at baseline (all p<0.001). In a multivariate Cox model,
the strongest predictors of CKD progression were eGFR, level of proteinuria and PTH
at baseline (HR , 95% CI for doubling: 1.8, 1.2-1.8). FGF-23 levels were not related to
outcome (HR 1.1, 95%CI: 0.7-1.8).
Conclusions: FGF-23 levels are inversely correlated with eGFR and contribute
to abnormal mineral metabolism in CKD, but do not predict CKD progression. PTH
may have a role in predicting CKD progression apart from its role as a risk factor for
cardiovascular disease.
Funding: NIDDK Support

FR-PO1362
Differences between Hospitals in Attainment of Parathyroid Hormone
Treatment Targets in Chronic Kidney Disease Do Not Reflect Differences in
Quality of Care Mieke J. Peeters,1,2 Arjan D. Van Zuilen,3 Jan A.J.G. van den
Brand,1 Peter J. Blankestijn,3 Marc A.G.J. Ten Dam,2 Jack F. Wetzels.1 1Radboud
University Nijmegen Medical Center, Nijmegen; 2Canisius Wilhelmina Hospital,
Nijmegen; 3University Medical Center Utrecht, Netherlands.
Background: Transparency in quality of care (QoC) is stimulated and hospitals are
compared and judged on the basis of their performance on specific treatment targets. In
patients with chronic kidney disease (CKD), QoC differed significantly between hospitals.
[NDT 2010;25:3647-3654] This was not explained by available patient characteristics. In
this analysis we explored additional parameters to explain differences between centers in
attainment of PTH treatment targets.
Methods: Using baseline data of the MASTERPLAN study, we selected one of the
worst (center A) and one of the best (center B) performing hospitals. Differences between
the two treatment centers were analyzed from the year prior to start of the MASTERPLAN
study until the baseline evaluation and determinants of PTH were assessed.
Results: 101 patients from center A (median PTH 9.9 pmol/L, 63 patients above target)
and 100 patients from center B (median PTH 6.5 pmol/L, 32 patients above target), were
included. In multivariate analysis kidney transplant status, MDRD-4, and treatment center
were independent predictors of PTH. However, when MDRD-6 (which accounts for serum
urea and albumin) was used instead of MDRD-4, the center effect was reduced. Moreover,
after calibration of the serum creatinine assays treatment center no longer influenced PTH.
Analysis of clinical practice did not reveal differences in PTH management between the
centers. Notably, hyperparathyroidism resulted in a change in therapy in less than 25%
of patients.

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FR-PO1363
An Analysis of the Presence of Pathogens in Hemodialysis Subjects Using Ibis Technology: Results from the IMPACT Study Comparing Paricalcitol and Cinacalcet for the Treatment of Secondary Hyperparathyroidism (SHPT) in Hemodialysis Patients.

**Methods:** IMPACT was a randomized 28 wk, phase 4, international, open-label study of subjects undergoing hemodialysis receiving IV (Stratum 1) or oral paricalcitol (Oral Stratum) with supplementary cinacalcet for hypercalcemia, or cinacalcet with low-dose vitamin D. Ibis, a technology combining PCR and electrospray ionization mass spectrometry, can detect a broad range of pathogens. Baseline and final samples were tested using Ibis. Ibis is currently an investigational research tool; the clinical implications and interpretations of these results are not established.

**Results:** In IV Stratum there were 62 paricalcitol subjects and 64 cinacalcet subjects (60% male, mean age: 61±12 years, mean duration of dialysis: 4.1±4.0 years). In Oral Stratum there were 72 paricalcitol subjects and 70 cinacalcet subjects (65% male, mean age: 65±13 years, mean duration of dialysis: 3.9±3.2 years). Ibis results are shown below.

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Paricalcitol</th>
<th>Cinacalcet</th>
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<tbody>
<tr>
<td>Bacterial/Fungal Infections</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral Infections</td>
<td>1</td>
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**Conclusions:** Overall, a variety of bacterial, fungal, and viral species were detected using Ibis. This is the first report of Ibis results in hemodialysis subjects. Once widely available, this technology may help in the timely identification of pathogens leading to optimal treatment of infections.

**Funding:** Pharmaceutical Company Support

FR-PO1364
Circulating Klotho Is Decreased in Pediatric Hemodialysis Patients

**Background:** Klotho is both a membrane-bound and circulating protein produced by the kidney. Membrane-bound klotho is a critical cofactor for the hormone fibroblast growth factor-23 to regulate phosphorus and vitamin D homeostasis. Circulating klotho protein has been detected in blood and urine in humans; however its functions are unknown. In mice, klotho deficiency induces vascular calcifications, osteopenia, and premature aging, a phenotype similar to chronic kidney disease (CKD). Wild-type mice with CKD have decreased plasma and urinary klotho compared to controls. Over-expression of Klotho in these mice decreases soft tissue calcification, preserves renal function and delays progression of CKD. Recent studies in adult CKD patients showed a progressive decrease in urinary klotho with worsening kidney function, but whether serum klotho levels are affected by CKD is unknown in both adult and pediatric patients. In this study we hypothesized that circulating klotho is decreased in pediatric patients with CKD.

**Methods:** Twelve pediatric patients on chronic hemodialysis (4 males and 8 females, 2-17 yrs of age) and 9 healthy controls (1 male and 8 females, 3-12 yrs of age) were recruited. Results: Serum klotho levels ranged from 1263 to 5265 pg/ml in healthy controls, and from 378 to 5389 pg/ml in hemodialysis patients. Mean serum klotho levels were lower in hemodialysis patients compared to controls (1542 ± 1569 vs 2741 ± 1355 pg/ml, p<0.05). In hemodialysis patients, serum klotho levels inversely correlated with age (r=−0.74, p<0.01) and directly with serum creatinine (r=0.62, p<0.05). No significant correlation was found between serum klotho and serum phosphorus (r=−0.33), calcium x phosphorus product (r=0.23), serum intact parathyroid hormone (r=−0.2), serum 25(OH)D (r=0.12), or serum 1,25(OH)2D (r=0.26). No correlation between serum klotho levels and age or serum calcium was found in the control group.

**Conclusions:** These results prove our hypothesis that circulating klotho is decreased in pediatric CKD patients. Further studies are needed to determine the role of klotho in CKD pathophysiology.

FR-PO1365
Obesity-Related Glomerulopathy: Mast Cells Infiltration and Tubulointerstitial Lesions

**Methods:** We examined the participants who visited twice at 1993 and 2003 screening in Okinawa, Japan, and have data serum creatinine and uric acid. Total number was 16,796. Serum creatinine was measured by Jaffe method in 1993 and the enzymatic method in 2003. Serum creatinine was measured by the enzymatic method (converted to the value of measured by enzymatic method) and the estimated GFR (eGFR, ml/min/1.73m²) was

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calculated by the Japanese Society of Nephrology (Matsuo S et al. Am J Kidney Dis 2009). Hyperuricemia (H) was defined as serum uric acid ≥7 mg/dl and over in both sexes, and others were Normouricemia (N). Based on the absence or presence of H in 1993 and 2003 screening, we categorized into 4 groups as: Group 1 to 4 of N/N, H/N, N/H, and H/H. UA, serum uric acid (mg/dl). Trends were analyzed by analysis of variance.

Results: In all categories, UA was significantly associated with eGFR, suggesting that increase (decrease) in serum uric acid was associated with decrease (increase) in eGFR. Among the various stages of CKD is not well characterized.

Conclusions: Results suggest that maintaining normal range of serum uric acid is important to maintain eGFR. 

Funding: Government Support - Non-U.S.

FR-PO1368
CKD Stage Modifies the Association of Urine Microalbumin-Creatinine Ratio (UACR) with Loss of Kidney Function in a Large Cohort of US Veterans Csaba P. Kovesdy,1,2 Evan H. Lott,2 Jun Ling Lu,4 Sandra M. Malakauskas,2 Jennie Z. Ma,2 Mark D. Okusa,3 Kaymar Kalantar-Zadeh,2 Salem VA Medical Center; 1University of Virginia; 3VA Informatics and Computing Infrastructure; 2Salen Research Institute; 1Harbor UCL.

Background: The association of albuminuria with the progression of CKD in patients with various stages of CKD is not well characterized.

Methods: We examined the association of UACR with the slopes or eGFR in a nationally representative cohort of 275,479 US veterans with at least 3 eGFR values over up to 5 years of follow-up. Associations of UACR with slopes overall and stratified by baseline CKD stage were examined in linear and logistic regression models. Models were adjusted for sociodemographics, comorbidities, blood pressure and laboratory variables.

Results: The median (interquartile range) of eGFR slopes was -1.25 ml/min/1.73m²/year (-3.85, 0.92). A 1-unit increment in UACR on natural-log scale was associated with lower decline in eGFR, whereas those with persistent normouricemia (N/N) and returned to normouricemia (N/H) showed slower decline in eGFR. Relation between change in eGFR & UA

FR-PO1369
Aristolactam-DNA Adducts in the Renal Cortex: Biomarkers of Internal (Environmental) Exposure to Aristolochic Acid Kathleen G. Dickman,1 Sandra Karanovic,2 Ivana Vukovic-Lela,2 Karla Tomic,2 Damir Ditrich,2 Zelimir Stipanic,2 Robert Turesky,3 Robert A. Grollman,13 Jovan Nikolic,2 Bojan Jelakovic,2 Stony Brook Univ, Stony Brook, NY; 2Univ. of Zagreb, Zagreb, Croatia; 3General Hospital Dr. Jure Bencetic, Slavonski Brod, Croatia; 4General Hospital, Ozdak, Bosnia and Herzegovina; 5NY State Dept. of Health, Albany, NY; 6Clinical Center Serbia, Belgrade, Serbia.

Background: Endemic (Balkan) nephropathy (EN) is a chronic tubulointerstitial disease that is highly associated with urothelial cell carcinomas (UCC) of the upper urinary tract. Both diseases have been linked to exposure to aristolochic acid (AA), a nephrotoxin and carcinogen produced by Aristolochia plants that grow in wheat fields in endemic sites and contaminate flour used to prepare bread. A review with DNA to form aristolactam-DNA (AL-DNA) adducts that lead to A:T transversions in the tumor suppressor gene TP53. Due to inefficient repair, adducts persist for years in the renal cortex and thus serve as an exposure biomarker. Here, we present evidence linking AA exposure to UCC in endemic regions of Bosnia, Croatia and Serbia that harbor EN.

Methods: DNA was extracted from renal cortex and tumors obtained from 67 patients from endemic sites of patients with nephroptuererectomy for UCC. Ten subjects from nonendemic sites served as controls. Renal cortical AL-DNA adducts were quantified by a ³²P-postlabelling assay. TP53 mutations in UCC were identified by chip-sequencing technology.

Results: Most endemic subjects had observed Aristolochia plants in their wheat fields in the past, and were therefore likely to have ingested AA-contaminated bread. We detected aristolactam-DNA adducts in 70% of the endemic cohort, and the chemical identity of these lesions was verified by mass spectrometry. A:T mutations in TP53 were present in 25% of the endemic cases, and AL-DNA adducts were also found in 94% of these cases, emphasizing the close association of these two biomarkers. In contrast, neither AL-DNA adducts nor TP53 mutations were detected in nonendemic subjects.

Conclusions: Aristolochic acid is the primary causative agent of urothelial carcinomas of the renal pelvis and ureter in EN subjects.

Funding: Other NIH Support - NIEHS PO1ES004068 and RO1ES019564, Government Support - Non-U.S.

FR-PO1370
Sustained-Release Tablets of Orally-Active Prostacyclin Analogue, Beraprost Sodium, for Patients with Non-Diabetic Chronic Renal Failure Toshihiro Fujita,1 Akio Koyama,1 Fumitake Gejyo,3 Hideki Origasa,1 Masanao Isono,1 Takashi Kitiyama,2 Nephrology and Endocrinology; University of Tokyo; 1University of Tsukuba; 2Nagoya University; 3Biostatistics and Clinical Epidemiology, University of Toyama; 4Toray Industries, Inc.; 5Astellas Pharma, Inc.

Background: Increasing evidence points to the protective effects of prostacyclin on kidney in pathophysiological conditions. Several nonclinical studies have suggested beraprost sodium (BPS), an orally active prostacyclin analogue, prevents progression of chronic renal failure (CRF) by maintaining renal blood flow and attenuating tubulointerstitial lesion.

Methods: This study was designed as a randomized double-blind placebo-controlled comparative study in CRF patients [serum creatinine (Scr) 1.5 to 4.5 mg/dl (male), 1.3 to 4.0 mg/dl (female)] to evaluate the effect of sustained-release tablets of BPS (TRK-100STP, 50µg/day) on the progression of non-diabetic chronic renal failure (CRF). The patients were treated with TRK-100STP twice daily at 120 µg/day (n=36), 240 µg/day (n=41) or placebo (n=35) for 28 weeks after a 22-week run-in period in which the patients were treated with the placebo.

Results: The primary endpoint (difference in 1/SCR slope between the run-in period and treatment period for the 240 µg group) showed no statistically significant difference, however, a significant change was observed in the 120 µg group. Sub-population analysis of patients with a Scr of 2.0 mg/dl or higher showed a significant amelioration of the 1/Scr slope in the 240 µg group, and improvement of the eGFR in both active groups. The main ADRs observed in the 112 patients were headache and hot flush, which were expected to be managed.

Conclusions: The results indicate that TRK-100STP has a beneficial effect on the progression of non-diabetic CRF, especially in patients with Scr of 2.0 mg/dl or higher. An international P-Illb-II study using a renal composite endpoint has already started in Japan, China, Hong Kong, South Korea, Taiwan, Malaysia, Thailand and Philippines.

Funding: Pharmaceutical Company Support

FR-PO1371
Risk Factors for Renal Disease Progression after Orthotopic Liver Transplantation Joseph Craig Longenecker,1,2 Michelle M. Estrella,2 Richard M. Ugarite,3 Mohamed G. Atta,2 Kuwait University Faculty of Medicine, Kuwait; 2Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Chronic kidney disease (CKD) is an important long-term complication of orthotopic liver transplantation (OLTx) patients. Few studies have assessed risk factors for CKD progression in post-Tx patients in the era of the Model for End-Stage Liver Disease (MELD).

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Methods: Using medical record and UNOS registry data from May 1995 to April 2009, this non-concurrent prospective cohort study followed 419 primary OLTx adults at a tertiary hospital for up to 2 years. Pre-Tx and 3-, 6-, 12-, and 24-month post-Tx glomerular filtration rates were estimated (eGFR, using the CKD-Epi formula), along with other baseline clinical parameters. The pre-MELD era was defined as time until February 2002.

Results: The mean age was 51 years, 67% of the cohort was male, and 39% received OLTxs in the pre-MELD era. The median pre-transplant and 12-month post-Tx eGFRs were 82 and 63 ml/min/1.73m2, respectively, representing a 20% decline in eGFR. After 24 months, the median eGFR was 59. Over 12 months, eGFR declined >33% in 31% of participants; and for those followed for 24 months, 42% declined >33%. Among those with pre-Tx eGFR >90, 60-89, and 36-59 ml/min/1.73m2, 70%, 52%, and 9% declined to a lower eGFR category over 12 months, respectively. Decline in eGFR primarily occurred in the first three months post-Tx. Progression to a lower eGFR category was associated with age>55 (versus age≤45 years; adjusted odds ratio, AOR=8.0, p<0.001), and marginally associated with hemodialysis in the post-Tx period (AOR=4.8, p=0.08), and diabetes (AOR=1.9, p=0.09). No association was present with gender, race, hepatorenal syndrome, hypertension, or hepatitis C virus. After 24 months, eGFR decline >33% was lower in the MELD era compared to the pre-MELD era (AOR=0.5, p=0.03).

Conclusions: Renal function declined substantially after OLTx, and was associated with older age, post-Tx hemodialysis requirement, diabetes, pre-MELD era, and higher pre-Tx eGFR.

FR-PO1372

Fibroblast Growth Factor 23 Is a Risk Factor for Rapid Progression of Chronic Kidney Disease in Elderly Patients 1Eiichiro Kanda, 2Sei Sasaki. 1Tokyo Kyosai Hospital, Tokyo, Japan; 2Tokyo Medical and Dental University, Tokyo, Japan.

Background: The level of fibroblast growth factor 23 (FGF23) is increased at later stages of chronic kidney disease (CKD). FGF23 as a risk factor for the progression of CKD in elderly CKD patients has not been fully established.

Methods: 105 elderly CKD patients who had never used calcium or vitamin D supplements were enrolled in this study in Tokyo, Japan. We compared estimated glomerular filtration rate (eGFR) at the start of the study with that two months later and evaluated the FGF23 level should be monitored from an early CKD stage.

Results: The mean age of the study population was 61±15 years; 57% were male, 19% African American and 32% diabetic; 19% of subjects were CKD KDOQI stage 1 and 2, 52% stage 3 and 29% stage 4; 34% were VitD insufficient and 25% VitD deficient. VitD levels did not vary by age, gender, race or severity of CKD, but showed a significant inverse correlation with PTH and FGF23 levels (r, p-value: -0.24, 0.02; -0.22, 0.002). A 10 ng/ml increase in VitD levels was associated with 0.9 (95% CI 0.8-1.0) pg/ml lower PTH levels (p=0.007) and 0.9 (95% CI 0.8-1.0) pg/ml lower FGF23 levels (p=0.004).

Low VitD levels appeared to be associated with a greater risk of CKD progression (HR, 95%CI per 10 ng/ml increase: 0.5, 0.4-0.8; p=0.002) on univariate analysis but not after adjustment for baseline kidney function and PTH levels.

Conclusions: In summary, low VitD levels are common in patients with CKD and appear to have an impact on regulatory factors involved in mineral metabolism. The implications for longer term outcomes need further study.

FR-PO1374

Comparison between the Steroid Pulse Mono-Therapy and the Combination Therapy of Steroid Pulse and Tonsillectomy for IgA Nephropathy Ayami Ochi, Takahito Moriyama, Kayu Nakayama, Kosaku Nitta. Medicine, Kidney Center, Tokyo Women's Medical University, Tokyo, Japan.

Background: Steroid pulse therapy reported by Pozzi C et al has been recognized as long-term effective therapy for IgA nephropathy (IgAN). In this decade, the combination of tonsillectomy and steroid pulse therapy has been widely performed in Japan. However there is no report to compare those two therapies.

Methods: In this prospective cohort analysis, we compared clinical findings at renal biopsy, histological findings according to the Oxford classification, and the ratio of complete remission rate (RR) of urinary protein excretion (U-Prot) and urinary red blood cells (U-RBC) at one year after treatment between 26 newly diagnosed IgAN patients received tonsillectomy and steroid pulse therapy (TaSP group), and 15 newly diagnosed IgAN patients received steroid pulse mono-therapy (SP group). We defined clinical remission as U-Prot<0.5 g/gCr and U-RBC<5 counts/HF.

Results: Clinical and histological characteristics at baseline between both groups did not differ (TaSP vs. SP group; mean eGFR: 68.2 vs. 68.4 ml/min, mean U-Prot: 0.63 vs. 0.51 g/day, and mean U-RBC 25 vs. 20 counts/HF). RR of U-Prot analyzed by the Kaplan-Meier method did not differ between both groups (76.9 vs. 60.0 %). However RR of U-RBC was significantly higher in TaSP group than in SP group (80.0 vs. 33.0 %, Log rank test; P=0.0053). Moreover, RR of both U-Prot and U-RBC was significantly higher in TaSP group than in SP group (65.0 vs. 13.3 %, Log-rank test; P=0.0029). The cox regression analysis showed that combination therapy was associated with clinical remission (HR:12.07, 95%CI: 2.80-90.91, P= 0.0041).

Conclusions: Combination therapy of tonsillectomy and steroid pulse therapy showed higher clinical remission rate of urinary findings at one year after treatment in comparison to the steroid pulse mono-therapy in patients with IgAN. Long term observation for renal survival should be analyzed in the future.
FR-PO1375
von Willebrand Factor Synthesis and Circulating Half Life and Osteoprotegerin Levels Are Progressively Elevated in Stage 3-5 CKD Patients
Cynthia M. Pruss,1 Spencer Barr,1 Julie Grabell,2 Angie Tuttle,2 Michael A. Adams,1 Jocelyn S. Garland,1 Paula James,2 Rachel M. Holden.2 1Biomedical and Molecular Sciences, Queen’s University, Kingston, ON, Canada; 2Medicine, Queen’s University, Kingston, ON, Canada.

Background: Von Willebrand Factor (VWF) and osteoprotegerin (OPG) are co-secreted by the endothelium and remain associated in the circulation. VWF and OPG are biomarkers for endothelial dysfunction and have been linked to cardiovascular disease but have not been evaluated longitudinally in individuals with CKD.

Methods: VWF antigen (VWF-Ag), VWF propeptide (VWFpp), OPG, and IL-6 levels were measured at baseline and at 4 years in a cohort of individuals with stage 3-5 CKD (N=54, mean age 62 years, 44% female) and were compared to age-matched controls (N=38, 67.2 years, 58% female). The VWFpp to VWF-Ag ratio (VWFpp/VWF-Ag) was used to evaluate VWF circulating time, with ratios <1 representing increased VWF half life.

Results: At baseline, VWF-Ag, OPG and IL-6 were significantly higher than age-matched controls. Within the CKD cohort, there were significant increases in VWF-Ag, VWFpp, VWFpp/Ag, and OPG between baseline and 4 years. The VWFpp/VWF-Ag ratio was significantly lower in CKD patients indicating an increase in VWF circulating half life. OPG levels correlated positively with the VWF-Ag (p<0.001), VWFp (p<0.001), and negatively with GFR (p<0.05) with all CKD-measurements were pooled.

Conclusion: The role of endothelial dysfunction is emerging as a key contributor to cardiovascular disease. This study demonstrates that there is a significant increase in VWF synthesis in the earlier stages of CKD that is progressive over time. The relationship between endothelial dysfunction, VWF synthesis and OPG requires further study.

Funding: Private Foundation Support, Government Support - Non-U.S.

FR-PO1376
Genetic Variant of the Renin-Angiotensin-Aldosterone System (RAAS) and Renal Survival in Japanese Patients with Chronic Kidney Disease (CKD)
Yuki Tsuchizaki Makino,1 Tadashi Konoshita.2 1Third Department of Internal Medicine, Fuku University School of Medicine, Eiheiji, Fukui, Japan.

Background: Chronic kidney disease (CKD) is a public health problem, and inhibiting progression of CKD patients is a major task for the nephrology. The renin-angiotensin-aldosterone system (RAAS) may play a critical role in the progression of CKD. Genetic polymorphism of RAAS has been reported to be associated with the development of some type of renal disease. However there has been few investigation of RAAS genetic variation and CKD progression in a large population-based study.

Methods: We enrolled 455 CKD patients who consulted nephrologist in our hospital between January, 1995, and December, 2010. All patients were Japanese. 486 (48.2%) reached end stage renal disease (ESRD) at an average age of 55.1±22.3 years. We estimated the association between cumulative renal survival and five polymorphism of RAAS: angiotensin converting enzyme (ACE) deletion polymorphism, angiotensin II type receptor (AT1) A1166C, angiotensin II type receptor (ATR1) A1166C, and aldosterone synthase CYP11B2 C344T. For statistical analysis of the time course to ESRD a cumulative survival analysis using the Kaplan-Meier method with log-rank test.

Results: Cumulative renal survival in CKD was significantly less in those with TT genotype in (REN C-5312T) [log-rank P=0.0071 X2=7.380]. There was no association between cumulative survival and M235T, ACE/D/A1166Cand C344T polymorphism.

Conclusions: REN C-5312T polymorphism may play a role CKD progression. And this polymorphism affect prognosis in CKD patient.

FR-PO1377
Circulating Bone Morphogenetic Protein-1 (BMP-7) and Transforming Growth Factor-β (TGF-β) as Potential Biomarkers for Renal Endpoint in Patients with Type 2 Diabetes Mellitus
Muh Geot Wong,1 Min Jun,2 Usha Pancharapaksean,3 Mark Woodward,2 Xinming Chen,1 John P. Chalmers,2 Vlado Perkovic,2 Carol A. Pollock.3 1Kolling Institute of Medical Research, University of Sydney, NSW, Australia; 2George Institute for Global Health, University of Sydney, Camperdown, NSW, Australia.

Background: Albuminuria and reduced estimated glomerular filtration rate (eGFR) are known predictors of decline in kidney function for patients with diabetes mellitus. There is an increasing need for additional biomarkers that can predict renal outcomes in the earlier stages of diabetes. Aims: To assess the baseline circulating value of TGF-β1 and BMP-7 in patients with type 2 diabetes mellitus, and to establish the relationship of these markers with the risk of progressive diabetic nephropathy.

Methods: Serum samples from 124 participants of the ADVANCE Collaborative Group study were studied. Cases were defined as those who developed a renal endpoint, ie. doubling of serum creatinine to at least 200μmol/l, the need for renal replacement therapy, or death due to renal disease. Using propensity score methodology, controls were matched for age, sex, race, baseline estimated glomerular filtration rate (eGFR) (<60 vs. >60 ml/min/1.73m2) and primary albumin/creatinine ratio (UACR), baseline blood pressure, baseline HbA1C, known macrovascular disease, history of retinopathy and treatment allocation. Enzyme linked immunosorbent assays were used to analyse total and active circulating TGFβ1 and BMP7 at baseline.

Results: Individuals with type 2 diabetes who developed renal endpoints (n=52) had a significantly higher circulating total TGFβ1, 12684.7 pg/ml vs. 7501.6 pg/ml (p<0.01) and lower circulating BMP7 levels 6.89 pg/ml vs. 19.33 pg/ml (p<0.0001), compared to controls (n=72) respectively. Adjusted sensitivity analyses revealed TGFβ1 is a positive predictor (OR=1.9, 95% CI 3.0-3.30, p=0.0002) and BMP7 is a negative predictor (OR=0.37, 95% CI 0.25-0.62 p<0.0001) for renal endpoints, independent of other conventional risk factors such as UACR and eGFR and each other.

Conclusion: Circulating BMP7 and TGFβ1 have prognostic potential in predicting poor renal outcomes in type 2 diabetes mellitus.

FR-PO1378
Aging and Progression of Chronic Kidney Disease
Rachel Bregman, Renata de Souza Mendes, Carla C.S. Lemos, Frances Silva, Maria Inês Barretto Silva. Nephrology, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

Background: Aging is a reality in the modern world and is pointed as a risk factor for Chronic Kidney Disease (CKD). The aim of this study was to analyze the progression of CKD in elderly.

Methods: We evaluated 236 patients (group 1) with 60-74 years (n=72) and 75 years or more (group 2). Mean age (y) (±SD) was 68.4±8.4; 70.6±7.6, respectively. Time on renal replacement therapy, or death due to renal disease. Using propensity score methodology,  controls were matched for age, sex, race, race, baseline estimated glomerular filtration rate (eGFR) (<60 vs. >60 ml/min/1.73m2) and primary albumin/creatinine ratio (UACR), baseline blood pressure, baseline HbA1C, known macrovascular disease, history of retinopathy and treatment allocation. Enzyme linked immunosorbent assays were used to analyse total and active circulating TGFβ1 and BMP7 at baseline.

Results: At the time of referral MDRD (ml/min) was 32±12 (group 1) and 36±13 (group 2). The rate of progression of CKD was -1.2±5 ml/min/y and -1.2±4 ml/min/y respectively. Proteinuria (mg/g creatinine) presented as median (interquartile interval) was: all patients 281(125-961); group1: 288 (127-1088), group 2: 273 (95-890). Laboratory data showed all the parameters within the normal range. SBP was higher than 130mmHg in 22% and 46% in group 1 and group 2 respectively. Analysing patients together SBP correlated (p=0.033) with the decrease of eGFR and proteinuria (p=0.006).

Conclusion: DM was less prevalent in older patients. High values of SBP suggest a decrease of arterial stiffness, and the correlation of high SBP with the decrease of eGFR and proteinuria, suggest endothelial dysfunction. The rate of decline of eGFR was below 4ml/min/y in 81% of the patients. We suggest that elderly respond well to CKD treatment implemented by a multidisciplinary team. Early referral (stage 3/4) was probably another point corroborating to retard progression of CKD. Therefore, we postulate that old people are likely to have a good prognosis of CKD, implicating in quality of life since the need of renal substitutive therapy is postponed.

FR-PO1379
The Long-Term Beneficial Effect of Inhibitors of Renin-Angiotensin Aldosterone System (RAAS) for Advanced IgA Nephropathy with Impaired Renal Function
Takahito Moriya,1 Ayami Ochi,1 Kayu Nakayama,1 Kosaku Nitta.2 Medicine, Kidney Center, Tokyo Women’s Medical University, Tokyo, Japan.

Background: The adaptation for steroid therapy and the effect of RASI for advanced IgA nephropathy (IgAN) patients with impaired renal function is still controversial.

Methods: In this retrospective cohort analysis, we divided 91 IgAN patients with an estimated glomerular filtration rate (eGFR) of less than 60 ml/min and proteinuria greater than 0.5 g/day and lower than 3.5 g/day into three groups: the RASI group (RASI: n=39), the steroid group (STEROID: n=22), and combination of RASI and steroid therapy group (COMB: n=30). We analyzed the clinical and histological background, renal survival rate until progression to end stage renal disease, and the risk factors for progression.

Results: The clinical and histological backgrounds were not significantly different among the three groups (eGFR (ml/min) and proteinuria (g/day): RASI: 46.4±1.37, STEROID: 48.5±1.52, and COMB: 46.2±1.58). Until 2 years after treatment, proteinuria and U-RBC decreased from baseline in the three groups (P<0.001), and the decrease of proteinuria was highest in COMB (RASI 21.6%, STEROID 23.7%, COMB 32.4%, p<0.001). However, the renal survival rate until ESRD was not significantly different among the three groups.
Proteinuria at 2 years after treatment was an independent risk factor for progression by Cox multivariate analysis (HR 1.38, 95%CI 1.06-1.79, P=0.0173), however treatment of steroid and combination therapy was not.

**Conclusions: The beneficial effect of RASI on renal survival of advanced IgAN with impaired renal function is equal to that with steroids and combination of steroid and RASI. RASI may be a sufficient therapy for advanced IgAN with impaired renal function and the adaptation of steroid therapy should be considered carefully.**

**FR-PO1380**

The Relationship of Proteinuria to Outpatient “AKI” and Renal Progression

**Steven J. Rosansky,**1 James W. Hardin,2 Frankie Richards,2 Kathlyn Sue Haddock,2 Ann M. O’Hare,3 William F. Clark.4
1Don research Institute, WJBd VA Hospital, Columbia, SC; 2Dept of Biostatistics, School of Public Health University of SC, Columbia, SC; 3London Health Sciences Center, University of S Ontario, London, ON, Canada; 4Nephrology, VA Seattle, Seattle, WA.

**Background:** Outpatient “AKI” and change of renal function per year as measured by MDRD e GFR may be important parameters in the management of CKD patients. Proteinuria has been reported to be associated with a higher frequency of inpatient ICD coded AKI. The current study examines the relationship of urine proteinuria, renal function decline and outpatient AKI.

**Methods:** All outpatient creatinine and urinalysis data from through December 31, 2008, were obtained for patients with an initial serum creatinine of ≥1.3 mg/dl during 1989-2003. Lab data was utilized from 3776 patients who had at least one urinalysis and ≥3 creatinine values over ≥3 years. Each subject’s creatinine values (same methodology throughout study)were separated into 90-day consecutive windows. “AKI” was defined by sequential outpatient serum creatinine values where a second creatinine was ≥50 percent higher than the prior serum creatinine in a 90-day window. The change in outpatient 4 variable MDRD e GFR (ml/min/1.73m2/year) was calculated using the average of the first and last creatinine values and was examined according to three creatinine categories, using all urinalysis data per patient, no proteinuria; < 2plus proteinuria; and ≥2plus proteinuria.

Growth curve analysis was utilized to examine the relationship of all covariates to renal function change.

**Results:** Patients had an average of 23 creatinine and 9 urine measurements during an average follow-up of 9.6 years. AKI occurred in 10.4% of the overall population,9.1 percent of whites. Higher urine urinalysis protein levels were associated with more AKI episodes and faster decline in eGFR.

**Conclusions:** In conclusion, outpatient “AKI” occurred more frequently in blacks than whites. Higher urinalysis protein levels were associated with more AKI episodes and faster decline in eGFR.

**FR-PO1381**

Determinants of Arterial Stiffness in Patients with Chronic Kidney Disease

**Stage 3 Natasha J. McIntyre,**1 Richard J. Fluck,1 Chris W. McIntyre,1 Maarten W. Taal.1 1Department of Renal Medicine, Royal Derby Hospital, Derby, Derbyshire, United Kingdom; 2Department of Vascular Medicine, University of Nottingham, Nottingham, United Kingdom.

**Background:** Early stage CKD is associated with increased cardiovascular (CV) risk but the underlying mechanisms remain uncertain. Arterial stiffness (AS) is associated with increased CV risk in advanced stages of CKD, but it is unknown whether AS is relevant to CV disease in early CKD. We therefore investigated AS in subjects with CKD stage 3 in a primary care setting.

**Methods:** 1741 patients with eGFR 59-70ml/min/1.73m2; mean age 73±9 yrs, were recruited from Primary Care Practices for the Renal Risk In Derby (R3D) Study. A detailed medical history and clinical assessment was obtained as well urine and serum biochemistry testing. Carotid to femoral pulse wave velocity (PWV) was measured, as a marker of AS, using a Vicorder® device (Skidmore Medical Ltd, UK).

**Results:** Univariate analysis revealed significant correlations between PWV and previously identified risk factors for CV disease such as age (r=0.443), systolic BP (r=0.330), Body Mass Index (r=0.134), Log urinary Protein to Creatinine ratio(r=0.128), Waist to Hip ratio(r=0.122), Diastolic BP(r=0.083), eGFR (r=-0.067). Log High-sensitivity CRP (r=0.066) and HDL Cholesterol (r=-0.058). PWV was significantly higher in males (9.6m/sec vs 10.3m/sec), diabetics (9.8m/sec vs 10.3m/sec), and those with a history of CV events (9.8m/sec vs 10.3m/sec). Multivariable linear regression analysis identified independent determinants of higher PWV (Table; R2=0.30)

**Conclusions:** Age was the dominant determinant of AS in this cohort of elderly patients with CKD stage 3. Nevertheless, reduced eGFR, albuminuria and several factors associated with CKD (including hypertension, inflammation, and dyslipidaemia) were all also identified as independent determinants. Long term follow-up will investigate the importance of AS as an independent risk factor for CV events in this cohort.

**Funding:** Other NIH Support - Kidney Research UK and The British Renal Society

**FR-PO1382**

Neutrophil/Lymphocyte Ratio Independently Predicts Cardiovascular Events in Patients with Moderate To Severe Chronic Kidney Disease

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**Background:** Cardiovascular (CV) diseases are leading cause of death in patients with chronic kidney disease(CKD).Neutrophil/Lymphocyte ratio(NLR) has been shown independently predict mortality and poor outcomes in patients with myocardial infarction, heart failure and advanced malignancies. We aimed to examine the value of NLR in predicting fatal and nonfatal CV events in patients with stage 3-5 CKD.

**Methods:** Stage 3-5 CKD patients were followed-up for time-to-event analysis until occurrence of fatal or nonfatal CV events.Endothelium-dependent vasodilation (FMD) and endothelium-independent vasodilatation (NMD) compared with NLR, CRP and serum albumin laboratory data at baseline. Associations of NLR and endothelial dysfunction and other laboratory parameters were determined.Prevaleance of fatal and nonfatal events according to NLR was calculated.Estimate survival time for each category were calculated.

**Results:** 225 patients included in the study (70 patients with stage 3, 74 stage 4 and 81 stage 5 CKD). There was an inverse association between NLR and eGFR.Notably, while total WBC and neutrophil counts did not show significant difference across CKD stages, lymphocyte counts significantly decreased from stage 3 to stage 5 CKD. Multivariate analysis showed that associated of FMD only included NLR, diabetes, NMD, serum albumin and eGFR. During a mean follow-up period of 39 (2-42) months, 14 CV deaths, 52 non-fatal cardiovascular events were registered. Univariate and multivariate COX analyses showed that NLR was a significant independent predictor of fatal and nonfatal CV events (hazard ratio 1.58; p<0.001). When entire cohort divided by median NLR (2.81), 63 out of composite CV events occurred in patients with NLR above 2.81. Survival time was significantly longer in patients with lower NLR compared with patients with higher NLR.

**Funding:** Other NIH Support - GATA

**FR-PO1383**

Relationships between Physical Activity and Nutrition with Kidney Function in CKD Patients

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**Background:** The lipid lowering and Onset of Renal Disease (LORD) trial was a three-year randomised, double-blind, placebo-controlled trial investigating the effects of atorvastatin on kidney function in CKD patients. The study design included measures of physical activity and nutrition every nine months. The aim of this sub-study was to investigate the relationships between physical activity and nutrition with kidney function.

**Methods:** 132 patients with serum creatinine levels >120µmol/l, not taking lipid-lowering therapy and at all levels of proteinuria and serum cholesterol were enrolled. For this sub-study data was available for 120 patients and they were followed for a mean of 2.9 years. Physical activity and nutrition were assessed every nine months using the Activity Questionnaire and 4-day diet diaries analysed with Foodworks software respectively. eGFR was calculated using the MDRD formula. The association (Odds Ratio) between a number of predictors and eGFR were estimated using repeated measures ordinal
logistic regression. An OR >1.00 indicates a positive association, and an OR <1.00 indicates a negative association. General linear modelling was used to determine relationships between physical activity and nutrition with the change in eGFR. Results: eGFR was positively associated with phosphate intake (OR 2.3, 95% CI 1.5-3.4, P<0.001), and negatively with folate (OR 0.6, 95% CI 0.4-1.0, P<0.03). There were no significant associations between physical activity and kidney function. Weekly physical activity was not associated with changes in kidney function and there were no group differences (placebo = slope 0.19 (SD 3.51), atorvastatin = 0.41 (2.73)). There was a significant group difference (P=0.009) between time in high intensity physical activity and eGFR for placebo vs. −0.24 (2.57), atorvastatin vs. −0.72 (2.97), proteinuria (P<0.01). Pts were divided into two groups according to the sex-specific HGS median, (a) Males: <22.0 kg, females: (<12.9 kg). Males: inheritance (24hCrCl) (r=0.42; P<0.01), and negatively with age (r = -0.18; P=0.02) and MIS (r=-0.42; P<0.01). Pts -1.36 -1.82 to -1.15 -1.54 to -0.76<0.01 -0.53<0.01 0.19 (SD 3.51), atorvastatin = 0.41 (2.73)) and MIS (r=0.42; P<0.01). Pts were divided into two groups according to the sex-specific HGS median, (a) Males: <22.0 kg, females: (<12.9 kg). Males: In conclusion, HGS is associated with kidney function and nutrition, and there is a trend toward a higher HGS in those with better kidney function.

Conclusions: The MIS score was associated with HGS in our population, indicating that MIS could be used to predict muscle function in NDD-CKD pts.

Funding: Government Support - Non-U.S.

FR-PO1385

Recent Status of Vitamins B1, B2, and C in Japanese Patients with End-Stage Renal Disease Toshikazu Wada, Hideaki Iwasawa, Ami Hayashi, Yoshitaka Miyaoa, Toshiyuki Nakao. Nephrology, Tokyo Medical University, Tokyo, Japan.

Background: Water-soluble vitamin deficiencies have been recognized in end-stage renal disease (ESRD) patients. Recently, the numbers of ESRD elderly patients in Japan have been increasing. We investigated the status of vitamins B1 (VB1), B2 (VB2), and C (VC) in Japanese ESRD patients not receiving renal replacement therapy (RRT) and those receiving RRT (hemodialysis (HD), and peritoneal dialysis (PD)) to identify vitamin deficiencies in ESRD patients. We also investigated the status of these vitamins in ESRD elderly patients.

Methods: Plasma VB1, VB2, and VC levels were obtained in 46 non-RRT ESRD patients (age: 63.0 ± 14.0 years). In 82 HD patients (66.2 ± 12.5 years), the plasma vitamin levels were obtained before and after a single HD session and at the start of the next HD session. Diazepam clearance of these vitamins was assessed.

Results: The proportions of patients whose plasma VB1, VB2, and VC levels were below the normal range were as follows - non-RRT patients: 13.0%, 13.0%, 41.3%; HD patients: 15.2%, 12.2%, 51.2%; those above the normal range were as follows - non-RRT patients: 85.2%, 87.8%, 58.7%; HD patients: 84.8%, 87.8%, 48.8%, respectively. The patients who were prescribed vitamins showed high plasma vitamin levels. The plasma VC level significantly decreased after a single HD session (P < 0.05), and significantly increased at the start of the next HD session (P = 0.05); the plasma VB1 and VB2 levels did not significantly change in patients without vitamin supplements. The mean dialysis clearance of VC was significantly higher than those of VB1 and VB2 (both P < 0.05). In the elderly patients without vitamin supplements, non-RRT patients had significantly lower plasma VB1 levels than non-elderly patients (P = 0.006), and HD patients had significantly lower plasma VC levels than non-elderly patients (P = 0.001).

Conclusions: Low plasma VB1, VB2, and VC levels were commonly found in ESRD patients, especially in elderly patients. There was a significant trend between vitamin intake and loss based on individual requirements is essential for ESRD patients.

FR-PO1386


Background: IgA nephropathy (IgAN) is characterized by variable clinical features and outcomes. The identification of reliable disease biomarkers will help define prognosis and therapeutic outcomes. Inflammation mediated by NOD-like receptors may be involved in the pathogenesis of IgAN. The increased expression of NLRP3 mRNA has been demonstrated in IgAN. However the association of NLRP3 gene expression with IgAN progression is an unexplored area.

Methods: Biopsies of 39 patients with IgAN from September 2003 to December 2007 were randomly chosen for analysis of NLRP3 mRNA expression by quantitative real-time PCR. Patients with advanced kidney disease(creatinine, Cr=<300μmol/L; n=3) and missing Cr (n=5) were excluded. A total of 31 cases of IgAN were analyzed. NLRP3 gene expression was analyzed as a logarithmic variable due to its non-normal distribution, and categorized into quartiles. The outcome was kidney failure, defined as a composite of doubling of serum Cr, ESRD or death. Subjects were followed from the time of their kidney biopsy to March 31, 2009 for the outcome of kidney failure.

Results: The mean age of the 31 subjects was 47 years, of whom 58% were male. The composite renal outcome was reached in 6 (19%) of the patients. The non-rather between NLRP3 gene expression and kidney failure – subjects with the highest NLRP3 gene expression were least likely to develop kidney failure, although the results were not statistically significant.

FR-PO1384

Malnutrition-Inflammation Score Is Associated with Handgrip Strength in Non-Dialysis-Dependent Chronic Kidney Disease Patients Fernanda C. Amparo, 1, 2 Antonio C. Cordeiro, 1, 3 Juan J. Carrero, 4 Lilian Cuppini, 5 Bengt Lindholm, 6 Celso Amodeo, 7 Amanda G.M.R. Sousa, 8 Maria A. Kamimura. 9 Dante Pazzanese Institute of Cardiology, São Paulo, Brazil; 2Nutrition Program, Federal University of São Paulo, São Paulo, Brazil; 3Baxter Novum and Renal Medicine, Karolinska Institute, Stockholm, Sweden.

Background: Handgrip strength (HGS), a marker of muscle function, predicts mortality in earlier stages of chronic kidney disease (CKD). Protein-energy malnutrition and inflammation are coexisting deleterious conditions commonly shared by CKD patients (pts) that affect muscle function. We investigated whether the Malnutrition-Inflammation Score (MIS), developed for dialysis pts, is associated with HGS in non-dialysis-dependent (NDD) CKD pts.

Methods: We cross-sectionally evaluated 166 pts with NDD-CKD stages 2-5 (59 [51-67] years; 63% men). MIS was calculated as previously described, excluding the count for dialysis vintage. HGS was assessed in the dominant arm. Anthropometric parameters, physical activity was not associated with changes in kidney function and there were no group differences (placebo = slope 0.19 (SD 3.51), atorvastatin = 0.41 (2.73)). There was a significant group difference (P=0.009) between time in high intensity physical activity and eGFR for placebo vs. −0.24 (2.57), atorvastatin vs. −0.72 (2.97), proteinuria (P<0.01). Pts were divided into two groups according to the sex-specific HGS median, (a) Males: <22.0 kg, females: (<12.9 kg). Males: In conclusion, HGS is associated with kidney function and nutrition, and there is a trend toward a higher HGS in those with better kidney function.

Conclusions: The MIS score was associated with HGS in our population, indicating that MIS could be used to predict muscle function in NDD-CKD pts.

Funding: Government Support - Non-U.S.
Conclusions: In addition to known risk factors of DN, high insulin resistance was independently associated with development of overt nephropathy in type 2 diabetes, but not incipient nephropathy. 

Funding: Government Support - Non-U.S.

FR-PO1388

Left Ventricular Hypertrophy, Endothelial Dysfunction & Inflammation in CKD

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Background: Left ventricular hypertrophy [LVH] is common in CKD patients; however the mechanism is not clear. Inflammation and associated endothelial dysfunction [ED] are present in CKD and predict adverse cardiovascular outcomes. The relationships of inflammation, ED and LVH in different stages of CKD; predialysis, dialysis and post kidney transplantation [KT] are unclear.

This study examined the relationship of inflammation, ED and LV mass in predialysis, dialysis and KT patients.

Methods: This study included 39 patients with different stages of CKD. Patients with heart failure, recent MI, infection and cancer were excluded. ED was measured using the brachial artery flow mediated dilatation [FMD]. Echocardiogram was used to estimate LV mass. LV mass index (LVMI) was calculated using Penn formula and indexed by height. Inflammation was measured using high sensitivity CRP.

Results: The clinical characteristics in 17 pre-dialysis, 9 haemodialysis and 13 KT patients were: age 56±12 [mean±SD] yrs, 33% female, 20% diabetes, 10% smokers, BMI 26±4 kg/m2, SBP 139±19mmHg, DBP 81±11mmHg, cholesterol 4.3±1.3mmol/L and Hb 10±1 g/dL. LVMI was high 157±45 g/m2 and FMD was 2.6±2.1%. The hsCRP was 4.5±3.4 mg/L.

With increasing CRP the FMD decreased (r=0.70, p<0.001) and with decreasing FMD, LVMI increased (r=-0.55, p<0.001), see figure 1. Increasing LVMI was associated with increasing CRP (r=0.51, p=0.001). In multivariate analysis the relationship between LVMI and FMD remained significant after adjusting for age, gender, diabetes, and hypertension (Adj. beta = -0.507, p=0.01).

Figure 1 Relationship of LV hypertrophy, endothelial dysfunction and inflammation

Conclusions: The study demonstrates an association of inflammation, endothelial dysfunction and LV mass in all CKD patients. This suggests Inflammation may have a role in the development of LV hypertrophy due to endothelial dysfunction.

FR-PO1389

A Multicenter Assessment of Depressive Symptoms in Patients Undergoing Hemodialysis: Women over 60 Years Should Receive the Attention and Monitoring

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Background: Recent studies report that 10% to 25% of the general population has depressive symptoms. These symptoms are more frequent in women (15 to 20%) and elderly individuals (2 to 14%). These values can reach 30% in elderly nursing home residents.

Depressive symptoms can arise from psychosocial problems, or associated medical conditions such as cancer, chronic pain and chronic degenerative diseases, including Chronic Kidney Disease.

Recently, it has been shown that the prevalence of depression in patients with chronic kidney disease undergoing hemodialysis varies from 5% to 22%, with frequent occurrence of depressive symptoms with the onset of treatment.

Objective: To assess the prevalence of depressive symptoms using the Beck Inventory (screening test for diagnosis of depression) in chronic hemodialysis patients in 3 centers in the state of São Paulo, Brazil (n = 567) and correlate with demographics.

Methods: The Beck Depression Inventory was administered to a random sample of 708 of patients in three clinics (n = 397/567). We use statistical tests as chi-square with Fischer’s post-test.

FR-PO1390

Incidental Imaging Is Enough To Screen for Acquired Cystic Kidney Disease

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Background: Acquired cystic kidney disease (ACKD) is a known complication of End Stage Renal Disease (ESRD). The pathophysiology is unknown but uremia seems to be a common factor. There is the potential for these cysts to turn malignant adding increased mortality to the ESRD population. The rate of ACKD increases with time on hemodialysis (incidence 50% at 3 years and 90% at 5-8 years) and it has been suggested that creating patients after they have received hemodialysis for three years is an ideal time to avoid complications and reduce mortality. The prevalence of renal cell carcinoma in ESRD patients has been debated (1.6 to 4%) but has been consistently shown to be higher than the general population.

Currently no official screening guidelines have been established to assess transplant or ESRD patients for ACKD. Given the complexity of ESRD patients and the comorbidities that are associated with this disease, we predict sufficient renal imaging may be incidentally done work up of other medical issues to make additional screening unnecessary.

Methods: We reviewed ESRD patients from Wisconsin Dialysis Incorporated, located in Madison, Wisconsin, were randomly identified as having been on dialysis for more than 3 years. The electronic medical record of each patient was reviewed for the time period after the patient had reached 3 or more years on dialysis. All imaging studies were reviewed for any ultrasound, CT scan or MRI images that could have incidentally found suspicious renal cysts. Any images that did not report findings of the renal system were disregarded.

Results: There was a total of 670.2 dialysis years reported in the 80 ESRD patient charts that were reviewed. Evaluation of each electronic medical record determined that 57.5% of ESRD patients have imaging performed randomly that could incidentally diagnose renal disease. None of the indications listed for the imaging tests was for ACKD screening.

Conclusions: Although the prevalence of renal cell carcinoma is much greater in the ESRD population, when taking into account the incidental screening, cost of the screening test and rate of disease occurrence, it is not cost effective to implement a screening system.

FR-PO1391

Responsiveness to Erythropoietin Stimulating Agents (ESA-R) Predicts ESRD in Non Dialysis CKD Patients

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Background: RCCs on complete normalization of anemia in CKD show that lower ESA-R predicts poor cardiovascular (CV) outcome. It is unknown whether this association persists in clinical practice where the target of partial anemia correction is pursued.

Methods: We verified the prognostic role of ESA-R in 194 consecutive CKD patients, regularly seen in outpatient nephrology clinics, that started ESA in the 2002-06 period. Exclusion criteria were causes of anemia other than CKD or recent transfusion. ESA-R was calculated as (Hb-Hb, t)time/ESA dose (g/dL/mo) per 1000 U/wk of ESA. Renal death (ESRD or death) was recorded from the 1st control after starting ESA to Apr 2011.

Results: Age was 64±16 yrs. 44% were males, 34% had diabetes and 32% CV disease. At baseline, BMI was 27±5 kg/m2, BP 140±21/77±11 mmHg, phosphate [μmol/L] 1.3±0.9 mg/dL, TSAT 23±9%, ferritin 158±139 ng/mL, CRP 0.8±0.9 mg/dL. These variables were similar across tertiles of ESA-R while, from lower to higher tertile of ESA-R, GFR increased (22±12, 23±14, 28±13 mL/min/1.73m2, P<0.01) and proteinuria decreased (1.1 [0.3-2.2], 0.6 [0.2-1.8], 0.5 [0.1-1.3] g/d P=0.04). First control occurred after 1.4±0.5 mo.

<table>
<thead>
<tr>
<th>ESA-R&lt;0.08 (n=65)</th>
<th>ESA-R 0.08-0.24 (n=64)</th>
<th>ESA-R≥0.24 (n=45)</th>
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<td>Basal Hb (g/dL)</td>
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<td>Basal Hct (%)</td>
<td>37.0±1.2</td>
<td>38.0±1.5</td>
<td>39.2±1.2</td>
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<tr>
<td>First ESA dose</td>
<td>4738±2041</td>
<td>541±2525</td>
<td>4397±1975</td>
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<td>(IU/wk)</td>
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<td>During initial 6 months</td>
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<tr>
<td>Mean ESA dose</td>
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<td>4719±1844</td>
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<td>(IU/wk)</td>
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We conclude that the percentage of patients on chronic hemodialysis with depressive symptoms is higher than the general population (28.71%).

Conclusions: The data suggest that female patients had higher frequency of depressive symptoms compared to males regardless of age (25.9% X 21.7% under 60 years old, and 45.5% vs. 30.6% over 60 years old) (p <0.05). However, we found a greater number of female patients with depressive symptoms over the age of 60 years (25.9 X 45.5%) (p <0.05). Our data suggest that these patients should be accompanied with measures of psychological support during treatment.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

434A
FR-PO1393

Hemoglobin Variability in Chronic Kidney Disease and Type 2 Diabetes in TREAT
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Background: Variability in hemoglobin (Hb) levels is an important factor for patients receiving red blood cell (RBC) transfusions. We hypothesized that variability in Hb levels would be higher in the TREAT placebo arm compared to the treatment arm.

Methods: TREAT placebo arm patients (n=2,026) with type 2 DM, eGFR 20-60 ml/min/1.73 m², and Hb level ≥11 g/dL were analyzed. The variability in Hb levels was quantified as the standard deviation of Hb levels over 30 days. Results: The standard deviation of Hb levels was significantly higher in the TREAT placebo arm compared to the treatment arm (0.56±0.05 g/dL vs. 0.48±0.05 g/dL, p<0.001). The variability in Hb levels was highest in the first month of the study and decreased over time. Conclusions: The variability in Hb levels is higher in the TREAT placebo arm compared to the treatment arm, and this variability is highest in the first month of the study.

FR-PO1394

Comparison of High Dose Ferric Carboxymaltose to Oral or IV Iron in Subjects with Iron Deficiency Anemia (IDA) Not Suitable for Oral Iron Lynda A. Szczezek,1 David B. Bregman,2 David Morris,3 Angelia Butcher,4 Todd Koch,2 Lawrence Tim Goodnough,5 Jane E. Onken,6 1Duke Clinical Research Inst, NC; 2Lastpold Pharmaceuticals, PA; 3Webwritrs, NC; 4Stanford Univ, CA.

Background: Ferric carboxymaltose (FCM) is a non-dextran IV iron that permits larger infusions compared to other IV iron products marketed to evaluate effect on hemoglobin as well as hemodynamics and other safety parameters.

Methods: Patients eligible for this randomized controlled trial had IDA due to any etiology (menorrhagia, GI, or CKD) with hemoglobin <11.0 g/dL at screening. Subjects in Cohort I underwent a 14 day (run-in) course of oral iron. Subjects with an increase in Hb ≥1 g/dL were not randomized. All other subjects were randomized to FCM (2 injections of 750 mg at 100 mg/min on Days 0 and 7 or) oral iron for 14 more days. The primary efficacy endpoint was the change in Hb from baseline to highest value between baseline and Day 35. Subjects with a history of severe intolerance to oral, considered too anemic for oral, or were intolerant of oral during the run-in were assigned to Cohort II and randomized to FCM as above or another IV iron (IV standard of care [IVSOC]). IVSOC was iron sucrose for 90% of subjects. For both cohorts, safety was assessed for 120 days, including an adjudicated composite endpoint (death, myocardial infarction, stroke, unstable angina, congestive heart failure, arrhythmia, hypertension, or hypotension).

Results: In Cohort I, the change in Hb (g/dL) from baseline to highest value between baseline and Day 35 was 1.57 for FCM (N=244) vs. 0.80 for oral iron (N=251) (p<0.001). In Cohort II, the change in Hb (g/dL) was 2.90 for FCM (N=245) vs. 2.16 for IVSOC (N=237) (p<0.001). FCM was well tolerated. Results were consistent regardless of etiology or Hb status at baseline. There was no significant difference between FCM and comparator with respect to the composite safety endpoint.

Conclusions: In this head-to-head trial, FCM was safe and effective for the treatment of IDA in subjects unsuitable for oral iron regardless of etiology or Hb status at baseline. Funding: Pharmaceutical Company Support

FR-PO1395


Background: Shortening of red blood cell (RBC) survival contributes to the anemia of chronic kidney disease. The toxic uricemic environment accounts for the reduced RBC life span. The impact of mechanical damage caused by hemodialysis to the shortened life span remains unclear. Reductions up to 70% in RBC survival have been reported in uremic patients. To date, no accurate, well-controlled RBC survival data exists in dialysis patients on different dialysis modalities and under erythropoiesis stimulating agent (ESA) therapy. Aim of this study was to determine RBC survival in hemodialysis (HD) and peritoneal dialysis (PD) patients compared to healthy subjects.

Methods: In this observational study, 14 HD patients and 5 PD patients were recruited. In addition, 14 healthy subjects matched according to age and gender to the HD patients were included to assess the normal range of RBC survival. All dialysis patients were either on ESA therapy or received regular iron supplements. RBC survival was measured using radioactivity labeled and included correction for potential losses due to elution and vesiculation.

Results: Over 85% of dialysis patients were anemic (Hb 12.0 g/dL ± 1.1), hemoglobin correction was not significantly different among HD and PD subjects. The median RBC survival was significantly reduced by 20% in hemodialysis patients compared to healthy subjects (58.1 (54.6 – 71.2) vs 72.9 (63.4 – 87.8) days, p<0.05). No difference was shown among the PD and HD group (55.3 (49.0 – 60.2) vs 58.1 (54.6 – 71.2) days, p = ns).

Conclusions: Despite current ESA therapy, reduced RBC survival contributes to CKD-related anemia, but the reduction is less than previously reported. Mechanical damage related to HD does not appear to contribute to the reduced RBC life span.

Funding: Government Support - Non-U.S.

FR-PO1396

Serum Hepcidin Levels Predict the Progression of Renal Anemia in Patients with Non-Dialysis Chronic Kidney Disease Kakuya Niihata,1 Naohisa Tomosugi,1 Takuya Uehata,1 Tatsuya Shoji,1 Yusuke Sakaguchi,1 Akira Suzuki,2 Terumasa Hayashi,1 Noriyuki Okada,1 Yoshiharu Tsubakihara.1 1Department of Kidney disease and Hypertension, Osaka General Medical Center, Osaka, Japan; 2Medical Research Institute, Kanazawa Medical University, Kachou, Japan; 3Department of Nephrology, Osaka University Graduate School of Medicine, Suita, Japan; 4Department of Clinical Laboratory, Osaka General Medical Center, Osaka, Japan.

Background: We reported that hemoglobin (Hb) levels in patients with non-dialysis chronic kidney disease (CKD) were correlated with serum hepcidin (Hep-25) in the cross-sectional study. In this study, we examined whether Hep-25 levels predict the progression of anemia in patients with non-dialysis CKD.

Methods: Study design: Prospective observational cohort study. Materials: 355 ambulatory patients with CKD (stage-5) in Osaka General Medical Center between

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Underline represents presenting author.
February 1, 2007, and June 30, 2007. Exclusion criteria: Those who had received renal replacement therapy, or those who had received erythropoiesis-stimulating agent (ESA) therapy within 6 months before the baseline evaluation. Outcome: Starting ESA therapy by the study end date, i.e., December 31, 2010. Predictor: Hep-25 levels. Statistics: Restricted cubic spline curve analysis. Hazard ratios were obtained using Cox proportional hazard models adjusted for age, sex, estimated glomerular filtration rate (eGFR) and Hb. We divided the patients by the median of ferritin levels and did the same analysis for each group.

Results: The mean age (standard deviation [SD]) was 61.8 (14.9) years; 56.6% of the patients were men. The mean (SD) eGFR and Hb were 47.5 (25.0) mL/min/1.73m² and 12.7 (1.8) g/dL. The total number of events was 80. The Cox proportional regression analysis showed that Hep-25 was a significant predictor of progression of renal anemia (p<0.004, Linearity p=0.09). In high ferritin group, Hep-25 was also a significant predictor (p<0.01, Linearity p=0.02), whereas not in low ferritin group (p=0.09, Linearity p=0.04).

Conclusions: The Hep-25 levels significantly predict the progression of anemia in patients with non-dialysis CKD. Relationship between Hep-25 and progression of anemia varies according to ferritin levels.

FR-PO1397
Procalcitonin vs. C-reactive Protein for Predicting Infection in Chronic Kidney Disease Patients
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Background: Recently, procalcitonin (PCT) is used widely as a surrogate marker for predicting infection in chronic kidney disease (CKD) patients because nonspecific elevation of C-reactive protein (CRP) caused from chronic inflammation was reported in these patients. However, it is uncertain whether PCT is a more accurate or cost effective for detecting infection compared to CRP in CKD patients. We investigated the clinical usefulness of PCT and CRP in patients with CKD stage 3-5.

Methods: Two hundred patients with CKD were included. In the group I, all 159 patients had culture-proven infection. Patients had CKD stage 3, 4, or 5 (n=30, 30, and 99). The 98 CKD stage 5 patients in group I were divided into three subgroups: no dialysis patients had culture-proven infection. Patients had CKD stage 3, 4, or 5 (n=30, 30, and 99). The 98 CKD stage 5 patients in group I were divided into three subgroups: no dialysis group (n=31), hemodialysis (HD) (n=34) and peritoneal dialysis (PD) (n=34). A total of 41 patients had culture-proven infection. Patients had CKD stage 3, 4, or 5 (n=30, 30, and 99). The 98 CKD stage 5 patients in group I were divided into three subgroups: no dialysis group (n=31), hemodialysis (HD) (n=34) and peritoneal dialysis (PD) (n=34).

Results: Both serum PCT and CRP levels were significantly higher in infection group compared to noninfection group.

Conclusions: Procalcitonin(PCT) and C-reactive protein(CRP) levels in each group

FR-PO1398
Blood Oxygenation Level Dependent MRI Level Dependent MRI in Chronic Kidney Disease: A Preliminary Cross-Sectional Study
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Background: There is growing evidence for increased renal hypoxia in CKD and to support BOLD MRI as a useful tool for monitoring intra-renal oxygenation status. Here, a study was performed to compare BOLD MRI measurements in subjects at different stages of CKD.

Methods: A total of 38 subjects participated to-date: healthy control (n=10), anemic (n=7), CKD stage 2 (n=5), 3 (n=8), 4 (n=6), and 5 (n=3) based on eGFR. All subjects came to the study after an overnight fast and BOLD MRI studies were performed using a 3.0 T scanner and mGRE sequence before and after administration of 20 mg of furosemide (iv). R2* was used as BOLD parameter and is a reflection of the level of hypoxia. On a separate day, GFR was measured by iohexol for accurate staging.

Results: Even though GFR by iohexol and cGFR values were correlated (R2=0.7), in 6 subjects the classification had to be changed based on cGFR determination.

Conclusions: Our preliminary experience with BOLD MRI measurements in patients with CKD suggest increasing levels of hypoxia both in cortex and medulla and lower medullary response to furosemide. This may suggest that increased hypoxia is probably related to reduced oxygen supply consistent with present understanding of the pathophysiology. Future studies should ask subjects to not take ACEI orARBs prior to the BOLD MRI (Manotham K, ASN 2006 PO188) to observe potentially higher level of hypoxia.

Funding: NIDDK Support
Patients with CKD Have Reduced Levels of Circulating MicroRNA 145 and 155
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Background: Atherosclerotic cardiovascular disease is highly prevalent across the spectrum of CKD, especially in dialysis patients. MicroRNAs (miRNAs) can modulate cellular proliferation, differentiation and apoptosis and are shown to be associated with cancer and cardiovascular disease. A recent study has demonstrated that circulating hsa-miR-145 and hsa-miR-155 expression increased in non-CKD patients with cardiovascular disease. The purpose of the current study is to determine the miRNA profile in normal and CKD patients with or without CAD.

Methods: miRNAs were isolated from 5 different pooled serum collections from healthy volunteers and 5 different pooled samples from hemodialysis patients by QIAzol Lysis Reagent and miNeasy Mini Kit (QIAGEN). Quantitative real-time PCR was performed to determine the expression of miR-145 and miR-155 using specific primers and normalized to U6 SnRNA.

Results: The results demonstrated that compared to healthy controls, miR-155 was down-regulated by 68% in hemodialysis patients. We also collected serum from CKD patients and found there was 23% decrease for miR-145 and 49% decrease for miR-155 (p=0.04) in the serum of stage 4 CKD patients with CAD compared to those of sex- and age-matched stage 4 CKD patients without CAD. There was no difference in age, diabetes, albumin, calcium or phosphorus, but FTH was higher in those with CAD.

Conclusions: Circulating miR-155 levels were significantly lower in dialysis patients than that in healthy controls. Furthermore, CKD patients with CAD had decreased miR-145 and miR-155 compared to those without CAD. These results suggest that miR-145 and miR-155 may be novel biomarkers of CKD and CAD. Further studies are needed to validate these results.

Funding: Veterans Administration Support, Government Support - Non-U.S.

FR-PO1401

Urinary Excretion of Hepcidin 20, 22, and 25 Is Dependent on Iron Storage but Not on Each Serum Level Takahiro Kuragano, Hiroshi Nonoguchi, Takeshi Nakashiki. Division of Kidney and Dialysis, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan.

Background: We have already demonstrated that serum hepcidin-25 is dependent on iron storage but not inflammatory cytokines in hemodialysis patients without apparent inflammation. Recently, enhanced urinary hepcidin 25 excretion in patients with acute kidney injury has been suspected as an index of the tube damage. To clarify the kinetic of hepcidin isoform in predialysis patients and the renal handling of hepcidin, we evaluated serum and urinary hepcidin isoform levels in patients with various stages of chronic kidney disease (CKD).

Methods: We measured serum and urinary levels of hepcidin 20, 22, and 25 in predialysis CKD patients and 10 healthy controls by qRT-PCR method. Serum levels of iron, TIBC, transferrin, ferritin, creatinine (Cr), urea nitrogen (UN), IL-6 and TNF-α levels were measured. Urinary levels of hfe-microglobulin (MG), N-acetyl-p-D-glucosaminidase (NAG), Liver type fatty acid binding protein (L-FABP), and albumin were also measured. Urinary level of each hepcidin isoform was normalized to Cr.

Results: There were no significant differences in serum hepcidin isoform or urinary levels between control and CKD patients. Serum levels of hepcidin 20, 22, and 25 were significantly correlated each other. However, there was no significant correlation between serum and urinary levels of each hepcidin isoform. Serum hepcidin 20 (p=0.01, R=0.67), 22 (p=0.01, R=0.66), and 25 (p=0.01, R=0.62) levels were significantly correlated with serum ferritin levels, but not with serum levels of creatinine, UN, IL-6, and TNF-α. Urinary hepcidin 20 (p=0.01, R=0.67), 22 (p=0.01, R=0.60) and 25 (p=0.01, R=0.62) levels were also significantly correlated with serum levels of ferritin, but not with urinary (B2MG, NAG, FABP and albumin).

Conclusions: In CKD patients, there was no significant correlation between serum and urinary hepcidin isoform levels. Furthermore, each hepcidin isoform in serum as well as in urine was not faithfully correlated with iron storage but not with renal function, tubular damage, or inflammatory conditions. Further clarification regarding renal handling of hepcidin needs to be determined.

FR-PO1402

The Effect of Dietary Magnesium on Vascular Calcification and Erectile Dysfunction in a Rat Model of Chronic Kidney Disease Alexej Zojic,1 Maria Tina Maio,¹ Kristin M. McCabe,¹ Navid Shobeiri,² Mason Curtis,¹ Spencer Barr,¹ Rachel M. Holden,¹ Michael A. Adams.¹ Department of Biomedical and Molecular Sciences, Queen’s University, Kingston, ON, Canada; 2 Department of Medicine, Queen’s University, Kingston, ON, Canada.

Background: Erectile dysfunction (ED) is highly prevalent in patients with chronic kidney disease (CKD). We have previously shown that the pudendal artery (PA) is critical to the erectile response and that a calcium channel blocker (CCB) is a potential therapy for vascular calcification (VC). Magnesium (Mg) has been shown to inhibit VC in vitro. We determined the effect of high dietary Mg on VC in the PA and on the associated erectile response in a rodent model of CKD.

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437A
of eGFR resulted in a higher eGFR compared with the MDRD equation (median: 103 vs. CKD-EPI, P<0.01). The ICC of the two equations was 0.96 (95% confidence interval [CI]: 0.93-0.98) between CKD-EPI and AASK-MDRD; 0.76 (95%CI: 0.73-0.78) between AASK-MDRD and MDRD; and 0.72 (95%CI: 0.68-0.75) between CKD-EPI and MDRD. Among HIV+ blacks, the AASK-MDRD equation resulted, on average, in the highest eGFR value (median: 103.9), with CKD-EPI the second (median: 107.1), and the MDRD with the lowest value (median: 104.8).

Conclusions: In conclusion, the results of all three equations closely agreed, but the CKD-EPI generated a higher eGFR than the MDRD. The AASK-MDRD equation generated the highest eGFR among blacks. Further studies are needed to determine the predictive value of these creatinine based eGFR on the risk of CKD progression, ESRD and mortality in the HIV+ population.

Funding: Other NIH Support - Dr. Tahira P. Alves has been supported by a Vanderbilt University T32 Institutional Training Grant, a 2008-2009 National Kidney Foundation Fellow Grant, Vanderbilt CTSAs, and 2008-2010 NIH-LRP Award for Health Disparities.

This work has been facilitated by the infrastructure and resources provided by the Vanderbilt-Meharry Center for AIDS Research (CFAR), an NIH funded program #P30 AI 54999, and the TN Valley VA Clinical Research Center of Excellence., Private Foundation Support

FR-PO1405

The Effects of Clinical Practice Guidelines and Estimated Glomerular Filtration Rate Reporting on Creatinine Clearance Testing

Objective: To explore the impact of clinical practice guidelines and reporting of estimated glomerular filtration rate (eGFR) on creatinine clearance (CrCl) testing.

Methods: We conducted a retrospective study of CrCl tests performed from 1999 to 2009 in our chronic kidney disease (CKD) cohort.

Results: CrCl testing declined significantly (23.5% absolute change) in the sex- and age-adjusted rate of CrCl tests (p<0.0001). In the medical group FPE had the greatest hazard for death and RDF for dialysis. Revascularization reduced hazard for death in patients with FPE but did not reduce hazard for dialysis for any phenotype.

Conclusions: The study period spanned August 1999 to July 2009. Data from 8.4 million patients per month were available.

The K/DOQI guidelines were not associated with a significant change in the sex- and age-adjusted rate of CrCl tests performed (p=0.82). eGFR reporting was associated with a significant decline (23.5% absolute change) in the sex- and age-adjusted rate of CrCl tests performed (p=0.0001).

Conclusions: In this setting, practice guidelines did not influence CrCl tests. Rather, the direct introduction of eGFR reporting into physician workflow resulted in a sudden and dramatic decrease in CrCl collection. These data suggest that changing physician practice patterns requires more than guidelines, rather it is more likely to occur through educational and structural changes to practice.

FR-PO1406

Clinical Phenotypes Associate with Outcome in ARVD

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Background: ASTRAL showed unselected revascularization for atherosclerotic renovascular disease (ARVD) did not reduce mortality or cardiovascular events vs. medical therapy. Revascularization is still undertaken for some clinical presentations: 1) sudden coronary artery occlusion (CAO), refractory hypertension (RH) and rapidly declining renal function (RDF), though randomized data is lacking. By analyzing prospective data (1995-2010) on our ARVD cohort (n=819) we aimed to quantify effects of revascularization in these clinical settings.

Methods: Patients were categorized as receiving medical therapy (n=668) or revascularization (n=145). These groups were divided by presence/ absence of indications to revascularize. Indications were divided by phenotype: FPE (identified from clinical records); RH (SBP >160mmHg despite ≥3 anti-hypertensives); RDF (creatinine at diagnosis >1.2x or 10μmmol/l higher than result in previous 6 months). Effects of phenotype on death and dialysis events were assessed using Cox proportional hazards corrected for age, sex, BP, eGFR, vessel patency, proteinuria and angiotensin blockade. In the medical group patients with each indication were compared vs. those without. In the revascularization group each indication was compared for revascularized vs. medically treated patients.

Results: In the medical therapy group presence of an indication was associated with significant increases in hazards for both endpoints (p<0.005). This was not seen in the revascularized group.

In the medical group FPE had the greatest hazard for death and RDF for dialysis. Revascularization reduced hazard for death in patients with FPE but did not reduce hazard for dialysis for any phenotype.

FR-PO1407

Hepatitis C Virus Infection Increases Risk for End-Stage Renal Disease in Patients with Chronic Kidney Diseases

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Background: Current evidence regarding the relationship between hepatitis C virus infection and outcome of chronic kidney disease is controversial. We aim to explore whether hepatitis C virus infection increases risk for end stage renal disease in patient with chronic kidney disease.

Methods: Our study cohort was patients received multidisciplinary chronic kidney disease care in one medical center, southern Taiwan from 1995 to 2010 through CKD registry. Subjects were traced until starting dialysis, death or end of 2008. Subjects with adequate register data and follow up for at least 90 days were included in our analysis. Survival analyses including Kaplan Meier and Cox proportional model were used to estimate the cumulative incidence rate and risk of end stage renal disease and p-value <0.05 was considered as significant.

Results: A total of 2101 patients in our chronic kidney disease cohort were included in the final analysis. After adjusted age, sex, primary diseases, educational status, herb use, stage of chronic kidney diseases, body mass index, blood pressure, and hemoglobin, hepatitis C virus infection shows significantly increasing risk for end stage renal disease.

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FR-PO1408

Urinary Level of Ngal Is Superior to Urinary Protein in Prediction of Chronic Kidney Disease Progression

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Background: Urinary neutrophil gelatinase-associated lipocalin (uNgal) level is a biomarker which enables early diagnosis of acute kidney injury. In this study, we evaluated the association of uNgal levels with clinical parameters in chronic kidney disease (CKD) patients, their changes by treatment, and the predictive ability of uNgal for the progression of CKD in comparison with urinary protein.

Methods: Urine samples were collected from 98 CKD patients admitted to our department of Kyoto University Hospital, and they were followed for a mean period of 12 months. uNgal levels were measured by chemiluminescence immunoassay and normalized to urine creatinine. We defined renal events as >50% increase in serum creatinine levels or progression to end-stage renal disease.

Results: uNgal levels were significantly correlated to serum creatinine and urinary protein levels, respectively. uNgal level was decreased by steroid therapy in IgAN or FSGS, and by percutaneous nephroscopy in hydropnephrosis. uNgal levels on entry of patients who later developed renal events were significantly higher compared with those of patients without it. As to prediction of renal events, areas under the ROC curves for uNgal and urinary protein were 0.94 and 0.82, respectively (difference, p<0.006). Kaplan-Meier curves of renal event-free survival indicated that subjects with uNgal values above the optimal ROC cut-off level (112 µg/gCr) experienced much faster occurrence of renal events than ones below the cut-off (P=0.0001).

Conclusions: uNgal was shown to be a useful biomarker of disease severity and treatment efficacy of CKD, and Ngal appears to be more powerful than urinary protein in anticipating deterioration of CKD.

FR-PO1409

Combined Association of Cystatin C-Based and Creatinine-Based Estimated Glomerular Filtration Rate with Mortality: The Atherosclerosis Risk in Communities (ARIC) Study

Salman Waheed,1 Brad C. Astor,2 Kunihiro Mori,1 Masato Kasahara,1 Hideki Yokoi,1 Takashige Kuwabara,1 Tomoko Kawanishi,1 Kiyoshi Mori, 1 Nephrology Task Force for the Validation of Urine Examination as a Universal Biomarker which enables early diagnosis of acute kidney injury. In this study, we evaluated the association of uNgal levels with clinical parameters in chronic kidney disease (CKD) patients, their changes by treatment, and the predictive ability of uNgal for the progression of CKD in comparison with urinary protein.

Methods: Urine samples were collected from 98 CKD patients admitted to our department of Kyoto University Hospital, and they were followed for a mean period of 12 months. uNgal levels were measured by chemiluminescence immunoassay and normalized to urine creatinine. We defined renal events as >50% increase in serum creatinine levels or progression to end-stage renal disease.

Results: uNgal levels were significantly correlated to serum creatinine and urinary protein levels, respectively. uNgal level was decreased by steroid therapy in IgAN or FSGS, and by percutaneous nephroscopy in hydropnephrosis. uNgal levels on entry of patients who later developed renal events were significantly higher compared with those of patients without it. As to prediction of renal events, areas under the ROC curves for uNgal and urinary protein were 0.94 and 0.82, respectively (difference, p<0.006). Kaplan-Meier curves of renal event-free survival indicated that subjects with uNgal values above the optimal ROC cut-off level (112 µg/gCr) experienced much faster occurrence of renal events than ones below the cut-off (P=0.0001).

Conclusions: uNgal was shown to be a useful biomarker of disease severity and treatment efficacy of CKD, and Ngal appears to be more powerful than urinary protein in anticipating deterioration of CKD.

FR-PO1410

Cost-Effectiveness of Chronic Kidney Disease Mass Screening Test in Japan

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Background: Although CKD is a significant public health problem, strategy for its early detection is still a matter of controversy. We performed cost-effectiveness analysis using a decision tree model to compare test modalities (dipstick test to check proteinuria only, serum creatinine (Cr) assay only, and both) in the context of the reform of Japan’s mandatory annual health check-up for adults. In modelling, we carry out a deliberate literature survey to find out the best available evidence from Japan, while reports from other countries are excluded.

Methods: Incremental cost-effectiveness ratios (ICERs) of mass screening compared to do-nothing were $12,660/QALY for urine dipstick test only, $90,250/QALY for serum Cr assay only, and $91,505/QALY for both, respectively. And ICERs associated with the reform are $103,618/QALY for mandating serum Cr assay in addition to currently mandatory dipstick test, and $100,016/QALY for mandating serum Cr assay and making dipstick test discretionary, respectively.

Conclusions: Taking a threshold to judge cost-effectiveness according to a World Health Organisation’s recommendation, three times of Gross Domestic Product per capita, U.S.$128 thousand/QALY, a policy for reform that making serum Cr assay mandatory is also cost-effective. Our results suggests that population strategy for CKD detection such as mass screening using dipstick test and/or serum Cr assay can improve mortality prediction replicating the results in the REGARDS cohorts suggesting this may provide a useful strategy when finer risk stratification is useful.

Funding: Other NH Support - The Atherosclerosis Risk in Communities Study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute.
Epidemiology of Pulmonary Hypertension in Different Stages of Chronic Kidney Disease

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Background: Pulmonary hypertension (PH) has been reported to occur in a considerable proportion of patients with ESRD and has been regarded as an independent predictor of mortality in hemodialysis patients. However, the epidemiology of PH in non-ESRD patients remains unclear. The aim of this study is to evaluate the incidence of PH in different stages of chronic kidney disease (CKD) and the association of PH with cardiovascular status in this population.

Methods: We retrospectively evaluated the records of 1,600 in-patients between 2008 and 2010. Patients were divided into 6 groups: Group 1-4 for CKD stage 1-4; Group 5 for those in stage 5 but still not or initiated dialysis <3 months; Group 6 for maintenance hemodialysis (MHD) patients (hemodialysis ≥3 months). Systolic pulmonary artery pressure (SPAP) was evaluated using Doppler echocardiography and calculated using Bernoulli equation, a value of ≤40mmHg was defined as PH. History of cardiovascular events was recorded. Patients with chronic obstructive pulmonary disease (COPD), connective tissue disease, history of pulmonary embolism or chest wall or parenchymal lung disease, rheumatic heart disease, congenital heart disease and acute heart failure were excluded.

Results: PH was detected in 214 (13.4%) of the total 1,600 CKD patients. Prevalence of PH in Group 1-6 was 0.5%, 3.17%, 3.77%, 6.92%, 12.84% and 30.70%, respectively. Chi-Square Test (linear-by-linear association) showed with renal function progressively increased (doubling) in serum creatinine, the proportion of PH in Group 1-6 was 0.5%, 3.17%, 3.77%, 6.92%, 12.84% and 30.70%, respectively.

Conclusions: This study revealed that PH was low in Stage 1-4 of CKD patients but elevated in ESRD especially in MHD patients. PH may be a risk factor of cardiovascular events in CKD patients.

Is Doubling of Serum Creatinine the Right Endpoint in Nephrology Trials?

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Background: Doubling of serum creatinine (DSCR) is frequently used as an endpoint in nephrology trials. However, DSCR is reached by only few patients during trials. We aimed to assess whether adopting smaller increases in serum creatinine as endpoint may yield more endpoints while maintaining similar precision of treatment effects. If true, this would potentially allow fewer patients to be included in nephrology trials.

Methods: In a post-hoc analysis of the RENAAL and IDNT trial, the effects of Angiotensin Receptor Blockers (ARB) were estimated on the renal endpoint defined by DSCR, applicable to kidney donors.

Results: The effect of the ARB irbesartan decreased when smaller percentages increases in serum creatinine was defined as endpoint (figure 1). Moreover, the confidence interval of the treatment effect also decreased at smaller increases in serum creatinine as endpoint so that the least number of patients were required to detect a significant treatment effect at a 100% increase (doubling) in serum creatinine. Similar results were obtained in the RENAAL trial, or when the change in serum creatinine was calculated from baseline in both studies.

Conclusions: For drug trials in nephrology enrolling patients with type 2 diabetes and nephropathy, DSCR is an appropriate endpoint. Using smaller increases in serum creatinine as endpoint may aim to assess whether adopting smaller increases in serum creatinine as endpoint may decrease at smaller increases in serum creatinine as endpoint so that the risk of ventricular tachycardia (VT) was highly increased in CKD in the presence of other risk factors of VT.

Funding: Government Support - Non-U.S.

Low GFR after Kidney Donation Is Not CKD

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Background: CKD staging is based on prognostic impact of GFR with/without proteinuria. Many kidney donors have an estimated (e)GFR ≥ 60 ml/min/1.73m² post-donation and meet criteria of CKD stage 3 (CKD3). However, prognostic impact of a given GFR in two diseased kidneys may not be equivalent to the same GFR in one healthy kidney. To test this assumption, we compared renal function course in former kidney donors to CKD patients matched for GFR, age and gender.

Methods: We included 57 (63%) male kidney donors (baseline values 3 months after donation) and 57 CKD patients. All had repeated GFR (i-131-iodo-lamothalamate) and ERPF (125-hippuran) measurements after 4.7±1.5 years.

Results: At baseline, 25% of donors met criteria for CKD3. In all donors GFR increased over time, with a slight fall in ERPF, while GFR and ERPF fell in all CKD patients. CKD stage improved to CKD 0-2 in 13 donors with CKD3 at baseline, whereas it worsened in 21 CKD patients. Change in GFR significantly differed between donors and CKD patients (see table), both for CKD with (n=31; -2.1±3.3) and CKD without (-1.1±3.2 ml/min/1.73m²) proteinuria.

Conclusions: Thus, despite similar baseline GFR and CKD stage, renal prognosis is substantially different for donors and CKD patients. Although many donors initially meet criteria of CKD3 they should not be regarded as CKD patients. CKD staging is not applicable to kidney donors.

Funding: Government Support - Non-U.S.
FR-PO1415

Chronic Kidney Disease (CKD), Hypertension Control, and Risk of Obstructive Sleep Apnea (OSA) Brian J. Mussio, 1, 2 Loretta Simbartl, 2 Ralph Panos, 1, 2 Charulhas V. Thakar, 1, 2 Internal Medicine, University of Cincinnati, OH; 3Medical Service, Cincinnati VA, Cincinnati, OH.

Background: Obstructive sleep apnea (OSA) is a known cause of secondary hypertension, however, there are limited studies examining its prevalence in CKD, and its effect on hypertension control.

Methods: We conducted a prospective study in 211 patients at the Cincinnati Veteran’s Administration Medical Center’s CKD & Hypertension Clinic. All enrolled patients completed a Berlin Questionnaire and an Epworth Sleepiness Scale, validated screening tools for OSA and daytime sleepiness, respectively. Baseline demographics, comorbidities, laboratory data, and anti-hypertensive medications were recorded at the time of survey, whereas outpatient blood pressure readings were averaged for a time-period of one-year prior to the survey date. Univariate analyses were conducted using t-test, Wilcoxon test, and Chi-square tests.

Results: 99% of participants were male with a mean age of 63.7 ± 10.7, and mean creatinine of 2 mg/dl (± 1.3). Only 29% of patients had an average BP of < 130 or < 80 mm of Hg, and 80% of patients were taking ≥ 3 medications for hypertension. Based on the Berlin score, 142 (67%) participants were in the “high-risk” group for OSA, and 69 (33%) were in the “low-risk” group. Based on the 29 patients with a confirmed diagnosis of OSA, the sensitivity of the Berlin Questionnaire was 93%. Compared with the low-risk group, patients in high-risk group were younger (62.5 ± 10.1 vs. 66.2 ± 11.6, p = 0.02), had a higher prevalence of diabetes (51% vs. 36%, p < 0.04), and had higher Epworth scores (11.5 ± 6.5 vs. 7.1 ± 6.8, p < 0.001). Average blood pressures were similar in both groups, but the number of blood pressure medications required to achieve the blood pressures was higher in the high-risk group (3.2 ± 1.3 vs. 2.7 ± 1.2, p = 0.01).

Conclusions: OSA in veterans attending CKD/hypertension clinics may be under-recognized. Although blood pressure control was comparable, patients at high-risk for OSA required more antihypertensive medications. Whether OSA is a modifiable risk factor in hypertension management in CKD needs prospective investigation.

Funding: Private Foundation Support

FR-PO1416

Development and Validation of a Model To Predict ESRD in Elderly Patients with Advanced CKD Puja Goswami, 1 Paul E. Drazewski, 1, 2, 3 Denise C. Babineau, 1 Mahboob Rahman, 1, 2, 3 Medicine, University Hospitals Case Medical Center, Cleveland, OH; 2Nephrology & Hypertension, Case Western Reserve University, Cleveland; 3Center for Clinical Research, Case Western Reserve University, Cleveland, OH.

Background: KDOQI guidelines recommend preparation for renal replacement therapy (RRT) in all patients with a GFR<30. However, elderly patients are at increased risk for all-cause mortality and have a lower risk for ESRD. The goal of this study was to develop and validate a model to predict the 12 month risk for ESRD in elderly subjects with advanced CKD. Additionally, we assessed the ability of the model recently published by Tangri et al to predict ESRD in our elderly cohort.

Methods: We performed a retrospective cohort study at the Louis Stokes Cleveland VAMC. Eligible subjects were 65 years of age and older, had an outpatient GFR<30, and were followed for at least one year. The primary outcome was the need for RRT within 1 year of the index GFR. A Cox proportional hazards regression model was developed using backward step-down variable selection and bootstrap sampling. The accuracy of the model to predict ESRD was assessed using Harrell’s C-index.

Results: Out of 1,866 events included in the study, 77 patients developed ESRD. Risk factors for ESRD in the model were age (HR 0.47 [95% CI 0.25 to 0.58] and chronic kidney disease (HR 1.02 [95% CI 0.63 to 1.63]). The model had a C-index of 0.85, indicating excellent predictive ability. The C-index for the Tangri model was 0.78, indicating good predictive ability.

Conclusions: A model using commonly available clinical measures showed excellent predictive ability for ESRD at 12 months in elderly subjects with advanced CKD. Additionally, we validated the ability of the Tangri model to predict ESRD in an external cohort. These risk prediction models can be utilized by patients and physicians to make more informed decisions regarding the need for preparation for ESRD, such as vascular access placement.

Funding: NIDDK Support

FR-PO1417

Risk Factors for Progression in DM and Non DM CKD Patients after CKD Initiated Renal Replacement Therapy (RRT) Chung-Chang Ku, 1, 2 Chi-Chih Hung, 1 Shinn-Wei Hwang, 1, 2 H.C. Chen, 1, 2, 4 Nephrology, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan; 4Faculty of Medical Science, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Background: Taiwan has the highest incidence of end stage renal disease in the world. For decreasing the incidence of end stage renal disease and better care of chronic kidney disease patients, team of chronic kidney disease (CKD) was set up in our hospital since Dec 31, 2002. Despite of intensive intervention including education and diet modification, rapid progression was still found in many patients. This aim of this study is to evaluate the difference of potential risk factors to influence the progression of CKD in DM and non DM CKD patients.

Methods: Patients that ever joined this program from Dec 31, 2002 to Dec 31, 2008 were enrolled. Those patients followed up for more than one year with at least 3 data of serum creatinine after joining this program were enrolled. After calculate estimated glomerular filtration rate (eGFR), we can further calculate the slope.

The slope declines more than -1ml/min/1.73m²/year was defined as progression group and -5ml/min/1.73m²/year was defined as rapid progression (RP) group. By comparison with the demographic and laboratory data, we can get the potential risk factors for the progression of CKD. The statistical methods we use include chi-square test, student’s t test and logistic regression.

Results: 1018 patients (female: 445, male: 573, age: 63±14 y/o) were enrolled in this study. 335 were DM patients. 211 patients were rapid progression and 100 were DM patients.

Conclusions: Severity of proteinuria is still the most significant risk factors for CKD progression despite of intensive CKD education program.

Funding: Private Foundation Support

FR-PO1418

Therapeutic Plasma Exchange for Renal Indications in the Elderly: Ten Years Experience in One Center Emaad M. Abdel-Rahman, John S. Hayes, Jamison W. Chang, Rashheed A. Balogun. Division of Nephrology, University of Virginia, Charlottesville, VA.

Background: Elderly, above age 65 years, are growing in number. The structural and functional changes associated with aging place elderly at risk when challenged by extra corporal therapies as therapeutic plasma exchange (TPE). Aim: To compare renal indications and mortality associated with the use of TPE in elderly versus younger patients.

Methods: We retrospectively analyzed data on all patients who underwent TPE for renal indications at the UVA between January 1 2000 and June 30, 2010. Beside demographic and comorbidity data, we collected therapy specific data; procedure access, indications, side effects and mortality data.

Results: During this period, 621 patients underwent 4722 sessions of TPE. Of them 191 patients were elderly (30.7%) and they underwent 1289 sessions (27.3%) of TPE. Total of 101 patients (16.3%) underwent 593 sessions of TPE because of renal indications; 24 patients (92.3% white and 54% males) in the elderly cohort and 77 (90 % white, 58.1% males) in the younger. No statistical differences were observed in laboratory parameters, technique or comorbidies. Side effects of dyspnea and hypotension were documented in only two patients, both in the elderly cohort. Main indications of TPE in the elderly were glomerulonephritis (GN) followed by multiple myeloma (MM), with a trend towards more death in the elderly (p=0.07). The multivariable regression model which included age category, serum albumin and entrance serum creatinine was unable to predict mortality in this group of patients.

Conclusions: In our experience, renal indications for TPE in elderly are different than for younger patients, with GN being the main indication . TPE used for renal indications in older patients is relatively safe, with an increased occurrence of adverse events compared to younger patients. Trends towards death in the elderly may be multi factorial and not related to TPE alone.

Funding: Private Foundation Support

FR-PO1419

Clinicopathological Manifestation in Japanese Patients of Idiopathic Membranous Nephropathy with Each Era Shinji Kitajima, 1 Tadashi Toyama, 1 Kiyoki Kitagawa, 1 Kengo Furuichi, 1 Hitoshi Yokoyama, 2 Shuichi Kaneko, 2 Takashi Wada.1 1Division of Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; 2Division of Nephrology, Kanazawa Medical University Hospital, Uchinada, Ishikawa, Japan; 3Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa, Japan.

Background: The 20-year renal survival of idiopathic membranous nephropathy (JMN) in Japanese adults with nephrotic syndrome was reported around 60%. In this study, we evaluated the predisposing clinicopathological factors of patient survival with each era in Japan.

Methods: One hundred forty six Japanese patients (85 males and 61 females; mean age: 41.8±13.4 years) from 1965 to 2009 in Kanazawa University Hospital were evaluated in this study. The patients were followed for more than three years, or until renal or patient death. The patients were divided into three groups with each era; group 1 (1963-1979, 74 cases), group 2 (1980-1989, 44 cases), and group 3 (1990-2007, 28 cases). Clinicopathological features were evaluated for rate of remission, renal death, and patient death.

Results: Age of onset was higher in group 3 (group 1: 37.7 years, group 2: 52.5 years, group 3: 58.6 years). The rate of nephrotic syndrome was higher in group 3 (group 1: 41.8%, group 2: 72.7%, group 3: 91.1%). Major immunosuppressive therapy at onset with each era were cyclophosphamide in group 1 (38.8%), immunoglobulin in group 2

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Cardiac Biomarkers in Patients with Chronic Kidney Disease Not on Dialysis

Midori Hasegawa, Kyoko Kanayama, Yukio Yuzawa.

Cardiac Biomarkers in Patients with Chronic Kidney Disease Not on Dialysis

Ji-Young Choi,1,2 Se-Hee Yoon,1,2 Yu,1,2 Jang-Hee Cho,1,2 Mi-Kyung Jin,1,2 Owen Kwon,1,2 Chung-Hoon Kim,1,2 Yong-Lim Kim,1,2 Sun-Kyung Li,1,2 Xue-Wang Li,1,2 Xiaohong Fan, Jianfang Cai, Bixia Gao, Hang Li, Xuemei Zhang,1,2 Xiaowen Li,1,2

Background: The purpose of this study is to assess N-terminal pro-brain natriuretic peptide (NT-proBNP), high sensitivity troponin T (hsTnT), and urinary albumin creatinine ratio (UACR) as predictors of cardiac events in patients with chronic kidney disease (CKD) not on dialysis.

Methods: The levels of serum NT-proBNP, hsTnT, and UACR were measured in 413 ambulatory CKD patients not on dialysis whose estimated glomerular filtration rate was <60 ml/min/1.73 m². The patients underwent clinical follow-up for a median period of 17 months. Cardiac events were defined as cardiac death or hospitalization for acute coronary syndrome or for worsening heart failure.

Results: There were 33 cardiac events. The levels of NT-proBNP, hsTnT, and UACR were each divided into tertiles. Kaplan-Meier curves for cardiac events were clearly separated by the tertiles of hsTnT, NT-proBNP, and UACR levels. Tertiles of NT-proBNP (HR4.79, 95% CI 3.2-19.97), hsTnT (HR2.98, 95% CI 1.66-5.38), and UACR (HR2.71, 95% CI 1.64-4.48) were predictors of cardiac events by Cox regression analysis adjusted by age, gender, the presence of diabetes mellitus, and eGFR. Figure shows ROC curves for NT-proBNP (AUC 0.844; 95% CI, 0.782 to 0.907), hsTnT (AUC 0.795; 95% CI, 0.725 to 0.865), and UACR (AUC 0.714; 95% CI, 0.629 to 0.800) in predicting cardiac events. The best cut-off values were NT-proBNP of 121 pg/mL (sensitivity 72.7%, specificity 83.2%), hsTnT of 25.5 pg/mL (sensitivity 84.8%, specificity 68.2%), and UACR of 0.91 g/gCr (sensitivity 66.7%, specificity 71.6%), respectively.

Conclusions: NT-proBNP, hsTnT, and UACR are useful for risk stratification of cardiac events in CKD patients not on dialysis.

Risk Factors for Progression to CKD 3 in IgA Nephropathy

Chung-Hoon Yu,1,2 Jong-Hee Cho,1,2 Mi-Kyung Jin,1,2 Owen Kwon,1,2 Kyung-Deuk Hong,1,2 Ji-Young Choi,1,2 Se-Hee Yoon,1,2 Chan-Duck Kim,1,2 Yong-Lim Kim,1,2 Sun-Hee Park.1,2 Department of Internal Medicine, Kyungpook National University Hospital, Daegu, Republic of Korea; 2Clinical Research Center for End Stage Renal Disease, Republic of Korea.

Background: IgA nephropathy (IgAN) is the most common primary glomerulonephritis in Korea. To investigate the risk factors for progression, we retrospectively analyzed the data of IgAN from a single center in Korea.

Methods: Three hundred and twenty nine patients (M: F 173:156, mean age 34 ± 12 ) with biopsy-proven IgAN (January 2002 – December 2009) were available for analysis. Progression was defined as an development of CKD stage 3 (eGFR<60 ml/min/1.73m² by MDRD) or start of renal replacement treatment (RRT) due to ESRD. Cox regression analysis was used and presented as odd ratio (OR; 95% CI).

Results: Number of patients with episodic gross hematuria, microscopic hematuria with proteinuria, nephritic syndrome and hypertension as an initial clinical presentation was 42 (12.6%), 203 (61.7%), 5 (1.5%) and 60 (18%), respectively. At presentation, mean creatinine and protein-creatinine ratio (PCR) by spot urine was 0.99±0.08 mg/dl and 970±130 mg/g. Number of patients with glomerulosclerosis on pathology was 151 (45%). During mean follow-up of 43 months (range 12–101), 14 (4.3%) patients had begun RRT and 28 (8.2%) patients were diagnosed as CKD stage 3 and above. With univariate analysis, age at diagnosis (p=0.014, OR=2.25 (CI 1.01-1.09)), glomerulosclerosis(p=0.002, OR= 7.39 (2.07-29.98)) and PCR > 500mg/g(p=0.013, OR=6.67 (1.508-29.67)) were associated with development of CKD stage 3. Glomerulosclerosis (>0.01, OR=1.55 (1.07-2.26)) and hypertension (p=0.025, OR=3.55 (0.43-1.13)) were associated with start of RRT. With multivariate analysis, age at diagnosis, glomerulosclerosis and PCR ≥ 500mg/g were independent risk factors for development of CKD stage 3 and above.

Conclusions: This study suggests that age at diagnosis, hypertension, proteinuria more than 500mg/g and glomerulosclerosis on pathology are major risk factors for progression to CKD 3 and above in Korean patients with IgA nephropathy.

Other NIH Support - NIA, Veterans Administration Support

Factors Associated with Impaired Urinary Albumin Excretion in Chinese Rural Population

Xiaohong Fan, Jianfang Cai, Bixia Gao, Hang Li, Xuemei Zhang,1,2 Xiaowen Li,1,2 Xiaowen Li,1,2 Xue-Wang Li. Nephrology, Peking Union Medical College Hospital, Beijing, China.

Background: Obesity may be measured by five anthropometric indicators such as waist circumference(WC), waist-to-hip ratio(WHR), waist-to-height ratio(WHR), and so on. We assess to investigate the relative factors associated with UAE in Chinese rural population, especially obesity and uric acid.

Methods: The investigated subpopulation derived from an epidemiological study in Pinggu district, Beijing(n=992, 51.7±10.8 years old) was analyzed in this
study. Measurements included overnight urine collection for UAE, blood pressure, anthropometric indicators, lipids, fasting glucose, uric acid, and high-sensitivity C-reactive protein (hsCRP).

Results: In multiple logistic regression analysis, male, hypertension, diabetes, increased level of uric acid and hsCRP were significantly associated with albuminuria (UAE≥200µg/ 
min) but five anthropometric indicators were not independently related to albuminuria. If stratified by gender, hyperuricemia and hsCRP were significantly potential risk factors for albuminuria in females, but the significant associations were not detected in males. And abdominal obesity measured by WC/CR≥2.59 95%CI:1.26-5.33, P=0.03), WHR≥0.92, 95% CI:0.58-0.96, P<0.05 and BMI≥30.5, 95% CI:2.1-2.9, P=0.053) was significantly related to albuminuria in females after adjusted for age, diabetes and hypertension, but the association was not detected in males. Logistic regression for relative factors associated with UAE

<table>
<thead>
<tr>
<th>Population</th>
<th>Relative factors</th>
<th>OR value (95%CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>hypertension</td>
<td>2.9 (1.5-4.3)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>diabetes</td>
<td>3.1 (2.3-4.6)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>hyperuricemia</td>
<td>3.0 (1.6-5.5)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>elevated hsCRP</td>
<td>1.8 (1.1-2.9)</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>WoMen(N=529)</td>
<td>1.3 (0.9-1.9)</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>diabetes</td>
<td>4.5 (2.3-8.4)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>hyperuricemia</td>
<td>2.4 (1.4-4.4)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>elevated hsCRP</td>
<td>1.4 (1.1-1.7)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>Men(N=463)</td>
<td>1.2 (0.6-2.3)</td>
<td>0.38</td>
<td></td>
</tr>
<tr>
<td>diabetes</td>
<td>3.8 (2.5-5.9)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>hyperuricemia</td>
<td>2.7 (1.0-4.4)</td>
<td>&lt;0.01</td>
<td></td>
</tr>
<tr>
<td>elevated hsCRP</td>
<td>1.3 (0.6-2.8)</td>
<td>0.38</td>
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</tr>
</tbody>
</table>

Conclusions: As the potential risk factors, hyperuricemia and elevated hsCRP had the genetic-specific effect on UAE, especially in females, abdominal obesity was also significantly related to impaired UAE in females.

Funding: Government Support - Non-U.S.

FR-PO1424

Self-Reported Chronic Kidney Disease Prevalence – Results from the 2009 Michigan Behavioral Risk Factor Surveillance System
Lori Corteville,1 Chris Fussman,2 Elizabeth Edgemedan,2 Jerry Yee,2 Rajiv Saran,2 Michigan Department of Community Health, Lansing, MI;1 University of Michigan, Ann Arbor, MI;2 Henry Ford Hospital, Detroit, MI.

Background: The incidence of kidney failure in Michigan is among the highest in the nation. However, state-specific prevalence estimates for the earlier stages of chronic kidney disease (CKD) are lacking. We sought to estimate the prevalence of CKD as well as the frequency of testing for CKD in the Michigan population using the Michigan Behavioral Risk Factor Surveillance System (MiBRFSS).

Methods: The MiBRFSS is an annual, state-level, random-digit-dial telephonic survey of adults who are 18 years of age and older conducted in cooperation with the Centers for Disease Control and Prevention; goals include providing population-level estimates of health behaviors, knowledge and awareness. In 2009, >6,000 Michigan adults were asked four questions regarding their current kidney function and whether they had undergone a serum creatinine and/or urine albumin test in the past three years. Final survey results were adjusted to select for adjust for selection probability and to approximate the Michigan population.

Results: Initial results of this first MiBRFSS CKD module suggest that testing-for and awareness of CKD is low within the Michigan adult population. Overall, 3.5% of Michigan adults were aware they had been diagnosed with CKD, and fewer than half (38.6%) were aware they had been tested in the past three years. Final survey results were weighted to adjust for selection probability and to approximate the Michigan population.

Conclusions: We report the first measure of state-level CKD prevalence using the MiBRFSS. Our results indicate that self-reported prevalence and testing is low within the general population. We contend that state efforts to raise the levels of detection and awareness of CKD are warranted to reduce the number of adults in Michigan living with CKD-associated complications and kidney failure.

Funding: Other NIH Support - Centers for Disease Control and Prevention

FR-PO1425

Soluble RAGE, Glycated Hemoglobin, and C-reactive Protein as Risk Factors for Incident Chronic Kidney Disease: The Atherosclerosis Risk in Communities (ARIC) Study
Brad C. Astor,1 Marc Halushka,1 Ron C. Hoogeveen,2 Christie Ballantyne,2 Josef Coresh,1 Johns Hopkins University;1 Baylor College of Medicine.

Background: Advanced glycation end products (AGEs) and their cell-bound receptors (RAGE) have been implicated in the pathogenesis of atherosclerosis and chronic kidney disease (CKD). Circulating soluble RAGE (sRAGE) may act as a decoy to prevent the inflammatory processes initiated by RAGE activation. Alternatively, sRAGE level may be a marker of higher AGE levels, indicating increased risk of these complications.

Methods: We conducted a case-control study nested within the ARIC Study. To determine whether levels of sRAGE, glycated hemoglobin (HbA1c), and hsCRP predict incident ESRD over 18 years of follow-up. A total of 161 ESRD cases were frequency matched on sex, race, diabetes status and baseline eGFR (10 mL/min/1.73m2) to 141 controls.

Results: Median (IQR) eGFR was 65.7 (47.4-92.3) mL/min/1.73m2. Cases had higher mean systolic blood pressure (136 vs. 130 mmHg; p<0.001), were more likely to be using antihypertensive medication (64 vs. 52%; p=0.03), and had higher mean sRAGE (1240 vs. 1039 pg/mL; p=0.02) than controls. Higher sRAGE was significantly associated with odds of ESRD in minimally adjusted analyses. Higher sRAGE and HbA1c were associated with ESRD after full adjustment.

Odds Ratio (95% CI) of ESRD

<table>
<thead>
<tr>
<th>sRAGE quartile</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.46 ng/mL</td>
<td>1.0 (Reference)</td>
</tr>
<tr>
<td>0.46-0.71 ng/mL</td>
<td>1.9 (1.4-2.7)</td>
</tr>
<tr>
<td>0.71-1.06 ng/mL</td>
<td>1.8 (1.0-3.2)</td>
</tr>
<tr>
<td>&gt;1.06 ng/mL</td>
<td>1.5 (1.2-1.9)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, race, baseline eGFR, systolic blood pressure, antihypertensive medication use, current smoking, BMI, LDL, HDL, triglycerides. †Self-reported diagnosis or medication use.

Conclusions: Higher sRAGE and HbA1c are independent risk factors for ESRD.

Funding: NIHDDK Support

FR-PO1426

Soluble RAGE, Glycated Hemoglobin, and hsCRP as Predictors of End-Stage Renal Disease: A Case-Control Analysis in the Atherosclerosis Risk in Communities (ARIC) Study
Brad C. Astor,1 Marc Halushka,1 Ron C. Hoogeveen,2 Christie Ballantyne,2 Elizabeth Selvin,3 Josef Coresh,1 Johns Hopkins University;1 Baylor College of Medicine.

Background: Advanced glycation end products (AGEs) and their cell-bound receptors (RAGE) have been implicated in the pathogenesis of atherosclerosis and chronic kidney disease (CKD). Circulating soluble RAGE (sRAGE) may act as a decoy to prevent the inflammatory processes initiated by RAGE activation. Alternatively, sRAGE level may be a marker of higher AGE levels, indicating increased risk of these complications.

Methods: We conducted a case-control study nested within the ARIC Study. To determine whether levels of sRAGE, glycated hemoglobin (HbA1c), and hsCRP predict incident ESRD over 18 years of follow-up. A total of 161 ESRD cases were frequency matched on sex, race, diabetes status and baseline eGFR (10 mL/min/1.73m2) to 141 controls.

Results: Median (IQR) eGFR was 65.7 (47.4-92.3) mL/min/1.73m2. Cases had higher mean systolic blood pressure (136 vs. 130 mmHg; p<0.001), were more likely to be using antihypertensive medication (64 vs. 52%; p=0.03), and had higher mean sRAGE (1240 vs. 1039 pg/mL; p=0.02) than controls. Higher sRAGE was significantly associated with odds of ESRD in minimally adjusted analyses. Higher sRAGE and HbA1c were associated with ESRD after full adjustment.

Odds Ratio (95% CI) of ESRD

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<tr>
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<td>&lt;0.46 ng/mL</td>
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</tr>
<tr>
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<td>1.8 (1.0-3.2)</td>
</tr>
<tr>
<td>&gt;1.06 ng/mL</td>
<td>1.5 (1.2-1.9)</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, race, baseline eGFR, systolic blood pressure, antihypertensive medication use, BMI, LDL, HDL, triglycerides. †Self-reported diagnosis or medication use.

Conclusions: Higher sRAGE and HbA1c are independent risk factors for ESRD.

Funding: NIHDDK Support

FR-PO1427

Association of Serum Levels of and Genetic Variation in Inflammatory Genes with Decline in Renal Function: Results from the Chronic Renal Insufficiency Cohort (CRIC) Study
Jayanta Gupta,1 Peter A. Kanetsky,1 Nandita Mitra,2 Marshall M. Joffe,2 Harold I. Feldman,3 Muredach Reilly,4 Nicolas J. Guzman,5 Maria R. Wing,4 Vaidyanathapura S. Balakrishnan,3 Vallabh O. Shah,4 John W. Kusek,5 Dominic S. Raj,2 University of Pennsylvania;2 George Washington University; Tufts Medical Center;1 University of New Mexico;4 NIHDDK.

Background: Serum levels of inflammatory biomarkers and inherited variation in genes encoding these biomarkers previously have been associated with adverse renal outcomes. We investigated the association of serum levels of interleukin (IL) 6, IL1, IL1 receptor antagonist (IL1RA), transforming growth factor (TGF), high sensitivity C-reactive protein (hsCRP) and fibrogenesis and underlying genetic variation with progressive deterioration of renal function in CRIC study participants.

Methods: Biomarkers were determined in baseline serum samples. Genotypes for 266 corresponding SNPs were available from the IHTM-Broad/CARE chip. For each participant, we took five measures of eGFR across study visits. Estimates were determined separately among 1,638 white and 1,651 African-American subjects

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Underline represents presenting author.

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using a linear mixed effects model. Analyses were conducted with log-transformed measurements adjusted for age, gender, and baseline eGFR in genetic models, and also for proteinuria, diabetic status, blood pressure and smoking status in addition to other covariates in biomarker models.

Results: Serum level of hsCRP was significantly associated with decline in eGFR in all African American populations after correction for the false discovery rate (FDR, p <0.04). We noted no other associations with baseline serum measures among either the white or African-American group. Although several variants in various genes were nominally associated with temporal decline in eGFR (p <0.05), none retained significance after FDR adjustment.

Conclusions: hsCRP predicts deterioration of renal function in the African-American subgroup of the CRIC cohort. Individual sequence variants in selected inflammatory pathway genes were not associated with progression of kidney disease; however, these findings need to be further investigated using pathway-based analysis.

Funding: NIDDK Support

FR-PO1428

Even Mild Chronic Kidney Disease Increases the Risk of Adverse Events after Elective Cholecystectomy: Analysis of National Data from the American College of Surgeons

Strechar A. Mandayam,1 Linda W. Moore,2 Stephen L. Jones,2 Edward Graviss,2 Barbara Lee Bass,2 William E. Mitch,2 A. Osama Gaber.2 1Nephrology, Baylor College of Medicine, Houston, TX; 2Well Cornell Medical College, The Methodist Hospital, Houston, TX.

Background: Cardiovascular risks from CKD are well described but whether CKD Stage 3 (CKD3) increases the risk of unanticipated adverse events in adults undergoing elective surgery is unknown. We assessed whether CKD3 increases unanticipated adverse events after elective cholecystectomy.

Methods: We evaluated the ACS-NSQIP 2005-2007 database (a nationally representative dataset of surgical procedures and outcomes) for 30-day mortality and major complications following elective surgery. Pre-operative serum creatinine (SCr) and estimated GFR (CKD-Epi formula) were used to stratify patients into CKD3 (eGFR, 30-59) or NoCKD (eGFR<90).

Results: - Non-emergency cholecystectomy occurred in 18,260 cases: median age was 47 (18-89) years; 66.9% were white, 71% were women, 11.7% diabetic and 35.2% hypertensive. CKD3 was present in 5,542 (30.4%) with median SCr 1.2mg/dL, 0.9 to 2.5 compared to NoCKD (median Scr 0.7mg/dL, 0.4 to 1.3). Only 62 patients died but 638 had major complications. 30 day mortality occurred in 42 CKD3 pts vs 20 with NoCKD (p<0.0001). Major complications occurred in 5.9% of CKD3 and 2.5% of the NoCKD group (p<0.0001). Length of stay was also longer (2.2±1.4 vs 1.2±0.6 days; p<0.0001).

By multivariable logistic regression analysis, CKD3 was a significant risk factor for mortality and major complications (p<0.0007). The proportional hazard for mortality was 2.6 (95%CI, 1.5-4.6; p<0.0007) with CKD3 of all pre-surgery risk factors.

Conclusions: The presence of even mild renal insufficiency increases the risk (> 2 fold) of major adverse outcomes following elective surgery. This raises the potential for more intensive evaluation in patients with CKD3.

Funding: NIDDK Support, Private Foundation Support

FR-PO1429

Progression of Risk Factors for Chronic Kidney Disease in Zuni Indians

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Background: The Zuni Kidney Project (ZKP) has described the epidemic of kidney disease in the Zuni Indians in a population based cross-sectional study identifying high prevalence of incipient and overt albuminuria in both diabetic and non-diabetic subjects. The subsequent investigation of Genetics of Kidney Disease in Zuni Indians (GKDZI) described the heritability of kidney disease and its intermediate phenotypes in a study of extended Zuni families.

Methods: In order to test the hypothesis that the risk factors for CKD would progress over time, we performed an analysis of a subset of GKDZI subjects who participated in both ZKP and GKDZI.

Results: Five hundred and twenty nine individuals who participated in GKDZI were studied at 2 time points at a mean interval of 6.7 years (range 2.5-9.9 years). Forty eight percent of this cohort was female, mean age at the 1st study point was 30.8 years. The table shows the progression of CKD and its risk factors over time.

Conclusions: The prevalence of incipient and overt albuminuria in both diabetic and non-diabetic subjects. This analysis of a cohort of individuals from the ZKP/GKDZI studied at 2 time points over up to 9 years shows a progression of CKD3 and its risk factors including diabetes and obesity. These findings reinforce the need for interventions to modify these risk factors for CKD progression in this high risk population, particularly amongst the young adult Zuni.

Funding: NIDDK Support, Clinical Revenue Support

FR-PO1430

A Novel Within-Person Design To Study the Relationship between Concurrent Risk Factors and CKD Progression

Liang Li,1 Bo Hu,1 Lawrence J. Appel,2 Brad C. Astor,2 Julia Lewis,2 Michael S. Lipkowitz,2 Robert D. Toto,1 Xuelei Wang,1 Jackson T. Wright,2 Tom H. Greene,2 1Cleveland Clinic; 2Johns Hopkins, Vanderbilt; Georgetown, University of Texas Southwestern; 6CWRU; 7U Utah.

Background: GFR decline in CKD is often assumed linear over time. We recently showed that over 9-12 yrs follow-up, a substantial portion of 1094 African American Study of Kidney Disease (AASK) pts had nonlinear eGFR patterns, which may include periods of rapid GFR decline or stable/improving GFR. Concurrent risk factors from the two periods on the same patient can be compared to study their relationship with CKD progression, removing confounding from baseline characteristics.

Methods: Penalized splines were used to estimate nonlinear eGFR trajectories. For each patient, we define a stable/improving period to be at least 3 yrs with eGFR slope no steeper than 3ml/min/1.73m2/yr and total decline<4.5 ml/min/1.73m2/yr (red segment in figure) and a rapid decline period with slope steeper than 3 and total decline>12 ml/min/1.73m2/yr (yellow). Concurrent factors, measured every 6-12 months, are compared between the two periods with ANOVA.

Results: 105 AASK pts had both a stable/improving period and a rapid decline period. Rapid GFR decline was associated with higher serum sodium (p=0.021), serum CO2 (p=0.012), creatinine (p<0.001), urine protein/creatinine ratio (p<0.001), hospitalization rate (p=0.032), and lower urine urea nitrogen (p=0.0014), but not with urine sodium (p=0.69) and serum glucose (p=0.67).

Conclusions: This design is a new way to study relationship between concurrent risk factors and CKD progression, which avoids confounding from patient specific factors. It may yield useful insight into the pathophysiology of biomarkers and other concurrent risk factors with the acceleration/deceleration of CKD progression.

Funding: NIDDK Support

FR-PO1431

Hypoalbuminemia, Mortality and Chronic Kidney Disease (CKD) among Participants of the REGARDS Study

Rebecca J. Schmidt,1 Bethany S. Pellegrino,2 Suzanne E. Judd,2 Paul Muntner,2 David G. Warnock,2 Brian D. Bradbury,2 Orlando M. Gutierrez,2 William M. McClellan,2 1West Virginia University, Morgantown, WV; 2University of Alabama, Birmingham, AL; 3Amer, Thousand Oaks, CA.

Background: The predictive value of hypoalbuminemia in patients with end stage renal disease (ESRD) is well known. The prevalence and prognostic implications of low serum albumin (SA) in the general population and in patients with earlier stages of CKD are less clear.

Methods: The association between SA, CKD and income was examined among 20,106 subjects from REGARDS, a population-based study designed to identify factors leading to stroke in the Southeastern US.

Results: Hypoalbuminemia, defined as SA < 3.8 g/dL (10th percentile), was found in 10.2% of females (8.2% of males), 12.4% of blacks (7.3% of whites), 15.1% of diabetics and 10.9% of hypertensives. Mean (SD) SA was 4.17 g/dl (0.33). Females were 28% more likely to have low SA than males, blacks 89% more likely than whites; diabetes 77% and hypertensives 23% more likely than those without either diagnosis. Low SA was 28% and 26% more likely in subjects without a high school education or with an annual income <$20,000, respectively. In both racial groups, the likelihood of having a low SA was >30% higher in those with low income or education. The prevalence of low SA rose with age reaching 17.3% for age >75.

Abnormal renal function associated with low SA; subjects with GFR <45 ml/min were twice as likely to have low SA; urine albumin-creatinine ratio (ACR) ≥ 30 mg/g posed
76% greater odds of having low SA. The association between low SA and both GFR and ACR persisted after adjusting for age, race, sex, comorbid illness and income. Low SA was associated with a mortality rate of 13.8% vs 5% among those with higher SA, HR (95% CI) = 2.18 (1.90, 2.50). Higher mortality persisted after controlling for age, race, sex, hypertension, diabetes and income, adjusted HR (95% CI) = 2.03 (1.77, 2.33).

Conclusions: Our findings suggest that hypohbuminemia is associated with the presence of CKD and survival in patients with CKD. Hypohbuminemia is of further study as a biomarker of nutritional and socioeconomic status, as well as of inflammation in progressive CKD.

Funding: Pharmaceutical Company Support

FR-PO1432
Comparison of Two-Stage and One-Stage Meta-Analyses: An Example of eGFR-Mortality Association for CKD-PC Collaborators Yingying Song, Kumiko Matsushita, B. Khan Mahmoodi, Brad C. Astor, Josef Coresh, Mark Woodward, Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Background: Individual participant data (IPD) meta-analysis provides precise statistical estimates. Two approaches to meta-analyze IPD are currently used. The 1-stage approach fits a regression model to a pooled dataset including all studies. The 2-stage approach fits models in individual studies and meta-analyzes the estimates. However, their comparability is not well described. We compare these methods for eGFR-mortality association in the CKD Prognosis Consortium (CKD-PC).

Methods: For the 1-stage method, we fitted a Cox stratified model, allowing each study to have a unique baseline hazard but assuming a common hazard ratio (HR) for eGFR across studies. For the 2-stage method, we first fitted a Cox model in each study, and then meta-analyzed HRs using a fixed-effect model (same true HR for eGFR across studies) and a random-effects (allowing some variance of true HR) model. eGFR was fitted as linear splines in all models.

Results: In a sample of 10 of 46 cohorts joining CKD-PC (58,790 participants and 8,369 deaths), these methods gave nearly identical estimates except for eGFR <30 (figure).

Conclusions: The two-stage and one-stage meta-analyses provided nearly identical statistical estimates. Two approaches to meta-analyze IPD are currently used. The 1-stage approach fits a regression model to a pooled dataset including all studies. The 2-stage approach fits models in individual studies and meta-analyzes the estimates. However, their comparability is not well described. We compare these methods for eGFR-mortality association in the CKD Prognosis Consortium (CKD-PC).

Funding: FIDDK SupportNIA

FR-PO1434
The Association of klotho Polymorphism with Disease Progression and Mortality in IgA Nephropathy Gano Je Lee,1 Eunah Lee,1 Un Sil Jeon,1 Heui Jung Pyo,2 Ho Jun Chiu,3 Hyun Seong Kim,3 Young Soo Kwon,1 Department of Internal Medicine, Korea University School of Medicine, Seoul, Korea; 2Department of Internal Medicine, Seoul National University Bundang Hospital, Bundang, Gyeonggi-do, Korea; 3Progressive Renal Disease and Medical Informatics and gEnomics Research (PREMIER) members.

Background: IgA nephropathy is most common in primary glomerulonephritis causing end stage renal disease (ESRD), and vasculopathy is known to involve disease progression. Klotho, a gene related to aging, has been reported to play a role in atherosclerosis and cardiovascular dysfunction. We investigated whether klotho gene polymorphism affect clinical course of IgA nephropathy.

Methods: The data registered for the Progressive Renal disease and Medical Informatics and gEnomics Research (PREMIER) study which enrolled the patients with biopsy proven IgA nephropathy from 34 hospitals and clinics were analyzed. Two single nucleotide polymorphisms for klotho gene, G395A of promoter region and C1818T of exon 4, were examined using Taqman PCR assay, and investigated the association of genotypes of klotho with the progression of IgA nephropathy and patients survival.

Results: Among 1079 patients, clinical data from 978 patients confirmed whether alive or dead were analyzed. The allele frequency was 0.174 for A allele of G395A and 0.184 for T allele of C1818T complied with Hardy-Weinberg equilibrium. Death was observed more frequently in A allele carrier of G395A polymorphism (0.7 vs 2.6 % in GG vs GA+AA, p=0.04). Proportion of patients who were progressed to ESRD treated with dialysis also tended to be higher in A allele carrier of G395A polymorphisms (p=0.07), and renal survival was worse in same group (p=0.04). In subgroup analysis of CKD stage I to III patients at enrollment, more patients progressed to CKD stage IV and V in T allele carrier of C1818T polymorphism (6.5 vs 11.1% in CC vs CT+TT, p=0.04).

Conclusions: Klotho gene polymorphism was associated with patients’ survival and disease progression of IgA nephropathy. The exact role and mechanism of Klotho protein in IgA nephropathy should be studied further in the future.

FR-PO1435
Factors Contributing to Suboptimal Initiation of Dialysis Despite Early Nephrologist Referral Stephanie A. Hughes,4 Sheldon W. Tobe,5 Philip McFarlane,2 David C. Mendelsohn,3 Medicine, Sunnybrook Health Sciences Centre, North York, ON, Canada; Medicine, St. Michael’s Hospital, Toronto, ON, Canada; Medicine, Humber River Regional Hospital, Weston, ON, Canada; Queen’s University, Kingston, ON, Canada.

Background: Early referral to a nephrologist improves dialysis outcomes. The STARRT trial recently demonstrated that many patients still wait more than 12 months prior to initiation of dialysis. However, we know little about the factors associated with suboptimal initiation of dialysis in patients who referred to a nephrologist at least 12 months prior to commencement of RRT.

Methods: The methodology is a retrospective chart review. At each of three Toronto centers, charts of consecutive RRT patients were identified. Charts were reviewed from 1st to December 31st 2010. Information was collected from initial referral to a nephrologist until initiation of RRT. Preliminary data from a single center is presented.

Results: 88 incident RRT patients were studied. 52.2% were followed by a nephrologist for more than 12 months prior to initiation of dialysis. Of this group, 47.8% started dialysis with a central venous catheter, 37% with an arteriovenous fistula and 15.2% with a peritoneal catheter. Suboptimal starts occurred in 52.5% of patients receiving more than 12 months of median follow up of 2.63 years. After multivariable adjustment, the highest quintile of eGFR as well as highest and lowest quintiles of eGFRc were significantly associated with all-cause mortality.

Association of Mortality with Klotho Function

<table>
<thead>
<tr>
<th>Klotho Function</th>
<th>Mortality Rate per 100 person yrs</th>
<th>Unadjusted Hazard Ratio (95% CI)</th>
<th>Adjusted* Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.89-1.08</td>
<td>3.6</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>0.90-1.02</td>
<td>3.8</td>
<td>1.37 (0.72, 2.54)</td>
<td>1.31 (0.72, 2.30)</td>
</tr>
<tr>
<td>1.01-1.15</td>
<td>4.8</td>
<td>1.53 (0.72, 2.45)</td>
<td>1.05 (0.57, 1.98)</td>
</tr>
<tr>
<td>1.16-1.39</td>
<td>5.5</td>
<td>1.44 (0.80, 2.59)</td>
<td>1.14 (0.62, 2.07)</td>
</tr>
<tr>
<td>≥ 1.40</td>
<td>1.1</td>
<td>0.12 (1.83, 5.30)</td>
<td>0.91 (1.39, 3.38)</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, race, hypertension, diabetes, smoking, body mass index, prevalent cardiovascular disease, prevalent heart failure, low density and low density lipoprotein, and C-reactive protein.

Funding: NIDDK SupportNIA
Use of an Electronic Medical Record, To Examine the Factors Associated with the Progression of Chronic Kidney Disease in Referred Patients in Australia Neil Bouvaille, 1, 2 Henry R. Moody, 2 Anna Kemp, 1 Robert G. Fassett, 2 Craig L. Nelson, 3 Eugenia Pedagogos, 4 Helen G. Healy, 4 George Jack Mangos, 4 Geoffrey S. Kirkland, 5 Troy D. Kay, 5 David A. Waugh, 6 University of Western Australia, Australia; 7e-|electron|ronic Kidney Disease National Audit Alliance, Australia (e|kid|NA|A), Australia.

Background: Despite the implementation of best practice guidelines, patients with chronic kidney disease (CKD) still progress. The aim of this study was to examine the utility of an electronic medical record (EMR), used by a number of Nephrology practices throughout Australia, to explore the factors that are associated with progression of CKD.

Methods: This was a retrospective study utilising Audit4 (Software 4 Specialists, Australia), which is used by over 40 nephrology practices around Australia. Patients were included if they had a minimum of 2 serum creatinine measurements at least 90 days apart. Baseline was the time of the first entry of the patient into Audit4. Patients on renal replacement therapy at baseline were excluded. Rate of change in estimated glomerular filtration rate (eGFR) was the primary outcome.

Results: 1327 patients were included, mean eGFR at baseline was 37.4±0.7 mL/min/1.73m2 with a follow-up of 17.7 months (range 7 to 26 months). The change in GFR was -0.8±0.26mL/min/1.73m2/year. Univariate analysis demonstrated that women, smoking, erythropoiesis stimulating agent (ESA) use and high serum phosphate were associated with a greater decline in eGFR. Multivariate analysis showed that the factors associated with a more rapid decline in eGFR included: gender, age, ESA use, use of phosphate binders, and baseline eGFR (r2=0.11).

Conclusions: Additional modelling demonstrated that hypertension (n=597, r2=0.15) and albuminuria (n=311, r2=0.12) were not predictors of progression in the Nephrology care setting. A reduction in the number of variables used reduced the number of participants considerably due to missing data.

Conclusions: Our results demonstrate that the retrospective use of this EMR may result in inadequate data quality that could potentially bias the results. Mechanisms to increase the rigor of prospective data collection are required to enable EMRs to perform adequately for research.

FR-PO1437 Moderate Renal Dysfunction as an Independent Predictor of Impaired Preference-Based Health-Related Quality of Life: The 3rd Korean National Health and Nutrition Examination Survey Yun Jung Oh, 1 Choon Sook Lim, 1 Yong Su Kim, 1 Dong Ki Kim. 1 Internal Medicine, Seoul National University Hospital, Seoul, Korea; 2Internal Medicine, Seoul National University Boramae Hospital, Seoul, Korea.

Background: Only a few large-scale studies have investigated the association between health-related quality of life (HRQOL) and renal function. This study aimed to assess the impact of renal function on the HRQOL of the general population.

Methods: We analyzed data for 5,555 adults, aged 19 years or older, from the Korean National Health and Nutritional Examination Survey 2005. The Euro-Qol-5D (EQ-5D) score was used to evaluate HRQOL. The population was stratified in 3 groups according to the estimated glomerular filtration rate (eGFR): group 1, eGFR ≥ 90; group 2, 90 > eGFR ≥ 60; group 3, 60 > eGFR ≥ 30 mL/min/1.73 m2. Individuals with more advanced renal dysfunction were excluded from the analysis.

Results: In this study population, parameters concerning the subjects’ socioeconomic status (marital status, educational status, occupation, income and residential space) and psychological status (degree of stress, and quality of sleep) were all deteriorated with declining eGFRs. Moreover, the score of EQ-5D in patients with eGFR less than 60 mL/min/1.73 m2 was significantly lower compared to the other groups. On multivariate analysis, eGFR less than 60 mL/min/1.73 m2 was an independent determinant of impaired HRQOL after adjustment for age, gender, health-related behaviors (smoking and alcohol intake), socioeconomic and psychological variables, and other medical comorbidities including diabetes, hypertension, metabolic syndrome, ischemic heart disease, and cerebrovascular disease (odds ratio, 1.455; 95% CI: 1.020-2.074; p = 0.038). In subgroup analysis for patients with eGFR less than 60 mL/min/1.73 m2, eGFR was independent determinants for reporting problems in the mobility dimension of EQ-5D questionnaire (odds ratio, 3.583; 95% CI, 1.579-8.131; p = 0.022).

Conclusions: This study strengthens evidence that moderate renal dysfunction is an important predictor of HRQOL and also suggests that even the patients with moderately decreased eGFR should be managed to improve its global health outcome.


Background: Blood pressure (BP) variability as measured by 24 ABPM is an independent risk factor for cardiovascular (CV) events in the general and in the hypertensive population. Recent observations in essential hypertensives indicate that also BP variability during consecutive visits predicts a high CV risk. No study investigated the relationship between visit to visit BP variability and the risk of CV events in CKD patients.

Methods: We investigated the relationship between visit to visit BP variability (expressed in terms of standard deviation (SD)) during consecutive visits (from 2 to 7 visits at 6-months intervals) in 792 patients with stage 3-4 CKD (eGFR range: 29.15 mL/min/1.73 m2) over an average follow-up of 31 months.

Results: During the follow-up, BP was on average 131/76 mmHg with a SD of 18/11 mmHg. The SDs of systolic BP (SBP) were directly related to the corresponding average values of SBP during the follow-up [r=0.31, P<0.001] and closely associated to the presence of CV morbidities (P<0.001). During the follow-up, 104 patients had CV events. On univariate Cox regression analyses, the SD of SBP predicted a high risk of CV events (HR [5 mmHg increase]: 1.34, 95% CI: 1.10–1.27, P<0.001) while no relationship was found between the SD of diastolic BP and the same outcome. In a multiple Cox model including the SDs [HR (5 mmHg): 1.22, 95% CI: 1.05-1.1, 47, P=0.03] and the corresponding average values of SBP [HR (5 mmHg): 1.10, 95% CI: 1.05-1.16, P=0.03] both variables significantly predicted CV events. Data adjustment for Framingham risk factors did not modify the association between the SD of SBP and CV outcomes [HR (5 mmHg): 1.22, 95% CI: 1.02-1.47, P=0.03] while the average value of SBP lost its prediction power for these outcomes after multivariate data adjustment (P=NS).

Conclusions: Visit to visit BP variability may be a stronger risk factor for CV events than average SBP in CKD patients. Assessment of visit to visit SBP variability may be useful for risk stratification in CKD patients.

*On behalf of the MAURO working group

Funding: Government Support - Non-U.S.
Results: In the whole population, the mean 25-hydroxyvitamin D concentration was 18.9 ng/mL, lower than the 20-25 ng/mL considered adequate (male 20.6 ng/mL; female 17.6 ng/mL). The serum level was lowest at the age range of 20-39, and increased, reaching its peak at the age of 60-69 years. Serum 25-hydroxyvitamin D level was lower in participants with albuminuria and higher in persons with obesity (BMI ≥25 kg/m²). Vitamin D status was not associated with hypertension, diabetes mellitus, ethnicity, and history of cardiovascular diseases. After adjustment for age, gender, BMI, hypertension, diabetes mellitus, dyslipidemia, participants with vitamin D deficiency had an increased risk of albuminuria (odds ratio 1.66 [95% confidence interval (CI) 1.36-2.02; p<0.001]).

Conclusions: A lower 25-hydroxyvitamin D level may be associated with higher risk of albuminuria, but cardiovascular diseases or eGFR was not associated with vitamin D status.

FR-PO1441

Development of Diagnostic Panel for Diabetic Kidney Disease Mysore Keshavmurthy Phanshik,1 Nileshkumar Shah,1 Sarah Yates,1 Paul J. Roderick,2 Scott Harris,3 Marta Lapsley,1 Mark Edward Dockrell4 1SWIT Institute for Renal Research, London, United Kingdom; 2Public Health, Southampton University, Southampton, United Kingdom.

Background: Albuminuria and eGFR are used to detect and monitor progression of diabetic kidney disease. A proportion of diabetic patients with CKD do not have albuminuria and progression may or may not occur with worsening albuminuria. In this work, we investigated urinary biomarkers in 400 patients with diabetes and correlated their levels with stages of CKD.

Methods: Urine samples were collected from 400 diabetic patients, 388 were analysed. eGFR≤60 = 204 patients, eGFR>60 = 184; Albumin/creatinine ratio ≤ 3 = 186 and >3 = 202 patients. We measured markers and mediators of renal injury- inflammatory cytokines (IL6, IL1β), TNFα and MCP1, markers of proximal tubular injury (RBP) and matrix protein Fibronectin (Fn) using Luminex and ELISA.

Results: Urinary RBP and IL1β correlated (Kruskal-Wallis test, p<0.001; IT test for trend in the ordinal eGFR categories, p<0.001) with CKD stages but not NAG, IL1β, TNFα and MCP1. MCP1 showed significant correlation with CKD stages when albuminuria was included in the analysis. In a subset urinary Fn, cadherin 2 & 6 (proximal tubule markers) and TGFβ 1, 2 & 3 were measured. Compared to the healthy controls (n=20, F5.59;13.7 ng/ ml) urinary Fn levels were elevated in diabetic patients without CKD (eGFR ≥60, ACR<5); 9.5±5: 2.6 ng/ml. There was progressive increase in excretion of Fn with increasing stages of CKD. TGFβ isoforms or cadherin 2 did not correlate with disease; however cadherin 6 was associated with severity of CKD. Cadherin 6 was undetectable in healthy controls (n=12) and was detected in the urine of all patients with progressive CKD.

Conclusions: Urinary levels of IL 6 and RBP correlate with CKD stages in diabetic patients both in the presence and absence of albuminuria. Urinary Fn excretion is elevated in diabetic patients without any other evidence of kidney disease suggesting it could be an early marker of diabetic kidney disease. Cadherin 6 appears to be a sensitive marker to detect and monitor progression of kidney disease in diabetic patients. Further analysis is in progress.

Funding: Pharmaceutical Company Support

FR-PO1442

Prevalence of Comorbidities among Veteran Chronic Kidney Disease Patients Nеха Nainani,1 Nilang G. Patel,1 James W. Lohr,2 Pradeep Arora.1,2 1Department of Medicine, SUNY, Buffalo, NY; 2Nephrology, VAMC, Buffalo, NY.

Background: Understanding the relationship between CKD and other chronic diseases is important to develop a public health policy to improve outcomes. In this study, we sought to describe the prevalence of comorbid conditions in a cohort of veteran patients.

Methods: We conducted a retrospective cohort study of 97,451 patients seen in primary care clinic in VISN 2 network over 7 years to determine the prevalence of CKD and other chronic diseases. Those with AKI were sicker and had worse comorbid conditions.

Results: We conducted a cohort study of 97,451 patients at 111 Veterans Affairs (VA) medical centers. We compared the prevalence of comorbidities in VA vs other data bases like NHANES, KEEP and Medicare

Conclusions: Veterans have a much higher burden of coexisting comorbidities with CKD. On further analysis patients above age of 65, Veterans had much higher prevalence of CKD and peripheral vascular disease compared to the KEEP and NHANES dataset. Among patients below 65 years of age, only 10.6% of patients had CKD. 36% patients had vascular disease in the CKD group as compared to 13.88% in the non CKD group. The prevalence of myocardial infarction, congestive heart failure and peripheral vascular disease was much higher in the patients with CKD vs the non CKD group.

Prevalence of Comorbidities among CKD patients >65 years

<table>
<thead>
<tr>
<th>VAMC</th>
<th>VA/MCC</th>
<th>KEEP</th>
<th>KEEP</th>
<th>NHANES</th>
<th>KEEP</th>
<th>NHANES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-CKD</td>
<td>Non-CKD</td>
<td>Non-CKD</td>
<td>Non-CKD</td>
<td>Non-CKD</td>
<td>Non-CKD</td>
<td>Non-CKD</td>
</tr>
<tr>
<td>Number of Patients</td>
<td>74.7%</td>
<td>25.3%</td>
<td>43.6%</td>
<td>56.4%</td>
<td>44.2%</td>
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</tr>
<tr>
<td>CAD</td>
<td>26.9%</td>
<td>18%</td>
<td>21%</td>
<td>34.6%</td>
<td>26.7%</td>
<td>16.8%</td>
</tr>
<tr>
<td>DM</td>
<td>12.9%</td>
<td>13.8%</td>
<td>7%</td>
<td>11.3%</td>
<td>13.8%</td>
<td>4.2%</td>
</tr>
<tr>
<td>PVD</td>
<td>16.9%</td>
<td>9.7%</td>
<td>1.3%</td>
<td>3%</td>
<td>8.5%</td>
<td>4%</td>
</tr>
<tr>
<td>CVA</td>
<td>13.6%</td>
<td>8.7%</td>
<td>11.1%</td>
<td>7.8%</td>
<td>12.4%</td>
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</tr>
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<td>IHTN</td>
<td>76.6%</td>
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<td>69%</td>
</tr>
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<td>CC</td>
<td>22.4%</td>
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<td>16.6%</td>
<td>13.2%</td>
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<tr>
<td>DM</td>
<td>34%</td>
<td>22.6%</td>
<td>45.1%</td>
<td>37.4%</td>
<td>21.4%</td>
<td>12.1%</td>
</tr>
</tbody>
</table>

Conclusion: This study demonstrates that the presence and severity of septic AKI varies significantly based on the site of infection.
Methods: Based on the data from routine health check-ups in tertiary university hospitals during 2003-2009, 112,115 adult subjects were identified. Serum creatinine levels were calibrated to an assay traceable to isotope-dilution mass spectrometry. The eGFR was calculated using MDRD equation. PU was determined by urine dipstick test and defined as being trace or more.

Results: The majority of subjects (96.9%) had an eGFR of 60 mL/min/1.73m2 or greater and 79.7% of subjects were under 60 years of age. Over 39.9±20.7 months, 498 (0.4%) subjects died and 72 (0.1%) died for cardiovascular cause. Compared with those with eGFR of ≥105 mL/min/1.73m2, subjects with eGFR 90-104 mL/min/1.73m2 did not have increased risk for all-cause mortality after adjusting for confounders. However, the hazard ratio (HR) for death was 1.67 for subjects with eGFR 75-89 (95%CI, 1.17-2.38); 1.98 for those with eGFR 60-74 (95%CI, 1.36-2.88); 3.65 for those with eGFR 60-90 (95%CI, 2.31-5.77). In addition, PU revealed as an independent factor for death (HR, 1.25; 95%CI 1.01-1.54). For cardiovascular death (CVD), subjects with eGFR 75-104 did not show increased risk compared with those with eGFR ≥105. The HR for CVD was 6.04 for those with eGFR 60-74 (95% CI, 1.39-26.37), 17.01 for those with eGFR ≥60 (95% CI, 3.63-79.77). Also, PU was associated with CVD (HR, 1.81; 95%CI, 1.07-3.05).

Conclusions: By using MDRD equation with standardized serum creatinine, the adjusted rate of all-cause mortality was higher in subjects with eGFR 75-89 mL/min/1.73m2 compared with those with eGFR of ≥105 mL/min/1.73m2 and the adjusted risk of CVD was higher in subjects with eGFR 60-74 mL/min/1.73m2. In addition, PU more than trace was an independent risk factor for death from all-cause and CVD.

FR-PO1446

GFR Variability and Death among Patients with Stage 3 CKD Robert M. Perkins,1,2 Amanda C. Bengtzer,1 Ion D. Bucaloiu,2 H. Lester Karchner.1 Center for Health Research, Geisinger Health System, Danville, PA; 2Nephrology, Geisinger Health System, Danville, PA.

Background: Fluctuations in kidney function may herald hemodynamic compromise. We hypothesized that GFR variability predicts death among those with CKD.

Methods: All adults receiving primary care at Geisinger Health System between 2005 and 2007 who had stage 3 CKD and a minimum of 4 outpatient eGFR values within 24 months were enrolled. Subjects were excluded for any prior ESRD, CHF, metastatic cancer, or immunosuppressive therapy. The slope of eGFR change over time was calculated. Mean absolute residual value was calculated, where residual was the difference between observed and predicted eGFRs. Quartiles of the mean absolute residual were used for stratification criteria. Subjects were followed until death or study end (March 31, 2011). An adjusted Cox proportional hazard model was developed to estimate the association between eGFR variability and death, the primary study outcome, while controlling for potential confounders.

Results: 3355 patients met entry criteria. Median (IQR) follow-up was 3.9 (3.5-4.1) years. Those patients with the highest (Q4) eGFR residual, relative to those with the lowest (Q1) were more likely to be younger and have diabetes, and to have been hospitalized in the prior 6 months; they were also more likely to have higher HDL and baseline eGFR levels. Unadjusted mortality rates (Q4 vs. Q1) were 40.8 vs. 30.0 deaths/1000 PY.

Conclusions: Multivariable adjusted Cox proportional hazard ratios for death by quartile of eGFR variability among patients with baseline stage 3 CKD.

FR-PO1447

Clinical Impact of the Examination of the Ocular Fundus for CKD Patients without Atherosclerosis Risk Factors Yoshinari Yasuda,1 Kyoshi Shibata,1 Sadao Suzuki,1 Sawako Kato,1 Shoichi Maruyama,1 Enyu Imai,1 Seiichi Matsuo,1 1Nephrology/CKD Initiatives, Nagoya University Post Graduate School of Medicine, Nagoya, Japan; 2Nagoya City University, Nagoya, Japan.

Background: In recent years, chronic kidney disease (CKD) has been increasingly highlighted as a risk factor for dialysis and cardiovascular diseases. Although arteriosclerosis plays an important role for CKD onset and progression, clinical significance of the examination ocular fundus (EOF), a marker for arteriosclerosis, has not elucidated yet. Thus we analyzed relationship between EOF and CKD, especially among CKD without arteriosclerosis risks.

Methods: The study subjects were 3,464 men and 3,251 women, who underwent health check including the EOF in Kasugai City Medical Care Center in 2008. Estimated GFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 mL/min/1.73m2 and/or with proteinuria were diagnosed as CKD. EOF abnormality was diagnosed by abnormal findings by Keith-Wagner and/or Scheie classifications. Multivariable odds ratios for CKD were calculated using logistic regression adjusted for age, sex. In 3,470 subjects without arteriosclerosis risks of hypertension, dyslipidemia or hyperglycemia, multivariate odds ratios and specificity for CKD were also calculated.

Results: EOF abnormalities were observed in 602 cases (8.97%), whose eGFR was significantly lower than those without. Multivariable analysis revealed that age, male, obese, dyslipidemia and EOF abnormality were significant factors for CKD. Among subjects without arteriosclerosis risks, EOF abnormalities were found in 138 cases (4.00%), and EOF was significant risk factor for CKD by multivariate analysis. Multiple logistic regression analysis of the risk factor for CKD

FR-PO1448

Mortality Risk Factors as a Function of CKD Cohort Definition: Implications for Outcome Analyses Robert M. Perkins,1,2 Jennifer Sartorius,1 Walter Stewart,1 1Center for Health Research, Geisinger Medical Center; 2Nephrology, Geisinger Medical Center.

Background: Most retrospective cohort studies examining associations between CKD co-morbidities and mortality have enrolled mixed prevalent and incident populations. The influence of differential length-biased sampling on estimates of mortality risk associated with various covariates is unknown.

Methods: We analyzed all adults receiving primary care in an integrated healthcare system in central Pennsylvania with at least one outpatient estimated GFR value between January 1, 2004 and December 31, 2009, and stratified them non-exclusively by incident (at least two CKD-EPI eGFR values < 60 mL/min/1.73m2, separated in time by at least 90 but no more than 730 days, with at least one prior value ≥ 60 mL/min/1.73m2), prevalent (at least two CKD-EPI eGFR values ≤ 60 mL/min/1.73m2, separated in time by at least 90 but no more than 730 days, with no prior values ≥ 60 mL/min/1.73m2), and mixed incident-prevalent categories. Patients with AKI at baseline, ESRD, or metastatic malignancy were excluded. Separate adjusted Cox proportional hazard models for each cohort were developed to identify factors independently associated with death.

Results: 32,596 subjects met study criteria. 12,578 were unclassifiable, largely due to having only a single eGFR value. Median follow-up across stratified groups ranged from 3.5-5.2 years. Mortality rates in the incident, prevalent, and mixed groups was 30.4, 49.9, and 42.4 deaths per 1000 PY, respectively. No covariates demonstrated discrepant risk directionality; many (age, gender, smoking status, ACEI/ARB use, CHF, AKI, proteinuria, and serum albumin) demonstrated consistent and significant hazard estimates across all cohorts. While a history of diabetes and myocardial infarction each independently predicted death among the prevalent population, these covariates were not independently associated with an increased risk of death in the incident cohort. Unique in the incident cohort, baseline BMI and eGFR did not independently predict death.

Conclusions: CKD cohort definitions influence mortality rates and mortality risk estimates for traditional covariates, with implications for risk modeling and prognostication.

Funding: Pharmaceutical Company Support
Association between Hepatitis B Virus Infection with High Alanine Aminotransferase Levels and Low Renal Function: A Cross-Sectional Study in a Representative Sample of Chinese Jianfang Cai, Xiaohong Fan, Bixia Gao, Xuejiao Liu, Lili Liang, Xuemei Li, Xuewang Li. Nephrology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences, Beijing, China.

Background: Population-based studies failed to ascertain the association between hepatitis B virus (HBV) infection and renal disease, which is clinically observed. Our study aimed to re-test this association by considering alanine aminotransferase levels (ALT).

Methods: We tested a representative sample of 6925 Chinese adults aged 30 to 75 years for levels of serum hepatitis B surface antigen, ALT, creatinine, urinary albumin-creatinine ratio, and potential risk factors for chronic renal dysfunction. Elevated ALT was defined as an ALT ≥ 1.25 times upper limit of normal proposed by AASLD. The participants were divided into HBV carriers with elevated ALT (HBV+/ALT+), HBV carriers with normal ALT (HBV+/ALT-), and non-carriers (HBV-/ALT+). We then reorganized into HBV+/ALT+, non-carriers with elevated ALT (HBV-/ALT+), and persons with normal ALT (HBV-/ALT-). General linear model was used to calculate and compare mean eGFR in different groups and odds ratios were estimated by using logistic regression.

Results: With Bonferroni correction for multiple comparisons (α = 0.017), group HBV+/ALT+ had a multivariate-adjusted estimated glomerular filtration rate (eGFR, mL/min/1.73 m2) that was 4.4 lower than that of HBV+/ALT- (95% CI, 7.6 – 1.1; P = 0.004), 4.8 lower than that of HBV+/ALT+ (95% CI, -8.1 to -1.4; P = 0.002), and 4.2 lower than that of HBV-/ALT+ (95% CI, -7.5 to 1.0; P = 0.005). Estimated GFR didn’t differ between HBV+/ALT+ and HBV-/ALT+. Neither did that between HBV+/ALT- and HBV-/ALT+. An eGFR less than 60 was 3.75 times (95% CI, 1.11 to 12.73, P = 0.032) times as likely to occur in carriers with elevated ALT as non-carriers. HBV+/ALT+, HBV+/ALT-, and HBV-/ALT+: didn’t differ in the probability of having albuminuria (10.9% vs. 7.5% vs. 10.8%, P = 0.39).

Conclusions: HBV carriage with elevated ALT levels was associated with reduced renal function. HBV infection may impair renal function paralleling liver injury and preceding HBV-associated nephropathy.

Funding: Government Support - Non-U.S.

Total Serum Free Light Chains Independently Predict Survival in Patients with Stage 3 Chronic Kidney Disease

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Background: Approximately 50% of all chronic kidney disease (CKD) patients have moderate renal impairment (Stage 3, GFR 30-59ml/min/1.73m²) and are generally managed in primary care practices. Tools which allow appropriate risk stratification of this population are required to identify those individuals at risk of progressive renal failure or cardiac events. This study evaluated the prognostic value of polyclonal free light chains (FLCs) in CKD stage 3 patients.

Methods: Patients were recruited from primary care practices, as part of the Renal Risk in Derby study. FLCs were measured with the Freelite™ assay and established normal ranges were used (κ: median 7.3mg/L, range 3.3-19.4mg/L; λ: median 12.7mg/L, range 5.71-26.3mg/L). Total FLCs and κ/λ ratios were also calculated. Time to death was assessed using Kaplan Meier and Cox regression analysis.

Results: At baseline, total FLCs were elevated in 383/1741 (23%) patients (>50mg/L). To date, 52 patients had died. Of these deaths, 23/52 (44%) had abnormally elevated FLCs (median: 46.6mg/L, IQR: 24-71) vs alive patients (median: 36.02, IQR: 19.12). Total FLCs were significantly associated with early mortality (p<0.001). Patients with FLCs >50mg/L had shorter overall survival compared to patients with <50mg/L (p<0.001). In a univariate analysis, the following markers were associated with death: FLCs >50mg/L, gender, CRP, age, calcium, and HDL. Multivariate analysis identified FLCs >50mg/L, age, and CRP to be associated with reduced time to death. Interestingly, eGFR and urinary albumin-creatinine ratio, two factors widely reported as predictors of death, were not associated suggesting FLCs may be a more sensitive predictor of death.

Conclusions: To conclude, total FLCs provide independent prognostic information on the survival of patients with CKD stage 3. Further work will determine how FLCs can be prognostically used to manage these patients.

Funding: Pharmaceutical Company Support

Are Chinese Herbs a Risk Factor for Progression to End-Stage Renal Disease in Patients of Newly Diagnosed Chronic Kidney Disease?-- A Population-Based Study

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Background: The impact of Chinese herbs on renal function in patients of chronic kidney disease remains controversial. We searched claimed data of National Health Insurance (NHI) to study the ESRD outcome in newly diagnosed CKD patients in Taiwan.

Methods: Newly diagnosed CKD patients, based on sets of ICD-9 codes were screened from 1997 to 2008. Patients who took Chinese herbs for over 90 days after first diagnosis of CKD were categorized as Herb-used group and else as Control group. Time period from diagnosis as CKD to first dialysis, date of withdraw from NHI or to the end of 2008 was traced in each patient. Patients who started dialysis were considered as event and else as censored. Survival analyses were used, and p <0.05 was considered as statistically significant.

Results: There were 39,620 newly diagnosed CKD patients in Herb-used group and 66,795 patients in Control group. After adjusted by age, sex, diabetes mellitus, index year, Charlson Index, and urbanization of residence, the Herb-used group significantly had a reduced risk to end-stage renal disease than Control group.

Table 1. Risk for ESRD in newly diagnosed CKD patients by Cox regression analysis (n=105,725)

<table>
<thead>
<tr>
<th>Age Category</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-44</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>45-64</td>
<td>2.52</td>
<td>2.29-2.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>≥65</td>
<td>2.19</td>
<td>1.97-2.43</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Sex</td>
<td>male</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>female</td>
<td>1.22</td>
<td>1.14-1.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chinese herb</td>
<td>used</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>control</td>
<td>1.00</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>used</td>
<td>0.56</td>
<td>0.52-0.61</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

All variables (age, sex, diabetes mellitus, index year, Chinese herb used, Charlson index, and urbanization of residence) was including in the model

Conclusions: The preliminary results of this study show a beneficial effect of Chinese herbs use for the outcome of ESRD, which is contrary to the detrimental effect of herbs containing aristolochic acid. Since one-third of newly diagnosed CKD patients in Taiwan still took Chinese herbs, thus the research on the therapeutic effect of Chinese herbs on kidney disease is encouraged.

Long-Term Antiproteinuric Effect of Spironolactone Translates into Slowing in the Progression of Renal Failure


Background: The efficacy of spironolactone (SL) to reduce proteinuria has been confirmed in several studies. However, information about whether this effect is persistent or transient and its possible repercussions on renal outcomes is lacking. Aims of the study were: 1) to analyze the influence of long-term treatment with SL on the slope of GFR in patients who maintain proteinuria >1 g/d in spite of ACEI (16 p, 19.5%), ARB (49 p, 56.3%) or ACEI+ARB (21 p, 24.1%) combination, 2) to analyze if the short-term antiproteinuric effect of SL is sustained over time and 3) safety and side effects of long-term SL treatment.

Methods: Prospective observational single-center study was performed. 87 patients (59 m/28 f, mean age 58.4 ± 15.3 yr, 62% with GFR <60 ml/m) who maintain proteinuria > 1 g/d in spite of ACEI (43-77), and 69% p had a proteinuria reduction >50%. GFR slope before the first mo of therapy was 24.5 ± 15.7 months (1-84). SL 25 mg/d (12.5-25) was added to previous treatment. Mean follow up 449A
Screening for CKD in Mexico. Targeting High-Risk Populations
Guillermo G. García,1 Alfonso Gutierrez Padilla,1 Alberto Barajas,1 Martha Mendoza,1 Ma del Mar Gonzalez,1 Marcello Tonelli.2
1Nephrology, Hospital Civil de Guadalajara, Guadalajara, Mexico; 2Of Alberta, Canada.

Background: Chronic non-communicable diseases, such as obesity, diabetes mellitus, hypertension, and CKD, have become a major public health problem in Mexico. Since 2006, we pioneered screening people at risk for the presence of CKD using mobile units that travel to rural and urban communities of Jalisco.

Methods: Participants were informed of risk factors for CKD, but all consenting adults without known CKD were included. Trained personnel collected demographic and clinical data, and obtained blood and urine for serum chemistry and dipstick urinalysis. GFR was estimated with the MDRD formula. CKD was defined as per KDOQI guidelines.

Results: Between September 2006 and December 2009, 9,619 adults were screened in the mobile units. Results are compared with those of Mexico’s National Health and Nutrition Survey (NHNS) 2006.

FR-PO1454
A Simple Estimation of Serum Bicarbonate Concentration in CKD Stage 5 Patients
Tetsuya Makita,1 Yujiro Yamaoka,2 Sayako Maeda. Internal Medicine, Otsu Red Cross Hospital, Otsu, Shiga, Japan.

Background: To evaluate bicarbonate concentration is essential in daily clinical practice for CKD patients. Blood gas analysis is usually used in Japanese medical facilities for this purpose, where to measure CO2 content of serum sample is uncommon. However, routine use of a blood gas analyzer at outpatient clinic bears some difficulties because it requires additional techniques and costs. Recently, Hirose et al. founded a linear correlation for this purpose, where to measure CO2 content of serum sample is uncommon. However, the existing equations have not been evaluated in people of South Asian descent.

Methods: We measured GFR (mGFR) using the gold standard of urinary clearance of inulin in 581 South Asian men and women in the communities of Karachi, Pakistan, including 40 patients from renal clinics. Standardized creatinine assay was used. The performance of the MDRD Study and CKD-EPI equations was assessed as bias (median difference between measured and estimated GFR), precision (interquartile range of the differences), accuracy (percent of estimated GFR values that are within 30% of measured values, P30), and the root mean square error (RSME) on the log scale. 95% CI were computed via the bootstrap method using 1000 replications.

Conclusions: We conclude that: 1) Our program has successfully targeted high risk populations for CKD; 2) the prevalence of CKD and its risk factors is higher in screeners examined by these units as compared with the general population; 3) a program to use mobile units to deliver a protocol-driven care for this high-risk population will start shortly.

Funding: Private Foundation Support

FR-PO1455
How Reliable Is Estimation of Glomerular Filtration Rate in Type 2 Diabetes?
Yan Liu,1 Cheng Wang,2 Tan-Qi Lou.1 Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.

Background: Type 2 diabetes is a recognized public health problem worldwide. We compared two estimates with standard golden rectangle formula (GFR), measured by the 

Methods: A total of 207 type 2 diabetic patients were recruited. Serum creatinine (SC) level was measured by the enzymatic method. Average sGFR was 47.8 ± 26.3 ml/min per 1.73 m2. In this study, GFR was predicted by Cockcroft-Gault-equation, 6-variable MDRD estimation, 4-variable MDRD equation and MCO equation.

Results: The agreement of all the equations exceeded the prior acceptable tolerances defined as 60 ml/min per 1.73 m2. Accuracies with a deviation less than 30% of all the equations were less than the prior acceptable tolerances defined as 70%. When compared the precision, bias as well as accuracy of estimated GFR (eGFR) with GFR, GFR estimated by Cockcroft-Gault-equation showed better results. Detailed performances are listed in the table.

Conclusions: We conclude that a program has successfully targeted high risk populations for CKD; 2) the prevalence of CKD and its risk factors is higher in screeners examined by these units as compared with the general population; 3) a program to use mobile units to deliver a protocol-driven care for this high-risk population will start shortly.

Funding: Private Foundation Support

FR-PO1456
Performance of GFR Estimation Equations in a South Asian Population
Saleem Jessani,1 Andrew S. Levey,2 Lesley Stevens Inker,3 Rasoob Bux,4 Christopher R. Marriott,5 Christopher H. Schmid,2 Tazeen H. Jafar,5 1Aga Khan University, Karachi, Pakistan; 2Tufts Medical Center, Boston, MA; 3University de Saint-Etienne, France.

Background: Ethnic differences in the performance of glomerular filtration rate (GFR) estimation equations have been observed, perhaps in part from non GFR determinants of serum creatinine. However, the existing equations have not been evaluated in people of South Asian descent.

Methods: We measured GFR (mGFR) using the gold standard of urinary clearance of inulin in 581 South Asian men and women in the communities of Karachi, Pakistan, including 40 patients from renal clinics. Standardized creatinine assay was used. The performance of the MDRD Study and CKD-EPI equations was assessed as bias (median difference between measured and estimated GFR), precision (interquartile range of the differences), accuracy (percent of estimated GFR values that are within 30% of measured values, P30), and the root mean square error (RSME) on the log scale. 95% CI were computed via the bootstrap method using 1000 replications.

Conclusions: We conclude that: 1) Our program has successfully targeted high risk populations for CKD; 2) the prevalence of CKD and its risk factors is higher in screeners examined by these units as compared with the general population; 3) a program to use mobile units to deliver a protocol-driven care for this high-risk population will start shortly.

Funding: Private Foundation Support

FR-PO1457
CKD-EPI without Adjustment for Black Race Is the Best Method for Estimating Glomerular Filtration Rate in Adult Patients with Sickle Cell Disease
Marie Courbebaeix,1 Jean-Antine Ribel,1 Gilles Chatellier,1 Dominique Prie,1 Dominique Eladari,2 Jacques Pouchot,4 Gerard Friedlander,3 Jean-Benoit Arlet,1 Nephrologie et Dialyses, Hoipital Tenon, AHPH, Paris, France; 2Biotherapie, Hospital Necker, AHPH, Paris, France; 3Explorations Fonctionnelles, Hospital Necker, AHPH, Paris, France; 4Medecine Interne, Hopital E Vogueles Georges Pompidou, Paris, France; 5Informatique Hospitaliere, Hopital E Vogueles Georges Pompidou, Paris, France.

Background: The aim of our study was to determine the best equation to estimate glomerular filtration rate (GFR) in adult sickle cell disease (SCD) patients.

Methods: Since 2007, all adult SCD patients on a steady state had GFR measurement by inulin clearance. Five equations were tested to estimate GFR: Cockcroft-Gault, MDRD-v4 and CKD-EPI equations with and without adjustment for black race. Measured GFR and estimated GFRs were compared according to Bland and Altman method.

Conclusions: We conclude that a program has successfully targeted high risk populations for CKD; 2) the prevalence of CKD and its risk factors is higher in screeners examined by these units as compared with the general population; 3) a program to use mobile units to deliver a protocol-driven care for this high-risk population will start shortly.

Funding: Other NIH Support - Fogarty International Center

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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renal failure (CRF) in 18.8%. Micro or macroalbuminuria were detected in 65%, 50% and 28% of patients with normal GFR, CRF and hyperfiltration, respectively (p < 0.04). Among the 5 equations tested, the CKD-EPI equation without adjustment for racial group had both the lowest bias and the greatest precision and the difference with the gold standard decreased with increasing GFR values, whereas it increased with the Cockroft and Gault and MDRD CRF equations.

Conclusions: SCD adult patients have a high rate of true glomerular hyperfiltration which is frequently associated with albuminuria. In this population, CKD-EPI equation without adjustment for black race is the best method for estimating GFR. The validity of this equation in black people of non American origin and in Black-Americans with low BMI should be evaluated.

FR-PO1458

Performance of GFR Estimating Equations in an HIV Population  Lesley Stevens Inek,1 Zipporah Krishnasami,1 Hiba Graham,1 James Hellingter,1 Maia Leippo,1 Andrew S. Levey,1 Aghogho A. Okparavero,1 Christopher H. Schmid,1 Leppo,1 Andrew S. Levey, Aghogho A. Okparavero, Christopher H. Schmid, Hocine Tighiouart, Christina M. Wyatt.1 1Tufts Medical Center; 2University of Alabama at Birmingham; 3Mt Sinai Hospital; 4Gilead Sciences Inc.

Background: The performance of GFR estimating equations using serum creatinine (cr) or cystatin C (cys) has not been extensively tested in people with HIV.

Methods: We evaluated the performance of CKD-EPI cr, cys, and cr-cys GFR estimating equations compared to measured GFR (mGFR) using plasma clearance of iohexol in 200 HIV-positive patients on stable antiretroviral therapy. Assays for cr and cys are standardized to certified high-level reference materials. Precision was evaluated by bias (median difference between measured and estimated GFR), precision (interquartile range, IQR, of the difference), and accuracy (percentage of estimated GFR that are greater than 30% of the mGFR).

Results: Of 200 patients, 125 were on tenofovir disoproxil fumarate (TDF) and 75 were not. Mean age was 41 ± 7 years and 73% were male. Mean Cr and cys clearance was 86 ± 23 (range 23–175) mL/min per 1.73 m2. There was no difference in cys clearance between patients on and off TDF. There was no difference in bias, precision and accuracy between patients on and off TDF for cr and cys based equations, but a large difference in precision and accuracy for the cys equation.

Conclusions: The performance of GFR estimating equations using serum creatinine (cr) or cystatin C (cys) has not been extensively tested in people with HIV. Cys based estimating equations are less accurate in patients on TDF due to some people having large under and overestimates of mGFR. It is not known whether this is due to differences in cys generation, kidney handling, or extra-renal elimination.

Funding: Pharmaceutical Company Support

FR-PO1459

Combination Biomarkers for Glomerular Filtration Rate Estimating Equations May Obviate Ethnicity Adjustments in a Multi-Ethnic Asian Population Boon Wee Toe, Evan J.C. Lee. Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

Background: Estimates of glomerular filtration rate (eGFR) are improved using serum creatinine (Scr), serum cystatin C (CysC) and/or beta-trace protein (BTP) in combination with demographic data. We hypothesize that ethnicity is not a significant factor when multiple biomarkers are used for eGFR prediction. We assess eGFR using standardized Scr, BTP and CysC by nephelometry. We measured timed filtration rate (mGFR) using 3-sample plasma clearance of 99mTc-DTPA, calculated by the slope-intercept method, with body surface area normalization (du Bois) and Brochner-Mortensen correction. We fitted models developed using linear regressions of combinations of Scr, CysC, BTP and demographic data using Multiple Classification Analysis (MCA) and Bayesian Information Criteria (BIC) to select the best models. We assess the equations by considering the median bias between eGFR minus mGFR, precision (inter-quartile range) root mean square error (RMSE), and percentage accuracy (P15) within 15% of mGFR.

Results: Population means: age 53.5 ± 15 years, Scr 1.44 ± 0.7 mg/dL, CysC 1.26 ± 0.66 mg/L, BTP 1.34 ± 0.86 mg/L, mGFR 66.7 ± 33.3 mL/min/1.73m2. In all models, ethnicity is not significant.

Funding: Pharmaceutical Company Support

FR-PO1460

Estimation of Glomerular Filtration Rate in Lupus Nephritis: Which Formula More Accurately Reflects Actual GFR? Diuangnut Pripawat,1 Pongpija Tuchinda,2 Thonnapong Thongpraparn, Somkit Vasuvattakul.1 1Division of Nephrology, Department of Medicine, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand; 2Division of Nuclear Medicine, Department of Radiology, Siriraj Hospital, Mahidol University, Bangkok, Thailand.

Background: Estimation of glomerular filtration rate (GFR) is widely used in clinical practice. Creatinine is hypertected by injured tubules of lupus nephritis (LN). Routine measurement of creatinine clearance may overestimate GFR. We, therefore, assessed the performance of the formulas for GFR estimation: Modification of Diet in Renal Disease (4-v MDRD); 4-v MDRD with the ethnicity factor as established for Chinese population [4-v MDRD (Chinese)]; Cockcroft–Gault (CG); and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formulas, to determine the best formula reflecting actual GFR of LN patients.

Methods: In this cross-sectional study, we enrolled sixty-two LN patients at our renal clinic. 99mTc-DTPA renogram was used as a gold standard to measure GFR. Estimated GFR calculated by the 4-v MDRD, 4-v MDRD (Chinese), CG, and CKD-EPI formulas were compared with the measured GFR using 3 modalities: the intraclass correlation coefficient, bias, and proportion of estimated GFR within 30% of measured GFR (P30).

Results: The intraclass correlation coefficients between measured GFR and estimated GFR were 0.88 for 4-v MDRD, 0.81 for CG, 0.87 for CKD-EPI, and 0.77 for 4-v MDRD (Chinese) formula. All of the estimated GFR formula overestimated measured GFR by 3.4, 9.6, 9.1, and 17.7 mL/min/1.73 m2, for 4-v MDRD, CG, CKD-EPI, and 4-v MDRD (Chinese) formulas respectively. There were statistically significant difference of bias between the 4-v MDRD formula and the others. The 4-v MDRD formula also had the most accuracy (P30 = 93.5%). All formulas lacked precision in estimating GFR in the patients with measured GFR more than 60 mL/min/1.73 m2.

Conclusions: The 4-v MDRD, 4-v MDRD (Chinese), CG, and CKD-EPI formulas can be used for estimating GFR in lupus nephritis, especially among patients with the GFR less than 60 mL/min/1.73 m2. The 4-v MDRD formula is the most reliable and accurate method to reflect actual GFR.

Funding: Project 3866 01-1-0072

FR-PO1461

Prevalence of Chronic Kidney Disease in England: Findings from the 2009 Health Survey Paul J. Roderick,1 Jenny Mindell,1 Marilyn Roth,1 Beverley Matthews,2 Donal O'Donoghue.1 1University of Southampton, United Kingdom; 2NHIS Kidney Care; Salford Royal Foundation NHS Trust.

Background: Chronic kidney disease is a global public health problem because it is common and associated with cardiovascular risk. Prevalence estimates from national health or research surveys have been obtained from several developed countries but no such survey undertaken in the UK. This paper presents nationally-representative general population data from the Health Survey for England 2009 on the prevalence of CKD in adults in England.

Methods: The HSE 2009 was one of an annual series of national cross sectional surveys which uses multistage probability sampling to obtain nationally representative estimates. Sampling was stratified by region, and used postcode sectors and postcode address file to sample households. 4680 households were invited and 2832 (61%) participated; in these sample households. The CKD prevalence was 9%, higher in males (10% vs 8%) and with a strong inverse socio-economic in males.

Conclusions: Prevalence estimates from national health or research surveys have been obtained from several developed countries but no such survey undertaken in the UK. This paper presents nationally-representative general population data from the Health Survey for England 2009 on the prevalence of CKD in adults in England.

Funding: Project 3866 01-1-0072

Models

<table>
<thead>
<tr>
<th>Model</th>
<th>R2</th>
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<tr>
<td>Cr,cys, Scr, Age, Gender</td>
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<td></td>
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<tr>
<td>BTP, Scr, Age, Gender</td>
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<td>-191.7</td>
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<td>-247.5</td>
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<td>BTP, CysC, Scr, Age, Gender, Interaction*</td>
<td>0.902</td>
<td>-151.1</td>
<td>-240.6</td>
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</table>

*Interaction of age and cystatin C

The best equation included all biomarkers with demographics. The bias is 0.8, precision is 12.2, and RMSE is 12.3 (all mL/min/1.73m2). The P15 is 64.2%.

Conclusions: Using a combination of biomarkers with demographics may eliminate ethnicity as a significant factor in GFR prediction equations.

Funding: Government Support - Non-U.S.
FR-PO1462
Comparison of Creatinine- and Cystatin C-Based GFR Estimating Equations in Children with the Diagnosis of Systemic Lupus Erythematosus

Menenchu Gong,1 Xuemei Li,1 Xuewang Li,1 Hongmei Song,1 Yan Qin,1 Ke Zheng,1 Nephrology, Peking Union Medical College Hospital, Beijing, China; 2Internal Medicine, Peking Union Medical College Hospital, Beijing, China; 3Pediatrics, Peking Union Medical College Hospital, Beijing, China.

Background: There is no data about glomerular filtration rate, measured through plasma clearance of the exogenous markers, in the systemic lupus erythematosus (SLE) children. The objective of this study was to measure GFR and validate the GFR estimating equations in the SLE children.

Methods: Children diagnosed as SLE were enrolled. Single-compartment plasma clearance of [111m]Te-DTPA was measured and transformed into two-compartment clearance (mGFR). mGFR was normalized (nGFR) by BSA. eGFR was calculated using 9 different equations. Creatinine and cystatin C were measured using kinetic Jaffe method and immunoturbidimetry, respectively. Correlation and agreement between eGFR and nGFR and the accuracy of GFR estimation were compared.

Results: 23 children diagnosed as SLE were enrolled. The original Schwartz equation and CKD equation, compared with the other seven equations, produced eGFR with better correlation with nGFR (0.56 vs 0.52) and concordance correlation coefficient (0.53 vs 0.51), higher ratio of eGFR within nGFR±10% in Bland-Altman analysis, higher intraclass correlation coefficient (0.56 and 0.52) and 4.12 ml/min×1.73m²), narrower 95% LOA ([−61, 64] and [−40,47]), better performance correlation with nGFR (γPearson was 0.567 and 549, respectively), smaller bias (1.8 and 1.7 vs 2.6, p=0.044), but improved with the Chronic Kidney Disease Epidemiology Collaboration equation (4.0 vs 6.8, p=0.1).

Conclusions: In summary, eGFR using the MDRD equation provides a reasonably unbiased and accurate estimation of GFR in Indigenous Australians. Detailed assessment of fat-free mass may enhance accuracy of eGFR and is in progress.

Funding: Government Support - Non-U.S.

FR-PO1464
Performance of GFR Prediction Formulas in ADPKD and Type 2 Diabetes Patients: Role of Kidney Function and Demographic, Anthropometric and Clinical Patient Characteristics

Flavio Gaspard,1 Antonio Camnata,2 Fabiola Carrara,1 Claudia Cellà,1 Silvia Ferrari,1 Norberto Perico,1 Nadia Stucchi,1 Giuseppe Remuzzi,1,2 Piero Ruggenetti,1,2 Mario Negri Institute for Pharmacological Research, Bergamo, Italy; 3Azienda Ospedaliero Universitaria di Bergamo, Italy; 4University of Birmingham, UK; 5The George Institute; 6University of Queensland; 7University of South Australia, Australia.

Background: Prediction formulas have been developed for glomerular filtration rate estimation (eGFR) in subjects with reduced renal function. Whether and to which extent their performance is affected by kidney function, underlying renal disease and patient demographic, anthropometric and biochemical characteristics is poorly understood.

Methods: We evaluated the precision of GFR estimations by 14 formulas versus GFR measured by iohexol plasma clearance technique (mGFR) in 2 cohorts of ADPKD and type 2 diabetic patients matched by gender and GFR (difference between matched patients ≤1 mL/min/1.73m²). Performance was assessed considering bias, mean percent error (MPE) and accuracy.

Results: In 97 ADPKD and 97 matched diabetic patients, mGFR (81.4±26.4 vs. 81.7±26.2 mL/min/1.73m²) and serum creatinine (1.15±0.43 vs. 1.04±0.40 mg/dL) were similar. Compared to ADPKD patients, however, diabetics were significantly (p=0.05) shorter, heavier, older, more dyslipidemic and hypertensive. In the whole study group accuracy within ±10% error of all formulas ranged from 14.4 to 49.5%. In diabetics with GFR ≥90mL/min/1.73m² median accuracy was as low as 31%. Both bias (see Figure) and MPE showed a large and systematic GFR underestimation in this subgroup.

Conclusions: Performance of prediction formulas was similarly poor in ADPKD patients independent of kidney function and in diabetic patients with GFR >90mL/min/1.73m². Performance of prediction formulas was similarly poor in diabetics with higher GFR all formulas were fully unreliable, possibly because of the confounding effect of demographic, anthropometric and clinical parameters in this population.

Funding: Private Foundation Support

FR-PO1463
Accurate Assessment of Kidney Function in Indigenous Australians: The CKD equations in children with systemic lupus erythematosus (SLE) are not validated for Indigenous Australians. Therefore, this study aimed to evaluate the performance of CKD equations in Indigenous Australian children.

Methods: Children aged 6-17 years who were referred to the Renal Service at the Lady Cilento Children's Hospital were included. The equations evaluated were the Schwartz equation, the Cockcroft-Gault equation, the MDRD equation, theModification of Diet in Renal Disease (MDRD) equation, the Chronic Kidney Disease Epidemiology Collaboration (CKD-Epi) equation, and the Modification of Diet in Renal Disease (MDRD) equation.

Results: 11 children were included in the study. The median age of the children was 11 years (range 7-16 years). The median eGFR was 60 mL/min/1.73m² (range 34-93 mL/min/1.73m²). The median nGFR was 84 mL/min/1.73m² (range 50-115 mL/min/1.73m²). The median bias was 2.1 mL/min/1.73m² (range -17.8 to 17.8 mL/min/1.73m²). The median accuracy was 78% (range 52-99%). The median precision was 49.5% (range 14.4-82%). The median MPE was 6.8% (range 6.1-17.8%). The median bias was 2.1 mL/min/1.73m² (range -17.8 to 17.8 mL/min/1.73m²). The median accuracy was 78% (range 52-99%). The median precision was 49.5% (range 14.4-82%). The median MPE was 6.8% (range 6.1-17.8%).

Conclusions: The CKD equations evaluated in this study did not provide accurate assessment of kidney function in Indigenous Australian children. Further studies are needed to validate the CKD equations in this population.

Funding: Government Support - Non-U.S.
**FR-PO1465**

Epidemiologic Investigation of Chronic Kidney Disease in the District of Hulunbeier of Inner Mongolia Autonomous Region  
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1Division of Nephrology, Beijing Anzhen Hospital, Capital Medical University, Beijing, China; 2Division of Nephrology, Tian Tan Hospital Affiliated to Capital Medical University, Beijing, China; 3Division of Nephrology, People’s Hospital of Hulunbeier, Inner Mongolia Autonomous Region, China; 4Department of Clinical Laboratory, Peking University First Hospital, Beijing, China.

**Background:** To investigate the prevalence and risk factors of chronic kidney disease (CKD) in the general adult population in the Hulunbeier Prefecture, Inner Mongolia Autonomous Region where many minorities of north China live.

**Methods:** Sampling survey was performed in the residents aged 20 years and older in the Hulunbeier Prefecture. All investigated subjects were tested for urinary albumin, creatinine ratio (ACR); hematuria by microscopy of urinary sediment; and GFR estimated by serum Cr based eGFR. Those who exhibit higher serum Cr compared to true GFR without apparent causes such as particular medications changing urinary Cr secretion were defined as “Idiopathic hypercreatininemia (IHC).”

**Results:** Among 1,482 patients who were consulted to our nephrology clinic between 2005 and 2006, 66 patients were diagnosed with IHC (IHC, n=15) and all were males. Of these 66 patients, the changes in eGFR were retrospectively analyzed over five years after the expiration of six months from hemi-nephrectomy.

**Conclusions:** Preoperative view of the overall eGFR than patients 30ml/min/1.73m2. The rate of decrease in eGFR over 5 years was -0.7±4.5%/year, and renal function was 70.1±12.6 ml/min/1.73m2, 75.0±12.8 ml/min/1.73m2, and 80.0±12.9 ml/min/1.73m2, at six months after hemi-nephrectomy, 51.9±11.4 ml/min/1.73m2, at five years after hemi-nephrectomy were less than 30ml/min/1.73m2.

**FR-PO1466**

Assessment of Renal Function in African Americans (AAs) after Kidney Donation by Cystatin C and Creatinine Based Formulas Compared to 125I iothalamate Clearance  
Sunil Kumar Jain, 1 John M. Arthur, 1 Milos Budisavljevic.  
1Division of Nephrology, Dept of Medicine, Medical University of South Carolina, Charleston, SC; 2Division of Biostatistics and Epidemiology, Medical University of South Carolina, Charleston, SC; 3Division of Biostatistics and Epidemiology, Medical University of South Carolina, Charleston, SC; 4Division of Nephrology, Dept of Medicine, Medical University of South Carolina, Charleston, SC.

**Background:** AAs have 4 times higher prevalence of ESRD than Caucasians. Therefore, assessment of kidney function is of considerable importance in this patient population. kidney function is assessed most commonly by estimated glomerular filtration rate (eGFR) using creatinine based equation. Some studies indicate that measuring Cystatin C levels provide better estimate of GFR. We compared cystatin C (Cys C) and creatinine based formulas for GFR estimation with measured GFR in 33 AAs who donated their kidneys 5-27 years (mean=11.1 years) previously.

**Methods:** GFR was measured by 125I iothalamate clearance. Cystatin C was measured by Bio Vendor Human Cystatin C ELISA Kit. Pearson correlation was used for statistical analysis.

**Results:** The mean measured GFR was 76.1±18 ml/min/1.73m2. The correlation between measured GFR and cystatin C and creatinine based equations is presented in Table 1. 

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MDRD</th>
<th>MC</th>
<th>CG</th>
<th>CG-EPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bias (95% CI)</td>
<td>1.34 (14.15-14.54)</td>
<td>9.75 (7.73-11.72)</td>
<td>9.51 (9.29-9.83)</td>
<td>8.9 (7.9-10.0)</td>
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<tr>
<td>% Bias (95% CI)</td>
<td>15.1 (11.2-15.05)</td>
<td>5.67 (-7.8-3.4)</td>
<td>8.5 (-3.0-15.9)</td>
<td>8.6 (7.1-10.1)</td>
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<tr>
<td>Precision (95% CI)</td>
<td>22.5 (10.5-25.9)</td>
<td>21.5 (20.23-23.6)</td>
<td>34.1 (30.37-37.7)</td>
<td>30.3 (17.2-43.4)</td>
</tr>
<tr>
<td>Accuracy (95% CI)</td>
<td>80.1 (59.1-91.1)</td>
<td>83.9 (77.2-92.9)</td>
<td>87.4 (57.2-97.3)</td>
<td>92.1 (79.8-98.4)</td>
</tr>
<tr>
<td>Sens/Spec at 80 ml/min</td>
<td>72%/75%</td>
<td>85%/95%</td>
<td>44%/87%</td>
<td>64%/84%</td>
</tr>
<tr>
<td>Sens/Spec at 85 ml/min</td>
<td>78%/79%</td>
<td>100%/99%</td>
<td>51%/82%</td>
<td>71%/78%</td>
</tr>
<tr>
<td>Sens/Spec at 90 ml/min</td>
<td>86%/79%</td>
<td>81%/97%</td>
<td>59%/65%</td>
<td>78%/70%</td>
</tr>
</tbody>
</table>

**Conclusions:** The prevalence of CKD is 12.95% in the Hulunbeir Prefecture, Inner Mongolia Autonomous Region. Increased risk factors of CKD included increased age, increased waist, hypertension, abnormal blood glucose or lipid, and metabolic syndrome.
Results: Cystatin C 0.13 vs. 0.93, Group A vs. IHC, P<0.05, so as to a urinary protein excretion 24hr urinary protein excretion 190+/−2378mg vs. 38+/− 23mg Group A vs. IHC, P<0.05). The female ratio in IHC was significantly higher (female ratio 0.30 vs 0.60, Group A vs. IHC, P<0.05) and BSA tends to be smaller (BSA 1.68+/−0.2n2 vs. 1.64+/−0.2 m2, Group A vs. IHC, P=0.10).

Conclusions: In conclusion, prevalence of IHC is high (14.5%) among patients referred to renal clinic. Cystatin C based gFR can effectively rule out IHC. Higher female ratio and relatively smaller BSA imply that Cr metabolism rather than Cr production is related to this condition.

FR-PO1470 Prevalence of Chronic Kidney Disease in the Adult Population of Lusanne-Switzerland Bolen Ponti,1 Menno Pruijn,2 Pierre-Yves F. Martin,3 Michel Burnier,1 Vincent E. Mooser,1 Gerard Waerber,2 Murielle Bochud2,1
1Centre Hospitalier Universitaire Vaudois; 2GlaxoSmithKline.
Background: Chronic kidney disease (CKD) represents an important burden in the general population with increased cardiovascular morbidity and mortality. Population-based data are available in US and some countries of Europe but are lacking in Switzerland. We aimed to determine the risk factors and prevalence of CKD in the population of Lusanne, Switzerland.

Methods: This population-based study included 6184 Caucasians aged 35-75 years old between 2003 and 2006, of whom 2821 men and 3158 women had data for the present analysis. CKD was defined using KDOQI stages 1-5 according to estimated glomerular filtration rate (eGFR) and microalbuminuria. We compared CKD-EPI and MDRD equations to calculate eGFR and classify CKD.

Results: The prevalence of CKD using MDRD was 2.1%, 3.5%, 4.7% and 0.17% for stages 1, 2, 3 and 4, respectively. The corresponding prevalence using CKD-EPI was 2.5%, 3.2%, 4.5% and 0.17%. The prevalence of CKD (stages 1-5) was 10.4% using MDRD and 10.2% using CKD-EPI. Overlap between the two equations was 91% for stage 3 and 100% for stages 4-5. The prevalence of CKD (CKD-EPI) was 8.7% in non-diabetic and 28.9% in diabetic, 5.9% in normotensive and 17.4% in hypertensive subjects. It was 4.9%, 5.6%, 11.3% and 25.1% in persons aged 35-45, 45-55, 55-65 and 65-75 years, respectively. According to the Body Mass Index (BMI) prevalence was 7.0%, 17.7% and 17.2% for BMI<25, BMI 25-30 and BMI≥30kg/m2 respectively. In multivariate regression analysis, determinants of CKD were age per year (OR 1.06; 95% CI 1.01-1.07), female sex (OR 1.22; 95% CI 1.02-1.47), hypertension (OR 1.78; 95% CI 1.45-2.18), diabetes (OR 2.32; 95% CI 1.78-3.03) and BMI≥30kg/m2 (OR 1.33; 9.13-1.72).

Conclusions: The prevalence of CKD in the adult population of Lusanne is substantial, although lower than in US. CKD prevalence sharply increases after 55 years of age and is particularly high in diabetic, hypertensive and obese subjects. Our results suggest that screening strategies aiming at detecting CKD should focus on higher risk patients such as elderly, diabetic, obese and hypertensive ones.

FR-PO1471 Annual Incidence of Kidney Damage in General Population Kei Nagai, Chie Saito, Kunihiro Yamagata. Nephrology, Clinical medicine, University of Tsukuba, Tsukuba, Ibaraki, Japan.
Background: Several reported studies on the prevalence of proteinuria in mass screening were based on single-screening data. Therefore, we investigated consecutive screening results to determine the annual incidence of kidney damage in the general population.

Methods: The subjects were participants in an annual health examination held in Ibaraki, Japan, between 1993 and 2003. 314,354 males and 649,533 females, who underwent Ibaraki, Japan, between 1993 and 2003. 314,354 males and 649,533 females, who underwent screening were based on single-screening data. Therefore, we investigated consecutive screening strategies aiming at detecting CKD should focus on higher risk patients such as elderly, diabetic, obese and hypertensive ones.

Results: Of 831 CKD patients were enrolled. SC was measured by the enzymatic method. Average eGFR measured by 63+Te-DTPA GFR estimation was 45.4±27.3 (3.3-137.7) ml/min/1.73 m2. The patients' GFRs were estimated by Cockcroft-Gault-equation, MDRD1-equation, abbreviated MDRD-equation, reexpressed 6-variable MDRD equation, Cockcroft-Gault-equation, abbreviated MDRD-equation and reexpressed 6-variable MDRD equation were closer to the identical line. The median of difference of Cockcroft-Gault-equation, abbreviated MDRD-equation and reexpressed 6-variable MDRD equation were smaller. The median 6 absolute difference of Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation and Chinese-equation were smaller. Accuracy of Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation and MDRD1-equation were higher than those of the other equations. However, accuracies with a deviation less than 30% of all the equations were less than 70%. When compared the performance between eGFR and GFR in different stages of CKD, GFR estimated by Cockcroft-Gault-equation, reexpressed 6-variable MDRD equation, MDRD1-equation and reexpressed 4-variable MDRD equation showed better performance.

Conclusions: When SC was measured by the enzymatic method, this study highlights a limitation in the use of GFR estimation equations in Chinese CKD patients. Correspondence to: Prof. Lou Tan-qi

FR-PO1473 Prevalence of Chronic Kidney Disease in China Luxia Zhang1, Li Wang2, Wenke Wang3, Bicheng Liu4, Jian Liu4, Menghua Chen4, Qiang He5, Yunhua Liao6, Xueqing Yu5, Nan Chen7, Jianer Zhang7, Zhao Hu1, Fuyou Liu1, Haiyan Wang2. 2Peking University First Hospital; 3Sichuan Provincial People’s Hospital; 4Nanjing Second Hospital; 5Zhongshan Hospital; 6Renji Hospital; 7Affiliated Medical University of Xijing Medical University; 8Affiliated Medical University, Ningxia Medical University; 9First Affiliated Hospital, Zhejiang University; 10First Affiliated Hospital, Guangxi Medical University; 11First Affiliated Hospital, Sun Yat-sen University; 12Ruijin Hospital; 13Taihe Hospital; 14Qilu Hospital; 15Second Xiangya Hospital.
Background: Previous studies revealed a high prevalence of CKD among developing countries. However, there is no national survey of CKD incorporating both estimated glomerular filtration rate (eGFR) and albuminuria in a developing country with marked heterogeneity like China.

Methods: The present study is a cross-sectional survey among a representative sample of 47 204 participants in China. Participants were interviewed and were tested for albuminuria and reduced renal function. The crude and adjusted prevalence of indicators of kidney damage were reported.

Results: The adjusted prevalence of eGFR<60ml/min/1.73m2 and albuminuria was 1.7% (95% CI 1.5%-1.9%) and 9.3% (95% CI 8.7%-9.8%), respectively. The overall prevalence of CKD was 10.6% (95% CI 10.1%-11.2); therefore the number of patients with CKD in China is estimated to be 117.3 million. In rural area, the prevalence of eGFR<60ml/min/1.73m2 did not vary markedly with levels of economic development, while higher prevalence of albuminuria was observed in higher tertiles of GDP per capita. In urban area, lower prevalence of both eGFR<60 ml/min/1.73m2 and albuminuria was observed in sites with higher level of economic development.

Conclusions: Our study revealed that China is going to experience an enormous increase in the prevalence of CKD, especially for the rural residents, who comprised more than half of the population in China. The rapid surge in diabetes and hypertension, both of which are predicted to drive epidemics in CKD, will have profound socioeconomic and public health consequences in developing countries such as China.

FR-PO1474 Frequency of Mild Kidney Disease and Associated Risk Factors in Apparently Healthy Mexican Subjects Carlos Kornhauser. Department of Medical Sciences, University of Guanajuato, Leon, Guanajuato, Mexico.
Background: Chronic kidney disease (CKD) associates with a wide range of complications leading to a decreased quality of life. Mild kidney disease comprise the first three stages of CKD, being over 5 times more frequent than terminal kidney disease. Objective: We evaluated the frequency of CKD in stages 1, 2, and 3, by assessing GFR according to the MDRD equation, and the presence of microalbuminuria in apparently healthy people.

Methods: We did an epidemiological, cross-sectional study in 1160 apparently healthy adult subjects of both sexes, in the city of Leon, Mexico. Subjects were randomly selected. Clinical and anthropometric data were collected. Glucose, creatinine, uric acid,
total cholesterol, triglycerides, and HDL, LDL cholesterol fractions were measured in serum. A urinary creatinine and albumin were also assessed. Descriptive statistics, Anova, Kruskal Wallis, multiple regression and logistic regression test were performed. **p** < 0.05 was considered significant.

**Results:** Male and female genders were evenly distributed. Male’s average age was 40.6 (19-87) years and 45±11 in stage 5 (5%); stage 4, 3.5±7.1%. Stage 3 or worse CKD (11.6%) and smoking in 17.4%. Furthermore 729 (78%) were overweight (BMI >25 Kg/m2). CKD was defined using the MDRD guideline. Individual results were discussed with the subjects to be relayed to their primary physicians for follow up as needed.

**Conclusions:** CKD is very common in West Texas with a prevalence of 18.5% with a high prevalence of risk factors for CKD especially obesity. Awareness of the condition is dismally low underscoring the need for widespread screening and prevention by addressing the risk factors.

**Funding:** Government Support - Non-U.S.

<table>
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<th>Control group</th>
<th>P-value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
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<td>18-30</td>
<td>0%</td>
<td>0%</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>31-40</td>
<td>0%</td>
<td>0%</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>41-50</td>
<td>5.5%</td>
<td>0.0%</td>
<td>&lt;0.001</td>
<td>4.4 (2.9-6.7)</td>
</tr>
<tr>
<td>51-60</td>
<td>14.4%</td>
<td>0.3%</td>
<td>&lt;0.001</td>
<td>54.7 (7.4-404.2)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>31.1%</td>
<td>2.3%</td>
<td>&lt;0.001</td>
<td>11.5 (1.3-128.1)</td>
</tr>
</tbody>
</table>

**Discussion:** Chronic kidney disease, in particular the younger age groups. This may eventually lead to kidney failure but more importantly places these patients at increased risk of cardiovascular disease.

**FR-PO1477**

A Prospective Population Screening Study of Prevalence of CKD and Its Risk Factors: The Texas CKD Study

**Background:** Recent reports indicate a high prevalence of CKD in general population in the US (16.8%, NHANES 2006). However most such reports are based on retrospective sampling and the true prevalence of CKD remains unclear. Texas is estimated to have disproportionately high prevalence of CKD and ESRD and related healthcare costs. The Texas CKD study is an initiative funded by the Texas Department of Health to address the same.

**Methods:** The study was organized by Texas Tech University and conducted in the West Texas population with a goal to screen a total of 1000 adult subjects (age >21 yrs). The subjects were recruited by a random digit dialing (RDD) methodology with phone interviews and scheduling administered by the University of North Texas Survey Research Center. Appropriate approvals were obtained to comply with IRB and HIPAA regulations.

**Results:** The sample was adjusted to conform to rural urban mix, as well as age, race and sex ratios in the Texas adult population. Risk factors for CKD were noted by personal questionnaire while GFR was estimated using both MDRD and CKD-EPI formulae from serum creatinine values. Urine microalbumin and urine protein/creatinine ratio were also determined. CKD was defined using the MDRD-EPI guidelines. Individual results were discussed with the subjects to be relayed to their primary physicians for follow up as needed.

**Conclusions:** CKD is very common in West Texas with a prevalence of 18.5% with a high prevalence of risk factors for CKD especially obesity. Awareness of the condition is dismally low underscoring the need for widespread screening and prevention by addressing the risk factors.

**Funding:** Government Support - Non-U.S.
Endocrine-Metabolic Disorders in Patients with Chronic Kidney Disease

Jung-Ho Shin, Min-Jee Han, Youn-Su Park, Woojin Nam, Su Hyun Kim, Dong-Jin Oh, Suk-Hee Yu. Department of Internal Medicine, Chung-Ang University College of Medicine, Seoul, Korea.

Background: Endocrine-metabolic disorders are common in patients with chronic kidney disease. Several studies showed that the prevalence of endocrine-metabolic disorders is increased in patients with chronic kidney disease, and that could have important role in the prognosis of chronic kidney disease. In this study, we investigated the prevalence of endocrine-metabolic disorders in healthy person according to the renal function.

Methods: We retrospectively reviewed 948 adults selected from the Health Promotion Center at Chung-Ang University Hospital. Age, sex, height, weight, waist circumference, blood pressure, fasting glucose, lipid profile, serum creatinine and bone mineral density were evaluated. The glomerular filtration rate (GFR) was estimated by the Modification of Diet in Renal Disease (MDRD) formula. Decreased renal function was defined as an estimated GFR under 60 mL/min/1.73m² and metabolic syndrome was defined as per the International Diabetes Federation (IDF) 2006 criteria.

Results: The mean age was 47.1±9.3 years in men (276, 29.1%) and 50.8±9.7 years in women (672, 70.9%). There were 918 persons with normal renal function and 30 persons with decreased renal function. The prevalences of metabolic syndrome and osteoporosis were 13.8% (10.1% in men, 11.1% in premenopausal women and 27.0% in postmenopausal women) and 14.2% (9.8% in men, 11.1% in premenopausal women and 29.8% in postmenopausal women), respectively. The percentages of persons with metabolic syndrome and osteoporosis increased in persons with decreased renal function (P=0.002 and 0.0084). Subgroup analysis was conducted for men, premenopausal women and postmenopausal women groups. In premenopausal women, we found similar results, but in men and postmenopausal women, we found no difference of prevalence between persons with normal renal function and persons with decreased renal function.

Conclusions: Metabolic syndrome and osteoporosis were increased in persons with a decreased renal function in premenopausal women. However, there was no association between endocrine-metabolic disorders and decreased renal function in men and postmenopausal women.

FR-PO1480

Estimated Glomerular Filtration Rate Does Not Progressively Decline in the Healthy Elderly, Particularly in Females and Those with High Cholesterol Levels

Fumihiko Hinoshita, Maki Shibata, Manami Tada.

Background: Estimated GFR (eGFR) seems to be inappropriately underevaluated in the elderly healthy. Consequently, in a sweeping assumption, many of the elderly have been grouped as CKD stage 3 and considered as would-be candidates for HD in the future. Therefore, this study was performed to discern whether or not eGFR does progressively decline in the elderly with aging.

Methods: A total of 278 elderly people (286 males, 288 females) at the age of 60 or older (60 to 94 years old) without serious diseases were enrolled. They were living in one local community in central Tokyo. Clinical data including eGFR, BP, BUN, total cholesterol (TC), triglyceride, uric acid and urinalysis between 2008 and 2010 were annually collected. Details of 332,174 (57.3% of the total) participants with both serum creatinine and Cystatin C was evaluated among198 Taiwanese and 157 Koreans. All samples were measured in a single center, and sCr values were IDMS-traceable. This study was a part of Asian Collaborative Study for Creation of GFR Estimation Equation (ACOS-CG-FREE).

Results: The findings of the present study indicate that CKD and risk factors factor profiles based on eGFR and proteinuria among the general population.

Conclusions: Better accuracy was demonstrated in Japanese eGFR equation for Korean but not for Taiwanese, probably due to different body mass muscle.

FR-PO1481

Validation of MDRD and Japanese GFR Equation among Taiwanese and Korean: Approach To Set the Fundamental Scheme for eGFR Evaluation

Enyu Yoshinari, Masaru Horio, H.C. Chen, Yo Yung Lee, Enyu Imaji, Seiichi Matsuo.

Background: Glomerular filtration rate (GFR) is essential for CKD diagnosis and staging. The Modification of Diet in Renal Disease (MDRD) Study equation is globally well used, however ethnicity coefficient is not available for Asians. Japanese society of nephrology develop Japanese coefficient of 0.808 for MDRD equation, whereas Chinese and Korean coefficients were reported to be 1.233 and 1.09285, regardless of similar genetic and cultural background among Asians, probably due to different GFR measured units and lack of creatinine (Cr) standardization. Thus we validate MDRD and Japanese eGFR equations among Taiwanese and Koreans by inulin clearance (Cin), a gold standard for GFR, under the same protocol as in Japan and accurate sCr values. The study was a part of Asian Collaborative Study for Creation of GFR Estimation Equation (ACOS-CG-FREE). Urinary Cr excretion rate divided by body weight was compared to that of Japanese.

Conclusions: Better accuracy was demonstrated in Japanese eGFR equation for Korean but not for Taiwanese, probably due to different body mass muscle.

FR-PO1482

Risk Factor Profiles Based on Estimated Glomerular Filtration Rate and Dipstick Proteinuria among Participants of the Specific Health Check and Guidance System in Japan 2008

Kunitoshi Iseki, Tsuyoshi Watanabe.

Background: Endocrine-metabolic disorders are common in patients with chronic kidney disease (CDV), end-stage renal disease (ESRD) and mortality. Few studies, however, have examined the risk factor profiles based on eGFR and proteinuria among the general population.

Methods: The data of the newly developed nationwide screening program of the Specific Health Checkups and Guidance System (Tokutai-Kensin) initiated in 2008 were used in this study. The aim of this screening was targeting people 40 to 74 years of age to detect those with metabolic syndrome and to offer those services regarding lifestyle modifications that will lead to the reduction of diabetes mellitus (DM) and DM-related ESRD. Individual records of 580,000 participants in 69 cities and towns and 3 unions' cohorts throughout Japan were anonymously provided by calcium in the National Center for Global Health and Medicine, Tokyo, Japan.

Results: Details of 332,174 (57.3% of the total) participants with both serum creatinine and dipstick urine test data were analyzed. Mean (SD) age was 63.6 (8.3) years and 40.6% were men. The mean (SD) eGFR was 67.2 (17.7) mL/min/1.73m² and 5.4% had proteinuria. The prevalence of DM, hypertension, and history of stroke and heart disease was correlated with the combination of eGFR and degree of proteinuria.

Conclusions: The findings of the present study indicate that CKD and risk factors for CDV are quite common among middle-aged Japanese. CKD classification based on eGFR and proteinuria may be useful for predicting CVD, mortality rate, and ESRD in Japan.

FR-PO1483

The Choice of the CKD Definition and the Impact on the Chronic Kidney Disease Prevalence

Pierre Delanaye, Etienne Cavalier, Christophe R. Mariat, Jean-Marie H. Krzesinski.

Background: CKD definition is notably based on an estimated GFR fixed cut-off at 60 mL/min/1.73 m³. Such a fixed knot has already been criticized in the literature because GFR physiologically decreases with aging. In this work, we will illustrate the consequences of this fixed reference value on the CKD epidemiology.

Methods: Over a two years period, we tested 4208 voluntary subject aged of more than 50 years old for CKD with the measurement of an IDMS traceable creatinine. GFR was estimated with the MDRD stage 1/2 equation: either with a reference range fixed at 60 mL/min/1.73 m³ or according to age as defined in the Nijmegen

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
To age. This is particularly relevant in women and older population. This seems logical because both populations have physiologically lower GFR values. Not considering age in the CKD definition will overestimate the number of CKD patients, especially in women and older subjects.

FR-PO1484

The Effect of Ethnicity on C-reactive Protein Level in Patients with Diabetic Nephropathy

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Background: Studies show that ethnic differences alter the rate of progression of diabetic nephropathy (DN) and that African Americans (AA) have a more rapid course as compared to Whites. We postulated that elevated indicators of inflammation in AA may potentially explain this disparity possibly revealed by levels of inflammatory marker C-reactive protein (CRP).

Methods: We included CRP and other clinical indicators of DN in different ethnic groups in the data from the National Health and Nutrition Examination Surveys (NHANES) 1999-2004.

Results: We analyzed data for 1,637 adults aged >=20 years who were told to have diabetes or had fasting blood sugar >125mg/dl. Descriptive statistics characterized the subjects. We used Chi-square for categorical variables and t-test or ANOVA for continuous variables to test the statistical differences between the groups.

Results: The data indicate that CRP, urinary albumin, serum creatinine, HbA1c and fasting glucose were significantly higher in diabetic patients with albuminuria compared to patients without albuminuria (75.1±0.06mg/dl, 4.5±0.01mg/dl, 7.3±0.11% and 187.5±7.5mg/dl, 4.6±0.04, 9.6±0.31, 0.8±0.01, 7.2±1.40, 56±15.3 respectively; p<0.05). Estimated GFR was significantly reduced in diabetic patients with albuminuria (75.1±1min/1.73m²) compared to those without it (79.1±1min/1.73m²; p<0.004). There was no ethnic difference in the level of CRP and urinary albumin in patients aged 20-44 and >65, except among those without albuminuria, where AA had higher CRP compared to Whites (1.05±0.14 and 0.46±0.08 respectively) (p<0.01). However, in age group 45-64, for those without albuminuria, CRP and urinary albumin were significantly elevated in AA compared to Whites (p<0.01) but for those with albuminuria, only urinary albumin was significantly higher in AA compared to Whites (p<0.01). However, CRP was trended higher in AA compared to Whites (p=0.05).

Conclusions: Concentration of CRP is higher in AA compared to Whites aged 45-64. The results suggest that more advanced inflammatory processes might explain the more rapid progression of type 2 DN in AA patients in this age group and should be further examined.

FR-PO1485

Chronic Kidney Disease: Still an Unrecognized Health Issue?

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Background: Chronic kidney disease (CKD), especially end stage renal disease (ESRD), is not only associated with a higher morbidity and mortality but also with extremely high cost for the healthcare system. Early diagnosis and therapy of CKD could slow down the progression of the disease and minimize the prevalence of cost-intensive ESRD. Aim of this present study was to determine the prevalence of CKD in inpatients at a general internal ward and to evaluate the frequency of documented CKD according to ICD-10 coding at the Department of Internal Medicine, Graz, Austria.

Methods: Over a period of 4 months the data of 238 patients admitted to a general internal ward of the Department of Internal Medicine, Medical University Graz, Austria, were collected. We analyzed the prevalence of CKD, defined as eGFR<60ml/min/1.73m². eGFR was derived from the four-variable MDRD. Furthermore discharge letters were collected. We analyzed the prevalence of CKD, defined as eGFR<60ml/min/1.73m².

Results: A total of 161 subjects with CKD were identified (7.4% of the study population). Nearly half of the study population at least had an eGFR<60ml/min/1.73m². The median eGFR was 54.5±15.8 in women and 63.6±10.4 in men (n.s.). Nearly half of the study population at least had an eGFR<60ml/min/1.73m². The median eGFR was 54.5±15.8 in women and 63.6±10.4 in men (n.s.). The prevalence of CKD was significantly higher in AA compared to Whites (p<0.01). However, CRP was trended lower to Whites (1.05±0.14 and 0.46±0.08 respectively) (p<0.01). However, in age group 45-64, for those without albuminuria, CRP and urinary albumin were significantly elevated in AA compared to Whites (p<0.01) but for those with albuminuria, only urinary albumin was significantly higher in AA compared to Whites (p<0.01). However, CRP was trended higher in AA compared to Whites (p=0.05).

Conclusions: Chronic kidney disease is higher in AA compared to Whites aged 45-64. The results suggest that more advanced inflammatory processes might explain the more rapid progression of type 2 DN in AA patients in this age group and should be further examined.

Conclusion: The present analysis shows a high rate of undiagnosed CKD in hospital settings. Public awareness campaigns and targeted educational programs could have dramatic implications on the care, treatment and prevention of CKD and associated complications.

FR-PO1486

Survival in Patients Entering Renal Replacement Therapy in their First Month of Life – A World-Wide Collaborative Study

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Background: End-stage renal disease requiring RRT from the neonatal period is a very rare condition. Hence, little information is available regarding long-term treatment outcomes in these challenging patients.

Methods: Neonates who started RRT in their first month of life were included in the present study. Patients starting RRT since 2000 who were registered prospectively in the ESPN/ERA-EDTA registry were included in the study and periodically updated. The primary outcome was survival to 180 days after start of RRT. Secondary outcomes included the number of RRT modalities used and change in management over time.

Results: A total of 161 patients from 30 countries started RRT during the first month of life. They were followed for a median of 31 (IQR 8-47) months. Nearly all children started on PD (90%), while 15 started on HD and one patient with a transplant. Half of them started in their first week of life. The most important causes of renal failure were congenital anomalies of the kidney and urinary tract (62%), followed by cystic kidneys (14%) and tubular necrosis (9%). Within the first 2 years after start of RRT, there were 99 changes in treatment modality among 75 children, including 26 transitions from PD to HD, 19 from HD to PD, 22 transplants, and 3 recoveries of renal function, of whom 2 were only temporary. Sixteen children died after a median of 6 months, resulting in an estimated two-year survival of 86%.

Conclusions: While we cannot exclude potential selection bias from excluding patients in whom RRT was electively not initiated, this study demonstrates that current RRT technology remarkably good medium-term patient survival is achieved in those neonates in whom a decision for RRT is made. In addition to neurodevelopmental issues, this study might help physicians in deciding whether RRT is an option in neonates with severe renal failure.

FR-PO1487

Foord1 Is An Upstream Regulator of the Renin-Angiotensin System (RAS) during Metanephric Kidney Development

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Background: The RAS is critical in ureteric bud (UB) branching and metanephric kidney development. Mutations in the RAS genes in mice or humans cause a spectrum of congenital anomalies of the kidney and urinary tract (CAKUT). However, the mechanisms by which RAS gene mutations result in CAKUT are poorly understood. In this study, we tested the hypothesis that Foord1, a forkhead box transcription factor essential for normal kidney development, is an upstream regulator of the RAS during UB morphogenesis.

Methods: The effect of genetic inactivation of Foord1 on UB branching and RAS gene and protein expression was examined in embryonic (E15) and E15.5+/− and −/− kidneys in vivo and in mesenchymal (MK4) cells transfected or not with Foxd1 expression vector (1.0 µg plasmid DNA) in vitro. Angiotensinogen (AGT), renin, angiotensin I-converting enzyme (ACE), angiotensin (Ang) II receptor type 1 (ATIR) mRNA levels were determined by real-time qRT-PCR. Cellular distribution of AGT and renin proteins was examined by immunohistochemistry.

Results: The number of UB tips was decreased in Foord1−/− (n=4) compared with Foxd1+/− (n=6) metamorphs (14±2.1 vs. 28±1.3, p<0.05). Renin, ACE, ATIR mRNA levels as well as AGT and renin protein expression was decreased in Foxd1−/− compared with Foxd1+/−. Treatment of E13.5 Foxd1−/− kidneys with AT1R and did not alter AGT mRNA levels. Treatment of E13.5 Foxd1−/− kidneys with exogenous Ang II (10-5 M) for 24 hours increased the number of UB tips compared with media (control) (42±2.0 vs. 33±2.5, p<0.05).

Conclusions: In summary, RAS gene expression during metanephric development is differentially regulated by Foord1 at the transcriptional level. We conclude that the cross-talk between the RAS and Foxd1 plays an important role in UB morphogenesis and pathogenesis of CAKUT.

Funding: NIDDK Support
FR-PO1488

Association between Genetic Variation at FOXP3 Gene Locus and Renal Transplant Outcomes Jennifer A. McCaughan,1,2 Aisling E. Courtney,1,2 A.J. McKnight,1,2 Alexander P. Maxwell,1,2 1Department of Nephrology, Belfast City Hospital, Belfast, United Kingdom; 2Nephrology Research Group, Queen’s University, Belfast, United Kingdom.

Background: Regulatory cells have emerged as key coordinators of immune tolerance. A unique feature of these cells is the stable expression of the forkhead box transcription factor gene FOXP3 (Xp11.23). It is reported that transplant recipients with longterm graft survival demonstrate significantly higher FOXP3 expression than those with graft loss from chronic immunological injury. This study is the first to investigate whether genetic variation at the FOXP3 locus is associated with allograft or transplant recipient survival.

Methods: Genomic DNA was prospectively collected from recipient and donor pairs in first deceased donor kidney transplants. A total of 575 recipients and 516 donors were included with 354 (61%) recipients and 301 (58%) donors being male. Over 99% of both populations were White.

Genotyping data for FOXP3 single nucleotide polymorphisms (SNPs) with a minor allele frequency > 5% were downloaded from the International HapMap Project, a White population. Five SNPs met the criteria for a Hardy-Weinberg cutoff 0.001 and genotype rate > 95%. Seven Tag SNPs were genotyped using Sequenom iPLEX and called using MassARRAY TYPER. A single SNP was genotyped using Taqman technology. Genotypes and allele frequencies were analysed on a gender-specific basis using SPSS.

Results: One FOXP3 SNP within the donor genome was significantly associated with allograft survival [rs2280883, p = 0.038] and one with recipient survival [rs2294021, p = 0.031]. FOXP3 gene variants in the recipient genome were not significantly associated with either allograft or recipient survival.

Conclusions: Replication of this study in another cohort of donor-recipient pairs will establish if this association is robust.

FR-PO1489

Genetic Variation at Caveolin-2 Locus and Renal Transplant Outcomes Jennifer A. McCaughan,1 Aisling E. Courtney,1 A.J. McKnight,1 Alexander P. Maxwell,1,2 1Dept. of Nephrology, Belfast City Hospital; 2Nephrology Research Group, Queen’s University, Belfast.

Background: Caveolae are invaginations of the plasma membrane which are formed from a stable complex of proteins caveolin-1 and caveolin-2. Caveole have a key role in intracellular drug transport, cell growth, transmembrane signalling, and apoptosis. Single nucleotide polymorphisms (SNPs) within the caveolin-1 gene are significantly associated with renal allograft fibrosis and survival. The caveolin-2 gene (CAV2), located at 7q31.1, is also a plausible candidate gene implicated in renal allograft survival.

Methods: We performed SNP analysis on genomic DNA from 575 recipients and 516 donors in paired, first deceased kidney transplants. Prospective clinical data has been recorded from 1986 on all recipients. Over 99% of both populations were White.

Genotyping data for CAV2 SNPs with a minor allele frequency > 5% were downloaded from the International HapMap Project for a White population. Thirteen SNPs met the criteria for Hardy–Weinberg cutoff 0.001 and genotype rate > 95%. Four Tag SNPs were chosen using the pair-wise approach implemented in Haploviev. Five potentially functional SNPs were downloaded from the Ensemble Genome Browser.

Eight SNPs were genotyped using Sequenom iPLEX and called using MassARRAY TYPER. A single SNP was genotyped using Taqman technology. Genotypes and allele frequencies were analysed on a gender-specific basis using SPSS.

Results: One SNP in FOXP3 expression post transplantation appears to be associated with longterm allograft survival. It was an unexpected finding that FOXP3 variants in the donor genome were associated with recipient and graft outcomes. Replication of this study in another cohort of donor-recipient pairs will establish if this association is robust.

FR-PO1490

Genome Wide Methylation Analysis of 485,577 Features in a Renal Transplant Population Jennifer A. McCaughan,1,2 Aisling E. Courtney,1,2 A.J. McKnight,1,2 Alexander P. Maxwell,1,2 1Dept. of Nephrology, Belfast City Hospital; 2Nephrology Research Group, Queen’s University, Belfast, United Kingdom.

Background: Studies have highlighted that renal (dys)function is influenced by differential methylation at several loci. We investigated the DNA methylome by conducting a genome-wide association study in peripheral blood leukocytes.

Methods: Ninety-six individuals (recipients vs. donors) were matched for age and gender. DNA methylation was performed for a renal disease panel (ESKD), IgA nephropathy, chronic allograft nephropathy and survival outcomes. Methylation status was profiled in individuals by hybridisation to 450K Illumina methylation beadchips (Illumina Inc, USA). 90 samples and arrays passed stringent quality control; raw data were normalised and beta values calculated. Significantly up and down regulated genes for each comparison (p < 0.005) were considered for biological relevance by functional enrichment analysis using KEGG pathways.

Results: Quantitative methylation values were obtained at a single-CpG site level for 485,577 features, encompassing coverage of all desigable RefSeq genes, including protein coding, non-coding, and 3' and 5' regions of coding genes, and miRNA promoter regions. Experimentally defined genders matched each individual submitted for analysis and >99% duplicate concordance was observed.

Conclusions: We have identified differences in methylation profiles both globally, and at individual CpG sites, which are associated with ESKD and renal transplant outcomes.

FR-PO1491

Comprehensive Genetic and Epigenetic Investigations for Association of the Major Histocompatibility Complex Region in a Renal Transplant Population A.J. McKnight,1 Jennifer A. McCaughan,1,2 Aisling E. Courtney,2 Alexander P. Maxwell,1,2 1Centre for Public Health, Queen’s University of Belfast, NI, United Kingdom; 2Regional Nephrology Unit, Belfast City Hospital, Belfast, NI, United Kingdom.

Background: The prevalence of end-stage kidney disease (ESKD) continues to rise so that there is a delay to the transplantation of patients with long-term survival of renal grafts and transplant recipients are important goals. Genetic variation within the major histocompatibility complex (MHC) results in phenotypic variation expressed in human leukocyte antigens (HLA) that, along with non-HLA targets, affect renal function and transplant outcomes. We have previously reported association of MHC-related genes with diabetic nephropathy.

Methods: We have genotyped >3,000 maximally informative SNPs in the MHC region and MHC-related genes in 900 matched, White European, kidney transplant donors and recipients (maximum follow-up 238 months; median 69 months) using a combination of dedicated MHC panels (illumina Inc, USA) and sequenom-based genotyping. Complementary to this approach, next generation sequencing technology (Illumina) was performed to individually fine-map the entire MHC region for 10 participants. Association of variants with ESKD was evaluated using PLINK while short and long-term graft and recipient survival was evaluated using Kaplan-Meier plots and Cox regression models. Methylation status of CpG islands within 6p21 was available for 90 of the individuals genotyped.

Results: Greater than 97.5% completion was observed with >99% duplicate concordance. Genome-wide significant association (adjusted P<10-8) was identified for several loci. We also provide detailed coverage of the technically challenging 5 Mb region that includes HLA-A, B, C, DMA, DMA, DOA, DBO, DPA1, DPB1, DQA1, DQB1, DRA, DRB1, E, F, G, H, J, K, L, U and HLA-W genes.

Conclusions: Our comprehensive study highlights the complexity of the MHC region and provides important information for genes that are primarily involved in innate and adaptive immune systems. We have identified several key loci that influence renal function, these need validated in larger cohorts followed by functional experiments to elucidate the mechanisms.

FR-PO1492

APO1 Risk Genotypes are Enriched in African American Study of Kidney Disease and Hypertension (AASK) Participants, Particularly among Those With Kidney Disease Progression Cheryl Ann Winkler,1 Barry I. Freedman,1,2 Wen Hong Linda Kao,1 Carl D. Langefeld,2 Brad C. Astor,2 George W. Nelson,3 Mary E. Comeau,3 Donald W. Bowden,3 Jeffrey B. Kopp,3 Michael S. Lipkowitz,2,3 1SAIC, NCI, Frederick, MD; 2Wake Forest University, Winston-Salem, NC; 3Johns Hopkins University, Baltimore, MD; 4EDDC, Bethesda, MD; 5Georgetown University Medical Center, Washington, DC.

Background: Chromosome 22 variation explains much of the increased kidney disease risks in African descent individuals. The AASK study enrolled 1,194 subjects with hypertension and reduced glomerular filtration rate and examined the effect of blood pressure control on nephropathy progression, defined as serum creatinine >3 mg/dl (61 events) or ESKD (158 events).

Methods: We compared APO1 and MYH8 genotypes in 675 AASK subjects and 618 African American population controls. The control group, of whom 43% had hypertension, was recruited in southeastern USA and lacked a personal or family history of kidney disease. Mean African admixture was 0.89 in both cases and controls.

Results: Compared to the AASK risk allele carriers (APO1 G and MYH8 A), the APO1 risk allele carriers were 23.2% in cases compared to 11.6% in controls, OR 2.31, P = 7.1X10E-8. After adjusting for age and gender, the OR associated with having two risk alleles were as follows: for hypertensive kidney disease (all AASK cases) versus all controls, 2.3 (95% CI 1.7, 3.1), P = 6 X 10E-8; for hypertensive chronic kidney disease (all AASK cases) versus hypertensive controls, 2.2 (1.4, 3.7), P = 0.002; for AASK cases with baseline urine protein/creatinine ratio >0.2 g/g vs controls, 4.3 (3.0, 6.2), P=1X10E-14; and for AASK cases with progression versus controls, 4.1 (2.9, 5.9), P = 1.8X10E-14. After adjusting for APO1, the presence of two MYH8 E1 haplotype copies...
was associated with OR 1.7 (1.1, 2.4), P=0.01 for progression. There was no interaction between APOL1 risk allele status and ACE inhibitor use with regard to progression.

Conclusions: APOL1 risk allele prevalence is elevated among AASK participants with hypertension-attributed kidney disease. Two APOL1 risk alleles markedly increase the risk of kidney disease progression.

Funding: NIDDK Support, Other NIH Support - NCI

FR-PO1493
The Use of Homozygosity Mapping To Identify the Responsible Gene for Diffuse Mesangial Sclerosis, an Entity with Genetic Heterogeneity
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Background: Many hereditary kidney diseases display genetic heterogeneity manifesting as a common phenotype derived from mutations in a number of different genes. Diffuse mesangial sclerosis (DMS), a histologic variant of steroid-resistant nephrotic syndrome (SRNS) in the pediatric age group represents an example of this phenomenon, with 4 genes (NPHS1, NPHS2, PLCE1 and WT1) responsible for a third of all cases. Given that these 4 genes contain together 79 exons, diagnostic sequencing is expensive, and remains ineffective in about 70% of all cases.

Methods: Detecting the genetic basis of the disease in patients from consanguineous or ethnically-related families can be accomplished by the implementation of homozygosity mapping based on SNP microarray analysis. Localization of homozygous genomic loci “identical-by-descent” allows to restrict the number of known genes which may be associated with the disease in particular cases.

Results: We performed homozygosity mapping in an attempt to study a consanguineous Arab family with 6 offspring affected with SRNS, 4 of whom succumbed to the disease. In one child, the histologic diagnosis of DMS was made when he already had ESRD. Homozygosity mapping revealed 8 homozygous regions of 10 to 2 Mbp in length. Later detected in an unrelated family of the same ethnic background with two affected children, pointing to a founder effect.

Conclusions: This approach is economically sound when the total number of exons of the potentially relevant genes exceeds 40-50. We recommend the implementation of homozygosity mapping in complex consanguineous families affected by diseases characterized by genetic heterogeneity.

Funding: Clinical Revenue Support

FR-PO1494
Glucocorticoid Receptor Sensitivity in Patients with Focal Segmental Glomerulosclerosis
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Background: Glucocorticoids are the primary therapy used to treat FSGS though neither the target cell nor their mechanism of action in FSGS are known.

The aim of this study was to compile the clinical data, NPHS2 mutation and Glucocorticoid Receptor sensitivity in our patients with FSGS.

Methods: We evaluated 14 patients with FSGS. There were 4 Hispanics and 10 African Americans. 4 had ESRD. 11 patients were evaluated for NPHS2 mutations. Monocytes Glucocorticoid receptors sensitivity in our patients with FSGS.

Results: Patients with no response to Steroids and Immunomoduspression, who progressed to ESRD had Hypersensitive GR response. One patient who was steroid dependent had normal GR sensitivity.

Conclusions: We conclude that GR sensitivity testing may provide predictive value prior to deciding on starting steroid treatment and dose of steroids.

Funding: Private Foundation Support

FR-PO1495
Replication and Validation of MYH9/APOL1 Chronic Kidney Disease Risk Alleles in an Urban Tertiary Care Center: Implications for Personalized Medicine
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Background: Genetic variants in a region of chromosome 22 have consistently associated with nephropathy in African Americans and have been replicated in other populations. These have potential utility for genomic prediction of CKD risk. The Institute for Personalized Medicine Biobank is an electronic medical record-linked biorepository with up to 20,000 participants. Self-reported populations include ~40% Hispanics or Latinos (HA), ~30% African Americans (AA) and ~25% European Americans (EA). The prevalence of CKD stage 3 or higher (KDQI definition) is ~15%.

Purpose: To replicate previous genotype-phenotype associations and validate risk loci in our heterogeneous local population prior to potential clinical decision support implementation.

Methods: We are directly genotyping all Biobank participants for 12 SNPs in the APOL1/MYH9 region that have been associated with CKD. Preliminary genotyping was undertaken on 2052 participants (900 cases (cCKD), 1152 controls). Logistic regression analysis was performed in each population for CKD and CKD+diabetes using age, sex, age2 and the first two principal components (from genome-wide data) as covariates. Levels of statistical significance were determined by the Bonferroni correction or at p<0.05 for previously replicated loci.

Results: We have demonstrated that previously validated risk variants can be replicated within our local populations. In particular, the G1 alleles of APOL1 (rs60910145, rs73835319) are strongly associated with non-diabetic CKD in our AA population (OR’s 2.6 and 1.8 respectively, p<0.01) with a recessive model of inheritance, consistent with previous reports. We also replicated a previous association of an MYH9 E1 allele (rs8214840) with non-diabetic CKD in our HA (OR 2.43, p<0.05).

Conclusions: We anticipate that extended results from ~5000 AA and ~7000 HA patients will provide a compelling rationale for future studies to evaluate the impact of genomic prediction tools in the management of modifiable CKD risk factors.

FR-PO1496
A Combined Deletion of CFHR1 and CFHR3 Conveys Protection Against IgA Nephropathy, Jingyuan Xie,1 Zhaohui Wang,1 Weiming Wang,1 Hong Zhang,1 Yingyan Gao,1 Xinwei Zhang,2 Yifu Li,2 Ping Hou,3 Simone Sanna-Cherchi, 2 Zhaohui Wang,1 Weiming Wang,1 Hong Zhang,3 Nan Chen,1 Ali G. Ghavarhi,1 1Renal Department, Shanghai Ruijin Hospital, Shanghai Jiaotong University, Shanghai, China; 2Division of Nephrology, Columbia University, New York City, NY; 3Renal Division, Peiking University First Hospital, Institute of Nephrology, Peiking, China.

Background: In a recent GWAS, we detected a major susceptibility locus for IgAN within the complement factor H (CFH) gene cluster on chr.1q32. The top SNP, rs6677704, tags a common deletion of CFHR1 and CFHR3 genes (CFHR1,3). The goal of this study was (1) to directly type CFHR1,3A to determine whether it accounts for the association a with multiplex ligation-dependent probe amplification (MLPA) and quantitative PCR. Association analyses were performed with PLINK v1.07 and UNPHASED 3.1.3.
Results: The CFHR1,3A and rs667604 variants were in strong LD (r2=0.90, D'=0.98) and both had a strongly protective effect on IgAN (rs6677604 OR=0.61, p=1.6×10^-6; CFHR1,3A OR=0.58, p=2.1×10^-7). After conditioning on rs6677604, CFHR1,3A had an independent protective effect (OR=0.51, 95%CI: 0.26-1.00, p=0.05). In contrast, after conditioning on CFHR1,3A, rs6677604 was no longer significant (95%CI: 0.59-1.27, P=0.7). We also identified 3 rare single-gene CNVs (CFHR3A, CFHR1A and CFHR1 duplication, freq=0.8-2%), but these CNVs were not associated with risk of IgAN.

Conclusions: The CFHR1,3A variant explains the rs6677604 association signal, strongly suggesting that this deletion is the functional allele at the CFH locus. The absence of association of single-gene CNV’s suggests a synergistic effect of the CFHR1 & CFHR3 deletions on the alternative complement pathway.

Funding: NIDDK Support

FR-PO1497

Genome-Wide Linkage Scan of Japanese Families with IgA Nephropathy
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Background: A genetic predisposition of IgA nephropathy (IgAN) has been suggested by the familial clustering of the disease. Previously, genome-wide linkage analysis of IgAN revealed several susceptibility loci including 6q23-25 (RGAN), 4q26-31, 1q12-22, and 2q36. However, no causative gene mutations underlying these linkage loci has been identified. From the point of view of genetic heterogeneity of familial IgAN, oligo(poly)genic and multiple susceptibility gene model for the disease are proposed.

Methods: In this study, we investigated 10 Japanese multiplex families in which multiple members are affected by biopsy proven IgAN. A total of 62 subjects (24 affected) were genotyped using Genome-Wide Human SNP Array 6.0. Multipoint linkage analysis was performed using SAGNHitLink, which is a program providing a useful pipeline to directly connect SNP data and linkage analysis program, enabling a high-throughput analysis.

Results: The genotypes of each sample were determined with a high overall call rate of over 99.5%. Parametric analysis with assumption of an autosomal dominant mode of inheritance, with estimated penetrance of 75%, yielded maximum heterogeneity LOD score of over 99.5%. By nonparametric analysis, eight regions of potentially interesting level of inheritance, with estimated penetrance of 75%, yielded maximum heterogeneity LOD score of over 99.5%. Parametric analysis with assumption of an autosomal dominant mode of inheritance, with estimated penetrance of 75%, yielded maximum heterogeneity LOD score of over 99.5%. By nonparametric analysis, eight regions of potentially interesting level of inheritance, with estimated penetrance of 75%, yielded maximum heterogeneity LOD score of over 99.5%

Conclusions: These results provide a support for genetic heterogeneity among families with IgAN, and novel candidate loci responsible for familial IgAN in Japan.

FR-PO1498

Coding Polymorphisms of Interleukin-22 Receptor Alpha-1 Contributing to the Development of Childhood IgA Nephropathy in Korean Population
Jin-Soon Suh,1 Byoung-Soo Cho.

Background: Interleukin (IL)-22 is a member of the IL-10 cytokine family and plays an important role in the immune response by activating certain tissue cells including kidney. In the present study, we investigated the associations between polymorphisms of IL-22R1 and IL-10R2 and childhood IgA nephropathy in Korean children.

Methods: We evaluated 194 pediatric patients with biopsy-proven IgAN and 287 healthy controls. Two single nucleotide polymorphisms (SNPs) in the coding region of the IL-22R1 gene [rs7395299 (missense, Arg45Pro)] and one SNP in the IL-10R2 gene [rs3795299 (missense, Lys47Glu)] were selected and genotyped by direct sequencing methods.

Results: Our control-analysis showed that genotypes of rs7395299 were associated with childhood IgAN in the codominant model (p=0.008, OR=95% CI=1.33 [0.91-1.95]) and in the recessive model [p=0.002, OR(95% CI)=0.28 [0.11-0.69]]. After Bonferroni correction, this association of rs7395299 with IgAN risk remained significant.

Conclusions: Polymorphisms in IL-22R1 may be associated with the development of childhood IgAN. Replication and functional studies are involved to better understand the mechanism by which these polymorphisms may contribute to the pathogenesis of IgAN.
Results: In the FSOS-HIVAN cohort, the risk-associated MYH9 SNP rs2413396 was tested for linkage signal with other APOL1 genotypes. In a AA - G2 - D1 (p = 0.006; G1 - AG - G2 - D1 p = 0.04). Considering all AA subjects in an analysis stratified on the APOL1 genotype, the MYH9 SNP was associated with renal disease (p<0.006, odds ratio 2.0, 95% confidence interval 1.4, 3.1). In the hypertension-attributed ESKD cohort, residual MYH9 effects were detected for three E1 haplotype SNPs under a recessive model: rs4821480 (P=0.029, OR=1.26), rs2032487 (P=0.036, OR=1.32), and rs4821481 (P=0.012, OR=1.40).

Conclusions: These results suggest that additional independent loci within the MYH9/ APOL1 extended linkage disequilibrium region on chromosome 22 are associated with renal disease.

Funding: NIDDK Support, Other NIH Support - NCI

FR-PO1504
Association of SLC2A9 with Serum Uric Acid and Renal Phenotypes in Zuni Indians

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1Genetics, Texas Biomedical Research Institute, San Antonio, TX; 2University of New Mexico, Albuquerque, NM; 3Dialysis Clinic Inc, Albuquerque, NM.

Background: Elevated serum uric acid (UA) levels are associated with gout, metabolic syndrome, heart and renal disease. UA levels are heritable in Caucasian, African American and Asian populations. Genome-wide association studies (GWAS) have demonstrated an association of SNPs in the solute carrier protein 2 family, member 9 (SLC2A9) gene with UA. The Zuni Indians are a small, relatively endogamous, tribe in NM.

Methods: We studied 1016 members of extended families who participated in the NIH funded, Genetics of Kidney Disease in Zuni Indians (GKDZI) study. The GKDZI seeks to identify genetic factors, which modulate susceptibility to renal disease and intermediate phenotypes. We conducted a GWAS using the Illumina Human1M-Duo v3.0 BeadChips, read on the Illumina BeadStation 500GX and analyzed with Genome Studio software. We used a linear regression-based association test under an additive model of allelic effect. We accounted for the non-independence of family members using a kinship variance component. Analyses were done in SOLAR.

Results: There was strong heritability of UA levels (h2 = 0.32 ± 0.07) (p < 0.001) and strong association of UA levels with solute carrier family 2 (facilitated glucose transporter), member 9 (SLC2A9) SNPs, rs6449213, rs938555, rs16890979, rs12498857, rs734553 and rs6832349 (p < 10-08). SLC2A9 encodes a UA transporter that mediates renal urate flux from proximal tubules. Minor allele frequencies of SNPs ranged from 32 to 49% and the mean effect sizes ranged from 3.6 to 4.3%. All SNPs except rs6449213 and rs12498857 were associated with higher UA levels. There were associations of SLC2A9 SNPs with urine albumin-creatinine ratio, serum creatinine and glomerular filtration rate (eGFR) (p < 0.05).

Conclusions: The strong associations of SLC2A9 variants with UA in Zuni Indians replicate findings in other populations. The association of SLC2A9 with renal phenotypes is a novel finding.

Funding: NIDDK Support

FR-PO1505
Genetic Correlation between Urinary Calcium and Heritable Mandibular/Maxillary Hypoplasia in a Congenital Rat Line

Krista L. Lewandowski,1 Guy M. P. Perry,1 Robert J. Reid,1 Jyotirmoy Nandi,1 David A. Bushinsky,2 Steven J. Scheinman.1 1Medicine, SUNY Upstate Medical University, Syracuse, NY; 2Medicine, University of Rochester, New York.

Background: In a hypercalciuric congenic rat line derived from Genetic Hypercalciuric Stone-forming rat, we detected a de novo mutation (pug) with marked facial abnormalities (Figure 1), including: i) fusion of the coronal and sagittal sutures and ii) hypoplasia of the mandible, premaxilla and maxilla. These features have sometimes been associated with craniosynostosis and elements of calcium physiology in humans. We investigated possible genetic associations between the penetrance of this new mutation and urinary calcium excretion in our pedigree.

Conclusions: Genome-wide association studies of serum urate identify novel genomic risk loci for gout. Association with FEUA suggest that some, but not all, SNPs may be associated with higher serum urate levels by altering renal urate excretion.

Funding: NIDDK Support, Other NIH Support - NHLBI
NIA, Government Support - Non-U.S.
Figure 1. Facial abnormalities seen in pug (below), versus normal (above) rats

Methods: The rat pedigree consisted of 1589 rats over 14 generations, including 170 F1; pug-normal heterozygotes to test possible bias from assortative breeding. Weight at 8 weeks was measured, followed by 4 days of urine collection. The pug phenotype was scored as a binary trait by multiple observers.

Results: Heritability for all three traits was high and pug was highly genetically correlated with calcium excretion (Table 1). There was no difference in the correlation of urinary calcium with pug in F2 pug-normal hetero compared to the complete population (δ = 0.25±0.0039; P<0.05).

Table 1. Additive and dominant heritabilities (diagonal), and genetic correlations (above diagonal) for pug, calcium excretion, and 8-week weight (95% CI).

<table>
<thead>
<tr>
<th>Additive</th>
<th>pug</th>
<th>S.wk. wt.</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>pug</td>
<td>0.63 (0.04)</td>
<td>0.10 (0.08)</td>
<td>0.96 (0.04)</td>
</tr>
<tr>
<td>Ca</td>
<td>0.61 (0.04)</td>
<td>0.17 (0.04)</td>
<td></td>
</tr>
<tr>
<td>S.wk. wt.</td>
<td>0.31 (0.05)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dominant</th>
<th>pug</th>
<th>S.wk. wt.</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>pug</td>
<td>0.23 (0.02)</td>
<td>0.37 (0.01)</td>
<td>0.30 (0.01)</td>
</tr>
<tr>
<td>Ca</td>
<td>0.34 (0.02)</td>
<td>0.21 (0.04)</td>
<td></td>
</tr>
<tr>
<td>S.wk. wt.</td>
<td>0.15 (0.02)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Our results suggest the genetic association or linkage of calcium excretion with pug. Such a link could be clinically important for the genetic basis of craniosynostosis, and for renal complications of this and related disorders.

Funding: Private Foundation Support

FR-PO1506

Next Generation Resequencing of 40 Candidate Genes for Calcium-Based Kidney Stones in 810 Subjects Identifies Novel Allelic Variants and Shows Association of Urinary Calcium Excretion with Claudin14 Hakon R. Toka, 1,2 David B. Mount, 1 Martin R. Pollak, 2 Gary C. Curhan.1, 1Nephrology, Brigham and Women’s Hospital, Boston, MA; 2Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.

Background: Nephrolithiasis is a major cause of morbidity and a complex, multifactorial condition involving multiple genes along with environmental influences determining the likelihood of stone formation. Higher urinary calcium excretion is associated with increased risk and can be considered as a strong risk factor.

Methods: This study was designed to investigate the role of rare, functionally significant allelic variants affecting urinary calcium excretion and other relevant urinary solutes. N = 40 known candidate genes were sequenced in 810 participants recruited from the Nurses Health Study (NHS) and the Health Professionals Follow-up Study (HPFS). Subjects were selected based on availability of validated phenotypical data and 24-hour urine collection data. Novel approaches were utilized which included barcoding and pooling strategies, target gene enrichment with RainDance technology and next generation sequencing.

Results: Most samples showed excellent sequencing data with deep coverage of 20x or more. Over 1600 potential SNPs were detected. Several strategies were applied to remove false positive variants. None of the novel variants showed statistically significant association between the high and the low urinary calcium group and were observed mostly in single subjects only. Analysis of more common, known variants suggested an association of Claudin14 (CLDN14) with low urinary calcium excretion. Although, this effect of CLDN14 was previously not observed in our GWAS study using samples from both, NHS and HPFS (unpublished data), an Icelandic study has previously shown association of CLDN14 with kidney stones.

Conclusions: Validation studies in larger sample sets will be necessary to confirm the association of CLDN14 with urinary calcium excretion. Further phenotypic analysis and functional studies of selected novel allelic variants identified in this study may shed light on molecular mechanisms leading to nephrolithiasis in a subset of patients in the general population.

Funding: Other NIH Support - Program Project

FR-PO1507

A Single Nucleotide Polymorphism in the Aqp11 Gene Is Associated with an Increased Risk for Chronic Kidney Disease in Patients with Diabetes David Peter Choma, Eric G. Neilson, Raymond C. Harris, Elena E. Tchekneva. Nephrology and Hypertension, Vanderbilt University School of Medicine, Nashville, TN.

Background: Aquaporin-11 is a novel aquaporin family member. Disruption of the murine Aqp11 gene causes severe proximal tubular injury and renal failure. A G662A single nucleotide polymorphism (SNP) in the human Aqp11 gene results in Gly102Ser substitution in a functionally important domain. The purpose of this study was to determine if individuals carrying the Aqp11 G662A SNP are at higher risk for developing chronic kidney disease (CKD).

Methods: This was a retrospective case control study. Patient data and DNA samples were obtained from the Vanderbilt DNA Databank (BioVU) and associated de-identified medical record. Caucasian patients greater than 18 years of age with exposure to intravenous hyperosmolar contrast by either ICD9 or CPT code were identified as potential study patients. Cases were defined as having AKI by creatinine elevation or ICD9 code. Control patients were those not having an AKI event. Covariates included age, gender, and diabetes.

Patients were defined as having CKD if the patient had least 2 creatinine values separated by at least 90 days that were greater than or equal to 1.5mg/dl. Risk for either AKI or CKD was assessed as an odds ratio.

Results: Patients with diabetes carrying the SNP were at increased risk for any AKI event (OR 1.834; 95% CI 1.047-3.212; ch2 4.507, p=0.034) and for AKI within 7 days following contrast exposure (OR 1.412; 95% CI 0.599-3.329; ch2 0.623; p=0.435). An increased risk for any AKI was not observed in patients without diabetes (OR 1.087; 95% CI 0.836-1.414; ch2 1.081; P=0.582). There was a strongly significantly increased risk for CKD in patients with diabetes associated with the SNP (OR 2.778, 95% CI 1.254-6.152; ch2 6.620; p=0.02) that was not seen in non-diabetes CKD and control subpopulations.

Conclusions: Patients with diabetes and whom carry the Aqp11 SNP are at higher risk for an AKI event and for developing CKD. These data suggest the Gly102Ser substitution in the Aqp11 protein results in altered function and susceptibility to chronic renal injury in stress-induced conditions present in diabetes.

Funding: NIDDK Support, Other NIH Support - Vanderbilt CTSA grant UL1 RR024975-01 from NCRR/NHLBI

FR-PO1508

Modeling Study of Human Chloride Channel 5 Mutations in Japanese Families with Dent’s Disease Suggests a Structure-Function Relationship Akira Ashida, 1 Daisuke Yamamoto, 2 Takashi Sekine, 3 Takashi Igarashi, 4 Motoshi Hattori, 1 Hiroshi Tamai. 1 Department of Pediatrics, Osaka Medical College, Takatsuki, Japan; 2Biomedical Computation Center, Osaka Medical College, Takatsuki, Japan; 3Department of Pediatrics, Osaka Medical College, Toho University, Tokyo, Japan; 4Department of Pediatrics, Graduate School of Medicine and Faculty of Medicine, The University of Tokyo, Tokyo, Japan; 5Department of Pediatric Nephrology, Tokyo Women’s Medical University, Tokyo, Japan.

Background: Dent’s disease, an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalcinuria, and nephrolithiasis is caused mainly by inactivating mutations in the human chloride channel 5 (BCLC-5) gene. Molecular models of the extracytoplasmic domain and transmembrane domain of the protein have been discussed from the crystal structures of two bacterial chloride channels. Additionally, the X-ray crystal structure of the cytoplasmic domain of BCLC-5 has been established, thereby allowing us to construct a model of this domain and examine the role of its mutations.

Methods: We examined 114 Japanese cases of Dent’s disease and identified BCLC-5 mutations in 69 cases, among which 26 missense mutations were subjected to molecular modeling analysis.

Results: The locations of the mutated residues were distributed around the three structural sites: 1) around the chloride or proton conduction pathway (12 mutated residues), 2) the subunit interface that would be buried during dimer formation (7 mutations), 3) the cytoplasmic domain that would regulate transport by binding to ATP (4 mutations).

Conclusions: The missense mutations identified in Japanese families can be classified into three clusters according to the structural sites at which they occur, each playing an important role in the Cl−/H+ activity of BCLC-5.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

462A
Sickle Cell Trait Is Not Associated with ESRD Susceptibility in African Americans
Barry I. Freedman, 1 Pamela J. Hicks, 2 Carl D. Langefeld, 1 Lingyi Lu, 1 Anthony J. Bleyer, 1 Jasmin Divers, 2 Patrick H. Nachman, 2, 3 Matthew David Morgan, 1 Lorraine Harper, 1 Richard Borrows. 2
1 Department of Internal Medicine-Nephrology, Wake Forest School of Medicine, 2 Department of Biochemistry, Wake Forest School of Medicine, 3 Department of Public Health Sciences, Wake Forest School of Medicine; 4 Department of Internal Medicine-Nephrology, University of North Carolina at Chapel Hill.

Background: Conflicting reports exist as to whether sickle cell trait (HbAS) is a risk factor for development and progression of nephropathy. To determine whether African Americans with HbAS are at increased risk for nephropathy, genetic association was assessed between HbAS and end-stage renal disease (ESRD).

Methods: Hemoglobin S (HBS), non-muscle myosin heavy chain 9 (MYH9) and apolipoprotein L1 (APOL1) nephropathy risk variants were genotyped in 3258 unrelated African Americans; 1085 with non-diabetic ESRD, 996 with type 2 diabetes (T2D)-associated ESRD, and 1177 non-nephropathy controls. Interactions between APOL1 and MYH9 risk variants and HBS were assessed using case-only and case-control centered two-way logistic regression interaction analyses.

Results: HbS genotypes met Hardy Weinberg Equilibrium expectations in both cases and controls. HbAS genotype frequencies were 8.7% in non-diabetic ESRD cases, 7.1% in T2D-ESRD cases, and 7.2% in non-nephropathy controls. Age, gender, and admixture-adjusted p-values for HbAS association with non-diabetic ESRD were p=0.34 (odds ratio [OR] 1.16; 95% confidence interval [CI] 0.85-1.60, dominant); p=0.96 for T2D-ESRD (OR 1.01; 95% CI 0.70-1.50, dominant); and p=0.74 for all-cause ESRD (combined non-diabetic and T2D-ESRD cases versus controls; OR 1.05; 95% CI 0.79-1.40, dominant). No evidence of APOL1 or MYH9 interactions with HbAS was detected.

Conclusions: Sickle cell trait was not associated with diabetic or non-diabetic etiologies of ESRD in a large sample of African American residing in the southeastern U.S. Sickle cell trait does not appear to predispose to progressive nephropathy.

FR-PO1511
The Influence of Some Polymorphisms on Development of AA Amyloidosis
Zuzana Potysova, 1 Romana Rysava, 1 Jitka Stekrova, 1 Vladimir Tesar. 1
1 Department of Nephrology, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic; 2 Department of Genetics, Charles University in Prague, First Faculty of Medicine and General Teaching Hospital, Prague, Czech Republic.

Background: Available data suggest an association between presence of AA (secondary) amyloidosis and MCP-1 (monocyte chemotaxant protein-1) and MIP-1α (macrophage inflammatory protein-1 alpha) genes polymorphisms and an impact of polymorphisms in exon 3 of SAA1 (serum amyloid A 1) gene on the incidence of AA amyloidosis in different populations.

Methods: DNA and serum specimens of patients with AA amyloidosis (43), rheumatoid arthritis (RA) without amyloidosis and healthy control group (100) were investigated by using PCR, RFLP and ELISA methods. Kruskal-Wallis and χ2-square tests were used for statistical data evaluation.

Results: Significantly more frequent occurrence of 1.1/1.1 genotype in SAA1 was recorded in AA amyloidosis group compared to RA group as well as in control group (p<0.001). Distribution of neither 1/1/1 genotype nor another one did not vary among RA and control group. No significant difference in distribution of another genes was recorded among all three groups. Serum concentration of SAA was statistically significantly higher in AA amyloidosis group and also in RA group compared to healthy controls (p<0.001). Serum concentration of MCP-1 was statistically significantly higher in AA amyloidosis group compared to RA group (p<0.05). Concentrations of MIP-1α were markedly higher in both groups of patients compared to healthy controls (borderline to statistical significance).

Discussion: Homozygosity of the 1.1 haplotype in SAA1 gene could be a risk factor for development of AA amyloidosis in Caucasian population. Our unique findings of higher serum concentration of MCP-1 in the AA amyloidosis group compared to RA group could advert to riskiness of another factors. This could have therapeutic consequence – earlier and more assertive therapy of underlying diseases in patients with appropriate genotype in order to prevent or interfere with occurrence of AA amyloidosis.

This work was supported by CSN 2008/02 and MZO 00032782 grants.

Funding: Government Support - Non-U.S.

FR-PO1512
Clinical Characteristics of PKD2 Gene-Linked Families Characterized by the Same Germ-Line Mutation Valentina Corradi, 1, 2 Fiorella Gastaldon, 1 Grazia Maria Virzi, 1, 2 Armando Vazquez, 1, 2 Manish Kaushik, 1 Dinna N. Cruz, 1, 2 Maurizio Clementi, 1 Claudio Ronco. 1, 2 Nephrology, St Bortolo Hosp, Italy; 2 Clinical Genetics and Pediatrics, University of Padova, Italy; 1 International Renal Research Institute Vicenza, Italy.

Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common inherited renal cystic disease. It is genetically heterogeneous (PKD1 and PKD2) with a significant intra-familial variability. ADPKD is characterized by “private mutations”: it is rare to identify the same germ-line mutation in different families. The aim of the study was to evaluate the clinical characteristics of PKD2 gene-linked families characterized by the same germ-line mutation.

Methods: Patients (pts) with ADPKD, by ultrasound criteria, were enrolled and followed prospectively. Complete clinical details were recorded, including family history and time of ADPKD diagnosis (t0). We applied linkage analysis to identify the gene involved. We used microsatellite markers (STR) Banking the 2 genes. Furthermore, we performed sequencing to identify mutations in PKD2 families. The eGFR was calculated with the 4-variable standardized-MDRD equation. The progression of CKD was determined by the change in eGFR per year. Data were shown as median (min;max).

Results: We identified 8 PKD2 gene-linked families characterized by the same disease haplotype and the same germ-line mutation (2533C>T) in all affected individuals (16 pts). At t0 their age was 33.5 (19;53) yrs and eGFR 78.5 (32;94) mL/min/1.73 m². After a median follow-up of 10.73 (8.06;32.25) yrs, eGFR was 70 (14/94) mL/min/1.73 m². Hypertension was present in 87.5% of pts, DM in 6.25%, kidney stones in 37.5%, and hepatic cysts in 62.5%. The change in eGFR per year was -0.70 (-7.01;+2.15) mL/min/1.73 m².

Conclusions: The identification of the same germ-line mutation in 16 pts belonging to 8 families indicate the presence of a common ancestral founder in our geographical area. We observed a considerable variability in CKD progression, within the same mutation. This could be explained by other clinical or genetic factors. We plan to analyze for other possible candidate genes that may be contributing to the observed variability in the ADPKD progression.

Funding: Support from the Fondazione Italiana di Nefrologia e Dialisi, Rome and from the Fondazione Italiana per l’Erbe, Rome.

References:
1. Calamia KM, et al. J Am Soc Nephrol 2010. The purpose of this study was to investigate the role of this gene variant in systemic vasculitis.
2. Moore et al. JASN 2010. The purpose of this study was to investigate the role of this gene variant in systemic vasculitis.
Methods: Since sex is a common element in this phenomenon, we hypothesized the existence of gender differences in coefficients of variation (CV) for paired 24-hour creatinine (Cr)-corrected measurements of urinary calcium (Ca), oxalate (Ox), citrate (Cit), uric acid (UA), sodium (Na), potassium (K), magnesium (Mg), phosphorus (P), ammonium (NH), chloride (Cl), urea nitrogen (UN) in 6,830 females and 9,135 males collected by the Litholink Corporation, Inc. 

Results: Females had significantly (P<0.05) higher CVs than males for Na, Mg, P, Cl, K and NH post-Bonferroni correction (Tab. 1).

<table>
<thead>
<tr>
<th>Product</th>
<th>CV male (SE)</th>
<th>CV female (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>0.19 (0.004)</td>
<td>0.18 (0.003)</td>
<td>0.001</td>
</tr>
<tr>
<td>Mg</td>
<td>0.16 (0.003)</td>
<td>0.15 (0.003)</td>
<td>0.001</td>
</tr>
<tr>
<td>P</td>
<td>0.14 (0.003)</td>
<td>0.13 (0.002)</td>
<td>0.001</td>
</tr>
<tr>
<td>CI</td>
<td>0.19 (0.004)</td>
<td>0.18 (0.003)</td>
<td>0.001</td>
</tr>
<tr>
<td>K</td>
<td>0.13 (0.003)</td>
<td>0.12 (0.002)</td>
<td>0.008</td>
</tr>
<tr>
<td>NH</td>
<td>0.19 (0.004)</td>
<td>0.18 (0.003)</td>
<td>0.037</td>
</tr>
</tbody>
</table>

SE = standard error

There was no univariate effect of sex on Ca CV (P=0.2). Male and female loadings were similar over the first principal component (λ=2.94), but diverged over PC2 (λ=1.55) and PC3 (λ=1.33) largely for Ca, Mg, Na and Cl (Fig. 1).

Conclusions: Our findings support the hypothesis of sexual differences in residual variability in human renal phenotype. A full understanding of the genetics of urinary physiology needs to account for sexual and inherited variability and the factors underlying them.

Funding: Clinical Revenue Support

FR-POI154

Role for Mammalian Target of Rapamycin in Mediating Expression and Activity of a Disintegrin and Metalloproteinate 17 in Diabetic Kidney Disease

Diabetic nephropathy is a major complication of type 1 diabetes characterized by mesangial expansion, glomerulosclerosis, and interstitial fibrosis. The disease is associated with increased levels of inflammation, oxidative stress, and decreased insulin sensitivity. Mammalian target of rapamycin (mTOR) is a central regulator of cellular growth, metabolism, and stress responses. In this study, we investigated the role of mTOR in the development of diabetic nephropathy.

Methods: Male and female Sprague-Dawley rats were treated with streptozotocin to induce diabetes. Serum and urinary markers of renal function were measured. Renal tissue was harvested for mRNA and protein analyses. Western blotting was performed to measure the expression and phosphorylation status of mTOR and its downstream targets.

Results: Male rats showed a significant increase in mTOR phosphorylation at 4 weeks post-diabetes induction compared to control rats. In contrast, female rats did not show any significant change in mTOR phosphorylation. Furthermore, female rats had significantly reduced levels of fibronectin compared to male rats, indicating a protective role for sex hormones in diabetic nephropathy.

Conclusions: Our study highlights the sex-specific role of mTOR in the development of diabetic nephropathy. The protective effects of sex hormones in female rats may provide insights into sex-based differences in disease progression.

Funding: Clinical Revenue Support

FR-POI154

Netrin-1 as a Novel Diagnostic Biomarker of Chronic Kidney Disease

Netrin-1 is a neurophinsulin that plays a crucial role in the development of the central nervous system. In addition, it has been identified as a potential biomarker for various diseases, including chronic kidney disease (CKD). In this study, we evaluated the role of Netrin-1 as a diagnostic biomarker for CKD.

Methods: A total of 30 patients with different stages of CKD were included in the study. Blood samples were collected, and Netrin-1 levels were measured using ELISA. The patients were divided into four groups based on the stages of CKD: normoalbuminuria, microalbuminuria, macroalbuminuria, and renal dysfunction.

Results: Netrin-1 levels were significantly higher in the macroalbuminuria group compared to the other groups. The levels of Netrin-1 were also significantly higher in the renal dysfunction group compared to the other stages.

Conclusions: Netrin-1 can be used as a novel diagnostic biomarker for CKD. Further studies are needed to validate these findings and evaluate the clinical utility of Netrin-1 as a diagnostic tool.

Funding: Clinical Revenue Support

FR-POI154

Reversibility of Microvascular Tortuosity after Simultaneous Pancreas-Kidney Transplantation

Microvascular tortuosity is a common feature of diabetic nephropathy and is associated with the progression of kidney disease. SPK transplantation is a potential treatment for diabetic nephropathy, but the reversibility of microvascular tortuosity after SPK is not well understood.

Methods: A retrospective study was conducted on patients who underwent SPK transplantation. Pre-and post-transplant microvascular images were obtained using optical coherence tomography (OCT). The OCT images were analyzed to quantify the degree of microvascular tortuosity.

Results: Microvascular tortuosity was significantly reduced after SPK transplantation, indicating the potential reversibility of microvascular tortuosity.

Conclusions: SPK transplantation can lead to the reversal of microvascular tortuosity, providing a potential treatment option for diabetic nephropathy.

Funding: Clinical Revenue Support

FR-POI154

IV expression was enhanced in the KC of diabetic animals. ADAM 17 protein expression, enzymatic activity, mTOR phosphorylation and collagen IV expression in the KC and in isolated PT were abolished with rapamycin treatment. In contrast to the findings in KC and in PT, the enzymatic activity of ADAM 17 was significantly decreased in isolated glomeruli of diabetic animals and was not affected by rapamycin treatment.

Conclusions: Collectively these data suggest a mechanism whereby mTORC1 mediates the activity and expression of ADAM 17 and collagen IV accumulation in the kidney cortex/proximal tubular kidney compartment. Moreover, the data suggest a differential role for ADAM 17 activity in kidney cortex and in proximal tubules as compared to the glomerular compartment.

Funding: NIDDK Support, Veterans Administration Support

FR-POI154

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Netrin-1 is a neurophinsulin that plays a crucial role in the development of the central nervous system. In addition, it has been identified as a potential biomarker for various diseases, including chronic kidney disease (CKD). In this study, we evaluated the role of Netrin-1 as a diagnostic biomarker for CKD.

Methods: A total of 30 patients with different stages of CKD were included in the study. Blood samples were collected, and Netrin-1 levels were measured using ELISA. The patients were divided into four groups based on the stages of CKD: normoalbuminuria, microalbuminuria, macroalbuminuria, and renal dysfunction.

Results: Netrin-1 levels were significantly higher in the macroalbuminuria group compared to the other groups. The levels of Netrin-1 were also significantly higher in the renal dysfunction group compared to the other stages.

Conclusions: Netrin-1 can be used as a novel diagnostic biomarker for CKD. Further studies are needed to validate these findings and evaluate the clinical utility of Netrin-1 as a diagnostic tool.

Funding: Clinical Revenue Support

FR-POI154

Reversibility of Microvascular Tortuosity after Simultaneous Pancreas-Kidney Transplantation

Microvascular tortuosity is a common feature of diabetic nephropathy and is associated with the progression of kidney disease. SPK transplantation is a potential treatment for diabetic nephropathy, but the reversibility of microvascular tortuosity after SPK is not well understood.

Methods: A retrospective study was conducted on patients who underwent SPK transplantation. Pre-and post-transplant microvascular images were obtained using optical coherence tomography (OCT). The OCT images were analyzed to quantify the degree of microvascular tortuosity.

Results: Microvascular tortuosity was significantly reduced after SPK transplantation, indicating the potential reversibility of microvascular tortuosity.

Conclusions: SPK transplantation can lead to the reversal of microvascular tortuosity, providing a potential treatment option for diabetic nephropathy.

Funding: Clinical Revenue Support

FR-POI154

Clinical Revenue Support

2. John W. De Fijter, 1 Yves C. Gorin, 1 Jeffrey L. Assaad Antoine Eid, 1 Yves C. Gorin, 1 Jeffrey L. Barnes, 1, 2 Hanna E. Abboud, 1, 2 Medicine/Nephrology, UT Health Science Center at San Antonio, San Antonio, TX; 2 South Texas Veterans Administration, San Antonio, TX.

Background: Diabetic kidney disease (DKD) is characterized by extracellular matrix (ECM) accumulation. However, the mechanisms involved have not been completely identified. A disintegrin and metalloproteinase 17 (ADAM 17) cleaves growth factors involved in matrix accumulation. This study examined the role of ADAM 17 in matrix accumulation in the kidney cortex (KC) of a type 1 diabetic rat model.

Methods: Diabetes was induced in Sprague Dawley rats with streptozotocin and tissue was isolated at six weeks after the induction of diabetes for western blot analysis, and a fluorometric-based assay for ADAM 17 activity. A subgroup of diabetic rats was treated with 10mg/kg of the mTORC1 inhibitor rapamycin, administered by intraperitoneal injection three times per week.

Results: MTOR phosphorylation on serine residue 2448 was enhanced in the KC of diabetic rats. ADAM 17 protein expression was significantly increased in the KC of type 1 diabetic rats by immunohistochemical staining and western blot analyses. ADAM 17 enzymatic activity in the KC and in proximal tubules (PT) isolated from the KC of the diabetic animals was also significantly increased suggesting a role for ADAM 17 in DKD. Collagen type IV expression was enhanced in the KC of diabetic animals. ADAM 17 protein expression, enzymatic activity, mTOR phosphorylation and collagen IV expression in the KC and in isolated PT were abolished with rapamycin treatment. In contrast to the findings in KC and in PT, the enzymatic activity of ADAM 17 was significantly decreased in isolated glomeruli of diabetic animals and was not affected by rapamycin treatment.

Conclusions: Collectively these data suggest a mechanism whereby mTORC1 mediates the activity and expression of ADAM 17 and collagen IV accumulation in the kidney cortex/proximal tubular kidney compartment. Moreover, the data suggest a differential role for ADAM 17 activity in kidney cortex and in proximal tubules as compared to the glomerular compartment.

Funding: NIDDK Support, Veterans Administration Support

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FR-PO1517

Nephroprotectin: A Novel Protein Associated with Diabetic Nephropathy --Proteome Analysis of Isolated Glomeruli from Autopsy and Immunohistochemical Study

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Background: Proteome analysis of glomeruli of renal biopsy is difficult, since sufficient quantities of glomeruli are rarely available. Formalin-fixed paraffin-embedded (FFPE) kidney tissues of autopsies contain enough amounts of glomeruli for proteome analysis. In order to identify a novel protein expression reflecting diabetic glomeruli, proteome analysis was performed, using FFPE kidney tissues of autopsy.

Methods: We conducted proteome analysis of laser-captured dissected glomeruli from FFPE kidney tissues of patients with diabetic nephropathy (n=10) and those of non-diabetic patients (n=10), using the isobaric tagging reagent iTRAQ, QSTAR Elite LC-MS/MS system, and Ingenuity Pathway Analysis (IPA). To validate the results of proteome analysis, we performed immunohistochemistry of 93 autopsies of type 2 diabetic patients.

Results: There were a total of 100 proteins that were differently expressed in glomeruli of diabetic patients, compared to those of non-diabetic patients. Based on the results of IPA, 31 renal and urological disease-related proteins were detected. Among them, nephroprotectin, an integrin αvβ8 ligand which functions as assembly of extracellular matrix, was up-regulated in diabetic glomeruli (1.25 folds increase). Immunohistochemical analysis revealed that nephroprotectin was highly expressed in mesangial expansion and nodular glomerulosclerosis of diabetic patients, but not in glomeruli of non-diabetic patients. There was a significant positive correlation between glomerular sclerosis index and the percentages of nephron-positive glomeruli of diabetic patients (r=0.89, p<0.0001, n=93).

Conclusions: The present study demonstrated, for the first time, increased nephroprotectin expression in diabetic glomeruli, suggesting an important role of nephroprotectin in the development of diabetic nephropathy. Our study also showed that proteome analysis with FFPE kidney tissues is a useful tool for investigating glomerular disease.

FR-PO1518

Advanced Glycation End Products (AGEs) Activating Endoplasmic Reticulum Stress (ERS) Induces Renal Tubulopneumocyte Cell Senescence

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Background: Renal tubulopneumocyte cell (RTCP) senescence plays a critical role in the development of diabetic nephropathy(DN). Our study focuses on the role of ERS in RTCP senescence induced by AGEs in DN.

Methods: In vivo: Eighteen diabetic patients (60 to 75 yr) were classified into two groups: Early clinical DN (n=8) and advanced clinical DN (n=10), and renal carcinoma as normal control (n=10). All samples were examined using SA-β-Gal, ATF4, p16, p21, and SA-HF by immunohistochemical staining. The relationship between p47phox and collagen Iα1 mRNA levels in renal biopsy samples from control subjects and subjects with diabetic nephropathy was determined in kidney biopsies from normal and diabetic subjects.

Results: Deletion of the gene for p47phox reduced NFκB oxidative activity, superoxide generation, oxidative stress, and profibrotic gene expression in glomeruli from diabetic mice and led to a reduction in urinary albumin excretion, renal and glomerular hypertrophy, and mesangial matrix expansion in the diabetic mice. High glucose-induced NFκB oxidative activity and pro-fibrotic gene expression was attenuated in primary MC from mice with a deletion in the p47phox gene. There was a positive correlation between p47phox and collagen 1 mRNA levels in normal biopsy samples from control subjects and subjects with diabetic nephropathy.

Conclusions: Deletion of the gene for p47phox attenuates diabetic nephropathy in MC, due in part to a decrease in the mesangial cell response to high glucose.

Funding: Government Support - Non-U.S.

FR-PO1520

Alikiren in Combination with Valsartan Improve Type 1 Diabetic Nephropathy in Mice

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Background: The current study was undertaken to investigate if combination therapy with aliskiren, a direct renin inhibitor, with valsartan, an angiotensin type 2 receptor blocker, provides additive protective effects in 1 diabetic nephropathy in mice.

Methods: Insulin deficiency and hyperglycemia were induced with streptozotocin (STZ, 40mg/kg/day) injection in 21/1 mice for five days. The mice were treated with either aliskiren (25 mg/kg/day), valsartan (8 mg/kg/day), or combined aliskiren and valsartan for 4 weeks. Western blots, immunofluorescence and qPCR was used to examine protective effects of the treatment against diabetic nephropathy.

Results: Combined treatment with aliskiren and valsartan significantly attenuated albuminuria (125 ± 10 % in controls, 209 ± 29 in diabetes, and 128 ± 20 µg/mg in combination, p<0.05) and urine nephrin excretion (81 ± 16 in controls, 183 ± 23 in diabetes, and 112 ± 30% in combination, p<0.05). This was associated with prevention of the reduced production of progenitor cell markers nephrite WT1, podocin, and synaptopodin in glomeruli of diabetic mice. The combination also markedly decreased 1) profibrotic growth factors: renal transforming growth factor-beta and PAI-1 expression, 2) proinflammatory cytokines: tumor necrosis factor-alpha, MCP-1, and CD68, and 3) neutral lipid (oil red o) accumulation. Single treatment with either aliskiren or valsartan provided marked, but less beneficial effects on all the above-mentioned parameters than combination.

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Conclusions: The combination of alikverin and valsartan therefore protects against diabetes by multiple mechanisms, and seems to be a promising therapeutic strategy for diabetic nephropathy.

Funding: Pharmaceutical Company Support

FR-PO1522

Amelioration of Albuminuria and Tubulointerstitial Inflammation in STZ-Induced Diabetic TLR4-Deficient Mice 1Miao Lin,2Wan Hsin Yu,1Hao-Jia Wu, Loretta Y.Y. Chan,3Joseph C.K. Leung,2Kar Neng Lai,1Sydney C.W. Tang,1Medicine, Hong Kong University, Hong Kong, China.

Background: We recently showed that tubular Toll-like receptor 4 (TLR4) expression was elevated in renal biopsies of histologically proven diabetic nephropathy (DN) and correlated with interstitial macrophage infiltration and HbA1c level. But the role of TLR4 in DN remains speculative. This study aims to study the role of TLR4 by using STZ-induced diabetic TLR4-deficient mice.

Methods: TLR4+/− mice and their wild type littersmates (TLR4+/+ on C57BL6 background at 10-12 weeks old underwent uninephrectomy (Unx) or sham operation, and were rendered diabetic by intraperitoneal injections of STZ. In vivo, the effect of an anti-TLR4 neutralizing antibody on high glucose (HG)-induced human proximal tubal epithelial cell (PTEC) inflammation was examined.

Results: At 12 weeks of diabetes, TLR4+/− mice significantly demonstrated ameliorated albuminuria and serum creatinine independent of blood glucose levels. This functional improvement was accompanied by substantially decreased F4/80+ macrophage infiltration into the tubulointerstitium and downregulation of cortical CCL-2, ICAM-1, and IL-1β expression. At the signaling level, tubulointerstitial phosphorylated NF-κB/p65 activation into the tubulointerstitium and downregulation of cortical CCL-2, ICAM-1, and IL-1β expression. The signaling pattern in TLR4−/− mice was similar to non-diabetic controls. Effective JNK inhibition was demonstrated at week 16. Furthermore, there was no difference in supernatant lactate measured in any of the treatment groups (mean 20.2 ± 1.10, P>NS). Incubation with IL-1β at 10.20 ng/ml and corticosterone at 0.1 and 0.5 micromolar concentrations yielded a dose-dependent increase in PCK1 activity and glucose production.

Conclusions: These results suggest that IR in uremia occurs partly through abnormal elevated GC-directed gluconeoegenesis as a manifestation of increased inflammatory responses. We also demonstrated a novel and easily reproducible in vitro model of IR in uremia.

Funding: Government Support - Non-U.S.

FR-PO1525

Intervention with JNK Blockade in the Early Phase of Type 1 Diabetic Nephropathy 1David J. Nikolic-Paterson,2Andy Lim,3Frank Yuanfang Ma,4Elayne Oozio,5Morag Young,5Brydon Bennett,6Glenn Friedman,7Gregory H. Te Velthoven,8Diabetes and Cardiovascular Medicine, Kings College London, London, United Kingdom; 2Biomedical Research Centre, Medical School, Manchester University, Manchester, United Kingdom; 3Prince Henry’s Institute of Medical Research, Clayton, Victoria, Australia; 4Celsius, San Diego, California.

Background: The c-Jun amino-terminal kinase (JNK) signalling pathway is activated in human kidney diseases, including diabetic nephropathy. Recent studies in animal models have shown that blockade of JNK signaling can suppress the development of renal injury in experimental glucerulonmeritis and acutely reduce mean arterial pressure and vascular resistance in normal rats. The aim of this study was to determine whether inhibition of the JNK signaling plays a role in the development of diabetic nephropathy and in regulating hypertension, which exacerbates diabetic nephropathy.

Methods: Diabetes was induced in spontaneously hypertensive rats (SHR) using streptozotocin. After 16 weeks, rats were randomized into 7 groups. 6 of these groups received the JNK inhibitor,SP600125 (20-50μmol/kg/d) or vehicle control for 10 weeks. The group that did not receive any treatment was assigned as baseline. Rats were assessed for hypertension and progression of renal damage.

Results: At week 16, diabetic rats showed increased kidney JNK activation compared with non-diabetic controls. Effective JNK inhibition was demonstrated at week 26 by reductions in c-Jun phosphorylation. CC-930 did not affect blood pressure, kidney hypertrophy, glomerular hyperfiltration, podocyte loss, glomerular fibrosis or albuminuria. CC-930 reduced macroporphes and cc12 mRNA levels in diabetic kidneys. In contrast, CC-930 exacerbated albuminuria at week 26, which was associated with reduced glomerular mRNA levels of the podocyte-specific molecules, nephrin and podocin.

Conclusions: JNK inhibition does not prevent the progression of early diabetic renal injury in hypertensive rats, which contrasts with the ability of JNK inhibition to suppress albuminuria and injury in experimental glomerulonephritis.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1526

Serum and Urine Metabonomic Profiling Reveals the Metabolic Feature of ddM/dB Mice with Diabetic Nephropathy Yongehun Ge, Mengjie Li, Juye A, Xufang Wang, Guangli Wang, Zhi-Hong Liu. 1Research Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing, China; 2Laboratory of Metabolomics, Key Laboratory of Drug Metabolism and Pharmacokinetics, China Pharmaceutical University, Nanjing, China.

Background: Metabonomics is a systematic tool for quantitative measurement of metabolites and has the potential to identify novel markers that characterize the development of diabetic nephropathy (DN) and are involved in metabolic perturbation.

Methods: We utilized diabetic dd/bM mice (n=14) as a DN model and non-diabetic ddM mice (n=14) as normal control, and profiled their serum and urinary metabolites using GC/TOF/MS-based metabolomics platform. Principal component analyses of the GC/TOF/MS data revealed distinct metabolic profiles of ddM/db mice and dbM/db mice.

Results: The identified discriminatory metabolites between dd/bd and dbM/db suggested a perturbed TCA cycle (malate, citrate, succinate, aconitate), lipid metabolism, glycolysis, urea cycle (arginine, creatine, glycine, alanine) and amino acids turn over. During the development of diabetes and DN (i.e., 6, 8, 10, 12 and 16 weeks of age), dbM/db showed clearly a metabolic pattern of their scores plotting away from the controls. The dd/db mice were characterized with extremely high level of TCA intermediates at 6 weeks, which was in sharp decline, and prominent elevation of free fatty acids in serum from 8 to 16 weeks of age. The drop of TCA intermediate level in serum at 8 weeks of age (compared with 6 weeks) indicated insulin resistance and a marked down-regulation of glycosylis; in contrast, TCA intermediates in urine did not change accordingly. We have also found

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serum lysine, and, to less extent, several other amino acids, were increased significantly at 8 weeks of age, in parallel with urinary albumin excretion.

Conclusions: In conclusion, diabetic db/db mice manifested a significant decrease of TCA intermediate and a significant increase of Lysine in serum at 8 weeks of age. Further studies would be important to determine if these changes also occur in DN patients.

FR-POI527
Comparison of Renoprotective Effects of Alternate Medication Versus Enalapril
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Background: To investigate the renoprotective effects of alternate medication, (+)-catechin (C) using in vivo and in vitro models of diabetes.

Methods: In vivo: Five groups of rats were used in the study lasting 12 weeks. Group 1 and 2 were the non-diabetic rats (n=12/group) treated with and without C respectively. Group 3 and 4 (n=12/group) were diabetic rats treated with and without C respectively. Group 5 (n=5) were diabetic rats treated with ACEi, enalapril. The dose of C was 30 mg/day and ACEi was 10 mg/kg. Urine was collected and analyzed for albumin and endothelin-1 (ET-1). Plasma was collected and analyzed for creatinine. Protein expression of fibronectin was decreased by two-fold in diabetic rats treated with C compared to the diabetic control db/db mice. More severe albuminuria and renal lesions were noted in the db/db mice treated with both of them compared with those of db/db mice treated with either GNQWF1 or A7R. These changes were associated with inactivation of PI3K-Akt-eNOS pathway. In contrast, GNQWF1- and A7R-induced albuminuria and histopathological changes were not observed in any db/m groups. In HUVECs, high glucose media containing VEGFRs inhibitors induced more apoptotic cell death than did high-glucose media without VEGFRs inhibitors, in association with inactivation of PI3K-Akt-eNOS pathway.

Results: There were no differences in FBSand HbA1c levels in all db/db groups. Diabetes significantly suppressed the VEGFR-1 and increased VEGFR-2 expressions in the kidneys. VEGFR-1 and VEGFR-2 expressions were completely inhibited by GNQWF1 in a glucose-dependent manner. VEGFR-1 and VEGFR-2 expressions were completely inhibited by GNQWF1 in a glucose-dependent manner. Also, in endothelial cells, C treatment significantly reduced high glucose induced ROS. Also, in endothelial cells, C treatment significantly reduced high glucose induced apoptosis.

Conclusions: Our findings suggest that C has strong renoprotective properties in diabetic nephropathy. In vivo studies show that C decrease albumin and ET-1 excretion in diabetic rats. In vitro studies show that the mechanisms of C action might be related to antioxidant properties leading to reduced ROS and apoptosis.

FR-POI528
ONO-1301, a Sustained-Release Prostacyclin Analog, Ameliorates Renal Alterations in a Mouse Type 2 Diabetes Model through Its Direct Protective Effects on Mesangial Cells

Background: Diabetic nephropathy is the most common pathological disorder predisposing ESRD, and novel therapeutic approaches are required. ONO-1301 is a novel sustained-release prostacyclin analog possessing thromboxane A2 synthase inhibitory activity. Therapeutic efficacies of ONO-1301 in experimental models of pulmonary hypertension, pulmonary fibrosis and myocardial ischemia has been reported, and we recently reported the therapeutic efficacies of slow-release ONO-1301(SR-ON0) in experimental rat type 1 diabetic nephropathy model. Here, we examined the therapeutic effects of intermittent administration of SR-ON0 on diabetic nephropathy in the obese type 2 diabetes mouse as well as its direct effects on mesangial cells.

Methods: Db/db mice, a model of obese type 2 diabetes, received subcutaneous injections of either SR-ON0(3mg/kg) or vehicle buffer every 3 weeks. Animals were sacrificed at 16 weeks of age. Cultured mouse mesangial cells(MeS13) were stimulated with high ambient glucose(HG; 25 mM) in the presence of ONO-1301(1-100 nM) for 6hrs or 24hrs. Clinical parameters, kidney weight, glomerular volume and mesangial matrix index were examined, and immunohistochemistry, immunoblot and real-time PCR was performed.

Results: SR-ON0 treatment did not affect obesity or hyperglycemia, but significantly ameliorated albuminuria, glomerular hypertrophy, the increase of mesangial matrix index, glomerular accumulation of type IV collagen, F4/80+ macrophages, TGF-beta1, alpha-SMA and MCP-1 in db/db mice compared with vehicle treated group. SR-ON0 treatment reduced the increase of oxidative stress(nitrotyrosine and MDA) in db/db mice. In MeS13 cells, ONO-1301 suppressed the increase of TGF-beta, type IV collagen, alpha-SMA, MCP-1 and fibronectin induced by HG(immunoblot and real-time PCR).

Conclusions: Taken together, these results suggest the potential therapeutic efficacy of intermittent administration of SR-ON0 in diabetic nephropathy through its direct protective effects on mesangial cells.

FR-POI529
Effects of VEGF-R1 or VEGF-R2 or Both VEGF-R1 and VEGF-R2 Inhibition on Diabetic Nephropathy in db/db Mice
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Background: Interventions to manipulate vascular endothelial growth factor (VEGF)- VEGF receptors axis may be therapeutic tools in diabetic nephropathy. In this study, we examined the renal effects of anti-flt-1 hexamer (GNQWF1; VEGF-R1 inhibitor) or anti-alk-1 heptamer (ATLWPR; A7R, VEGF-R2 inhibitor) or both of them in db/db mice.

Methods: The db/m and db/db mice were treated with GNQWF1 or A7R peptide or both of them for 12 weeks.

Results: There were no differences in FBSand HbA1c levels in all db/db groups. Diabetes significantly suppressed the VEGF-R1 and increased VEGFR-2 expressions in the kidneys. VEGF-R1 and VEGFR-2 expressions were completely inhibited by GNQWF1 in a glucose-dependent manner. VEGFR-1 and VEGFR-2 expressions were completely inhibited by GNQWF1 and A7R-induced albuminuria and histopathological changes were not observed in any db/m groups. In HUVECs, high glucose media containing VEGFRs inhibitors induced more apoptotic cell death than did high-glucose media without VEGFRs inhibitors, in association with inactivation of PI3K-Akt-eNOS pathway.

Conclusions: The blockade of VEGF-R1 or VEGF-R2 or both using GNQWF1 or A7R peptide caused glomerular injury related to the inactivation of PI3K-Akt-eNOS pathway resulting in the oxidative stress-induced apoptosis in type 2 diabetic nephropathy.

FR-POI530
Metabolic Syndrome Due to Deletion of the Gene Encoding Canonical Transient Receptor Potential 1 (TRPC1) Channel: A Novel Model Induced by Hyperphagia & Associated with Key Organ Dysfunctions
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Background: TRPC superfamily of cation channels includes certain members implicated in obesity & diabetes. TRPC1 expression is reduced in diabetes, but the relationship is unclear. Since null mice are fatter, we asked if TRPC1 deficiency produces insulin resistance & glucose intolerance & if hyperphagia is vital.

Methods: Metabolic studies were done on male mice at 2 mon & 4-22 mon, using insulinometer, insulin ELISA, & lipids enzymatic assays.

Results: At 2 mon, null mice were 20% fatter, non-fasting glucose (171 vs 98 mg%) higher, & liver 36% heavier. At 7 mon, liver echo was denser, suggesting steatosis. Random glucose (135 vs 100 mg%) at 9 mon & fasting glucose (109 vs 72 mg%) at 10 mon were higher. Null mice ate more food (3.3 vs 1.4 g/d) & calorie (19 vs 12 kcal/d). By 1 yr, they stayed 20% fatter as hyperglycemia & liver hyperdensity persisted. Glucose tolerance test (IP 2 mg/g) showed sustained hyperglycemia, 2-fold higher for 3 h & 65% higher by the 6h. Plasma insulin was 2.5-fold higher throughout. Fasting insulin (23 vs 7 µU/ml) & glucose (7 vs 4 mM) were elevated, due to severe insulin resistance (IR) (8 vs 1 by homeostatic model assessment (HOMA). HOMA beta cell function (β) (90 vs 99%) was normal. Null mice had elevated fasting total (153 vs 118 mg%) & LDL cholesterol (94 vs 59 mg%) & triglyceride (111 vs 36 mg%). At 13 mon, HOMA IR (5 vs 1) stayed high & HOMA β (84 vs 105%) normal. Hyperinsulinemia was absent from 6 to 20 mon by tailcuff or intrarterial readings. Present were cardiomopathy, renal failure & endothelial dysfunction. Caloric restriction corrected excess weight, elevated glucose & cholesterols, implicating hyperphagia.

Conclusions: 1) TRPC1 deficiency produces all the features of metabolic syndrome except hyperphagia. 2) Hyperphagia is pathogenic. 3) The null mice are useful in studying the associated complications.

Funding: NIDDK Support, Veterans Administration Support, Private Foundation Support, Clinical Research Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Glomerular Immunoglobulin Deposition Is a Feature of Human Diabetic Glomerular Injury and Immunoglobulin Deficient Mice Are Protected from Diabetic Albuminuria

Background: Glomerular immunoglobulin (Ig) deposition is observed in many animal models of diabetic nephropathy; however, its significance and relevance to human disease is unknown. This study aims to establish whether glomerular Ig deposition is a feature of human diabetic glomerulopathy and if it has a role in diabetic renal injury in mice.

Methods: Biopsies were examined by assessment of Ig and complement deposition. Western blotting was used for quantification of TGF-β1, HIF-1α, PAI-1, and PAI-2. TGF-β1 was expressed as percentage of untreated wild type (WT) controls.

Results: A significant amount of IgG was detected in diabetic kidney biopsies compared to the WT. Glomerular IgM deposition was also observed in some nodules. In comparison, IgG showed a similar pattern, but with weaker intensity, and was detected in 61% of cases. All diabetic wild type mice displayed glomerular deposits of Ig and C3, which were absent in diabetic mu-chain-/- mice.

Conclusions: Glomerular Ig deposition is a feature of patients with diabetic glomerulosclerosis and diabetic albuminuria. Our findings in Ig-deficient mice suggest a pathologic role for Ig in diabetic albuminuria.

Funding: Government Support - Non-U.S.

Kidney Toll Like Receptor 4 Activation Mediates Injury in the Early Diabetic Kidney

Background: Toll Like Receptor 4 (TLR4) ligands, which activate TLR4 resulting in inflammation in early DN. Here we hypothesize that TLR2 signaling is integral to the development of DN.

Methods: WT and TLR2-/- mice were subjected to intraperitoneal injection of STZ. Samples were harvested at week 6, 12 and 24 post-injection. Renal tubular epithelial cells (TEC) exposed to 25mM glucose for 12 hours were also examined.

Results: WT and TLR2-/- mice developed equivalent diabetes. WT diabetic mice developed significantalbuminuria from week 6 versus controls (ACR: 182.3±30.0 vs 46.4±9.2mg/mmol (wk6); 402.4±145.9 vs 371.8±8.8mg/mmol (wk12); 377.5±118.8 vs 41.3±10.2mg/mmol (wk24); p<0.01), which was reduced in TLR2-/- mice (ACR: 117.0±33.4mg/mmol (wk6); 224.9±93.9mg/mmol (wk12); 254.0±93.4mg/mmol (wk24); p<0.001). TLR2-/- diabetic mice also showed no difference in the glomerular matrix fraction or tubular damage between diabetic and non-diabetic kidneys, whereas TLR2-/- mice showed early lethality associated with malnutrition. Notably, oxidative stress-induced apoptosis contributes the pathogenesis of diabetic nephropathy. We investigated the role of TLR expression on the diabetic nephropathy using Bis haploinsufficiency (Bis-HT) mice after 20 weeks of diabetes induced by streptozotocin.

Methods: We treated antioxidant tempol starting after 12 weeks of diabetes for 8 weeks to see the antioxidant effect on the retardation of the progress of renal damage and restoration of the renal phenotypes associated with Bis expression.

Results: After 20 weeks of diabetes, there was an increase in Bis levels in diabetic Bis-WT DM mice compared to Bis-WT mice in the kidneys. In contrast, there was a significant decrease in Bis expression in diabetic Bis-WT (Bis-HT DM) mice compared to Bis-WT DM mice even under the same degree of hyperglycemia. Serum creatinine and albuminuria were increased in the Bis-HT DM mice compared to those of Bis-WT DM mice. More glomerular matrix expansion, TGF-β1 and HIF-1α expression, and tubulointerstitial fibrosis were also noted in the Bis-HT DM mice related to increases in apopotic glomerular and tubular epithelial cells, accompanying with decreases in Bis and TGF-β1 and HIF-1α expression levels. Notably, oxidative stress-induced apoptosis contributes the pathogenesis of diabetic nephropathy. Using a prototypical mouse model of human diabetes mellitus type 2 (Leprdb/Leprdb), we demonstrated that Bis expression has a protective effect on diabetic nephropathy and that its effect may be explained, at least in part, by preserving of anti-oxidative function.

Toll Like Receptor 4 Activation Mediates Injury in the Early Diabetic Kidney

Background: Catalytic iron (CatFe) participates in the generation of powerful reactive oxygen species that propagate diabetic nephropathy (DN). Chelation may stabilize these histopathologic lesions.

Methods: Using a prototypical mouse model of human diabetes mellitus type 2 (Leprdb/Leprdb), we tested the efficacy of the iron chelator deferiprone in stabilizing mesangial matrix expansion and glomerular basement membrane (GBM) thickening. Using male mice, 200 µg iron in 25 µl of 1% BSA in PBS. 12 weeks following iron overload, 2 batches of 6 animals for the control and untreated groups each, the latter received deferiprone dissolved in drinking water (125mg/kg body weight) starting at age 4.5 weeks till euthanasia at 28 weeks.

Results: While the mean blood glucose was lower in the treatment compared with the control group (50.9±18.0 vs 98.49 ± 68.55 (p<0.01)), body weight was better preserved (59.2±2.29 vs 54.15 ± 8.81g). Deferiprone predictably decreased the 24-hour urinary CatFe excretion (CatFe/Creatinine ratio) which average for treatment group, batch, time, and their two-way interactions in a repeated measures analysis of variance (ANOVA, using Least Square Means (LSM) gave values of (p=0.0001) at 6wk, 12wk and 24wk, respectively. The latter received deferiprone dissolved in drinking water (125mg/kg body weight) starting at age 4.5 weeks till euthanasia at 28 weeks.

Results: While the mean blood glucose was lower in the treatment compared with the control group (50.9±18.0 vs 98.49 ± 68.55 (p<0.01)), body weight was better preserved (59.2±2.29 vs 54.15 ± 8.81g). Deferiprone predictably decreased the 24-hour urinary CatFe excretion (CatFe/Creatinine ratio) which average for treatment group, batch, time, and their two-way interactions in a repeated measures analysis of variance (ANOVA, using Least Square Means (LSM) gave values of (p=0.0001) at 6wk, 12wk and 24wk, respectively. The latter received deferiprone dissolved in drinking water (125mg/kg body weight) starting at age 4.5 weeks till euthanasia at 28 weeks.

To conclude, the result demonstrate that Bis protein has a protective effect on diabetic nephropathy and that its effect may be explained, at least in part, by preserving of anti-oxidative function.
Ets-1 Acetylation Maintains Persistent Expression of microRNA-192 in Kidney Glomerular Mesangial Cells after Transforming Growth Factor-beta1 Treatment  Mitsu Kato, Mei Wang, Jung Tak Park, Sumanth Puttha, Lindal L. Lanting, Rama Naratjanak. Diabetes, Beckman Research Institute of City of Hope, Duarte, CA.

Background: microRNAs (miRNAs) are involved in accumulation of extracellular matrix proteins in diabetic kidney glomerular mesangium. microRNA-192 (miR-192) is upregulated by Transforming Growth Factor beta1 (TGF-b) in a Smad3 and p53 dependent manner in early stages and plays a role in renal fibrosis. Although potential Ets-1 binding sequences are found in the promoter region of miR-192 and Ets-1 is an important factor in kidney injury, the role of Ets-1 has not been fully studied.

Methods: RNA levels were examined by Realtime PCR, protein, phosphorylation or acetylation levels by Western blotting, staining of phospho-p300 in mouse glomeruli by immunohistochemistry, promoter activities by luciferase reporter assay, binding of Ets-1 and acetylation of histone H3 on the miR-192 promoter by Chromatin-immunoprecipitation assay.

Results: TGF-b activated Akt kinase which phosphorylates p300 (phospho-p300S1834) that acetylates Ets-1 and Histone H3K9/14 to regulate miR-192 expression through Ets-1 binding sites. This lead to persistent expression (>24hr) of miR-192 in mouse kidney glomerular mesangial cells. Luciferase reporter assays with miR-192 promoter constructs showed that the Ets-1 site is critical for the TGF-b response. Significant increase of phospho-p300S1834 levels were detected in glomeruli from the type 2 diabetes mice (db/db). Interestingly, in glomerular mesangial cells derived from Ets-1 knockout mice, basal miR-192 levels were higher and the persistent increase in TGF-b-induced miR-192 was lost. Furthermore, Ets-1 siRNA treatments increased miR-192 levels and inhibited the TGF-b-induced persistent increase of miR-192.

Conclusions: These results demonstrate a negative regulatory role for Ets-1 in miR-192 expression and demonstrate that the dissociation of acetylated Ets-1 (via TGF-b activated Akt/p300 pathway) from the miR-192 promoter region may allow a sustained and persistent expression of miR-192 in glomerular fibrosis. Thus Ets-1 may be a key regulator of fibrosis associated with diabetic nephropathy by controlling the expression of miR-192.

Funding: NIDDK Support

Insulin Modulates TRPC6 Channels in Podocytes: Possible Role in Stabilizing the Glomerular Filtration Barrier  Stuart E. Dryer, Jochen Reiser, Alessia Fornoni. ‘University of Houston, Houston, TX; ‘University of Miami, FL.

Background: Insulin signaling to podocytes is essential for normal function of the glomerular filtration barrier, but the effects of insulin on podocyte physiology are not well understood. TRPC6 channels are expressed in podocyte foot processes, and mutant forms of these channels can lead to glomerular disease.

Methods: Insulin modulation of TRPC6 channels was assessed by whole-cell recordings and cell-surface biotinylation assays in immortalized podocyte cell lines. Reactive oxygen species (ROS) were measured using fluorometric assays. Urinary albumin/creatinine ratios and cell-surface biotinylation assays in immortalized podocyte cell lines. Reactive oxygen species (ROS) were measured using fluorometric assays. Urinary albumin/creatinine ratios and cell-surface biotinylation assays in immortalized podocyte cell lines.

Results: Insulin caused a robust increase in macroscopic SKF96365- and La3+-sensitive cationic currents in podocyte cell lines. Insulin also increased steady-state surface expression of TRPC6 channels. These effects occurred in less than 15 min but were maximal after 24 hr. The effects of insulin on TRPC6 trafficking were blocked by siRNA against the TRPC6 channel, which is known to be a type of second messenger for this effect. Modulation of podocyte TRPC6 channels, their steady-state surface expression. This effect requires generation of ROS, which appear to initiate and accelerate diabetic nephropathy, especially development of albuminuria, in diabetic milieu.

Methods: We used endothelial specific NOX2 transgenic (NOX2TG) mice, AKITA type diabetic (AKITA) mice, NOX2TG crossed with AKITA mice, and wild type (WT) mice. NOX2TG was generated in which NOX2, gp91 phox of NADPH oxidase, under the control of Tie2 promoter, was overexpressed in the endothelium. All mice were back-crossed into C57BL/6J. These mice were sacrificed at 6 and 12-week-old of ages for molecular and histological analysis. We applied the in vivo live imaging techniques with multi-photon laser microscopy and various sizes of FITC labeled dextrans to analyze alterations in permeability of glomerular capillary walls in disease conditions.

Results: Urinary albumin excretion was increased only in NOX2TG-AKITA but not in WT and AKITA at 6-week-old. At 12-week-old, serum creatinine level was significantly elevated only in NOX2TG-AKITA but not AKITA and WT. No significant morphological changes were detected in glomeruli from all groups by light microscopic examinations. But slight degree of structural changes in podocytes and mesangial cells were observed only in NOX2TG-AKITA under the electron microscope. The in vivo live imaging techniques revealed increased filtration of 40kDa dextran in glomeruli in AKITA and NOX2TG-AKITA, but not in WT. Moreover, increased permeability of larger molecules, 70kDa dextran, were detected in NOX2TG-AKITA. Lecitin staining was decreased along glomerular endothelium in NOX2TG-AKITA.

Conclusions: Activation of endothelial NADPH oxidase in hyperglycemic milieu initiated and accelerated diabetic nephropathy characterized by development of albuminuria and hyperfiltration of macromolecules.

Funding: Other NIH Support - Israel Science Foundation (ISF), Government Support - Non-U.S.
FR-PO1540
Maxacalcitol Prevents Progression of Endothelial Dysfunction in Rats with Diabetic Nephropathy
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Background: Endothelial function is a powerful surrogate marker of cardiovascular risk in patients with end-stage renal disease (ESRD) or diabetic nephropathy, and can be evaluated by flow-mediated dilatation (FMD). FMD is reportedly correlated with serum 25-hydroxyvitamin D levels in ESRD patients, and restored by nutritional vitamin D in patients with type 2 diabetes. There is evidence that vitamin D receptor activators (VDRAs) might protect against cardiovascular event directly or through protecting against endothelial dysfunction, but the mechanisms remain unclear.

Methods: This study aimed to assess whether the FMD vasorelaxation (MCA) could prevent decrease of FMD in spontaneously diabetic Torii (SDT) rats, a non-obese, type II diabetes rat model with hyperglycemia and proteinuria (from 20 weeks of age). FMD was measured in rats as changes in femoral arterial diameter after 5 min ischemia.

Results: FMD was lower in SDT rats than in Sprague-Dawley (SD) rats (% diameter change [means±S.E.]: SD, 12.8±2.1%; SDT, 8.3±1.2%; n=6). Treatment with MXA (0.2 or 0.6 µg/kg/day, i.p. 3 times/week for 10 weeks) significantly prevented the decline of FMD without hypercalciuria or decreased blood glucose level (MXA 0.2, 15.4±2.4%; MXA 0.6, 16.7±2.4%; n=4 or 6); with insulin, FMD also returned to normal level (17.6±3.4%; n=6) but with decreased glucose level.

To clarify the mechanism of MCA, we evaluated the affects of anti-reactive oxygen species (ROS) on human atherogenic endothelial cells (HCAECs). High glucose significantly increased ROS generation in HCAECs, and MCA significantly inhibited ROS generation by supporting p22phox expression. Supporting such a mechanism, p22phox subunit of NADPH oxidase, related to oxidative stress, was increased in femoral arteries of SDT rats.

Conclusions: In conclusion, in rats with diabetic nephropathy, MCA prevented endothelial dysfunction without hypercalciuria or decreased blood glucose by ameliorating oxidative stress.

FR-PO1541
Low Nitric Oxide Bioavailability Upregulates HB-EGF Expression in eNOS Knockout Diabetic Mouse Kidney
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Background: Diabetic nephropathy is the leading cause of end-stage renal disease, and the involvement of proangiogenic factors such as VEGF-A has been reported. We previously reported the renoprotective role of exogenous Vasotheclin (VASH-1), a negative feedback regulator of angiogenesis and vascular maturation factor, in mouse models of diabetic nephropathy (Diabetes, 2009, AJP-Hys-Penal, 2011). VEGF-A and decreased angiotensin (Ang)-1,2 ratio are associated with inflammation. In the present study, we aimed to evaluate the potential role of endogenous VASH-1 to regulate diabetic renal alterations.

Methods: Type 1 diabetes was induced in male VASH1 heterozygous knockout mice (VASH1+/-) or wild-type (VASH1+/+) mice by intraperitoneal injection of streptozotocin (STZ, 50 mg/kg) for 5 consecutive days. Mice were sacrificed on week 16 after inducing diabetes.

Results: Renal histological alterations were not observed in non-diabetic VASH1+/- mice. Although hyperglycemia, blood pressure or hyperfiltration were not altered, renal hypertrophy, glomerular hyper trophy, albuminuria, glomerular accumulation of type IV collagen and mesangial matrix, and glomerular monocyte/macrophage infiltration, were significantly exacerbated in the diabetic VASH1+/- mice compared with diabetic VASH1+/- mice. Renal levels of transforming growth factor (TGF)-beta1, VEGF-A and Ang-2 (immunoblot) were significantly increased, and the levels of Ang-1 was reduced in the diabetic VASH1+/- mice compared with the diabetic wild-type mice.

Conclusions: These results suggest that endogenous VASH-1 may exert renoprotective effects in type 1 diabetes, via regulation of inflammation and fibrosis partly through regulating VEGF-A and Ang-1,2 ratio, thus implicating its potential to serve as a novel therapeutic reagent for diabetic nephropathy.

FR-PO1544
Sipaipterin Improves eNOS Function and Attenuates Renal Injury in db/db Mice
Huiying Cheng, Xiaofeng Fan, Raymond C. Harris. Department of Medicine, Vanderbilt University School, Nashville, TN.

Background: Our previous studies have demonstrated a role for impaired eNOS activity in the development of diabetic nephropathy (DN).

Methods: To investigate the effect of sipaipterin (Sep), a cofactor for endothelial nitric oxide synthase and a stable precursor of tetrahydrobiopterin (BH4), on DN, we administered Sepippterin (0.1 mg/day by gavage) or the NO precursor, L-arginine (L-arg) (100 mg/kg/day) in a type II diabetic model, db/db mice for 8 weeks (from 26 to 34 weeks).

Results: Neither Sep nor L-arg significantly affected the hyperfiltration (GFR: 290±20 µl/min. in control; 394±92 in untreated db/db; 431±52 in Sep and 394±19 in L-arg respectively), but both of the treatments reduced urine albuminuria (album/cre-unintreated: 759±281, Sep: 40±7 and L-arg: 163±37 µg ml⁻¹Cr⁻¹ respectively, n=6-8, p<0.05). Mesangial expansion was not affected by treatment, but Sep or L-arg decreased GBR thickness (control: 154±6; db/db: 355±23; Sep: 180±13; L-arg: 219±13 nm; n=3). After Sep or L-arg, urinary isoprostane, a marker of oxidative stress, were significantly less (2.6±0.62 and 3.4±0.2 ng 8-isopGF2α/7 µg Cr respectively, compared with untreated db/db (5.6±0.05), although they were still higher than control (1±0.1). Neither immunohistochemistry nor immunoblotting indicated any significant alteration of glomerular eNOS monomer expression, but impaired eNOS dimerization was partially reversed by Sep or L-arg (dimer: 0.53±0.14; db/db: 0.23±0.08; Sep: 0.47±0.07 and L-arg: 0.40±0.13 respectively), indicating recovery of eNOS uncoupling after treatment. In addition, there was decreased phosphorylation of eNOS at Ser 1179 in db/db mice, which was partially restored by Sep or L-arg.
CTGF Is Overexpressed in BTBR ob/ob Mice and Inhibited upon Reversal of Diabetic Nephropathy

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**Background:** BTBR mice with the ob/ob leptin deficiency mutation develop progressive diabetic nephropathy (DN) that resembles its human counterpart (JASN 2010;15:333-42). Connective tissue growth factor (CTGF, CCN2) is strongly upregulated in renal fibrosis and is an important factor in the progression of DN. It is not known if renal CTGF expression is dependent on the diabetic state or if it is regulated by other factors. We investigated CTGF expression in BTBR ob/ob mice both during progression of DN and during reversal of diabetes and DN.

**Methods:** Cohorts of female diabetic BTBR ob/ob mice (n=6) and normoglycemic BTBR wild-type (WT) mice (n=6) were followed for 24 weeks. In a third cohort, leptin was administered by osmotic minipumps for 6 weeks, starting at age 18 weeks (n=6). In a fourth cohort, enalapril was given orally for the same period (n=6). CTGF expression was assessed by immunostaining and quantified by digital image analysis.

**Results:** In the kidneys of BTBR WT mice, CTGF was only focally present in podocytes and mesangial cells (mean positive glomerular area 1.7% ± 1.2). In BTBR ob/ob mice, glomerular CTGF was strongly increased (18.6%±4.6; P<0.001) and localized in podocytes, endothelial cells, mesangial cells and -matrix, and parietal cells. Treatment with leptin resulted in remission of diabetes and reversal of DN as reported previously (ASN abstract 2010) with corresponding decrease of CTGF (7.4%±1; P<0.05). Treatment with enalapril did not produce reversal of DN or decreased CTGF (20.5±10.4; P<0.71).

No significant CTGF staining was observed in the tubulointerstitium of BTBR WT or BTBR ob/ob mice.

**Conclusions:** CTGF is upregulated in glomeruli of BTBR ob/ob mice with DN. Treatment with leptin, but not enalapril, inhibits CTGF in association with reversal of the functional and structural kidney damage of DN. These data indicate that the expression of CTGF in DN may be closely linked or even dependent on the presence of a diabetic milieu, and that achieving normoglycemia may prevent the deleterious fibrosis mediated by CTGF.

**Funding:** Other NIH Support - MPPC

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**Mechano-Growth Factor Induces the Mesangial Cell GLUT1 Glucose Transport System**

**Mimnhi Xiang, 1 Kathleen O. Heilig, 1 Leighton R. James, 2 Joana Panini, 2 N. Stanley Naham, 1 Charles W. Heilig. 1 Medicine, University of Florida College of Medicine-Jacksonville, FL; 2 Anesthesiology, University of Florida College of Medicine-Jacksonville, FL; 3 Medicine, Georgia Health Sciences University, Augusta, GA.

**Background:** Recently we reported in preliminary form the expression of Mechano-Growth Factor (MGF) in mouse glomeruli and primary culture mesangial cells (MC). Glomerular MGF was increased in the glomerular mesangium of both Type 1- and Type 2 diabetic mice. Here we describe responses of cultured mouse MC to MGF-overexpression and to 20mM high glucose, including GLUT1 glucose transporter expression, GLUT1 transcription, glucose uptake rates, and extracellular matrix (ECM) protein expression.

**Methods:** MGF overexpression in primary culture mouse MC via a MoMuLV retroviral vector, 3H2-Deoxyglucose (3H2-DG) uptake rates, Western analyses for GLUT1 and ECM proteins; MC exposure to 8 vs 20mM high glucose x 5d, GLUT1-luciferase reporter assays for transcription.

**Results:** MGF was detectable in control MC (MC-EV) transduced with the empty MoMuLV vector. Multiple clones of MGF-sense transduced MC (MGF-S) were obtained with overexpression of MGF protein at 3-fold control, P<0.005. In comparison, 20mM high glucose treatment of MC-EV increased MGF protein 2.6-fold. In 8mM glucose, immunoblotting of cells revealed diffuse expression of MGF in MGF-S, as opposed to perinuclear localization of MGF in MC-EV. GLUT1 transcription via the promoter + Enhancer-2 was increased 2.3-fold in MGF-S, with 2.5-fold increased GLUT1 protein (P < .05), and 3-fold increased glucose uptake (P < .05). Fibronectin (FN) protein was increased 1.6-fold in MGF-S.

**Conclusions:** 1. MGF-overexpression in MC induced GLUT1 transcription, GLUT1 protein expression, and glucose uptake, and FN ECM protein. 2.High glucose similarly increased MC MGF protein. 3. MGF is a MC protein induced by high glucose and diabetes, and may play a role in diabetic glomerulosclerosis in vivo.

**Funding:** Private Foundation Support

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**Regulation of Stress Granule Formation through Diabetes and RACK1 Expression**

**Michael Merchant, Michelle T. Barati. Medicine-Nephrology, University of Louisville, KY.**

**Background:** Stress granules are transient cytoplasmic aggregations of protein and RNA, formed following stressors including heat, osmotic, and oxidative stress. They represent a compensatory attempt of the cell to triage and sequester important proteins and messenger RNA during stress but preceding ERAD. Previous studies indicated alteration to stress granule metabolism in parenchyma of patients with early diabetic nephropathy (DN). This current study addressed the hypothesis that elevated urinary glucose and/or protein may alter stress granule proteome.

**Methods:** Cultured immobilized human proximal tubules (HK2) cells were exposed to high glucose and/or albumin concentrations, then fractionated to enrich stress granules. Hypertensive rats were identified based on renal fibrosis and hypertension. Soluble and granule-enriched HK2 lysate fractions, human renal biopsies, and isolated mouse tubules were subjected to immunoblot or confocal analysis for constitutive and conditionally associating stress granule proteins. HK2 cells were treated with thapsigargin, a pharmacologic inducer of ER stress, to characterize the recruitment of proteins into stress granule structures.

**Results:** The receptor for activated protein C kinase-1 (RACK1) was recruited into stress granule containing fractions by diabetic stressors. Expression of RACK1 in renal biopsies from diabetic patients transiently increase with early DN and then decreased with advanced DN. RACK1 expression in isolated mouse tubules of non-diabetic and diabetic mice decreased with diabetes and age. Treatment of HK2 cells with the ER stress inducer, thapsigargin, resulted in 1) constitutive TIA-1 and G3BP co-localization to granular structures and also a transient and significant re-localization of RACK1 to granule structures.

**Conclusions:** Sequestration of RACK1 into stress granules has been shown to inhibit cell apoptosis, by scaffolding key signaling proteins (eg protein kinase C). Stress granule triage of RACK1 by HK2 cells in diabetic conditions suggests an early attempt of the cell to survive the diabetic stressors. Data from human biopsy and isolated mouse tubules suggests this role of RACK1 may be lost with disease progression and/or age.

**Funding:** NIDDK Support, Other U.S. Government Support
FR-PO1550

Functional Analysis of miR-30c, miR-26a and miR-379 in Podocytes and Their Potential Roles in Diabetic Nephropathy: Jisun Paeng, Hideki Yokoi, Masashi Mukoyama, Kiyoshi Mori, Masato Kasahara, Takakage Kawanari, Hirono Fukuoka, Akira Sugawara, Katsuya Makino, Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan; Academic and Children's Renal Unit, University of Bristol, Bristol, United Kingdom.

Background: MicroRNAs (miRNAs) are small non-coding RNAs that downregulate mRNA levels. We have reported that connective tissue growth factor (CTGF) is involved in the progression of diabetic nephropathy, and that the natriuretic peptide (guanyl cyclase-A) pathway has a protective role against diabetic nephropathy. Recently, we showed that 48 miRNAs are changed with the stimulation of TGFβ1 on immortalized human podocytes by microarray analysis. Among them, we focused on miRNAs targeting CTGF (i.e., miR-30c and miR-26a) and GC-A (i.e., miR-379). We transfected their mimics or inhibitors into podocytes by using nucleofection and examined target mRNA expression. We studied the role of miR-26a using its mimics in inhibiting TGFβ1-CTGF pathway on podocytes with the stimulation of TGFβ1. Finally we examined expression of these miRNAs in glomeruli in type 2 diabetic db/db mice.

Methods: We measured the expression of miR-30c, miR-26a, and miR-379 in podocytes from db/db mice. For functional analysis, we transfected the mimics or inhibitors of miR-30c, miR-26a, and/or miR-379 into podocytes. Expression of CTGF and GC-A was quantified by real-time PCR.

Results: Transfection of miR-30c mimic inhibited CTGF mRNA and col1a1 and col4a3 mRNAs. Transfection of miR-26a mimic reduced col1a1 and col4a3 mRNAs, and transfection of miR-379 mimic reduced CTGF, GC-A, and col1a1 mRNA levels. Transfection of miR-26a mimic significantly suppressed expression of col1a1 and col4a3 by 33% and 1.8-fold in control group, respectively and transfection of miR-379 mimic downregulated GC-A mRNA by 47%.

Conclusions: We showed that 48 miRNAs are changed with the stimulation of TGFβ1 on immortalized human podocytes by microarray analysis. Among them, we focused on miRNAs targeting CTGF (i.e., miR-30c and miR-26a) and GC-A (i.e., miR-379). Finally, we showed that these miRNAs may be involved in the progression of diabetic nephropathy through the CTGF-β1 pathway with the stimulation of TGFβ1.
diabetes have diabetic nephropathy (DN), oral hypoglycemic agents that have additional renoprotective effects beyond glucose lowering would be highly desirable.

Methods: HK2 cells (a human kidney PTC line) were exposed to control (5mM) or high glucose (30mM), 0.5 ng/ml transforming growth factor beta (TGFβ) +/- the SGLT2 inhibitor BI10773. After treatment cells were collected and assessed for SGLT2 levels. SGLT2 levels were assessed using ELISA.

Results: TGFβ but not high glucose increased SGLT2 expression. SGLT2 inhibition with BI10773 reduced HG induced toll-like receptor 2 and 4 as well as the transcription factors nuclear factor kappa B and activator protein 1 which promote inflammation and fibrosis in DN. Furthermore, BI10773 lowered HG induced collagen IV, an extracellular matrix protein as well as interleukin 6.

Conclusions: The SGLT2 inhibitor BI10773 reduces high glucose induced inflammation and fibrosis by blocking transport into the PTC. SGLT2 inhibition didn’t increase compensatory glucose transport through SGLT1. Although HG did not regulate the expression of SGLT2, the presence of TGFβ, a cytokine intrinsic to the development of diabetic nephropathy, may potentiate the ill effects of HG through upregulating SGLT2.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

FR-PO1555

Effects of Probiotic Ingestion on Renal Function and Oxidative Stress in Diabetic rats

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Background: Probiotics are defined as live microorganisms which could confer a health benefit, perhaps by interacting with the antioxidant system. Previous studies in our laboratory showed that after 8 weeks of DM induction in rats, the treatment with probiotic reduced oxidative stress and increased nitric oxide (NO), without significant changes in renal function.

The aim of this study is to assess the effects of early probiotic ingestion on the renal function and oxidative stress in diabetic rats.

Methods: DM rats were induced in male Wistar rats, with streptozotocin (45mg/kg, iv). The animals received probiotic (P) or its vehicle (C) (1.8mL/d) by gavage, in the 5th day of DM induction. Twenty-four hours urine was collected to determine thiobarbituric acid reactive substances (TBARS) (nmol/24h), NO (µmol/24h) and proteinuria (mg/24h). Before and after the treatment, 24 hour urine was collected to determine NOx levels.

Results: P x 32±8 x 9±.7) and decreased NO (4±3 x 23±2); plasmatic urea was higher in DM (61±8 x 32±2). All P<0.05. After probiotic treatment in DM group there was a reduction of TBARS (293±19 x 81±2) and proteinuria (32 ±8 x 9±.7) and decreased NO (4±3 x 23±2); plasmatic urea was higher in DM (61±8 x 32±2). All P<0.05. After probiotic treatment in DM group there was a reduction of NOx levels.

Some studies suggest the oxidative stress as well as the NO in the pathophysiology of diabetic nephropathy.

In fact, in this study, TBARS, an indicator of lipid peroxidation, was increased and NO was reduced in DM group. The use of probiotic attenuated these effects and, at the same time reduced plasmatic urea in these animals.

Conclusions: Our study suggests that the early utilization of probiotics can protect against the DM deleterious effects on kidneys, by controlling the oxidative stress and recovering NO levels.

Funding: Government Support - Non-U.S.

FR-PO1556

Hemoglobin Level and Survival in Hemodialysis Patients with and without Polycystic Kidney Disease: The Role of Administered Erythropoietin

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Background: Interventional trials indicate adverse outcomes when hemoglobin >13 g/dL is targeted in CKD patients who receive erythropoietin stimulating agents (ESAs). It is not clear whether high achieved hemoglobin with minimal or no ESA such as in some polycystic kidney disease (PKD) patients (pts) is also associated with poor outcomes.

Methods: 473 adult patients were examined to assess the association of hemoglobin-mortality association in a 6-year cohort of 110875 non-PKD and 2402 PKD hemodialysis pts across infrequent versus regular ESA therapy defined as ESA<25% of cohort time vs. otherwise, respectively.

Results: PKD and non-PKD pts were 58±13 & 62±15 years old & included 46% & 45% women. PKD pts had lower mortality than non-PKd within each hemoglobin level above 12 g/dL. In PKD pts, fully adjusted death HRs (95% confidence interval) of time-averaged hemoglobin increments <11.0, 12.0-<13.0, & ≥13.0 g/dL (reference: 11.0-<12.0 g/dL) for regular ESA therapy were 2.57 (1.48-4.48), 0.60 (0.43-0.82) & 0.81 (0.50-1.29), & for infrequent ESA therapy were 1.33 (0.74-2.38) & 0.64 (0.41-1.01) & 0.75 (0.47-1.20)

In non-PKD pts, a similar trend towards higher death with hemoglobin>13g/dL was noticed with regular ESA dosing, although infrequent ESA therapy level did not modify the U-shaped Hb-death associations.

Conclusions: Achieved hemoglobin >13.0 g/dL exhibits a trend towards higher mortality in hemodialysis pts, which is not observed in PKD patient subgroup with infrequent ESA therapy. Whether ESA therapy leads to mortality of high hemoglobin warrants additional studies.

Funding: NIDDK Support

FR-PO1557

Sustained Erythropoiesis (6-30 Months) by the EPODURE Biopump in Patients with Chronic Kidney Disease: Further Results of Phase I/II Proof of Concept Trial

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Background: Sustained delivery of EPO maintaining levels 1-5 fold of normal could reduce risks of hemoglobin variability yet achieve recommended Hb targets, avoid hemoglobin fluctuations and increase patient compliance. The goal of EPODURE is to provide > 6 months of sustained EPO delivery from a single treatment using autologous 30mm x 2mm dermis core biopsies excised from the patient’s skin under local anesthesia and converted in days into “biopump” EPO production units by introducing the EPO gene into cells of the intact explant. We reported (ASN 2010) the results in 12 patients treated up to 12 months with EPODURE Biopumps.

Methods: We now report results in 16 (8 EPO-naive, 8 EPO-dependent) of a planned 18 CKD patients treated 6-30 months by 20, 40, or 60 IU/kg/day EPODURE implanted dose in an open label, dose ranging Phase I-II study in anemic CKD patients.

Results: SAFETY: No related SAEs were reported, EPO serum levels never exceeded 70μIU/ml, and all tests for anti-EPO antibodies were negative. Clinical feasibility was demonstrated, the brief procedure well tolerated.

EFFICACY: A single EPODURE administration elevated Hb levels for >3 mo in 14/16 and >6 mo in 10/16, maintained Hb between 10-12 g/dL in 14/16 for >3 mo and 9/16 for >6mo, with >70% mo.

Where Hb declined it correlated with decreasing EPO levels, which peaked at 3 days post implantation. We suspect possible decline in EPO output in some biopumps, possibly due to suboptimal implantation. Improved implantation methods are now under study.

Conclusions: EPODURE is safe at doses up to 65 IU/kg/day is safe, a single administration in most patients can elevate Hb levels for 3-30 months and in appropriate dose maintain Hb in 10-12 g/dl range for up to 30 months. Further refinement in implantation methods to further increase average duration are underway.

Funding: Pharmaceutical Company Support

FR-PO1558

Effect of Vitamin B12 and Folic Acid Supplementation on Erythropoietin Requirements To Maintain Hemoglobin Concentrations – Clinical and Economic Outcomes at 2 Years

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Background: The deficiency of vitamin B12 is known to cause erythropoietin resistance in hemodialysis (HD) patients. B12 is a middle molecule that is effectively removed by modern dialysis membranes, leading to an increased risk of B12 deficiency in HD patients. The aim of this prospective observational study was to assess the effect of intramuscular B12 and oral folic acid supplementation on hemoglobin concentrations and erythropoietin requirements in HD patients over 2 years.

Methods: All HD patients were assessed during the study period of January 2009 to January 2011 for erythropoietin requirement. Hemoglobin, B12, red cell folate and ferritin concentrations and transferrin saturation were also assessed. From January 2009 to January 2010 all HD patients with a plasma concentration of vitamin B12 less than 300 pmol/L were offered weekly injections of hydroxocobalamin 1000 micrograms (Neo-B12®; Hospira Australia) intramuscularly for 3 weeks and oral folic acid supplementation 0.5 mg daily for the course of the study. The B12 treatment was repeated if levels again fell below 300 pmol/L during the study period. No change in intravenous iron protocols occurred during the study period. Exclusion criteria included transplantation, change to peritoneal dialysis, death and loss to follow up during the study period.
Results: 48 HD patients were eligible. Average B12 concentration rose from 216 to 487 pmol/L (p<0.0001) and red cell folate from 928 nmol/L to 1696 nmol/L (p<0.0001). Average erythropoietin usage reduced from 11300 IU/week to 6300 IU/week (p<0.0005) to maintain an average haemoglobin of 116 g/L. The average transferrin saturation did not change during the study. The average ferritin rose from 670 to 880 mcg/L.

Conclusions: In this 2-year observational study, intramuscular vitamin B12 with oral folic acid supplementation reduced the average erythropoietin requirements in HD patients with a serum B12 concentration less than 300 pmol/L by 44% during the study period without a significant change in the average haemoglobin concentration. This translates to an annual cost saving of approximately $AUD4600 per HD patient in this group.

FR-PO1559

An Open, Randomized, Parallel Group, Multi-Center Study on the Prognosis of Hemodialysis Patients in Anemia Treatment by Combination Therapy with Iron and Vitamin C and Erythropoietin (ACTIVE Study) Takahiro Kuragano, Takeshi Nakanishi, Hyogo College of Medicine, Department of Internal Medicine, Division of Kidney and Dialysis, Nishinomiya, Hyogo, Japan.

Background: Clinical trials demonstrated that higher levels of hemoglobin (Hb) do not necessarily translate into improved mortality patients with chronic kidney disease. As such, there has been an increasing focus on individualized therapy in the renal anemia treatment. We evaluated the effect of newly proposed protocol for anemia therapy on adverse events in patients undergoing maintenance hemodialysis (MHD).

Methods: Study design: Randomized parallel group multi-center study. Study period: 3 years. Patients: 266 MHD patients. Intervention group: For obtaining target Hb (10.5-11.5 g/dL) and ferritin (300 ng/ml), doses of erythropoietin (EPO), iron and vitamin C (VC) were changed every month based on ferritin and Hb levels according to the ACTIVE protocol. Non-intervention group: Attending physician decided the doses of EPO and iron. Primary outcomes: Survival rate, hospitalization rate, infection and cardiovascular disease (CVD). Secondary outcomes: Comparison of Hb, ferritin, TSAT, and dose of EPO between the groups.

Results: A total of 45 composite events occurred (4 deaths, 31 hospitalizations, 3 infections, 7 CVD) during the period. There was no significant difference between the groups in the frequency of adverse events. The percentage of the patients who could maintain target Hb (≥75%) during the period. In the intervention group, Hb, TSAT levels, and dose of EPO of the intervention group were significantly higher, and ferritin level was significantly lower than in the non-intervention group.

Conclusions: The ACTIVE protocol modifying the doses of EPO, iron and VC according to monthly Hb and ferritin levels of individual MHD patient can stabilize Hb and ferritin levels within the target range, which in turn could contribute to the lower frequency of adverse events.

FR-PO1560

Vitamin D as a New Regulator of Iron Metabolism: Vitamin D Suppresses Hepcidin In Vitro and In Vivo Justine Bacchetta,1 Joshua Zaritsky,1 Thomas S. Lisse,1 Jessica L. Seo,1 Rene Chen,1 Elizabeta Nemeth,1 Tomas Ganz,1 Mark E. Westerman,2 Isidro B. Salusky,3 Mark Hiewson.1 1David Geffen School of Medicine at UCLA, Los Angeles, CA; 2Intrinsic LifeSciences, La Jolla, CA.

Background: Recent reports have shown improved hemoglobin levels and decreased ESA doses with vitamin D repletion in CKD patients. We examined the effects of vitamin D on hepcidin (Hep), the iron-regulatory hormone responsible for iron sequestration and anemia by posttranslational downregulation of ferroportin (FP), the sole known exporter of iron from cells to the systemic circulation. In CKD, decreased renal clearance and inflammation increase Hep levels.

Methods: We utilized nPCR techniques to assess Hep mRNA production and immunohistochemistry to stain for FP in peripheral blood mononuclear cells (PBMC) isolated from healthy donors and in monocytes (PDM) isolated from the dialysate of patients undergoing peritoneal dialysis.

Results: When treated with active 1,25-vitamin D (5 nM) or with precursor 25OH-vitamin D (100 nM) for 6 hours, PBMC and PDM showed decreased expression of Hep (fig). Chromatin immunoprecipitation revealed decreased recruitment of the RNA polymerase II within the promoter of the human Hep gene after treatment with 1,25D, pointing to direct effects of 1,25D on Hep transcription. Immunohistochemistry showed that PBMC and PDM expressed Fp, with membrane enhancement after treatment with 1,25D. Finally we observed a 50%-decrease in serum hepcidin (ELISA) which persisted for 72 hours in 7 healthy human subjects after a single oral dose of vitamin D (100,000 IU).

Conclusions: For the first time, these results in vitro and in vivo indicate that vitamin D is a potent suppressor of Hep in humans. These findings provide a clinically relevant mechanism by which vitamin D supplementation can improve anemia management in CKD.

Funding: NIDDK Support, Private Foundation Support

FR-PO1562

Novel Approaches To Treat Anemia in Chronic Kidney Disease Jodie L. Babish,1 Qifang Wu,1 Chia Chi Sun,1 Valentina Vaja,1 Delphine Meynard,1 Igor Theurl,2 Guenter Weiss,2 Herbert Y. Lin.1 1Program in Membrane Biology, Nephrology Division and Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, Boston, MA; 2Department of Internal Medicine, Clinical Immunology and Infectious Diseases, Medical University, Innsbruck, Austria.

Background: Anemia is prevalent in patients with chronic kidney disease (CKD). An excess of the iron regulatory hormone hepcidin may contribute to the anemia of CKD by limiting iron availability for red blood cell production. Hepcidin decreases iron absorption from the diet and promotes iron sequestration in macrophage stores by downregulating the iron exporter ferroportin. Hepcidin is thought to accumulate in CKD patients due to reduced renal clearance and inflammation. We have demonstrated a central role for the bone morphogenetic protein (BMP) signaling pathway, via the ligand BMP6 and the co-receptor hemojuvelin (HJV), in regulating hepcidin expression and systemic iron balance. While cell-surface HJV acts as a BMP co-receptor to increase hepcidin, soluble HJV (shHJV) inhibits BMP signaling.

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and hepcidin expression, presumably by sequestering BMP ligands. Importantly, proliferating cytotendons require BMP signaling to upregulate hepcidin expression, suggesting that BMP inhibitors may be promising candidates as hepcidin lowering agents to treat anemia of inflammation, including anemia of CKD.

**Methods:** Here, we investigate the selectivity of sHJV as a BMP inhibitor by quantitating its binding affinity for various BMP ligands using Surface Plasmon Resonance, and we test the effects of BMP inhibitors in 3 rodent anemia models.

**Results:** We show that sHJV has the highest binding affinity for BMP6, while it has lower affinity for BMP7 and does not bind BMP9. sHJV and the small molecule BMP inhibitor licopodol 1899 lower hepcidin expression in bone macrophage iron stores, and improve anemia in a PG-APS rat model of anemia of inflammation and a genetic model of anemia due to hepcidin excess (Tmprss6–/– mice). Finally, we investigate the use of these BMP inhibitors in an adenine rat model of anemia of CKD.

**Conclusions:** Together, our data suggest the possible utility of BMP inhibitors as hepcidin lowering agents to treat anemia of CKD.

**Funding:** NIDDK Support

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**FR-PO1563**

**Impact of Cholecalciferol Repletion on Erythropoietin Requirements in Vitamin D-Deficient Hemodialysis Patients: Pilot Data from a Randomized Controlled Trial**

**Anita Mehrotra,** Maria Krassilnikova, Brian D. Radbill, Peter S. Heeger. *Medicine, Mount Sinai School of Medicine, New York, NY.*

**Background:** Vitamin D deficiency is common in hemodialysis patients. Uncontrolled studies suggest that correction of Vit D deficiency with ergocalciferol (D2), cholecalciferol (D3), and calcitriol is associated with decreased erythropoietin (EPO) requirements.

**Methods:** To better characterize the relationship between Vit D deficiency/repletion and EPO requirements, we examined the impact of D3 repletion on EPO requirements in 79 Vit D-deficient (25OH-D <25 ng/mL) hemodialysis patients randomized to receive D3 (n=51) or standard of care (no repletion, n=28) in a 2:1 ratio. Patients randomized to treatment with D3 received 50,000 IU/wk to a goal 25OH-D of >35ng/mL, followed by 10,000 IU/wk. Changes in 25OH-D, hemoglobin (Hb), and EPO requirements were assessed at 3 months. EPO (Darbepeo) doses were adjusted by the nursing staff as per the dialysis unit protocol (for target Hb 10-12 g/dL) independent of D3 administration.

**Results:** Baseline demographic characteristics (age, race, sex) were similar between both groups, as were baseline Vit D levels (median 13.5 in treatment group vs 13.1 in control group, p=0.623), baseline Hb (mean 11.8 ±1.6 g/dL in treatment group vs 11.4 g/dL in control group, p=0.155), and baseline EPO requirements (median Darbepeo dose 40 units/wk in treatment group vs 50 units/wk in control group, p=0.262). 45 patients had 3 month follow-up data available. Patients randomized to D3 treatment had a rise in 25OH-D at 3 months (11.9 to 44.1 ng/mL, p<0.001, n=30), while patients randomized to the control group did not have any change in 25OH-D. 30 patients were available for Hb measurements at 3 months (n=15). Patients randomized to D3 did not experience hypercalcemia or other adverse events.

**Conclusions:** Our preliminary data from this ongoing randomized controlled trial suggest that treatment of Vit D deficient dialysis patients with D3 is safe, effective, and may result in lower EPO requirements. If these results are confirmed, the cost-savings may be significant.

**Funding:** Other NIH Support - T32 DK07757-12, Private Foundation Support

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**FR-PO1564**

**Semen Hemojuvelin and Ferritin Levels Are Tightly Correlated in CKD Patients**

**Adam Pantelis,** Panutelis Sarafidis, JoLanta Malyszyko, Iain C. Macdougall. 1 King’s College Hospital; 2Medical Academy, Białystok.

**Background:** Hemojuvelin (HJV) is a recently discovered protein involved in the cellular regulation of hepcidin. Both HJV and hepcidin are emerging as crucial players in the complex biology of iron homeostasis. It is known that membrane-bound and soluble cellular regulation of hepcidin. Both HJV and hepcidin are emerging as crucial players in the complex biology of iron homeostasis. It is known that membrane-bound and soluble forms of hepcidin exist, but their exact functions remain uncertain. HJV levels are known to be elevated in CKD and dialysis patients, but this is the first report of serum HJV levels in a renal population.

**Methods:** 93 patients were studied (31 HD, 31 transplant, and 31 CKD; age- and gender-matched). Blood samples were taken for measurement of serum HJV, hepcidin, ferritin, IL-6, CRP, and standard hematological parameters. HJV levels were measured using an ELISA (USCN Life Science Inc, Wuhan, China) by an operator blinded to the clinical details. HJV levels were determined by mass spectrometry, using hepcidin-25 as an internal standard.

**Results:** HJV levels (ng/mL) were highest in HD (2619±1445) followed by transplant (870±638), and then CKD patients (590±344). HJV and hepcidin were moderately correlated in CKD (r=0.641, p<0.001) and transplant (r=0.569, p=0.001), but not in HD patients (r=0.112, p=0.570). The correlation of HJV with ferritin was very tight in all 3 groups; CKD (r=0.918, p<0.001), transplant (r=0.969, p<0.001), HD (r=0.794, p<0.001). A correlation between HJV and Hb was seen only in transplant patients (r=0.407, p=0.028); HJV did not correlate with IL-6 or CRP.

**Conclusions:** Together, our data suggest the possible utility of BMP inhibitors as hepcidin lowering agents to treat anemia of CKD.

**Funding:** NIDDK Support

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**FR-PO1565**

**Similar Mortality in Hemodialysis Patients with Hemoglobin 9 to 9.9 Versus 10 to 10.9**

**A. Gul,** O. Myers, B. Horowitz, E. Bedrick, A. Harford, P. Zager. 1Internal Medicine, UNMHSC, Albuquerque, NM; 2Dialysis Clinic, Inc., Albuquerque, NM.

**Background:** High epoetin (EPO) doses have been associated with increased mortality in hemodialysis (HD) patients. This finding, in conjunction, with the ‘bundle’, have led opinion leaders to recommend reducing the target hemoglobin (Hb) from 10-12 to 9-11. However, prior to implementing a randomized controlled trial to assess the safety and efficacy of the proposed change, a preliminary comparative effectiveness study comparing outcomes in patients with Hb of 9.9-9.9 versus 10-10.9 is needed. The present study explored the hypothesis that all-cause mortality is similar in patients with Hb levels of 9.9-9.9 versus 10-10.9.

**Methods:** We studied an incident cohort of 8365 patients, who began HD in DCI facilities between 2006-2009 and survived ≥150 days. We used Cox models to assess the relationship between the most recent Hb value, excluding a 30-day lag, and mortality. Baseline covariates included age, sex, race, cause of ESRD and vintage. Time-varying covariates included pre-dialysis systolic blood pressure, albumin, creatinine, Kt/V, TSAT, ferritin, BMI, vascular access and iron dose.

**Results:** There were 1867 deaths. Mortality in patients with Hb 9.9-9.9 (HR = 0.97; 95% CI 0.97-1.00) was similar to the referent group (Hb 10.0-10.9). Mortality was lower among patients with Hb ≥11-11.9 (HR 0.76; 95% CI 0.67-0.87) versus the referent group. Conversely, mortality tended to be higher in patients with Hb <9.0 versus the referent group (HR = 1.20; 95% CI 0.92-1.57). In a sensitivity analysis, addition of EPO dose to the model did not significantly change the hazard ratios. EPO doses were highest in patients with Hb values < 9.0. EPO doses >20,000 units/week, were associated with increased mortality versus the EPO referent group (8,000-12,499 units/week) (HR = 1.18 95% CI 1.02 – 1.36).

**Conclusions:** Mortality among patients with Hb 9.9-9.9 was similar to that in the referent group (Hb 10.0-10.9). These observational data suggest that it may be safe and feasible to conduct a pilot study comparing Hb targets 9-11 versus 10-12. EPO resistance may be a significant risk factor for mortality.

**Funding:** Clinical Revenue Support

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**FR-PO1566**

**TSAT and Serum Ferritin Increases Observed in Ferric Citrate Clinical Trials May Lead to Dialysis Cost Savings**


**Background:** Ferric citrate, a novel, investigational phosphate binder for the treatment of hyperphosphatemia in dialysis patients (pts), has been shown in clinical trials to increase serum ferritin (SF) and saturated transferrin (TSAT) and reduce erythropoietin stimulating agents (ESAs) and intravenous (IV) iron (Fe) use. We developed a cost-offset model

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quantifying potential cost-savings associated with ESA and Fe reductions observed in moderate (M) or high (H) ESA use pts experiencing equivalent increases in Fe markers.

Methods: We constructed a cost-offset model of M(4500 to <9000U/session) and H(>9000U/session) ESA users over a 2 mo time horizon from a payer perspective. Unit costs for phosphate binders (lanthanum carbonate, sevelamer, calcium acetate), ESAs and IV Fe were derived from 14 published sources. Monthly ESA (M=16,400U; H=47,400U) and Fe (M=132mg, H=61mg) dose reductions resulting from concurrent TSAT (≥10%) and SF (15-25%) increases were derived from a DaVita database. We assumed equal phosphorus outcomes and price for ferric citrate and comparator binders.

Results: Given equivalent phosphorus outcomes and price, ferric citrate would potentially generate monthly cost savings of M=$123 and H=$135 due to reductions in ESA (M=$102, H=$268) and IV Fe (M=$52, H=$47); reducing ESA costs by 15% and 21% per mo, respectively. Ferric citrate would potentially save $630/high-use pt/mo. For the average dialysis clinic with 80 pts and 50% M/H ESA users, the monthly savings would be $17,500 for these pts. When the expected per-session reduction in ESA and IV Fe dose was reduced by half, the expected cost savings would be reduced proportionally.

Conclusions: These results indicate that the rises in TSAT and SF observed with the investigative drug ferric citrate in clinical trials may provide significant reductions in monthly ESA and IV Fe costs, generating meaningful savings under the Medicare bundled dialysis payment. Sustained rises in TSAT and SF over a longer duration would potentially lead to considerable annual cost-savings.

Funding: Pharmaceutical Company Support

FR-PO1567

The Impact of Frequent In-Center Versus Conventional Hemodialysis on Anemia: The Frequent Hemodialysis Network Trial Daniel B. Ortm,1 Alan S. Kligier,2 Rita Suri,3 Mohanad Akram Rashid,4 Anjan Rastogi,5 Manjula Kurella Taneja,6 John T. Daugirdas,7 Tom H. Greene,3 Nathan W. Levin,8 The FHN Trial Group.1 1Case Western Reserve U.; 2St. Raphael and Yale U.; 3U. of Western Ontario; 4UCAL; 5Cleveland Clinic; 6U. of Illinois; 7U. Utah; 8Renal Research In.; 9NIDDK.

Background: Management of anemia in hemodialysis (HD) patients remains complex and costly. The Frequent HD Network (FHN) Daily prospective, randomized trial demonstrated significantly reduced left ventricular hypertrophy and improved physical-health composite scores for patients receiving 1 year of 6 times/week HD (6X) compared to conventional HD (3X).

Methods: ESA and IV Fe administered were reported as total 4-week dose. We report treatment comparisons of changes from B to month 12 obtained using mixed effects models, with log transformations applied to ESA and IV Fe. Results: Hb increased slightly in the 6X (n=125) group vs. 3X (n=120) (0.3; CI 0.0 to 0.6, p=0.02), but both groups had mean Hb levels in the appropriate clinical range at month 12 (11.7 to 12.0 mg/dL). Geometric mean ESA-equivalent dose declined 16% in the 6x, 95% CI: 38% to 1%, p = 0.12). There were no significant differences in IV Fe or Fe stores between the groups.

Conclusions: In the FHN Daily trial, the frequent HD intervention had no significant effect on either ESA or ESA/Hb. Consistent with some observational studies, the 6X intervention did lead to a small increase in Hb level. The implications of this finding remain under investigation.

Funding: NIDDK Support, Other U.S. Government Support

FR-PO1569

Treatment with Hemodiafiltration (HDF) Improves ESA Responsiveness over 12 Months in Patients with High ESA Resistance without a Concomitant Reduction in Predialysis Hepcidin-25 (Hep25) Levels Neelke C. Van Der Weelen,1 Muriel Grooteman,2 Peter J. Blankestijn,2 Michiel Bots,2 Marinus A. Van Den Doper,1 Claire H. Den Hoed,3 Albert H. Muzzarrie,2 Merco Nube,1 Erik L. Penne,1 Jack F. Wetzels,2 Dorine W. Swinkels,2 Pieter M. Ter Wee.1 1IU Medical Center, Amsterdam, Netherlands; 2University Medical Center, Utrecht, Netherlands; 3Maasstad Hospital, Rotterdam, Netherlands; 4Radboud University Medical Center, Nijmegen, Netherlands.

Background: In chronic Hemodialysis (HD) patients with high resistance to erythropoiesis stimulating agents (ESA), increased non-significantly in both groups (HDF: 4.3 mg/L [-0.1 to 8.7]; HD: 1.4 [-3.9 to -0.15]; mean [95%CI]) and the soluble transferrin receptor (sTfR, -0.33 mg/L [-0.56 to -0.1]; mean [SD]) increased non-significantly in both groups (HDF: 4.3 mg/L [-0.1 to 8.7]; HD: 1.4 [-3.9 to 6.6]; p=0.40). The Hep25/sTfR ratio increased in the HDF group (p=0.02) and remained unaltered in HD patients.

Conclusions: These data suggest either that ESA resistance is not primarily mediated by Hep25, or that treatment with HDF results in a temporarily decrease in Hep25 levels resulting in improved iron availability, ultimately leading to a new balance between Hep25 and sTfR.

The same patterns in iron and ESA doses and Hb were observed across demographic and clinical subgroups, but there were important differences between subgroups in the amount of iron and ESA received. These differences were most notable among black versus white and shorter versus longer dialysis vintage subgroups.

Conclusions: Anemia management patterns have changed markedly between 2002-2008 with a steady increase in IV iron use even after declines in ESA dose and Hb. The clinical impact of these changes need further study.

Funding: Pharmaceutical Company Support

FR-PO1568


Background: Current data on patterns of anemia management, particularly in regard to iron therapy, are lacking in the hemodialysis (HD) population. Such information will further our understanding of changes in anemia management in response to changing clinical guidelines and safety concerns raised from high hemoglobin (Hb) target studies using erythropoiesis-stimulating agent (ESA) therapy; and will provide baseline data for future studies examining the impact of the new dialysis bundled payment system on anemia management.

Methods: USRDS data (2002-2008) on prevalent, Medicare HD patients receiving ESA therapy were examined. For each patient, receipt of intravenous (IV) iron, total IV iron dose, total ESA dose/month, and Hb values were determined. These data were then summarized by calendar quarter (percentage or mean) and plotted for the entire sample and by demographic (sex, age, race) and clinical (cause of ESRD, dialysis vintage) subgroups.

Results: Among ~250,000 HD patients/year, quarterly iron use increased from approximately 65% in 2002 (Q1) to 78% in the 2008 (Q4). Mean quarterly iron dose increased from approximately 525 mg in 2002 to 675 mg in 2008. Mean quarterly ESA dose/month increased from 2002 to 2006 and then began to decline. Similar patterns were observed for Hb values.

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FR-PO1570

Rate of Hemoglobin (Hb) Decline Following Peginesatide Dose Interruption

Anatole Besarab,1 Francesco Locatelli,2 Steven Fishbane,1 Nathan W. Levin,1 Carol Franciscio,2 Hong-Ye Gao,2 Vandana S. Mathur,2 Alex Yang,2 Anne-Marie Duliege,2 Krishna R. Polu,2 *AFIX-01-12 and -14 Peginesatide Study Groups; 1Affymax, Inc., Palo Alto, CA.

Background: The rate of Hb decline after ESA dose interruption is likely due to intrinsic factors such as RBC lifespan rather than the effect of the ESA used on erythropoiesis (Locatelli, 2001). Similar rates of Hb decline are reported for various ESAs (Barany, 2007), despite differences in pharmacokinetics. Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based ESA that is designed to specifically stimulate the erythropoietin receptor. This analysis characterizes Hb decline after ESA dose interruption in patients on dialysis.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials (N=1608) assessing safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in hemodialysis patients (EMERALD 1 and 2). Doses were titrated to Hb levels of 10-12 g/dL. Protocol-specified thresholds for dose interruption (delay by <21 week) due to elevated Hb levels were: Hb ≥ 13.3 g/dL or ≥12.5 g/dL for peginesatide and ≥12.5 g/dL for epoetin. The rate of Hb decline was estimated from linear regression after a dose interruption occurred.

Results: This analysis included 210 of 1066 patients on peginesatide and 327 of 542 patients on epoetin who had ≥2 protocol-specified dose interruption (median Hb = 13.3 g/dL or ≥12.5 g/dL at time of dose hold, respectively). The rate of median Hb change was −0.35 g/dL/wk for peginesatide and −0.40 g/dL/wk for epoetin (Figure). Mean time from dose interruption until resumed dosing was ∼3 wks for both groups (>75% of patients reintiated the ESA within 4 wks).

Figure. Change in Median Hb After First Dose Interruption

Conclusions: Rates of Hb decline after dose interruption were similar for peginesatide and epoetin.

Funding: Pharmaceutical Company Support

FR-PO1571

Safety Results from Two Phase 3 Studies of Peginesatide Treatment for Anemia in Hemodialysis (HD) Patients

Francesco Locatelli,1 Steven Fishbane,1 Iain C. Macdougall,1 Andrzej Wiecek,1 Adrian Constantin Covic,1 Hina Patel,2 Daniel S. Cooper,2 Helen Tang,2 Minjia Chen,2 Anne-Marie Duliege,2 Martha Mayo,1 Krishna R. Polu,2 *AFIX-01-12 and -14 Peginesatide Study Groups; 1Affymax, Inc., Palo Alto, CA.

Background: Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that acts via stimulation of the erythropoietin receptor. Key adverse events (AEs) including those associated with the ESA class in HD patients are reported here.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials evaluating the safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in HD patients (EMERALD 1 and 2). A primary analysis of cardiovascular (CV) events adjudicated by an independent Event Review Committee showed similar rates in the peginesatide and epoetin groups; here nonadjudicated CV events and ESA class AEs were evaluated.

Results: A similar number of patients in the peginesatide and epoetin groups had AEs (94.6% vs 93.0%), serious AEs (53.7% vs 57.0%), nonadjudicated CV AEs, and ESA class AEs (Table). Adjusted on-study mortality rates were also similar for the two groups (8.7 vs 9.5 deaths per 100 patient follow-up years). No clinically relevant differences in laboratory parameters, including platelet counts, or blood pressure levels were observed between treatment groups.

Conclusions: Rates of Hb decline after dose interruption were similar for peginesatide and epoetin.

Funding: Pharmaceutical Company Support

FR-PO1572

Greater Rise in Percent Hemoglobin < 10 g/dL among Black Patients with Implementation of the New US Bundled Dialysis Payment System: Initial Results from the DOPPS Practice Monitor

Ronald L. Pisoni,1 Douglas S. Fuller,1 Justin M. Albert,1 Brian Bieber,2 Brenda W. Gillespie,2 Hal Morgenstern,2 Friedrich K. Port,1 Francesca Tentori,1 Marc Turenne,1 Bruce M. Robinson,2 1Arbor Res Collab for Hlt, Ann Arbor, MI; 2Univ of MI, Ann Arbor, MI.

Background: A new bundled Medicare ESRD prospective payment system (PPS) for dialysis services was implemented in the US in January 2011. The US Government Accountability Office (GAO) urged prompt monitoring of the PPS, cautioning that some patients may be adversely affected by provider responses to the PPS. We report initial trends in anemia management in blacks vs. non-black with PPS implementation.

Methods: The Dialysis Outcomes and Practice Patterns Study (DOPPS) launched the nationally representative DOPPS Practice Monitor (DPM; www.dopps.org/dpm) for timely, public reporting of US hemodialysis (HD) practice trends during implementation of the PPS and quality incentive program (QIP). Linear regression analyses were based on monthly cross-sectional data from US dialysis units between July 2010 to Feb 2011.

Results: During the 8 months, mean Hgb level fell by 0.4 g/dL in blacks vs 0.1 g/dL in other patients (p=0.01). The proportion of patients with Hgb <10 g/dL rose from 6.6% to 11.3% in blacks vs little change in other patients (p=0.02). Mean EPO dose declined similarly among black vs other patients (−204 vs −164 U/mo; p=0.76). The proportion of patients prescribed erythropoietin use rose 1.3-1.5%/mo in both groups; mean IV iron dose remained steady and did not differ by race. >98% of EPO was given by IV during each month.

Distribution of monthly anemia measurements in US-DOPPS

Month Mean Hgb [g/dL] Hgb <10 [g/dL] % CV and ESA [g/dL] % Mean EPO [U/mo]

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean Hgb</th>
<th>Hgb &lt;10</th>
<th>CV and ESA</th>
<th>Mean EPO</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2010</td>
<td>10.9</td>
<td>0.3</td>
<td>1.5%</td>
<td>4.9</td>
</tr>
<tr>
<td>Aug 2010</td>
<td>11.0</td>
<td>0.4</td>
<td>1.6%</td>
<td>5.6</td>
</tr>
<tr>
<td>Sep 2010</td>
<td>11.1</td>
<td>0.5</td>
<td>1.7%</td>
<td>5.7</td>
</tr>
<tr>
<td>Oct 2010</td>
<td>11.2</td>
<td>0.6</td>
<td>1.8%</td>
<td>5.8</td>
</tr>
<tr>
<td>Nov 2010</td>
<td>11.3</td>
<td>0.7</td>
<td>1.9%</td>
<td>5.9</td>
</tr>
<tr>
<td>Dec 2010</td>
<td>11.4</td>
<td>0.8</td>
<td>2.0%</td>
<td>6.0</td>
</tr>
<tr>
<td>Jan 2011</td>
<td>11.5</td>
<td>0.9</td>
<td>2.1%</td>
<td>6.1</td>
</tr>
<tr>
<td>Feb 2011</td>
<td>11.6</td>
<td>1.0</td>
<td>2.2%</td>
<td>6.2</td>
</tr>
</tbody>
</table>

Conclusions: During this PPS transition period, changes in anemia management have led to a greater rise in % of patients with Hgb <10 g/dL and larger decline in mean Hgb in black vs other patients. Continued monitoring of these practice trends and effect on transfusion rates and other clinical outcomes is warranted.

Funding: Pharmaceutical Company Support

FR-PO1573

The Safety of Feraheme® (ferumoxytrol) in Hemodialysis Patients at Three Dialysis Chains over a One Year Period

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Background: Feraheme® (ferumoxytrol) is an IV iron approved in June 2009 for the treatment of iron deficiency anemia (IDA) in adult patients with chronic kidney disease (CKD). The aims of this study was to characterize the safety profile of ferumoxytrol as administered in a real-world setting.

Methods: Adverse events (AE) following treatment were tracked by the medical staff at each of the sites across the three dialysis chains. Any patient who received any dose of ferumoxytrol from January through December 2010 was included in this analysis. To standardize the reported AE terms, AEs were coded using a standard drug safety coding
convention (MedDRA Version 13). All AEs were cross-checked against post-marketing safety reports, received by AMAG to ensure all AEs were captured.

Results: Overall, 8,666 CKD patients were administered a total of 33,358 doses of ferumoxytol at these three dialysis chains. The table below displays the rates of these three AE categories calculated on a per-patient and on an event-per-exposure basis.

<table>
<thead>
<tr>
<th>Total Subjects (N=8,666)</th>
<th>Total Exposures (N=33,358)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects (%)</td>
<td>Events (%)</td>
</tr>
<tr>
<td>AEs</td>
<td>108 (1.25)</td>
</tr>
<tr>
<td>Serious AEs</td>
<td>18 (0.21)</td>
</tr>
<tr>
<td>AEs leading to drug discontinuation</td>
<td>49 (0.57)</td>
</tr>
</tbody>
</table>

The overall rates of AEs, SAEs, and AEs leading to ferumoxytol discontinuation were low. The most common AEs (≥2 subjects) were hypotension (0.12%), hyperkalemia (0.06%), dyspnea (0.05%), and loss of consciousness (0.03%). The frequency or severity of AEs did not increase among patients receiving two or more courses of ferumoxytol.

Conclusions: Based on a one year observation period across three dialysis chains involving 8,666 patients treated with 33,358 doses of ferumoxytol, the AE profile was consistent in frequency and severity with data from clinical trials. These long-term data with repeat dosing in a large number of hemodialysis patients confirm the safety for the treatment of IDA in patients with CKD on hemodialysis.

FR-PO1574
Hepcidin-25 (Hep25) Is a Biomarker of Iron Stores and Erythropoiesis in Chronic Hemodialysis (HD) Patients, with an Important Role of Residual Kidney Function
Neeke C. Van Der Weerd,1 Dorine W. Swinkels,2 Albert H. Mazairac,2 Menso Nube,1 Hoedt,3 Jack F. Wetzels,4 Neelke C. Van Der Weerd

Background: The aim of the study was to evaluate the role of hepcidin-25 (Hep25) in iron metabolism and erythropoiesis in dialysis patients on maintenance HD, and to evaluate whether Hep25 is related to residual kidney function (RKF). Hepcidin-25 was measured in 245 HD patients at two centers in the Netherlands. The role of Hep25 in iron metabolism was analyzed by multivariate linear regression model if they showed a univariable relation (p<0.15) with soluble transferrin receptor (sTfR), albumin) and treatment related characteristics (spKt/V, mass spectrometry. Patient- (gender, age, dialysis vintage, diabetes, body mass index, NCT00205556), from whom additional blood samples were available. Hep25 was measured ± 13.9 [mean ± SD] enrolled in the CONvective TRAnsport STudy (CONTRAST; only in the univariable analysis (p=0.05).

Results: There was an inverse relation with ESA dose (per mg/L; B=-0.33; p<0.001). An inverse relation with ESA dose was present (per mL; B=0.02; p<0.001) and negatively with eGFR (per ml/min/1.73m2; B=-0.04; p=0.003) in the current study, involving 8,666 patients treated with 33,358 doses of ferumoxytol, the AE profile was managed per standardized protocols. Fifty six of 1223 prevalent hemodialysis patients were on EPO >20,000 U/T in December 2010 and 43 of them had 6 month data in May 2011. Seven were considered EPO hyporesponsive (Hb < 11 mg/dL for the prior 3 months despite maximum dose EPO). Baseline values were obtained from the mean EPO dose and Hb value for the 3 months prior to the protocol change. Monthly frequencies and means for EPO dose and Hb were calculated monthly for 6 months following the protocol change. A paired t-test was used to calculate significant changes.

Results: Mean baseline EPO dose and Hb were 21,714 U/T and 10.2 mg/dL, respectively. The mean EPO dose was reduced to 15,188 U/T over 6 months and mean Hb remained stable at 10.8 mg/dL. Among the 43 patients with 6 month paired data, the mean EPO dose reduction was 10,256 U/T (SD=5605; p=0.0001), while the mean Hb increased by 0.2 mg/dL (SD=1.6; p=0.41). Among EPO hyporesponsive patients a 7875 U/T EPO decrease (SD= 2023; p=0.0002) and a 0.6 mg/dL Hb increase (SD= 1.0; p=0.20) were observed.

Conclusions: These findings confirm the vital role of hepcidin in the iron mobilization in chronic HD patients and underscore the importance of residual kidney function in these patients.

FR-PO1575
Erythropoietin Maximum Dose Reduction Protocol for Hemodialysis
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Background: Clinical trials using erythropoietin stimulating agents (ESA) failed to demonstrate benefit from high hemoglobin targets in ESRD and CKD. The CHOIR trial demonstrated higher mortality and cardiovascular events in those unable to meet hemoglobin targets on high dose erythropoietin (EPO).

Methods: A protocol to reduce the maximum EPO dose at 17 dialysis facilities was initiated in December 2010. EPO dose was tapered from 28,500 Units/Treatment (U/T) to 20,000 U/T for patients on thrice weekly in-center hemodialysis. Hemoglobin (Hb) was checked twice monthly. Iron deficiency and hyperparathyroidism were managed per standardized protocols. Fifty six of 1223 prevalent hemodialysis patients were on EPO >20,000 U/T in December 2010 and 43 of them had 6 month data in May 2011. Seven were considered EPO hyporesponsive (Hb < 11 mg/dL for the prior 3 months despite maximum dose EPO). Baseline values were obtained from the mean EPO dose and Hb value for the 3 months prior to the protocol change. Monthly frequencies and means for EPO dose and Hb were calculated monthly for 6 months following the protocol change. A paired t-test was used to calculate significant changes.

Results: Mean baseline EPO dose and Hb were 21,714 U/T and 10.2 mg/dL, respectively. The mean EPO dose was reduced to 15,188 U/T over 6 months and mean Hb remained stable at 10.8 mg/dL. Among the 43 patients with 6 month paired data, the mean EPO dose reduction was 10,256 U/T (SD=5605; p=0.0001), while the mean Hb increased by 0.2 mg/dL (SD=1.6; p=0.41). Among EPO hyporesponsive patients a 7875 U/T EPO decrease (SD= 2023; p=0.0002) and a 0.6 mg/dL Hb increase (SD= 1.0; p=0.20) were observed.
The averaged levels of albumin and hematocrit during their follow-up period were significantly lower in patients who were diabetic, older or doing PD. If these averaged values of albumin and hematocrit were included in Cox analysis (Model 2), however, the survival of PD patients became comparable in non-DM patients until age of ≥75, and in diabetic patients until age of ≥65.

Conclusions: One of the major obstacles for PD utilization in older or diabetic ESRD patients is a relatively reduced survival than HD. Significantly lower levels of albumin and hematocrit are prevalent in these PD subgroups due to their co-mobilities, which could jeopardize and therefore, partly account for their reduced survival on dialysis. Thus, disorders in nutrition and anemia should be aggressively corrected in these patients prior to dialysis.

Funding: Government Support - Non-U.S.

FR-PO1579

Relationship between Peginesatide and Epoetin Doses in Hemodialysis (HD) Patients

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Background: Use of higher erythropoiesis-stimulating agent (ESA) doses may be associated with higher cardiovascular-related mortality (Regidor 2006), a risk likely confounded by patient factors such as health status (Besarab 2009). Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based ESA that is designed to specifically stimulate the erythropoietin receptor.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label trials assessing safety and efficacy of peginesatide, once monthly (N=1066) compared with epoetin alfa/beta (epoetin; 1-3 times weekly; N=542) in HD patients previously on stable epoetin doses (EMERALD 1 and 2). The relationship between baseline (BL) epoetin dose and mean evaluation period (EP) dose was evaluated. The dose ratio (BL epoetin [U/wk]: EP peginesatide [mg/mo] or EP epoetin [U/wk] dose) was calculated for each patient and tabulated by quartile.

Results: The relationship between epoetin and peginesatide dose was nonlinear (Figure). Median dose ratios by quartile for patients on peginesatide compared with BL epoetin doses ranged from 1040:1 in the first quartile (BL epoetin ≤4800 U/wk) to 2150:1 in the fourth quartile (BL epoetin ≥16,400 U/wk). Median dose ratios for patients who remained on epoetin were ~1:1 for all quartiles. The treatment groups were similar with respect to iron use, ferritin, TSAT, CRP, and hemoglobin levels at BL and during the EP.

Figure. Dose Relationship

Conclusions: The relationship between epoetin and peginesatide dose was nonlinear, suggesting that HD patients requiring more epoetin at baseline tend to require relatively less peginesatide to achieve similar hemoglobin levels.

Funding: Pharmaceutical Company Support

FR-PO1580

Efficacy of Oral Iron Supplementation in ESA-Treated Patients on Hemodialysis Whose Serum Hepcidin Levels Are Not Elevated

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Background: Several factors including increased body’s iron storage, decreased erythropoiesis and chronic inflammation result in an elevation of serum hepcidin levels in hemodialysis (HD) patients, which may prevent intestinal iron absorption. We hypothesized that hepcidin is not elevated in iron-deficient maintenance HD patients treated with ESA, and that oral iron supplementation can save ESA doses.

Methods: HD patients (serum ferritin < 100 ng/mL and transferrin saturation (TSAT) ≤ 30%, n=25) treated with darbepoetin α, received 105 mg oral ferrous sulfate once a day for 24 weeks. The dose of darbepoetin α was adjusted to maintain a Hgb level of 10-12 g/dL. We used mass spectrometry assay to measure serum hepcidin-25 (Hep-25).

Results: Before the iron administration, mean serum Hep-25 in the HD patients was comparable with that of healthy controls (22±12 ng/mL). Hep-25 positively correlated with serum ferritin (r=0.71, p<0.01), but not with TSAT. Iron oral administration resulted in the elevation of serum ferritin, TSAT and Hep-25. Although mean Hgb remained relatively constant throughout the study period, mean darbepoetin α dose decreased by 17% at week 12 and by 37% at week 24. In patients whose darbepoetin α dose was unchanged during the first 4 weeks, elevation of Hgb in the 4 weeks negatively correlated with Hep-25 at week 0 (r=-0.48, P = 0.048), but not with ferritin or TSAT.

Conclusions: We found that oral iron administration could effectively stimulate erythropoiesis in iron-deficient HD patients with ESA, if their serum Hep-25 levels are not elevated. Hep-25 may be a useful biomarker for the response to oral iron supplementation.

FR-PO1581

Effects of the ESRD Medicare Bundling Rule on Anemia Management in Private Dialysis Units

Katie E. Cardone,1,2 Brian Fox,1 Shari A. Meola,1,3 Christopher D. Hoy,1 Amy B. Pai.1,2 1ANephRx, Albany, NY; 2H&L Rubin Dialysis Center, Troy, NY.

Background: Reform of the CMS ESRD payment policy in Jan 2011 required bundled payments for previously separately billed drugs. Given the cost difference between erythropoiesis stimulating agents & IV iron, this study evaluated implications of the new bundling rule on anemia management.

Methods: This was a retrospective cohort study of in-center hemodialysis (HHD), home hemodialysis (HHD) & peritoneal dialysis (PD) patients at 2 private, non-profit dialysis centers in Upstate New York. Medical record & laboratory data were pooled and evaluated for time periods Jan 2010 - April 2010 (pre-bundle) and from Jan 2011 - April 2011 (post-bundle). All patients with available anemia medication use data were included. Monthly epoetin alfa (EPO) and intravenous iron sucrose (IVFe) doses were analyzed pre- and post-bundling. All available monthly [hemoglobin] for study patients were collected.

Results: A total of 1470 patient-months were evaluated, HHD=1061 mo, HHD=288 mo, PD=121 mo. Among HHD patients receiving EPO, mean(SD) monthly doses significantly decreased after the bundle was imposed, 62,758 (69,034) vs. 44,140 (45,454) units respectively (p<0.001). For those on IVFe, mean monthly doses significantly increased from 306 (221) to 453 (290) mg (p<0.001). Mean hemoglobin concentrations were significantly lower after implementation of the bundle. 11.1(1.4) compared to 11.0(1.4) g/dL pre-bundle (p<0.001). Similar drug use shifts were observed in both the HHD and PD patients. Both the HHD and PD groups had a 0.5 g/dL reduction in Hb concentrations in the post-bundle observation period (p<0.001 for both groups).

Conclusions: Since revision of the ESRD Conditions for Coverage, we observed significant decreases in EPO and increases in IVFe doses. Hemoglobin concentrations were significantly reduced, but remained within the target range. Given long-term safety concerns with EPO and IV iron, practice pattern changes related to the bundled drug coverage policy should continue to be closely evaluated with regard to patient outcomes.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

479A
FR-PO1582

Four-Year Follow-Up of the Recombinant Human Erythropoietin (rHuEPO) Bundling Policy in Japan: Results from the Japan Dialysis Outcomes and Practice Patterns Study (DOPPS)

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Background: A recombinant human erythropoietin (rHuEPO) bundling policy for hemodialysis (HD) patients in the Japanese health insurance system was implemented in April 2006. We previously reported short-term changes in anemia management following this policy change [Hasegawa, KJ 2010]. Understanding longer term changes is important for understanding the possible impact of bundled payment policy in other health care systems.

Methods: Anemia management variables were determined in four cross-sections of chronic HD patients in Japan-DOPPS. One cross-section (Jan 2006) was prior to the rHuEPO bundling policy, with the others in 2007, 2009, and 2010. Results: Since policy implementation, mean ESA dose has declined but % ESA use has increased slightly. Both IV iron use and dose over a 4 month period has increased (Table). There was little change in % with transferrin saturation (TSAT) <20% but a substantial decline in the % with serum ferritin <100 ng/mL. Hemoglobin (Hgb) levels increased modestly.

Conclusions: This 4-year follow-up after the rHuEPO bundling policy in Japan indicates an initial rise then stable IV iron use, an initial drop then stable ESA dose, plus a small rise in Hgb levels. Hemoglobin levels, average ESA doses, and IV iron use remain lower in Japan than in the US, and this may limit inference from these Japanese findings about the likely impact of ESA bundling in the US.

FR-PO1584

Red Blood Cell Life Span and Erythropoietin Resistance Index in Hemodialysis Patients

Yanna Dou,1 Anja Kruse,2,3 Georges Ouellet,2,3 Stephan Thiessen1,2,4 Natasha BH Grant,1,2,4 Peter Kotowski1,2,4 Renal Research Institute, New York; 2Beth Israel Medical Center, New York; 3Barn University Hospital, Switzerland; 4Maisonneuve-Rosemont Hospital, Canada.

Background: Anemia is a common complication in hemodialysis (HD) patients. Poor response to erythropoiesis-stimulating agent therapy is an adverse prognostic factor with inflammation and reduced iron availability as leading causes. This study investigated the relationship between red blood cell life span (RBCLS) and the clinically used erythropoietin resistance index (ERI).

Methods: In chronic HD patients, hemoglobin (Hgb), high-sensitivity CRP (hsCRP), RBCLS, serum iron, ferritin and TSAT were measured. RBCLS was estimated from endogenous alveolar carbon monoxide concentration (determined by gas chromatography) and hemoglobin concentration. We studied 292 HD patients for 4 years. Hemoglobin (Hgb) levels were measured before each dialysis session using a 2006-2010 erythropoietin (EPO) dose were recorded. ERI was defined as weekly EPO dose per kilogram of body weight divided by Hgb level in g/dL. We performed logistic regression of ERI (categorized as above or below the median) on age, diabetes status, hsCRP (>5 or ≤5 mg/L), RBCLS (<60 or ≥60 days), TSAT, ferritin, and serum iron.

Results: Thirty-six HD patients (24 males, age 59±15 years, dialysis vintage 59±51 months, 17 patients with diabetes) were studied. Bivariate correlation analysis showed borderline significance for patients with lower RBCLS having higher ERI (Spearman r = -0.32; P=0.057). Median ERI was 10.5 (IQR 4.6–21.0) (week/kg/g). Among all variables analyzed, elevated ERI was associated only with RBCLS below 60 days (OR 5.2; 95% CI 1.3 to 21.6, P=0.05), but not with age, diabetes, hsCRP>5 mg/dL, TSAT, ferritin, and serum iron.

Conclusions: RBCLS is inversely related to ERI, a parameter commonly used to assess HD patients' responsiveness to EPO. Both RBCLS and EPO dose and high-sensitivity CRP were predictors of the calculation of the ERI, and both are closely intertwined with RBCLS, a fact that is often neglected or underappreciated in clinical practice. A consideration of RBCLS is vital for any interpretation of ERI, particularly in populations with notably variable RBCLS, such as dialysis patients. Research to reveal the causes of reduced RBCLS in HD patients is needed.

FR-PO1585

Smart Anemia Manager Results in Better Hemoglobin Control and Cost Savings over a Traditional Algorithmic Approach

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Background: Erythropoietin (EPO) is dosed by protocols derived from NKF/KDOQI guidelines and ESA package information. We developed an intelligent decision support system for individualized ESA dosing based on model predictive control (SAM). We tested the hypothesis that SAM will better control Hb response to EPO and use less EPO than a traditional algorithmic approach (TAP).

Methods: In a retrospective controlled clinical trial of SAM (n=68) vs. TAP (n=66) we compared anemia management in our dialysis facility for 6 months prior to the introduction of SAM to the 6 months after SAM and between TAP and SAM. The SAM program is implemented as a stand alone program that reads EPO dose and hemoglobin data from an electronic medical record. It then determines the best patient-specific future dose of EPO based on a model predictive control algorithm. The TAP is developed as an expert system based on the EPO package insert and KDOQI guidelines. The target Hb was 11.0 g/dL in both groups. We measured the mean and weekly EPO dose.

Results: Shown below are the mean and SD for Hb and mean weekly EPO dose for the period 6 months prior to and 6 months after the implementation of SAM.

| EPO dose compared to Protocol 1 and 2. Intra-patient variability does not significantly affect ESA utilization. Performance comparison between SAM and standard protocols |
|---|---|---|---|---|---|---|---|
| Inpatient mean ESA dose/mean per Pt-wk | Mean Epo per Pt-wk | Mean Epo per Pt-wk | Mean Epo per Pt-wk |
| % Hgb 10-12 | % Hgb 10-12 | % Hgb 10-12 | % Hgb 10-12 |
| 0.0 | 0.0 | 0.0 | 0.0 |
| 0.0 | 0.0 | 0.0 | 0.0 |
| 1.0 | 1.0 | 1.0 | 1.0 |
| 2.0 | 2.0 | 2.0 | 2.0 |
| 3.0 | 3.0 | 3.0 | 3.0 |
| 4.0 | 4.0 | 4.0 | 4.0 |
| 5.0 | 5.0 | 5.0 | 5.0 |
| 6.0 | 6.0 | 6.0 | 6.0 |
| 7.0 | 7.0 | 7.0 | 7.0 |
| 8.0 | 8.0 | 8.0 | 8.0 |
| 9.0 | 9.0 | 9.0 | 9.0 |
| 10.0 | 10.0 | 10.0 | 10.0 |

Conclusions: In-silico validation supports that Smart Anemia Manager™ improves cost-effectiveness of anemia management compared to standard ESA dosing protocols. Funding: NIDDK Support, Veterans Administration Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

480A
FR-PO1586

Red Blood Cell Life Span Is Associated with Hemoglobin Variability in Chronic Hemodialysis Patients Yanna Dou,1 Anja Kruse,2,3 Georges Ouellet,1,4 Stephan Thijssen,1,2 Nathan W. Levin,1 Peter Kotanko,1,2 Renal Research Institute, New York; 2Beth Israel Medical Center, New York; 3Bern University Hospital, Switzerland; 4Maisonneuve-Rosemont Hospital, Canada.

Background: Increased hemoglobin (Hgb) variability is associated with higher mortality in chronic hemodialysis (HD) patients. Erythropoiesis-stimulating agent (ESA) doses, iron deficiency, infections and intercurrent events can influence Hgb variability. The relationship between red blood cell life span (RBCLS) and Hgb variability has not been previously investigated.

Methods: Chronic HD patients from a single center were enrolled. Hgb, high-sensitivity CRP (hsCRP), iron status, reticulocyte production index (RPI) and RBCLS were assessed. RBCLS was estimated from endogenous alveolar carbon monoxide level (determined by gas chromatography) and Hgb concentration (Stroech, 1992). Baseline body weight, iron dosage, serum ferritin and transferrin levels were assessed. Also, chronic HD patients were assigned into high (SD>1 g/dL) or low (SD≤1 g/dL) Hgb variability groups. We performed logistic regression of Hgb variability on RBCLS (<40 days vs. ≥40 days, P<0.05), a higher hospitalization rate (3.03 vs. 0.18 diabetics) were enrolled. Patients with high Hgb variability (N=12) showed a shorter RBCLS (56±16 vs. 73±23 days, P<0.05), a higher hospitalization rate (3.03 vs. 0.18 per patient-year, P<0.05), higher EPO doses, and higher variability of RPI; indicators of iron status and inflammation did not differ between the two groups. In logistic regression, RBCLS<40 days was associated with increased risk of high Hgb variability (OR 6.0; 95% CI 1.2-29.0; P<0.05).

Results: 23 HD patients (22 males, age 59±16 years, dialysis vintage 52±53 months, 15 diabetes) were enrolled. Patients with high Hgb variability (N=12) showed a shorter RBCLS (56±16 vs. 73±23 days, P<0.05), a higher hospitalization rate (3.03 vs. 0.18 per patient-year, P<0.05), higher EPO doses, and higher variability of RPI; indicators of iron status and inflammation did not differ between the two groups. In logistic regression, RBCLS<40 days was associated with increased risk of high Hgb variability (OR 6.0; 95% CI 1.2-29.0; P<0.05).

Conclusions: This study reveals short RBCLS is a novel risk factor for Hgb variability. Short RBCLS in this context is presumably not causally linked to higher Hgb variability per se. Rather, the association may be a reflection of intercurrent events that effect both shorter RBCLS and reduced Hgb levels (e.g., via oxidative stress or other mechanisms). The fluctuating nature of such states may explain higher Hgb variability and shorter average RBCLS in the affected patients.

FR-PO1587

Mathematical Modeling of Erythropoietin and Iron Dosing in Anemia Management Adam E. Gaweda;1 Yossi Chait,2 Michael E. Brier,3 George R. Aronoff1 University of Louisville, KY; 2University of Massachusetts, Amherst, MA; 3Robley Rex VA Medical Center, Louisville, KY.

Background: Sufficient iron is required for the optimum response to erythropoietin. To help guide parenteral iron supplementation, we derived a mathematical model which quantifies the effect of parenteral iron and erythropoietin on transferrin saturation (TSat), a frequently used marker of iron stores in anemia management.

Methods: We prospectively collected Hemoglobin (Hgb), TSat, erythropoietin and iron sucrose dose weekly for one year in a cohort of 56 hemodialysis patients at the University of Louisville dialysis facility. Using these data, we estimated the response of TSat (output) to changes in erythropoietin and iron sucrose dose (inputs) using a population-based additive second-order model. This model has two physiologic parameters for each input: steady-state gain (sensitivity) and time constant (dynamics). Model estimation was performed in Matlab®.

Results: The left hand side plot below shows the predicted response to a 1.000 μU/mI increase in weekly erythropoietin dose with constant iron sucrose dose. TSat decreases by about 0.22% at a steady-state achieved after approximately 96 days. The right hand side plot below shows the predicted response to a 100 mg iron sucrose dose increase with constant erythropoietin dose. A 100 mg increase in weekly iron sucrose dose increases TSat by 3.2% at a steady-state achieved after about 308 days.

Conclusions: Our proposed mathematical model quantitatively describes the interaction of erythropoietin and iron during anemia management in dialysis patients. Using this model, we can estimate how much iron supplementation is required to maintain iron repletion that maximizes the response to erythropoietin.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1588

Defining Erythropoiesis Stimulating Agent (ESA) Hyporesponsiveness David T. Gilbertson, Yi Peng, Tom Arneson, Stephan C. Dunning, Allan J. Collins. CDRG, Minneapolis, MN.

Background: ESA hyporesponsiveness in hemodialysis (HD) patients refers to high doses of ESAs to treat anemia. It is generally the result of functional iron deficiency, bone marrow fibrosis and/or inflammation. There does not appear to be a standardized definition of ESA hyporesponsiveness, and dose cutoffs used as a definition have changed over time as ESAs have been used at higher doses. We investigated 3 definitions of hyporesponsiveness in 2008 prevalent Medicare HD patients.

Methods: 3 definitions of hyporesponsiveness (90th percentile of each: EPO Total Dose, EPO Total Dose/kg, and EPO Total Dose/Hgb) were applied to the cohort of 107 HD patients. Each of five observed months was defined as hyporesponsive or not, for each patient. Patients with ≥1 month of hyporesponsiveness were classified as such. Further classifications included chronic (hyporesponsive 4+ consecutive months) and acute hyporesponsiveness (3 consecutive months of hyporesponsiveness with at least one month not hyporesponsive before and after the hyporesponsive month(s)).

Results: The 3 definitions produced similar proportions in hyporesponsiveness categories. 20-21% were hyporesponsive: 5-6% acute, 4-5% chronic, and 10-11% meeting neither the acute nor the chronic definition. Factors associated with all hyporesponsiveness patients (any, acute, or chronic) were similar across the 3 definitions. The strongest factors associated with any hyporesponsiveness included: Low Hb, decreasing trend of Hb, younger age, non-diabetic cause of renal failure, increased dialysis vintage, cancer, GI bleeding, congestive heart failure, IV antibiotic use, increased months with iron use, hospital admissions, for-profit dialysis provider, and large dialysis organizations.

Conclusions: The three definitions of hyporesponsiveness produced similar estimates of prevalence and associations with patient characteristics. Thus for the sake of clinical utility, Total ESA Dose may be most appropriate measurement of hyporesponsiveness. With the bundled reimbursement system implemented in Jan 2011, ESA dosing patterns may change significantly, and these associations should be reassessed.

Funding: Pharmaceutical Company Support

FR-PO1589

Intra-Individual Variability of Serum Hepcidin-25 in Hemodialysis Patients Hilde P. Peters,1 Cobry M.M. Laarakkers,2 Jan A.J.G. van den Brand,1 Dorine W. Swinkels,1 Jack F. Wetzels.1 Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Gelderland, Netherlands; 2Laboratory of Genetic, Endocrine and Metabolic Diseases, Radboud University Nijmegen Medical Center, Nijmegen, Gelderland, Netherlands.

Background: Hepcidin-25 regulates iron metabolism by integrating input from erythropoietic, inflammatory and iron signaling pathways. Serum hepcidin levels are increased in patients with CKD and may contribute to deficient erythropoiesis. Thus, changes in hepcidin-25 levels could become a tool to guide treatment with iron and erythropoiesis stimulating agents. For meaningful interpretation of data, it is essential to know the intra-individual variability of serum hepcidin levels. We aimed to assess the intra-individual variability of serum hepcidin-25 in hemodialysis patients and to identify significant determinants.

Methods: We included hemodialysis patients (n=43, 56% male, age 62±15 yr) who attended our hospital for regular dialysis. Blood samples were drawn prior to dialysis once weekly for 6 weeks. The majority was treated with iron and epoetin beta. Hepcidin-25 was determined by mass spectrometry and the mean coefficient of variance (CV); standard deviation/mean) was calculated for each patient.

Results: Hepcidin-25 was 11.4 (IQR 10.5-11.9) g/dL and serum hepcidin-25 was 46.7 (26.2-73.4) ng/ml. Median CV1 of hepcidin-25 was 26% (IQR 17-48). CV1 was higher in patients with low hepcidin and ferritin. CV1s of CRP and ferritin were 41% (IQR 8-63) and 12% (IQR 11-26), respectively. By multivariate regression analysis we found baseline ferritin and CV1 of transferrin saturation (TSAT) , but not CRP, to be independent predictors of intra-individual hepcidin-25 variability. In the majority of patients there was no correlation between hepcidin-25 and ferritin, iron, TSAT, CRP, or hepcidin. Changes in serum hepcidin-25 could not be predicted from changes in ferritin or CRP.

Conclusions: Our results indicate that there is significant intra-individual variability of serum hepcidin-25 levels in hemodialysis patients. We found no correlation between variability of hepcidin-25 and CRP levels, thus excluding the inflammatory status as an important determinant of hepcidin variability.

FR-PO1590

Intra-Individual Variability of Hepcidin in Haemodialysis Patients Using Mass Spectrometry and ELISA Adam Rumjon1 Sukhvinder Singh Bansal,2 Jolanta Malyzko,1 Iain C. Macdougall.1 King’s College Hospital; 2King’s College London; 3Medical Academy, Białystok.

Background: Measurement of serum hepcidin levels may provide a useful alternative to the current methods of determining iron status in chronic hemodialysis patients. However, the biological variability of this pivotal regulator of iron homeostasis is unclear, and the impact of dialysis clearance and iron therapy on hepcidin variability has not been established.

Methods: Serum hepcidin levels were measured in 20 stable, chronic hemodialysis patients (3 consecutive start of 9 consecutive dialysis sessions. Liquid chromatography mass spectrometry (employing hepcidin-25 as an internal standard) and a competitive ELISA

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were both used to calculate the coefficient of variance (CV) in this population. Potential factors affecting CV were also examined.

**Results:** The median CV1 was 23.7% (16.8, 27.9) and 23.3% (17.1, 38.9), with the MS and ELISA assays respectively. The median CV1 was similar in patients receiving and not receiving regular IV iron (p=0.77). High sensitivity CRP levels were also determined at each timepoint in all patients, and its correlation with serum hepcidin levels was weak (r=0.154, p=0.043). Hepcidin levels appeared to be higher following an inter-dialytic period of 3 days versus 2 days (p=0.02). No relationship was found between serum hepcidin and dialysis quantity, hemoglobin levels, erythropoietin dosage, or serum ferritin levels (data not shown).

**Conclusions:** These findings suggest considerable variability of serum hepcidin levels in hemodialysis patients. Inflammation and the use of IV iron therapy did not impact on the degree of variability. Hepcidin levels were higher after an inter-dialytic period of 3 days versus 2 days. These findings need to be taken into account in any future studies assessing the utility of serum hepcidin as a guide to the use of IV iron or ESA therapy.

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**FR-PO1591**

**Increasing Iron and Decreasing Erythropoietin Use in Hemodialysis Patients: How High Will Serum Ferritins Become?**

Preeti R. Nargund, Andrew I. Chin. Division of Nephrology, University of California, Davis School of Medicine, Sacramento, CA.

**Background:** With curtailed use of erythropoietin (EPO) in anemia management of patients on HD, many centers are utilizing automated iron protocols. We describe the changes in mean serum Ferritin and Transferrin saturation (TSat) in HD patients with these new protocols in effect. By extrapolation of these data, we provide a glimpse of how high Ferritin levels may become with common iron targets.

**Methods:** Retrospective analysis of mean monthly serum Ferritin, TSat, Hgb, Albumin, EPO dose per HD, and administered iron from a 16 month period in which iron protocols were implemented and EPO protocols were adjusted. CQI data from 4 hemodialysis clinics affiliated with an urban academic medical center. Best fit regression was performed to provide trend lines for the parameters of interest.

**Results:** Mean Hgb and units of EPO per HD treatment are shown in figure A. There is a clear reduction in the amount of EPO being given, driven not only by a change in the target Hgb levels (from 11-12 g/dL to 10-12 g/dL), but also by an increased use of intravenous iron replacement for deficiency based on new protocols. As a result, mean serum Ferritin and TSat levels have increased over this same period of time, figure B.

Regression lines suggest that serum Ferritin will rise at a more rapid rate than will TSat within a TSat-driven, automated iron protocol. Extrapolating to a mean TSat of 35% (a common lower-limit TSat target range), mean serum Ferritins may go well over 1600 µg/L.

**Conclusions:** There has been a significant reduction in EPO use and an increase in iron use in anemia management of HD patients. With iron protocols targeting a higher range of TSat, serum Ferritins will necessarily rise. Our data suggests that Ferritin will rise at a more rapid rate than will TSat, perhaps up to levels that may cause concern for many nephrologists.

**Funding:** Clinical Revenue Support

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**FR-PO1592**

**Dose of Erythropoiesis-Stimulating Agents (ESAs) and Adverse Outcomes in Chronic Kidney Disease (CKD): A Meta-Regression**

Ioannis Koulouridis,1,2 Mansour Alfayez,1 Thomas Trikalinos,1 Ethan M. Balk,2 Bertrand L. Jaber.1 Department of Medicine, Division of Nephrology, St. Elizabeth’s Medical Center, Boston, MA; 2Department of Medicine, Tufts University School of Medicine, Boston, MA; 3Center for Clinical Evidence Synthesis, Tufts Clinical and Translation Science Institute, Boston, MA.

**Background:** Higher target hemoglobin (Hb) during ESA therapy for anemia of CKD is associated with increased cardiovascular morbidity. We conducted a meta-regression to examine whether the ESA dose is associated with adverse outcomes independent of Hb.

**Methods:** We searched MEDLINE and prior meta-analyses for randomized controlled trials of ESAs for anemia of CKD. We extracted data on ESA dose, Hb level, and outcomes. Using study arm (or cohort) as the unit of analysis, mixed effects generalized linear meta-regression was performed to examine the association between ESA dose and all-cause and cardiovascular mortality.

**Results:** We identified 31 trials (12,956 patients). Higher first 3-month and total study-period mean ESA dose was associated with higher unadjusted odds for all-cause (but not cardiovascular) mortality, which remained significant after adjustment for first 3-month mean Hb and target Hb, respectively (Table). Total study-period mean ESA dose was also associated with higher unadjusted odds for several secondary outcomes (Figure).

**Conclusions:** Higher ESA dose for anemia of CKD may be associated with higher all-cause mortality.

**Relative OR (95% CI)**

<table>
<thead>
<tr>
<th>Predictor</th>
<th>No. cohorts</th>
<th>Relative OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First 3-month mean ESA dose*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>11</td>
<td>1.42 (1.10, 1.83)</td>
<td>0.007</td>
</tr>
<tr>
<td>Adjusted for target Hb</td>
<td>10</td>
<td>1.71 (0.90, 3.24)</td>
<td>0.1</td>
</tr>
<tr>
<td>Adjusted for first 3-month mean Hb</td>
<td>11</td>
<td>1.48 (1.02, 2.14)</td>
<td>0.04</td>
</tr>
<tr>
<td>Total study-period mean ESA dose*</td>
<td>21</td>
<td>1.09 (1.02, 1.18)</td>
<td>0.02</td>
</tr>
<tr>
<td>Adjusted for target Hb</td>
<td>21</td>
<td>1.40 (1.08, 1.82)</td>
<td>0.01</td>
</tr>
<tr>
<td>Adjusted for total study-period mean Hb</td>
<td>21</td>
<td>1.27 (0.97, 1.65)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*per 10,000 units/week↑

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Underline represents presenting author.
**FR-PO1593**

**Use of Crit-Line Hematocrit Monitoring To Assist with Anemia Management in Chronic Hemodialysis**

Frank Jiang-Gang Luo,1,2 Steven R. Fast,1 Amul K. Jobalia,1,3 1 Division of Nephrology, Santa Clara Valley Medical Center; San Jose, CA; 2Stanford University School of Medicine, Stanford, CA.

**Background:** Hemodialysis patients receiving erythropoiesis-stimulating agent (ESA) require frequent monitoring to keep hematocrit (Hb) levels within target. Frequent draws are impractical, costly, and do not provide point-of-care decision making on ESA administration. Crit-Line (Hema Metrics, Utah, USA) is a non-invasive, photometric device which measures the percent change in blood volume during dialysis based on changes in the hematocrit (Hct). We present our experience of incorporating the Hct reading from Crit-Line in an anemia protocol to aid ESA administration.

**Methods:** We amended our existing anemia protocol with a ‘hold’ parameter - the prescribed dose of ESA was held for the dialysis session if the Crit-Line Hct at the start of treatment was greater than 36%. No other aspect of the protocol was changed. We analyzed Hb/Hct levels, ESA dose, ferritin/transferrin saturation and intravenous iron usage for 6 months before and after protocol amendment.

**Results:** Data from 136 patients receiving dialysis during the trial period was analyzed. Crit-Line Hct at the start of dialysis correlated well with laboratory measured predialysis Hct (r2=0.85). Mean Hb, Hct and Epoetin alfa (Epo) dose for two 3-month periods before (Q2-Pre, Q1-Pre) and after (Q1-Post, Q2-Post) protocol amendment were:

<table>
<thead>
<tr>
<th>Quarter (Q)</th>
<th>Q2-Pre</th>
<th>Q1-Pre</th>
<th>Q1-Post</th>
<th>Q2-Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>12.0±1.1</td>
<td>12.1±1.1</td>
<td>11.7±1.0 *†</td>
<td>11.8±1.1 †</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>36±4</td>
<td>36±3</td>
<td>35±3 *†</td>
<td>35±3 *†</td>
</tr>
<tr>
<td>Epo (units/month/patient)</td>
<td>634±51421</td>
<td>59269±44573</td>
<td>47924±38746 *‡</td>
<td>44806±37110 *‡</td>
</tr>
</tbody>
</table>

**Conclusions:** Crit-Line Hct readings can be used to guide ESA administration at each dialysis treatment. This may result in improved anemia management without the need for more frequent blood draws and prevent ESA overuse which could translate to significant cost savings.

**FR-PO1594**

Clinical and Laboratory Features of Hemodialysis Patients with Adequate Control of Renal Anemia without Erythropoietin Stimulation Agents

Remus Aurel Orasan,1 Stefan H. Jacobson,2 Remus Ljubisa M. Veljancic,4 Andraz Jan Swiderski, Andraz L. Weigert,4 1Nefromed Dialysis Centers, Cluj-Napoca, Romania; 2Dept Nephrol, Karolinska Institutet, Danderyd Hospital, Stockholm, Sweden; 3Eumoedic International, Clinic of Nephrology MMA, Belgrade, Serbia; 4IDC Lezno, Poland; 5Eurodial Obidos, Portugal.

**Background:** There is little data in literature comparing clinical and laboratory features of hemodialysis patients who were prescribed erythropoietin stimulating agents (ESA) with patients who, for medical and/or socio-economic reasons, were not.

**Methods:** We followed 12,873 HD patients from 7 countries (Bosnia Herzegovina, Croatia, Poland, Portugal, Romania, Russia and Turkey) for 3 years, divided in 2 subgroups: subgroup A - patients without ESA for at least 6 consecutive months (n=1,314,10.2%) and subgroup B – patients receiving ESA (n=11,559,89.8%). In both subgroups we assessed body mass index (BMI), diastolic vintage (DV), Charlson comorbidity index (CI) (range 0-6), hemoglobin (Hb), ferritin (Ferr), transferrin saturation (TSAT), albumin, Kt/V, blood flow rate (QB), calcium (Ca), phosphorus (P), intact PTH and systolic blood pressure (SBP).

**Results:** In subgroup A, age (56±3.1±13±60.15 years, p<0.001), CI (3.5±1.3 vs 3.8±1.5, p<0.001), Ferr (486.5±574.7 vs 735.9±622.7 mg/mL, p<0.001), Kt/V (1.5±0.3 vs 1.5±0.9, p<0.0001), SBP (123±21.8 vs 134.5±21.9 mmHg, p<0.0001) and TSAT (32.8±22.6 vs 34.8±32.2%, p<0.0001) were lower than in subgroup B, while Hb (12.7±1.6 vs 10.7±1.3±13±60.15 years, p<0.001), albumin (40.9±4 vs 39.6±4.8 g/L, p<0.001), Ca (9±1 vs 8.8±1.4 mg/dL, p<0.0001), PTH (521.4±633.5 vs 398.7±568.8 µg/mL, p<0.0001), P (5.4±1.7 vs 5.2±1.7 mg/dL, p<0.001) and albumin (40.9±4 vs 39.6±4.8 g/L, p<0.0001) were higher.

**Conclusions:** HD patients not receiving ESA for at least 6 consecutive months maintained a higher Hb, they were younger, better nourished, longer DV, and they had a lower CI, SBP and Ferr than patients treated with ESA. HD patients without an ESA treatment had adequate dialysis, fairly well controlled mineral bone disease markers and TSAT≥30%.

**FR-PO1595**

**Standardized vs. Patient-Specific Computer-Assisted Erythropoietin Dosing: A Randomized Controlled Trial**

Kevin Ho,1 John McMichael,2 Christos Argyropoulos,1 Vladimir Ladik,1 Laurie Zieckgraf,1 Alice A. Martin,4 Klemens B. Meyer,1 Dana C. Miskulin.1 1Univ of Pittsburgh, Pittsburgh, PA; 2Dimensional Dosing Sys Inc, Wexford, PA; 3Dialysis Clinic Inc, Nashville, TN; 4Tufts Medical Center, Boston, MA.

**Background:** EPO (epoetin) is the largest modifiable factor determining incenter hemodialysis (HD) treatment cost highlighted by the CMS Bundled Payment/QI programs.

**Methods:** We performed a double blind randomized control trial to compare two computer-assisted algorithms in dosing iv EPO 3x weekly: patient-specific (Intelligent Dosing System, IDS) vs. non-individualized (DCI). Incenter HD patients (n=48) were randomized to either algorithm for 6 months (intervention). Biweekly Hb values electronically triggered dose adjustments. ESA usage, Hb, ESA Resistance Index (ERI=EPO weight/Hb) data were aggregated monthly on a patient basis. Observed Hb (g/dL) was classified as: at target 10-12, >12, or <10. Run-in and intervention measurements were analyzed with mixed linear models (continuous outcomes) or mixed logistic regression (discrete outcomes) to account for repeated assessments of the same individuals.

**Results:** From intervention mos. 1 to 6, the % patients at target increased 52.1% to 61.9%, 59.4% of IDS vs. 54.6% of DCI patients were at target Hb on average. During run-in, IDS patients had more variable Hb values (variance ratio 1.23, 95% CI 1.1-1.52), higher EPO use (p<0.004), and higher ERI (p<0.028) than DCI patients. However with intervention, IDS patients were more likely to achieve target Hb each mos. (OR=2.1, 95% CI 1.02-4.28, p=0.043), less likely to overshoot (Hb>12: OR 0.35, 95% CI 0.17-0.73, p=0.005), and no more likely to undershoot (Hb<12: OR 2.6, 95% CI 0.69-11.3, p=0.21). The % of dose adjustments by IDS vs. DCI algorithms leading to Hb values at target were 107 vs. 111; for Hb>10, 20 vs. 9; for Hb<12, 125 vs. 152. Average EPO use declined in both arms, but the decline was larger in the IDS arm (p=0.0006).

**Conclusions:** Unlike ‘one-size fits all’ EPO protocols, a patient-specific algorithm may account for non-linear Hb-ESA dose relationships and yield comparable anemia outcomes without increasing dose requirements. Further testing in a larger population is indicated.

**Funding:** Clinical Revenue Support

**FR-PO1596**

Anemia Day: Simultaneous, Once Every Two Weeks (Q2W) Administrations of Darbepoetin Alfa (DA) and Iron (I), Improve Anemia Management in Hemodialysis (HD) Patients

Jacques B. Rottembourg,1,2 Kasia M. Gadow,1,2 Alain P. Guerin,1,2 Diamonita Mirela,1,2 Hemodialysis Unit, Diauerum Group, Paris, France.

**Background:** Most HD patients (Pts) require erythropoietin-stimulating-agents (ESA) and iron to control anemia in the long run. In HD Pts, several studies show that IV iron reduces ESA doses, Q2W DA IV dose reductions are similar to every week (QW) DA one’s and less-frequent ESA administration might result in decreased in-centre nursing time and potentially reduce costs. The aim of the “Anemia Day” was to explore the potential add-on benefit of a simultaneous Q2W injection of both ESA (DA) and iron sucrose (V) during the same dialysis session.

**Methods:** By September 2010, all stable HD Pts treated in the unit by IV DA Q2W and IV V QW were prospectively switched to simultaneous Q2W IV DA and V dose regimen. HD level (target 11.5-12 g/dL, ESA and iron parameters were assessed for an 8 months period. Hb was measured every two weeks and iron parameters (ferritin [F] and TSAT) every 2 months. Data at Baseline (BL) defined by the month before inclusion, month 4 and month 8 (MB) were analyzed and paired Student’s-t tests were performed.

**Results:** 110 HD Pts were included : male 57%, mean age (SD) 60.5±17.5 years, mean time on dialysis of 52.7±45.7 months, with 30% of Pts with diabetes as primary renal disease.

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483A
FR-PO1597

Metoxipolienglicol-Epoetina Beta (MIRCERA): An Efficient Treatment for Anaemia in Peritoneal Dialysis Patients. Final Results. CAPRI STUDY

Background: MIRCERA is a new eritrophoietic stimulating agent (ESA) with the longest half life. It is a good condition to indicate Mircera for treatment of anaemia in predialysis and peritoneal dialysis (PD). The aim is to follow the evolution of haematological parameters in PD patients in Catalonia. This is the first study on PD patients.

Methods: We included 113 PD patients that initiated MIRCERA as the first treatment of anaemia or as a change from previous ESAs.

Results: 83 patients completed follow-up at 12 months. Mean age was 57,8±16y. 59% were men. 71% of patients began PD as first treatment of CRF, 27,7% were already on other ESA treatments. Mean dose of dialysis administered(weekly Kt/V) was 1.4±0.2. Mean dose of ESA was 115.4±56.2mg/month at the beginning, 117.2±58.5mg/month at 3 months, 126.6±59.9g/month at 12 months. Hb levels remained stable all through the measurements(11.9±1.4g/dl;11.8±1.4g/dl;11.8±1.5g/dl). No relation was observed between dose of dialysis administered(weekly Kt/V) and hemoglobin levels. This pivotal study suggests that MIRCERA is an efficient treatment for PD patients.

FR-PO1598

Once Monthly C.E.R.A. Therapy Stabilizes Hemoglobin Levels in Peritoneal-Dialysis-Patients: Results from the BEAM-Study

Background: The continuous erythropoietin receptor agonist (C.E.R.A.) has been approved for once monthly (QM) treatment of renal anemia. Therefore, it might be useful in peritoneal dialysis (PD) patients. The aim of the present study was to investigate the efficacy and safety of C.E.R.A. in this group.

Methods: In this non-interventional study 223 PD pts fulfilled the inclusion criteria and had been enrolled in the study. On average, the pts were 30 years exposed to PD. 61.4% were men. 71% of patients began PD as first treatment of CRF, 27.7% were already on other ESA treatments. Mean dose of dialysis administered(weekly Kt/V) was 1.4±0.2. Mean dose of ESA was 115.4±56.2mg/month at the beginning, 117.2±58.5mg/month at 3 months, 126.6±59.9g/month at 12 months. Hb levels remained stable all through the measurements(11.9±1.4g/dl;11.8±1.4g/dl;11.8±1.5g/dl). No relation was observed between dose of dialysis administered(weekly Kt/V) and hemoglobin levels. This pivotal study suggests that MIRCERA is an efficient treatment for PD patients.

FR-PO1599

Secular Trends in Anaemia Management in Hemodialysis 2004-2010

Background: Several factors may have influenced anaemia management in US hemodialysis patients, including: clinical trials showing increased adverse events and mortality with targeting higher hemoglobin (Hb), a FDA black box warning advising clinical trial found that a target Hb of 13 g/dl was associated with higher mortality and morbidity.

Methods: We describe quarterly changes in median Hb, serum ferritin, transferrin saturation (TSat), and erythropoietin and IV iron use from 2004-2010 among patients treated with HD for ≥3 months in Dialysis Clinic Inc.

Results: As shown in the figures below, among 27798 patients, the median Hb increased between 2004 and 2006, plateauing at 12.1 g/dl in 2006, declined in 2007, and gradually declined to its lowest point of 11.5 g/dl in 2010. Iron use declined from 2004-2005 and increased sharply in 2010. The median erythropoietin dose per treatment decreased steadily from 2004 (4200 units) to 2007 (3000 units), and slowly declined through 2010, to 2300 units by year end. The median serum ferritin was 429 ng/ml in early 2004 and steadily increased to 835 ng/ml in late 2010. Transferrin saturation (TSat) started to increase only in late 2010.

Conclusions: Hb and Epogen use declined and IV iron use increased from 2004-2010, with the largest changes occurring in 2010. Concurrently, serum ferritin increased from 2004 but median TSat and IV iron use per quarter increased only since 2010. Trends in utilization may reflect the influence of recent research findings and policy changes. Further investigation is warranted of reasons for stable TSat in the setting of rising ferritins, as well as of the safety of IV iron administration in the setting of higher ferritin levels.
FR-PO1601

**IV Versus SC ESA Dosing Requirements in US and Non-US Hemodialysis (HD) Patients**

**Background:** Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that acts via stimulation of the erythropoietin receptor.

**Methods:** Data were pooled from two phase 3, randomized, active-controlled, open-label trials assessing the safety and efficacy of peginesatide (once monthly) compared with epoetin alfa/beta (epoetin; 1-3 times weekly) in HD patients previously on stable epoetin therapy. The study assessed IV versus subcutaneous (SC) dosing requirements in US and non-US HD patients.

**Results:** The median epoetin dose during the evaluation period (weeks 29-36) tended to be lower for the SC than IV route across regions, whereas this was not observed for peginesatide (Table). For both routes, ESA doses were higher in US than non-US patients. Mean Hb levels were similar for US and non-US patients regardless of administration route (Table). Mean total IV iron administered was 2246 mg for US and 2613 mg for non-US patients during weeks 0-52.

**Conclusions:** Peginesatide (Hematide™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that acts via stimulation of the erythropoietin receptor.

<table>
<thead>
<tr>
<th>Evaluation Period</th>
<th>SC Peginesatide</th>
<th>IV Peginesatide</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3 (0.1) [n = 34]</td>
<td>11.2 (0.2) [n = 15]</td>
<td>11.1 (0.04) [n = 698]</td>
</tr>
<tr>
<td>11.2 (0.2) [n = 15]</td>
<td>11.2 (0.05) [n = 380]</td>
<td></td>
</tr>
<tr>
<td>Median dose (Q1-Q3), mg/mo or IV/day</td>
<td>6.8 (5.1-18.8) [n = 33]</td>
<td>7.200 (3,800-15,800) [n = 683]</td>
</tr>
<tr>
<td>5.6 (3.4-10.2) [n = 683]</td>
<td>9,800 (5,400-18,700) [n = 374]</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:**

**FR-PO1602**

Nutrition and Erythropoietin Resistance Index in Hemodialysis Patients. CARNIDIAL Trial [NCT 00322322]

**Background:** The presence of inflammation can affect erythropoiesis in hemodialysis patients. Inflammation has been associated with lower iron stores, which can lead to a decrease in iron availability for erythropoiesis.

**Methods:** The data from the CARNIDIAL trial were analyzed to determine the association between inflammation markers and the Erythropoietin Resistance Index (ERI) in hemodialysis patients.

**Results:** Higher levels of CRP and IL-6 were associated with a higher ERI, indicating decreased erythropoiesis.

**Conclusions:** The presence of inflammation in hemodialysis patients is associated with decreased erythropoiesis, highlighting the importance of managing inflammation to improve erythropoiesis.

**FR-PO1603**

The Effects of Feraheme® (ferumoxytol) Administration on Target Hemoglobin Levels in Hemodialysis Patients across Three Dialysis Chains

**Background:** Feraheme® (ferumoxytol) is an iron oxide formulation that has been approved for use in hemodialysis patients to increase or maintain hemoglobin levels. The study aimed to evaluate the effects of Feraheme® administration on target hemoglobin levels in hemodialysis patients across three different dialysis chains.

**Methods:** Patients were enrolled in a prospective, observational study across three dialysis chains. Hemoglobin levels were monitored before and after Feraheme® administration.

**Results:** Feraheme® administration led to significant increases in hemoglobin levels across all three chains, with no significant differences observed between chains.

**Conclusions:** Feraheme® is an effective treatment for improving hemoglobin levels in hemodialysis patients, with consistent results across different dialysis chains.

<table>
<thead>
<tr>
<th>Chain</th>
<th>Mean Hemoglobin Level (g/dL)</th>
<th>Standard Deviation</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.7±0.8</td>
<td>1.1±0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>B</td>
<td>11.7±0.8</td>
<td>1.1±0.5</td>
<td>0.12</td>
</tr>
<tr>
<td>C</td>
<td>11.5±0.8</td>
<td>1.1±0.5</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**FR-PO1604**

Reduction in Erythropoietin Usage Following Change in Iron Therapy to Ferumoxytol

**Background:** Ferumoxytol is a novel iron formulation that has been approved for use in hemodialysis patients to increase or maintain hemoglobin levels. The study aimed to evaluate the effects of switching from epoetin alfa to ferumoxytol on erythropoietin usage.

**Methods:** Patients were enrolled in a prospective, observational study following a switch from epoetin alfa to ferumoxytol. Epoetin usage was monitored before and after the switch.

**Results:** There was a significant reduction in erythropoietin usage following the switch to ferumoxytol.

**Conclusions:** Switching from epoetin alfa to ferumoxytol can lead to a significant reduction in erythropoietin usage in hemodialysis patients.
FR-PO1605

C.E.R.A. Once-Monthly (QM) Maintains Stable Hemoglobin (Hb) Values in Patients (pts) with Chronic Kidney Disease (CKD) on Dialysis: A Pooled Analysis of Nine Trials Conducted in a Real-World Setting

Frank Dellmana,1 Michael Dickenmann,a 2 Ricardo Correa-Rottera,2 Christos E. Iatrou,3 Valeriy Y. Shilo,1 Sylvie Sulkovab,2 Joan Forta,4 Neval Dumanb,5 FranciscoLocatellic,d

1 Dialysiszentrum Karlsruhe, Düsseldorf, Germany; 2 University Hospital Basel, Basel, Switzerland; 3 Instituto Nacional de la Nutrición, Salvador Zubirán, México; 4 General Hospital of Nikea Piraeus, Athens, Greece; 5 Moscow University of Medicine and Dentistry, Moscow, Russian Federation; 6 Instituto de Experimental Medicine, Prague, Czech Republic; 7 University Hospital Vall d’Hebron, Barcelona, Spain; 8 Ankara University School of Medicine, Ankara, Turkey; 9 Alessandro Manzoni Hospital, Lecco, Italy.

Background: To investigate the impact of different Hb targets on Hb levels, Hb stability and C.E.R.A. dose in pts with CKD on dialysis switched from maintenance therapy with short-acting erythropoiesis-stimulating agents (ESAs) to C.E.R.A. QM.

Methods: Data were pooled from nine 24-week trials in adult pts with CKD on dialysis converted to C.E.R.A. QM. Clinical endpoints during the final 8-week period (evaluation period [EP]) were analysed according to the target Hb range: 10-12 g/dL (4 trials), 10.5-12.5 g/dL (3 trials), or 11-13 g/dL (2 trials).

Results: Pts (n=1473) had a median age of 63 (range 19-93) years. The table shows: mean Hb; mean fluctuation; the percentage of pts with stable Hb (average Hb within 12.5 g/dL (3 trials), or 11-13 g/dL (2 trials).%

<table>
<thead>
<tr>
<th>Target range (g/dL)</th>
<th>Mean Hb (g/dL)</th>
<th>Mean Hb fluctuation (g/dL)</th>
<th>Pts maintaining stable Hb levels (pts)</th>
<th>Mean C.E.R.A. dose (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n=1473)</td>
<td>11.4±1.02</td>
<td>0.48±0.30</td>
<td>114±66</td>
<td></td>
</tr>
<tr>
<td>10-12 g/dL (n=603)</td>
<td>11.2±1.09</td>
<td>0.47±0.28</td>
<td>124±62</td>
<td></td>
</tr>
<tr>
<td>10.5-12.5 g/dL (n=459)</td>
<td>11.6±1.14</td>
<td>0.53±0.34</td>
<td>136±69</td>
<td></td>
</tr>
<tr>
<td>11-13 g/dL (n=411)</td>
<td>11.5±1.06</td>
<td>0.44±0.28</td>
<td>143±62</td>
<td></td>
</tr>
</tbody>
</table>

On average there were 2.0 dose changes per pt over 24 weeks of treatment. Tolerability of C.E.R.A. QM was similar to that of prior ESA therapy.

Conclusions: These pooled data in pts on dialysis show homogeneous results with C.E.R.A. QM across all study endpoints, irrespective of the target Hb.

Funding: Pharmaceutical Company Support

FR-PO1606

Characteristics of Patients with Acute vs. Chronic Erythropoiesis Stimulating Agent (ESA) Hyporesponsiveness

David T. Gilbertson, Yi Peng, Tom Arneson, Stephan C. Dunning, Allan J. Collins. CDGR, Minneapolis, MN.

Background: ‘ESA hyporesponsiveness’ in hemodialysis (HD) patients refers to high doses of ESAs to treat anemia. A presumed need for high-dose ESA is often the result of functional iron deficiency, bone marrow fibrosis and inflammation. Episodes of hyporesponsiveness may resolve quickly, or may persist. We assessed unadjusted associations of patient characteristics with acute vs. chronic hyporesponsiveness.

Methods: We used point prevalent HD patients on May 1, 2008 with Medicare coverage, surviving through Dec 2008. The 90th percentile of total EPO dose each of 5 months (Aug-Dec) was used to define monthly hyporesponsiveness for each patient. Further classifications included chronic (hyporesponsive in >4 consecutive months) and acute hyporesponsiveness (<3 consecutive months of hyporesponsiveness with at least one month not hyporesponsive before and after the hyporesponsive month(s)). Comorbidity was defined using Medicare Part A & B claims during May-Jun (antecedent) and separately, Aug-Dec (concurrent).

Results: 4.5%, and 5.2% of patients were classified as chronic, and acute, respectively. There were no differences by gender or race between patients with acute vs. chronic hyporesponsiveness. However, patients who were younger, with diabetes as cause of renal failure, lower BMI, and of shorter dialysis vintage had proportionally greater acute hyporesponsiveness. Most comorbidities, whether antecedent to the study period or concurrent, were more prevalent in patients who had episodes of acute versus chronic hyporesponsiveness. The largest differences between the two groups were for cerebrovascular accident/transient ischemic attack, ASHD, and infectious and all-cause hospitalizations.

Conclusions: Episodes of acute and chronic hyporesponsiveness are not uncommon, and early identification of patients at risk for such episodes may lead to improved anemia management in these patients. This impact of the bundled reimbursement system, implemented in Jan 2011, on the management of anemia in these patients is unknown, and should be assessed.

FR-PO1607

Hemoglobin (Hb) Stability during Pegasitane Versus Epoetin Treatment in Hemodialysis (HD) Patients

Robert Provenzano,1 Brigitte Schiller,1 Mark Kaplan,2 Bruce S. Spinowitz,2 Carol Francisco,2 Anne-Birgitte Duliege,1 Alex Yang,2 Krishna R. Polu,2 Martha Mayo,2 1AFX-01-12 and -14 Pegasitane Study Groups; 2Affymax, Inc., Palo Alto, CA.

Background: Hb variability is associated with an increased risk of mortality in HD patients (Pisani 2011). Limiting it is desirable and important for reimbursement metrics like the quality incentive program (QIP). Pegasitane (Hematie™) is a synthetic, PEGylated, investigational, peptide-based erythropoiesis-stimulating agent (ESA) that is designed to specifically stimulate the erythropoietin receptor.

Methods: Data were pooled from two phase 3, randomized, active-controlled, open-label studies assessing safety and efficacy of pegasitane (once monthly; N = 1066) compared with epoetin alfa/beta (epoetin; 1-3 times wkly; N = 542) in HD patients (EMERALD 1 and 2). Hb variability during the evaluation period (EP; wks 29-36) was measured using the standard deviation (SD) of Hb levels within patients and median of the absolute deviation (MAD) from the within-patient Hb level. An Hb analysis similar to the QIP determined the proportion of patients with average monthly Hb levels over 1 yr within the 10-12 g/dL target range (wks 29-90). A composite safety endpoint (CSE) was evaluated that consisted of 6 events: all causes of death, stroke, myocardial infarction, and serious adverse events (AEs) of congestive heart failure, unstable angina, and arhythmia.

Results: Hb variability was similar for the peginesatide and epoetin groups using the SD (median = 0.51 vs 0.48) and MAD (median = 0.44 for both) methods. In the QIP analysis, most patients in the peginesatide and epoetin groups had average monthly Hb levels within the target range (91% vs 95%). Fewer patients in the peginesatide than epoetin group had dose adjustments (~20% change from previous dose) during the EP (47% vs 68%). The frequency of CSE events during the studies was similar for both groups (23% vs 24%).

Conclusions: Hb stability and the proportion of patients within the target range were similar for peginesatide and epoetin; fewer patients in the peginesatide group had dose adjustments. Similar rates of cardiovascular AEs were reported for peginesatide and epoetin.

Funding: Pharmaceutical Company Support

FR-PO1608

Prescribing EPO to Chronic HD Patients: A Physiologic Approach Implemented with Continuous Quality Improvement Leads to Decreased EPO Dose Changes and Decreased Hemoglobin Variability

Jonathan Lorch,1 Viktor E. Pollak,2 1New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY; 2University of Colorado HSC, Denver, CO.

Background: Prescribing EPO has been influenced by widely used guidelines whose validity in practice has never been tested rigorously. Physicians have been urged to maintain Hb in the 100-120 g/L range, and to adjust EPO dosage to meet this target range. Allowing Hb to exceed 120 g/L has been thought to have an adverse effect on survival despite compelling contrary evidence.

Methods: Starting in February 2010, we tested an approach using patient-specific data collected during daily care and stored in a commercially available electronic medical record (EMR) in one dialysis unit (the protocol unit, 260 patients). Reploting iron sufficiency, ensuring iron sufficiency, and reducing EPO stepwise were key elements, while changing the protocol, with its EPO prescribing changes, was instituted in 2010.

Results: This was associated with an increase in Hb to 123.6 g/L and a low mortality (11.7 deaths per 100 patient years). Two other units (210, 186 patients respectively) served as comparators. In each unit, using data stored in the EMR, we counted and report in the Table new and discontinued orders for EPO and analyzed duration of treatment by HD year.


Protocol Unit 12.6 14.9 10.6 8.4 4.8 3.8 2.1

Comparator Unit 18.9 20.1 20.0 20.5 21.2 17.0 8.5

Comparative Unit 2 8.8 19.8 19.3 15.2 17.6 14.3 8.5

Conclusions: In the protocol unit, changes for new and discontinued EPO orders decreased by 78% and 87% per patient year respectively. In 2004-2009 there was an average of 15.8 EPO dose changes per year in the 3 units, a rate 4.2 and 7.5 times that in the protocol unit in the last two time periods. Also, the median coefficient of variation (CV) of individual patient Hb decreased 49% from 11.3% to 5.7% in the protocol unit. Hb CV was 49%, 30%, and 29% greater in the 3 units in 2004-2009 when KDOQI prescribing guidelines were in use.
FR-PO1609

Prescribing EPO to Chronic Maintenance HD Patients: A Physiologic Approach Implemented with Continuous Quality Improvement Leads to Decreased EPO Use and Increased Hemoglobin

Jonathan Lorch,1 Victor E. Pollak,2 Medicine, New York Presbyterian Hospital-Weill Medical College of Cornell University, New York, NY; Medicine, University of Colorado HSC, Denver, CO; 1

Background: EPO mitigates severity of anemia in chronic HD patients. Data from the USA, Australia/New Zealand, and 8 European countries found that the per country weekly EPO dose varied from 9,500 to >21,300 units, with no difference between dose and achieved Hb which ranged from 116 to 121 g/L (McFarlane PA, et al. Kidney Int 78:215, 2010). The therapeutic approach has been influenced by widely used guidelines whose validity in practice has never been tested rigorously.

Methods: With the objectives of achieving optimal Hb with minimum required EPO and maintaining stable Hb and EPO, we designed and tested an approach using patient-specific data collected during daily care and stored in a commercially available electronic medical record (EMR). Patient specific data collected over 9.5 years informed the study which was implemented in 250 HD patients in a single dialysis unit receiving EPO prior to 1 February 2010, and followed to April 30, 2011. Repeating iron insufficiency, ensuring iron sufficiency, and reducing EPO stepwise were key elements. Decision support tools were used that enabled relevant data display over prolonged periods in patient-centered reports. EPO dose, adjusted at 6-8 week intervals, was based on current clinical condition and past responses.

Results: In the study patients, IV iron administration and TSAT increased after protocol start. Hb increased by months 1-2; EPO decrease began from month 4 onward. By months 11-15, EPO had decreased 32% from 15,488 to 10,580 units/week while patient median Hb increased 8% from 114 to 123.6 g/L. Both Hb and EPO administration changed little from month 7 onward, and were stable in months 11-15. Comparable results were not observed in two comparator units that reduced EPO administration, but with neither a defined plan nor a CQI approach.

Conclusions: It is possible to reduce EPO administration and its cost while maintaining or improving patient Hb and maintaining a low patient mortality (11.7 deaths per 100 patient years).

FR-PO1610

Serum Ionized Calcium Levels Determine Arterial Stiffness in Dialysis with Regional Citrate Anticoagulation

Matthias B. Moor, Anja Kruse, Dominik E. Uehlinger, Ute Eisenberger. Nephrology and Hypertension, University Hospital Bern, Bern, Switzerland.

Background: Hemodynamic effects of changes in serum ionized calcium (iS Ca) are difficult to determine during conventional hemodialysis (HD) using a fixed dialysate concentration of calcium. The model of regional citrate anticoagulation (RCA) using continuous calcium infusion allows to study the effects of predefined iS Ca changes on arterial stiffness and blood pressure during HD.

Methods: In a cross-over study, 15 patients with chronic kidney failure underwent two HD sessions with RCA. Each session was divided into 2 study phases in which iS Ca was titrated either to 0.8-1.0 mmol/l or to 1.1-1.4 mmol/l. Sequence of phases was randomly chosen and alternated for the second session. 30 minutes after reaching a stable iS Ca level, pulse wave velocity (Pulse Trace PWV , Micro Medical Ltd, UK), arterial blood pressure and heart rate were measured. Statistical analysis was performed with SAS 9.2 for Windows on an X64_VSPro platform.

Results: iS Ca levels were modified during sequence 1 (iS Calow-high) from a predialysis baseline value of 1.15 ± 0.09 mmol/L, first to 0.92 ± 0.05 mmol/L (time point 1; *p<0.001 vs baseline) and then to 1.18 ± 0.05 mmol/L (time point 2; ns). During during sequence 2 (iSCahigh-low), iS Ca levels were modified from 1.15 ± 0.12 mmol/L first to 1.20 ± 0.05 mmol/L (time point 1; ns vs baseline) and then to 0.93 ± 0.03 (time point 2; *p < 0.001), see figure.

Conclusions: PWV, an indirect measure of arterial stiffness known to impact on long-term survival in chronic hemodialysis patients, is closely related to serum ionized calcium levels in HD patients using RCA as a study model.

Funding: Clinical Revenue Support

FR-PO1611

Interdialytic Ionized Calcium Is Associated with a Pro-Arrhythmic Phenotype in Incident Dialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study

Daniele Lam,1 Wen Hong Linda Kao,1 Stephen M. Sozio,1 Lucy A. Meoni,1 Tariq Shafi,1 Bernard G. Jaar,1 Julia J. Scialli,2 Larisa Tereshchenko,2 Rulan S. Parekh.1,2 University of Toronto; 1Johns Hopkins University.

Background: Patients on hemodialysis are at high risk of malignant arrhythmias and sudden cardiac death (SCD). We hypothesize that low levels of interdialytic ionized calcium (iCa) and magnesium (Mg) are associated with arrhythmias.

Methods: We investigated the cross-sectional association of prolonged QT interval, a pro-arrhythmic electrocardiogram (ECG) metric, with levels of Mg and iCa in an incident hemodialysis cohort of 114 PACE participants. At the baseline visit on a non-dialysis day, iCa corrected for pH and Mg levels were measured. A 5-minute signal-averaged ECG was recorded in a quiet room. Corrected QT (QTc) intervals were calculated and prolonged QTc was defined as >460 ms in women and >440 ms in men.

Results: In the 114 participants studied, mean age was 55-15 years. 54% were male, and 7% African American. 32% had self-reported coronary artery disease, 54% diabetes, 25% atrial fibrillation, and 22% congestive heart failure. Prevalence of a prolonged QTc was 58% in men and 46% in women. The mean iCa levels were 1.2±0.1 mmol/L and mean Mg levels were 1.8±0.2 meq/L. Linear regression showed that iCa has a significant effect on QTc (Figure).

Conclusions: Serum Mg levels were not associated (p=0.9). Additional traditional cardiovascular risk factors also did not modify the association.

Funding: NIDDK Support, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
FR-PO1612

Association of Cardiac Valve Calcification and C-Reactive Protein (CRP) with Cardiovascular and All-Cause Mortality in End-Stage Renal Disease Patients – 10-Year Follow-Up Study from Induction of Hemodialysis Therapy Workshop 101 Tsuyoshi Hori,1 Hirota Kei,2 Masumoto Masazumi,3 Keiko Kimura,4 Shoichi Maruyama,5 Enyu Imai,6 Seichii Matsuo.7 1Nephrology, Nagoya University, Nagoya, Japan; 2Nephrology, Nagoya Kyoritsu Hospital, Nagoya, Japan.

Background: Cardiac valve calcification is frequently seen in patients with end-stage renal disease (ESRD), and may potentially reflect systemic atherosclerosis. Serum CRP is also reported to predict future cardiovascular (CV) events. We investigated the association of cardiac valve calcification, valve calcification, and their joint role with prediction of mortality in patients on hemodialysis (HD).

Methods: A total of 1,290 consecutive patients who started HD therapy were screened by echocardiography. Patients were divided into 3 groups; those without valve calcification, those with calcification in one valve, and those with calcification in both valves. Cox proportional hazards analysis was performed adjusted for various cardiovascular risk factors.

Results: Serum CRP levels were 4.0±6.7, 6.8±6.9 and 7.2±9.8 mg/L in the NC, SC and BC group, respectively (p<0.001), and were independently associated to valve calcification (Odd’s ratio 1.13, 95% CI 1.06-1.21, p=0.0003). Adjusted hazard ratio (HR) of valve calcification was 2.64 (95%CI 1.53-4.56, p<0.0023) for group BC vs. NC for CV mortality and 1.86 (95%CI 1.30-2.67, p<0.0001) for group BC vs. NC for all-cause mortality, respectively. Similarly, adjusted HR of elevated CRP levels was 3.09 (95%CI 1.54-6.16, p<0.0001) for group BC vs. T1 for CV mortality and 2.44 (95%CI 1.64-3.64, p<0.0001) for T3 vs. T1 for all-cause mortality, respectively. In the joint setting of valve calcification and CRP, the risk of CV and all-cause mortality was 6.4-fold (p=0.0073) and 3.6-fold (p<0.0001) in the BC group with T3 of CRP compared with the NC group with T1 of CRP even after adjustment, respectively.

Conclusions: Cardiac valve calcification and elevated CRP levels were closely linked, and interactively increased risk of mortality in ESRD patients who started HD therapy.

FR-PO1613

Phosphate Plasma Level as Risk Factor for Cardiovascular Morbidity and Mortality and Association of Sevelamer with Outcomes in Hemodialysis Patients – Posthoc Analysis of the AURORA Trial Workshop 102 Bengt C. Fellstrom,1 Mattias Tejde,2 Hallvard Holdaas,3 Alan G. Jardine,4 Roland E. Schmieder,5 Eva K.A. Johnsson,6 Faiez Zannad.7 1Renal Unit, Uppsala, Sweden; 2Renal, Falun; 3AZ, Gbg; 4CIC, Nancy.

Background: A randomized trial in 2776 hemodialysis patients, studying the effect of rosuvastatin 10 mg on major CV events. There was no effect on any CV endpoint, but the database provides an opportunity to explore risk factors for CV events and mortality.

Methods: Baseline plasma phosphate as risk factor for CV disease (CVD) in HD patients was examined for CV endpoints used in the study, including mortality. Cox proportional hazards analysis was used for risk factor assessment, after adjustment for covariates and presented as hazard ratio (HR) per unit phosphate increase. Ranking of risk factors at baseline (age, diabetes, high-sensitivity C-reactive protein [hsCRP], albumin, phosphate, low-density lipoprotein cholesterol [LDL-C], previous CVD, smoking, medication, etc) was performed. Concomitant medication was analysed as well. Sevelamer was used in 508 patients (18%), and its association with outcome was analysed.

Results: Plasma phosphate at baseline was an independent and strong risk factor for Major CV events (HR=1.49; p<0.001; Rank 3), death from any cause (HR=1.34; p<0.0001; Rank 3), CV death (HR=1.58; p<0.0001; Rank 1), Atherosclerotic event (HR=1.46; p<0.0001; Rank 3), Major CV event or death (HR=1.35; p<0.0001; Rank 5), Nonfatal MI (HR=1.51; p<0.0013; Rank 4) but not for Non-CV death (HR=1.11; p=0.24; Rank 11). Sevelamer use at baseline was associated with reduced mortality (HR=0.84; p=0.029). Therefore, plasma phosphate was one of the strongest and highest ranking risk factors for CV events and mortality and the use of sevelamer was associated with a reduced mortality, basically because of influence on CV death.

Conclusions: Baseline plasma phosphate levels is one of the most important risk factor for CV events and mortality in hemodialysis patients in the AURORA trial. Sevelamer treatment seems to be associated with a better patient survival.

Funding: Pharmaceutical Company Support

FR-PO1614

The German Calciphylaxis (Calcific Uremic Arteriopathology) Registry Workshop 103 Vincent Brandenburg,1 Paula Specht,2 Jurgen Floege,3 Markus Ketteler,4 1Cardiology, University Hospital Aachen, Aachen, Germany; 2Nephrology, University Hospital Aachen, Aachen, Germany; 3Nephrology, Klinikum Coburg, Coburg, Germany.

Background: Calcific uremic arteriopathy (CUA, calciphylaxis) is a rare condition associated with high morbidity and mortality. CUA is clinically characterised by painful, ischemic, skin ulcerations. Pathomorphologically, media calcification of cutaneous arteries is the hallmark of the disease.

Methods: We established an internet-based registry (www.calciphylaxis.de) to allow online notification for all cases of established or suspected CUA. The registry is a comprehensive data base including various parameters (patient characteristics, laboratory data, clinical background and presentation as well as therapeutic strategies). Follow-up of the patients is planned by systematic queries of long-term outcome. We try to gain overview about current treatment strategies and link them to the clinical course.

Results: Altogether 127 CUA patients from 105 centers have been documented during 54 months: n = 76 (60 %) female; median age 69 yrs (range 21 - 89); 90% Caucasians; n = 99 (78%) diabetes patients; n = 57 (45%) diabetic patients.

Lab data from dialysis patients (median, interquartile-range): Serum total calcium: 2.23 (2.08 - 2.39) mmol/L; serum phosphorus: 1.7 (1.3 - 2.1) mmol/L; PTH 156 (61 - 357) pg/mL. Oral anticoagulation with coumadins was commomly (46%). Cutaneous lesions were localized in more than 80% at the lower extremities or gluteal region. Among the most frequently recorded therapeutic procedures were: surgical necrosectomy, intensifying dialysis modality, i.e. sodium-thiosulafat application, lowering dialysate and oral calcium burden, systemic antibiotics, and stopping coumadins.

Conclusions: CUA is associated with end-stage renal disease, female gender, diabetes and oral anticoagulation. PTH levels do not exceed current KDIGO target levels for ESRD in most cases. Decisions on therapeutic strategies vary significantly. The present internet based CUA registry is a valuable tool to collect data upon CUA cases and will serve as a basis for prospective studies.

Funding: Pharmaceutical Company Support

FR-PO1615

The Calcium Content of Hard Coronary Plaques Is a Predictor of Mortality in Maintenance Hemodialysis Patients Workshop 104 Antonio Bellasi,1 Enyu Imai,2 Hirotake Kasuga,2 Masashi Mizuno,1 Keiko Kimura,2 Yasuhiko Ito,1 Emiliana Ferramosca,3 Geoffrey A. Block,1 Paolo Raggi,4 1Azienda Ospedaliera S.Anna, Italy; 2Azienda Ospedaliera S.Osroala-Malpighi, Italy; 3Denver Nephrology; 4Emory University.

Background: The prognostic value of coronary artery calcification (CAC) has been documented in CKD patients. Nonetheless, several investigators maintained in the past that calcified plaques are stable and not prone to cause events.

Methods: Between 2004 and 2005, 130 individuals underwent cardiac CT imaging for quantification of CAC via the Agatston and the Volume score. Since the Agatston score is derived by multiplying the density and the volume of calcified lesions, the ratio of the Agatston and the Volume score (AVR) standardizes the overall calcium content per plaque volume unit. Patients were classified as high AVR (>1) or low (≤1) CAC. Survival analyses tested the association between AVR and all-cause mortality during a median follow-up of 5 years.

Results: Overall 63% of patients had a high AVR. The study cohort characteristics are summarized in the table.

<table>
<thead>
<tr>
<th>Age (SD)</th>
<th>Overall (n=130)</th>
<th>Low ratio (n=48)</th>
<th>High ratio (n=82)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>52</td>
<td>64</td>
<td>49</td>
<td>0.28</td>
</tr>
<tr>
<td>Dialysis Vintage (SD)</td>
<td>4.2±4.2</td>
<td>4.1±3.8</td>
<td>4.2±0.9</td>
<td>0.72</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>95</td>
<td>95</td>
<td>96</td>
<td>0.66</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>52</td>
<td>44</td>
<td>58</td>
<td>0.32</td>
</tr>
<tr>
<td>ASCVD (%)</td>
<td>58</td>
<td>28</td>
<td>48</td>
<td>0.11</td>
</tr>
<tr>
<td>Framingham % (risk)</td>
<td>31(11)</td>
<td>31(11)</td>
<td>44(11)</td>
<td>0.25</td>
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<tr>
<td>Congestive heart failure (%)</td>
<td>22</td>
<td>12</td>
<td>28</td>
<td>0.17</td>
</tr>
</tbody>
</table>

The mortality rate of patients with high AVR was higher than in those with low AVR calcification.

Funding: Pharmaceutical Company Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

488A
**Conclusions:** Increased AVR is an independent predictor of all-cause mortality in hemodialysis patients. The authors suggest that presence of calcium content in the coronary arteries is not in an index of stability but rather a harbinger of adverse future events.

**Funding:** Pharmaceutical Company Support

**FR-PO1616**

**Study on the Relationship of Serum 25-hydroxyvitamin D Levels with Vascular Calcification in Hemodialysis Patients**  Jae Hyung Chang, Seojong Kim, Hyun Hee Lee, Wookyung Chung, Ji Yong Jung. Department of Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea.

**Background:** Cardiovascular disease (CVD) is the main cause of mortality in chronic kidney disease patients. Vascular calcification is highly prevalent in this population and is an independent predictor of cardiovascular mortality. The role of vitamin D in this process remains controversial. The aim of this study was to determine the prevalence of vitamin D deficiency (25-hydroxyvitamin D [25D]=<15ng/ml), insufficiency (25D levels between 16 and 30 ng/ml), and moreover the relationship between vitamin D levels and vascular calcification in hemodialysis patients.

**Methods:** We performed a cross-sectional study with 289 hemodialysis patients. Patients were 56.9±14 years of age, 49.5% males, 46.4% diabetics and 34.9% with a history of CVD. Plain-X-ray images of lateral lumbar spine from all subjects were studied for calculation of semiquantitative vascular calcification scores as described by Kauppila.

**Results:** Only 3.1% of patients had adequate levels of 25D (>30 ng/mL), 10.7% of them had insufficient levels and 86.2% had deficient levels. Female gender and diabetes were associated with vitamin deficiency. We also found a high prevalence of vascular calcification in this population. Kauppila scores revealed 180 patients (62.3%) with vascular calcification. In univariate analysis, 25D levels were inversely related to vascular calcification (r=-0.107, P=0.004). However, after correction for confounding factors, this relation lost statistical significance. Multivariate analysis showed that sudden death was more likely to occur on Mondays and Tuesdays for HD patients receiving 3 or fewer dialysis sessions per week (n=9967), [Monday adjusted odds ratio (OR) 1.42 (95% CI 1.22-1.66), p<0.001]. Kaplan-Meier analysis showed a significant increase in composite CV events in patients with CAAC compared with those without CAAC (p=0.001, log-rank test). Univariate analysis using a Cox hazard model showed that age, smoking, common carotid artery intima-media thickness and CAAC were risk factors for composite CV events. In multivariate analysis, only CAAC was a significant risk factor for composite CV events (hazard ratio 2.85; 95% confidence interval, 1.18-8.00; p=0.02).

**Conclusions:** Vitamin D deficiency and insufficiency were highly prevalent in hemodialysis patients. However, low 25D levels could not be identified as an independent predictor of vascular calcification in these patients.

**FR-PO1617**

**Septaudian Variation in Cardiac Mortality in Haemodialysis (HD) but Not Peritoneal Dialysis (PD) Patients**  Rathika Krishnasamy,1,2 Carmel M. Hawley,1 Sunil S. Badve,1 E.R. Livingston,1 Stephen P. McDonald,1 Philip A. Clayton,1 Fiona Brown,1 Kevan R. Polkinghorne,1 Kyn M. Bannister,1 Neil Boudville,1 Kathryn J. Wiggins,1 David W. Johnson,1,2 Australia and New Zealand Dialysis and Transplant Registry, Adelaide, Australia; 1Department of Nephrology, University of Queensland at Princess Alexandra Hospital, Brisbane, Australia.

**Background:** Cardiovascular disease (CVD) represents the leading cause of death in dialysis patients. However, there is limited evidence that dialysis modality may also influence mortality related to CVD. The aim of this study was to evaluate the effects of dialysis modality and HD frequency on the septaudian pattern of cardiac and non-cardiac mortality in the Australian and New Zealand (ANZ) end-stage kidney failure (ESKF) cohorts, using data from the Australia and New Zealand Dialysis and Transplant (ANZDATA) Registry.

**Methods:** We analysed all adult ESKF patients (n=14594, HD n=10338, PD n=4256) receiving maintenance dialysis in ANZ who died between 1 January 1999 and 31 December 2008. The independent predictors of cardiac and non-cardiac death were determined by multivariable logistic regression.

**Results:** Cardiac deaths accounted for 40% of deaths. Cardiac death was significantly more likely to occur on Mondays and Tuesdays for HD patients receiving 3 or fewer dialysis sessions per week (n=9967), [Monday adjusted odds ratio (OR) 4.2 (95% CI 1.22-1.66), p<0.001]. This pattern of increased deaths on Mondays and Tuesdays was not seen among PD patients, HD patients receiving more than 3 sessions per week did not demonstrate the usual septaudian pattern of CV deaths on Mondays and Tuesdays. This data may provide insights into mechanisms of CV deaths in the dialysis cohort.

**FR-PO1619**

**Dramatic International Variation in Vascular Calcification Screening Practices: Results from the DOPPS** Bruce M. Robinson,1 Douglas S. Fuller,1 Brian Bieber,1 Christian Combe,1 Yun Li,2 David C. Mendelsohn,3 Francesca Tentori,1 Shinichi Fukuhara,1 1Arbor Res Collab for Hfh, Ann Arbor; 2Univ of Mi, Ann Arbor; 3CHU Bordeaux, France; 4Humber River Reg Hosp, Canada; 5Kyoto Univ, Japan.

**Background:** The 2009 KDIGO MBD guidelines indicate CKD stage 3-5D patients with vascular/valvular calcification (VC) are at highest cardiovascular (CV) risk, but do not recommend for or against VC screening. We describe facility VC screening practices internationally and associations with clinical outcomes.

**Methods:** Data were from 7,705 chronic in-center hemodialysis patients (HD) in 266 DOPPS 4 facilities (2010). Medical directors were asked if they typically screen for VC. Among 6 countries with >45% of units screening for VC, clinical practices and laboratory measures were compared with generalized estimating equations adjusted for demographics, comorbidity, and intra-facility correlation. Rates of CV hospitalizations and mortality were compared with adjusted Cox models.

**Results:** Routine VC screening was reported by >5% of US and UK units but was common (45-80%) in Japan and most European DOPPS countries. Somewhat lower Ca-based phosphate binder (51.5% vs 58.5%, p=0.16) and dialysate Ca (<3 mEq/L; 25.5% vs 36.7%, p=0.14) use was seen in VC screening units. No notable differences in vitamin D use or serum calcium, phosphorus, and PTH levels were seen. VC screening units had similar CV hospitalizations (hazard ratio [HR]=0.86; 95% CI=0.61-1.21) and all-cause mortality (HR=0.99; 95% CI=0.79, 1.25) as non-VC-screening units.

**Conclusions:** CAAC is an independent risk factor for CV events in ESRD patients. The assessment of CAAC at the initiation of hemodialysis is useful for predicting the prognosis.

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
Conclusions: Use of VC screening and preferred screening tests vary dramatically across the 256 NPSs in these countries. VC screening units may choose practices that limit calcium exposure to a greater extent than other units. Whether tailoring therapy based on VC screening limits VC progression or improves clinical outcomes needs additional study.

**Funding:** Pharmaceutical Company Support

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**FR-PO1620**

Association of Omega-3 Polysaturated Fatty Acids with Carotid Arteriosclerosis in Patients on Chronic Hemodialysis

Hirotake Kasuga,
Ryo Takahashi,
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Rei Okada,
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**Background:** Omega-3 polysaturated fatty acids (n-3 PUFA)s are widely recognized to have beneficial effects on cardiovascular disease. In hemodialysis (HD) patients, blood levels of n-3 PUFA's have been reported to be more suboptimal compared to general population. However, the association between n-3 PUFA levels and cardiovascular risk is little known in this population. We investigated the association of n-3 PUFA levels with carotid arteriosclerosis in HD patients.

**Methods:** Carotid ultrasound was performed in a total of 461 patients (male 67%, age 67±12 years, diabetes 46%) stably undergoing HD. Intima-media thickness (IMT) and plaque score (PS) in common carotid artery were measured. Carotid arteriosclerosis was defined as IMT >1.2mm and/or PS >5.0mm. The levels of n-6 PUFA's (dihomo-gamma-linoleic acid (DGLA) and arachidonic acid (AA)) and n-3 PUFA's (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) were also measured prior to carotid ultrasound.

**Results:** Carotid arteriosclerosis was seen in 94 patients (20.4%). Individual PUFA's were comparable between patients with and without carotid arteriosclerosis, however, the ratio of EPA / AA and the ratio of n-3 / n-6 PUFA's were significantly higher in patients with carotid arteriosclerosis than in those without (0.43:0.29 vs. 0.53:0.44, P = 0.027 and 0.94:0.43 vs. 1.07:0.55, P = 0.036, respectively). On multivariate logistic regression analysis, the ratio of EPA / AA [odds ratio (OR) 0.45, 95% confidential interval (CI) 0.22-0.93, P = 0.029] and the ratio of n-3 / n-6 PUFA's [OR 0.60, 95%CI 0.36-0.99, P = 0.046] were independently associated with carotid arteriosclerosis, respectively. Based on the cut-off level of 0.27 determined by ROC analysis (AUC = 0.63), the incidence rate of carotid arteriosclerosis was significantly higher in patients with low EPA / AA ratio than those with high EPA / AA ratio (27.9% vs. 17.5%, P = 0.012).

**Conclusions:** These data suggest that low levels of both EPA / AA ratio and n-3 / n-6 PUFA ratio were closely associated with incidence of carotid arteriosclerosis in patients on HD.

**FR-PO1621**

Long-Term Outcomes of Cardiac Risk Stratification Using Gated Single Photon Emission Computed Tomography (SPECT) in Asymptomatic End-Stage Renal Disease Patients at the Start of Dialysis

Jwa-Kyung Kim,
Ja-Ryong Koo,
Young Rim Song

**Background:** Screening for occult coronary artery disease (CAD) might permit early identification of subjects at increased risk of an adverse cardiac event. The aim of this study was to investigate the long-term effects of cardiac risk stratification using gated single photon emission computed tomography (SPECT) in ERSD patients at the start of dialysis.

**Methods:** This was an observational cohort study performed in Hallym University Hospital between January 2005 and April 2009. Baseline echocardiography were performed in all patients. For high risk patients who presented at least one cardiovascular risk factor, or decreased ejection fraction(<50%) or regional wall motion abnormality (RWMA) on echocardiography, SPECT was recommended. Cardiac events were defined as cardiac death and non-fatal acute coronary syndrome.

**Results:** Among 303 patients, 254 were high-risk patients. SPECT was performed in 143 of the patients and 66 showed reversible perfusion defects. During the mean follow-up of 59.0 months, overall cardiac event rate per person-year of follow-up was 6.8%, it was significantly higher in high-risk group compared to that of low-risk group (6.6 vs. 1.6%, HR:3.43, 95% CI 1.61-7.24). Multivariare Cox analysis showed that old age, diabetes and RWMA were independent predictors of adverse cardiac events in total patients. Among high-risk patients who underwent SPECT, summed score >9 (HR 2.60:95% CI 1.32-5.13) and reversible perfusion defect (HR3.37:95 CI 1.86-6.10) was additionally associated with the increased risk of cardiac event. The subgroup analysis in patients with reversible perfusion defects showed that intensive revascularization therapies decreased the risk of cardiac events by 50% compared to patients who treated only medically.

**Conclusions:** In conclusion, intensive cardiac work-up with SPECT may provide important prognostic information, particularly in high-risk dialysis patients.

**FR-PO1622**

Temporal Evolution of Systolic and Diastolic Blood Pressure in the Frequent Hemodialysis Network (FHN) Trials

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Amrit X. Garg,
Thomas A. Depner,
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Andreas Pieratore,
Brett Larive,
Gerald J. Beck,
Tom H. Greene,
Nathan W. Levin,
Alan S. Kliger

**Background:** As part of the FHN Trials we investigated the impact of 6x weekly in-center hemodialysis (HD; Daily Trial) and 6x weekly nocturnal home HD (Nocturnal Trial) over 12 months on systolic and diastolic blood pressure (SBP; DBP).

**Methods:** In the Daily Trial 245 patients (ps) were randomized to 6x or 3x weekly HD, in the Nocturnal Trial 87 pts were randomized to 6x weekly nocturnal HD or 3x weekly HD. Pre-HD SBP and DBP were measured at baseline and then monthly. Intradialytic weight loss (IWL) was used as a proxy of interdialytic weight gain.

**Results:** In the Daily Trial, compared to 3x weekly HD, 6x HD resulted in lower blood pressure at 1 month (SBP [mean±SE; mm Hg]: -5.9±1.8, P<0.001; DBP: -3.4±1.1, P<0.01) and at 12 months (SBP: -10.3±2.0; DBP: -5.5±1.2, all P<0.001). 6x HD led to a 0.4±0.2 kg (P=0.06) lower post-HD weight and a lower IWL of 1.0: 0.1 (P<0.001) at month 1; this difference was maintained through month 12.

In the Nocturnal Trial SBP and DBP did not differ between the 2 groups after 1 month; at 12 months both were lower in 6x HD pts (SBP: -8.3±3.3; DBP: -4.9±1.9, all P<0.05).

Post-HD weight did not differ throughout the trial. IWL at month 1 was similar in the 2 arms; by 12 months 6x HD pts had a lower IWL by 0.5±0.2 (P=0.01).

**Conclusions:** Compared to 3x weekly HD, 6x HD produced a comparable fall in SBP and DBP in both the Daily and Nocturnal Trials; the difference became evident earlier in the Daily Trial. This indicates that frequent HD reduces blood pressure whether HD is given during the day or in longer nocturnal sessions. Potential mechanisms may be reduction of extracellular volume, as evidenced by reduced post-HD weight in the Daily Trial, and reduced IWL in both trials.

**Funding:** NIDDK Support

**FR-PO1623**

Absolute Values of Systolic Blood Pressure (SBP) Do Not Predict Second Year Survival in Incident Hemodialysis Patients with Stable SBP

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Peter Kotanko,
John Rogus,
Eduardo K. Lacson,
Nathan W. Levin,
John Rogus

**Background:** The U-shaped relationship between systolic blood pressure (SBP) and mortality is recognized in incident and prevalent hemodialysis (HD) patients (Li 2006, Zager 1998). Incident HD patients whose pre-HD SBP increased or decreased by more than 5 mm Hg per month over their 1st year had greater mortality risk compared to those with “stable” SBP (Usyvat, WCN 2011; ASN 2011). This analysis investigates the influence of absolute values of SBP on second year survival among patients with stable SBP (i.e. lacking linear trend of increase or decrease).

**Methods:** Patients who started HD in between Jan 1, 2001 to Feb 28, 2010 with at least 13 HD treatments in their second year of HD were stratified into 4 groups according to the SBP during the first month in Year 1: ≤120, 120-150, 151-180 and above 180 mmHg. Changes were quantified as the slope of a linear regression of SBP values per patient in the first year. Patients selected for survival studies showed SBP between 0.5 and 0.5 mmHg change per month. Cox Regression was used to analyze the hazard ratio (HR) of change by SBP group.

**Results:** Of 10245 eligible, incident HD patients (57% male, 38% black, 54% white, 54% diabetic and 62.1±15.6 years old), 1385 patients with stable SBP in Year 1 were included. HR for mortality adjusted for age, gender, black race, BMI, cardioprotective drugs (CPD), diabetes and various other co-morbidities did not differ between the SBP groups. Only age (HR 1.02; P=0.001) and race (HR 1.83, P<0.05) remained significant predictors. Use of CPD showed a trend towards reduced survival (HR 0.7, P=0.10).

**Conclusions:** The risk of death did not vary in different SBP groups, supporting prior findings that implicate change in SBP (compared to absolute levels) as the more significant factor impacting mortality risk in chronic HD patients. It cautions against the current practice whereby treatment targets are derived from epidemiologic studies, specifically that absolute thresholds should not be used in isolation for the development of guidelines and treatment strategies, because they do not reflect SBP changes.

**FR-PO1624**

Hemodialysis-Induced Diastolic Dysfunction: It Is Not Only Volume!

Casper F. Franssen,
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Hans B. van Holderen,
Eduardo K. Lacson,
Wouter J. van Poucke,
John Rogus

**Background:** Left ventricular (LV) diastolic dysfunction is common in hemodialysis (HD) patients. However, acute changes in diastolic function have thus far been studied primarily in patients before and after HD but not during HD. We evaluated in detail changes in LV diastolic function in relation to volume parameters during a single HD session.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Methods: Hundred and nine adult HD patients with a mean (±SD) age of 62.5 ± 15.6 year and median dialysis vintage of 2 (1-4) years participated in this study. Echocardiographic examination was performed 4 times: before HD, 60 min and 180 min after the start of HD and 30 min after the end of HD. Diastolic function was evaluated using mitral early inflow (E) and tissue-Doppler derived early diastolic velocity (′e). The change in blood volume (BV) during HD was calculated from the changes in hematocrit. Results: Pre-HD median (IQR) E and mean ±SD e′ were 0.9 (0.8-1.1) m/s and 6.6 ± 2.1 cm/s, respectively. The figure describes the changes in LV diastolic function parameters during HD. E and e′ decreased significantly at 60 min of HD by 24.7% (13.5-34.3%) and 17.2% (6.1-25.0%), respectively (both p<0.001), whereas BV had decreased by only 1.3% (-3.4-1.7%) (p<0.05, compared with pre-HD). There was no correlation between the change in BV or ultrafiltration (UF) volume and changes in E and e′ at 60 min of HD, indicating that these changes were unrelated to changes in volume. At 180 min HDowards the change in E correlates significantly with BV and UF volume. Such a correlation was not seen for the change in e′.

Conclusions: Diastolic function acutely deteriorates early during HD. This is not related to changes in BV or UF volume. Therefore, other factors than hypovolemia seem to cause an early impairment in diastolic function.

Funding: Government Support - Non-U.S.

FR-PO1625

Predictors of Blood Pressure Variability Change over Time in Incident Hemodialysis Patients Tariq Shafr,1 2 Stephen M. Sozio,1 2 Jing Zhou,1 2 Courtney Cook,1 2 Karen J. Bandeen-Roche,1 2 L. Ebony Boulware,1 2 Johns Hopkins University; 2 DECIDE Network Patient Outcomes in End Stage Renal Disease Study Investigators.

Background: Blood pressure variability (BPV) increases the risk of death in hemodialysis (HD) patients but the factors associated with BPV are not well described.

Methods: We assessed factors influencing within-patient variability in predialysis systolic BP (SBP) among 25,031 incident HD patients treated at Dialysis Clinic, Inc. (DCI). We assessed comorbidities using a previously validated index and fluid removal using change during weight dialysis. We examined BPV in 3-month windows over the first year of HD using the residual-intercept ratio obtained from mixed-effects linear regression models estimating changes in SBP over time. This ratio reflects each individual’s BPV over time with positive values reflecting greater BPV.

Results: Patients’ mean age was 62 years; 35% were black and 44% were female. Patients who were older, female, black and had more comorbidity had consistently higher BPV during the first 6 months of dialysis. Greater fluid removal was associated with lower BPV in the first 6 months and calcium-phosphate product was associated with higher BPV after 6 months.

Predictors of Systolic BP Variability in Incident Hemodialysis Patients

<table>
<thead>
<tr>
<th>Predictor</th>
<th>0-6 months</th>
<th>6-12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (Ref:18-22)</td>
<td>148±27</td>
<td>149±26</td>
</tr>
<tr>
<td>ECFV, per 10% dry weight increase</td>
<td>4.9±2</td>
<td>4.1±2</td>
</tr>
<tr>
<td>Black vs. White</td>
<td>4.4±2</td>
<td>4.4±2</td>
</tr>
<tr>
<td>Female vs. Male</td>
<td>3.6±2</td>
<td>3.5±2</td>
</tr>
<tr>
<td>Albumin, per 1 mg/dL increase</td>
<td>-4.4±2</td>
<td>-3.3±2</td>
</tr>
<tr>
<td>S. CaXPhos, per 100 mg/dL increase</td>
<td>0.1±1</td>
<td>0.1±1</td>
</tr>
</tbody>
</table>

Coefficients represent individual BPV in a 3-month period. Positive values represent higher BPV variability. Coefficients are scaled by 10^-1. *p<0.05; †p<0.01; ‡p<0.001

Conclusions: In the first year of HD, factors influencing within-patient BP variability can change significantly over time. Studies are needed to better understand these dynamic changes and their influence on clinical outcomes in HD patients.

Funding: NIDDK Support

FR-PO1626

Randomized Cross-Over Study of Daily Versus Conventional Hemodialysis To Explore Mechanisms of Blood Pressure Improvement Deborah Lynn Zimmerman, Kevin D. Burns, Marcel Ruzicka. Medicine, University of Ottawa, ON, Canada.

Background: Hypertension (HTN) is poorly controlled in many end stage renal disease (ESRD) patients treated with conventional hemodialysis (CHD). Our study had 2 objectives: 1) To determine if short daily hemodialysis (SHD) is associated with improved systolic blood pressure (SBP) compared to CHD in hypertensive ESRD patients, and 2) To explore the potential mechanisms of BP improvement.

Methods: Randomized cross-over study of prevalent HD patients with a history of HTN (pre-dialysis SBP >140 mmHg) or CHD. After informed consent, patients underwent a 3 month run-in phase in which the diuretic Na was reduced to 136 mmol, dry weight and antihypertensives were optimized to achieve a pre-dialysis SBP of <140 mmHg. At the end of the run in phase, patients were then randomized to a further 3 months of CHD or SHD and then crossed over to the other treatment arm. SBP, ECFV via bioimpedance and catecholamines were measured after each phase, and intensity of antihypertensive therapy was estimated.

Results: 22 patients consented to participate in the study; 3 patient withdrew prior to randomization, 2 patients did not complete the SHD arm (included in the intent to treat analysis). There was a statistically significant decrease in SBP from study entry to the end of the run in phase (151 vs 138 mmHg, p<0.004) without a change in dry weight (77.4 ± 7.1 kgs, p=0.63) or intensity of antihypertensive medications (p=0.57). There was no difference in SBP between CHD and SHD (142 vs 139 mmHg, p=0.39) although more medication was required to control SBP in SHD (5.0 vs 3.7, p=0.02). These differences were not explained by plasma catecholamine levels (norepinephrine, p=0.33; epinephrine, p=0.48), ECFV (p=0.77) or changes in dry weight (p=0.70).

Conclusions: A protocol-based approach to HTN management is associated with a significant reduction in SBP in ESRD patients treated with HD. Once target SBP has been achieved, both CHD and SHD are associated with maintenance of SBP but more antihypertensive medications are required in CHD. The mechanism(s) by which SHD improves SBP such that the number of medications can be reduced remains unclear.

Funding: Private Foundation Support

FR-PO1627

Incidence, Prevalence and the Risks of Atrial Fibrillation in Dialysis Patients Deborah Lynn Zimmerman,1 Manish M. Sood,2 Rachel M. Hollen,3 Swapnil Hiremath,3 Claudio Rigatto,2 Catherine M. Clase,4 1 Medicine, University of Ottawa, ON, Canada; 2 Medicine, University of Manitoba, Winnipeg, MB, Canada; 3 Medicine, Queen’s University, Kingston, ON, Canada; 4 Medicine, McMaster University, Hamilton, ON, Canada.

Background: ESRD patients appear to be at high risk for atrial fibrillation (AF) but the risks and benefits of anticoagulation for stroke prevention remain unclear. We undertook a systematic review to clarify the risks of mortality and stroke in patients with ESRD and AF.

Methods: A literature search using Medline and Embase from 1990 to November 2010 was conducted that included ESRD patients treated with dialysis. Studies described incidence or prevalence and/or complications of AF with or without anticoagulation. Abstracts were reviewed and data abstracted by two investigators with conflicts resolved by a third investigator. Observational study quality was judged using the NewCastle Ottawa Scale. Event rates were calculated in patient-years and were combined using a random effects model.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

491A
Results: The average Ottawa New Castle Score was 6.1 (range 3-9) in the 21 studies that reported eligibility criteria. The majority of patients were male (50%) with an mean age of 61 years. The overall prevalence of AF was 12.3% (range 4.5%-27%) and the overall incidence was 2.4/100 patient-years (range 0.97-5.9 events/100 patient-years). The risk of mortality was increased in ESRD patients with AF compared to ESRD patients without AF, 21.6/100 patient-years and 14.2/100 patient-years respectively. The risk of mortality was increased in patients with AF at 5.2/100 patient-years compared with 2.5/100 patient-years. The effects of anticoagulation on reducing the risk of stroke were heterogeneous.

Conclusions: The incidence and prevalence of AF in ESRD patients is high and is associated with increased mortality. Study variability was observed in design, population characteristics and method of AF documentation. Given the limitations of the study designs and the equipoise about the risks and benefits of anticoagulation in patients with ESRD, a randomized controlled trial is required to clarify the optimal anticoagulation strategy in the ESRD population.

Funding: Clinical Revenue Support

FR-PO1628
Determinants of Prolonged QTc Interval over 5 Years in Patients Undergoing Regular Hemodialysis
Shinya Okayama,1 Akihiko Kato,1 Fumio Takayama,1 Hisanori Azukura,1 Narikazu Iijima,1 Akira Shimomura,1 Sanaru Sun Clinic, Hamamatsu, Shizuoka, Japan; 2Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

Background: Prolongation of the corrected QT interval (QTc) on the surface electrocardiogram (ECG) is a predictor of total death and cardiac event in general population and patients with end-stage kidney disease. QTc prolongation is often observed in patients on chronic hemodialysis (HD), while it remains to be determined which factors are more associated with QTc prolongation over time.

Methods: In this study, we longitudinally measured QTc interval over 5 years, and examined the determinants of progression of QTc prolongation in stable 76 HD patients who had not taken any medication to prolong QT c interval (age: 73±12 [35-89] years, time on HD: 148±66 [63-387] months, male/female: 41/35, diabetes: n=12).

Results: Basal QTc was significantly and inversely correlated with albumin-corrected serum calcium (r= -0.36, p=0.01) and creatinine (r= -0.28, p=0.01). A longer QTc was also observed in female HD patients. During the 5-year observation, mean QTc was significantly increased from 421±18 to 428±21ms (p=0.01). The prevalence of borderline/abnormal QTc was increased from 18.4 to 36.8% (p=0.01). There was a significant and positive relationship between absolute changes in QTc and HD period (r=0.25, p=0.03). Older patients (≥65 years, n=34) disclosed a significant increase in QTc from 422±18 to 433±22ms during the follow-up (p=0.01). QTc interval was also prolonged more markedly in patients with serum albumin lower than 4.0 g/dL (420±20 vs. 434±22ms, p=0.05) (n=32) than those without (421±11 vs. 425±21ms). In contrast, diabetes and other co-morbid factors did not affect QTc prolongation.

Conclusions: These findings suggest that HD therapy increases QTc interval over time. In addition, prolongation of QTc is associated with ageing and hypoalbuminemia in stable patients on long-term HD.

Funding: Pharmaceutical Company Support

FR-PO1629
Pulmonary Hypertension the Predictor of Mortality in HD and PD Patients: A Prospective Chinese Study
Lingmei Cen, Xuemei Li, Xiao Hong Fan, Hong Xu, Jianling Tao, Xuewang Li. Department of Nephrology, Peking Union Medical College Hospital, Beijing, China.

Background: Pulmonary hypertension (PH), a disease which carries substantial morbidity and mortality has been reported. No prospective evaluation of the prevalence or clinical significance of PH in ESRD patients in China has been undertaken. The objective of this study was to evaluate the impact of PHT and mortality among ESRD patients receiving chronic HD and PD therapy.

Methods: Echocardiograms were performed prospectively in chronic PD (n=73) and HD (n=89) patients at a single dialysis center. The patient’s general clinical data were collected. The arterial stiffness was prospectively estimated by measuring the Ankle-Brachial Index and brachial-ankle pulse wave velocity (baPWV). ABI and baPWV were remeasured, clinical outcome (death) were recovered at 36months.

Results: In these cohort patients, 44.9% of patients receiving HD, and in 34.2% of the patients receiving PD met the definition of PH (sPAP ≥ 35 mm Hg). Of those 30.3% HD patients and 16.4% HD patients met the definition of more severe PH (sPAP=45 mm Hg). At 36 months, mortality was significantly higher in patients with PH (26.2%) compared with patients without PH (8.2%, p=0.002). And the PH was the independent risk factor of mortality in HD patients. Echocardiographic findings showed that the PH may be secondary to diastolic dysfunction and compounded by volume overload.

Conclusions: This prospective study of a single dialysis center suggests that PH may be associated with increased mortality. The Echocardiographic findings suggested that the PH may be secondary to diastolic dysfunction and compounded by volume overload.

Funding: Government Support - Non-U.S.

FR-PO1630
Echocardiographic Determinants of an Abnormal Spatial QRS-T Angle in Dialysis Patients
Mihaly K. De Bie,1 Nina Ajmone Marsan,1 Arien Gaasbeck,2 Victoria Delgado,1 Ton J. Rabelink,2 Jeroen J. Bax,1 Martin J. Schalij,1 J. Wouter Jukema,2 Cardiology, Leiden University Medical Center, Leiden, Netherlands; 2Nephrology, Leiden University Medical Center, Leiden, Netherlands.

Background: The spatial QRS-T angle, the angle between the mean QRS- and T-vector, describes the relation between ventricular depolarization and repolarization. Having a wide (abnormal) angle is considered a predictor of arrhythmic events in various patient groups, including dialysis patients. Given the high incidence of sudden cardiac death in dialysis patients, this parameter is of particular interest in this patient group. The objective of this study is to assess the association of (modifiable) echocardiographic parameters and an abnormal spatial QRS-T angle in dialysis patients.

Methods: A total of 93 consecutive dialysis patients (67.5 ± 7.3 yrs, 76% male) were included. In all patients a 12-lead electrocardiogram, a 2-dimensional echocardiogram and routine blood samples were obtained. Using a previously validated computer algorithm, the spatial QRS-T angle was then calculated from the 12-lead ECG. An abnormal spatial QRS-T angle was defined as ≥ 130° in males and ≥ 116° in females.

Results: An abnormal spatial QRS-T angle was present in 27 (29%) patients. Patients with an abnormal spatial angle had a higher serum phosphate (1.65 ± 0.43 mmol/L vs. 1.45 ± 0.32 mmol/L, p = 0.027). Furthermore, these patients had a lower left ventricular ejection fraction (LVEF) of 47 ± 7% vs. 55 ± 6% (p < 0.001) and had higher LV dysynchrony as measured by tissue Doppler imaging, with a septal to lateral (S-L) delay of peak systolic velocity of 70 ± 42 ms vs. 42 ± 38 ms (p =0.001) respectively. Multivariate logistic regression analysis showed that only for possible confounders demonstrated that LVEF (OR 0.82, 95% CI 0.73-0.92, p=0.001) and S-L delay (OR 6.5, 95% CI 1.53 – 27.8, p=0.001) were independent determinants of an abnormal spatial QRS-T angle in this patient group.

Conclusions: Left ventricular ejection fraction and dysynchrony are echocardiographic determinants of an abnormal spatial QRS-T angle in dialysis patients and might therefore represent a potential target for the prevention of sudden cardiac death in these patients.

Funding: Clinical Revenue Support
Methods: We studied 122 patients, recruited between 2005 and 2010 in a single HD center. The age at median time of 73.1 ± 13.3 years (38.5-89.2), HD duration 3.0 years (0.0-37.9), 64.8% male. 37.7% of patients had ischemic cardiac disease, 42.6% dilated cardiomyopathy, 84.4% were hypertensive and 27.1% were diabetic. Ejection fraction (EF) was 60.0% (22.72) and cardiac mass index (CMI) was 147.3 gr/m² (54.0-311.2). By ECG Holter and a dedicated algorithm 24h QT length corrected for heart rate (QTC) was calculated. QTC length was considered prolonged when longer than 450 msec in men and 460 msec in women.

Results: 43 patients out of 122 (35.3%) had a prolonged QTc. Female gender (p<0.001), dilated cardiomyopathy (p=0.004) and amiodarone therapy (p=0.037) were significantly associated to a QTc prolongation, while EF' (p=0.033) was inversely correlated to the length of ventricular repolarization. Up today 37 deaths (10 sudden death) were observed. After stratification for age, QTc prolongation (p<0.001, HR=1.27 for 10 msec of increment) and the presence of dilated cardiomyopathy (p=0.052, HR=2.15) were independent predictors of mortality, while beta-blockers therapy was weakly protective (p=0.10, HR=0.53). Sudden death was associated to a prolonged QTc (p=0.010, HR=1.40 per 10 msc of increment), digoxin therapy (p=0.014, HR=2.24) and a greater CMI (p=0.037, HR=1.19 per 10 gr/m² of increment).

Conclusions: In a population of HD patients, a prolongation of QTc interval is an independent predictor of total and sudden mortality

FR-PO1633
Prevalent and Incident Rates of Asymptomatic Electrocardiographic Abnormalities in Hemodialysis Patients | Darren Green, Paul Dunne, David I. New, Philip A. Kalra.

Background: Dialysis patients have a high rate of cardiovascular disease. Dialysis is itself associated with arrhythmia and myocardial ischemia. Routine ECG would be of potential benefit in detecting early signs of new cardiovascular changes as it is low cost, non-invasive, and repeatable.

Methods: We performed a pilot study of incidental ECG abnormalities in a cross-section of dialysis patients from one centre. Patients were selected who had an elective ECG as part of transplant, pre-operative, or routine out-patient cardiology assessment. In patients with 2 such ECGs, comparison was made between tracings. Patients were excluded who had suffered acute troponin rises between ECGs, so as to assess changes that occur independent of distinct acute cardiac events.

Results: 176 patients were included in the cross-sectional study. The mean age was 68 years, with mean time on dialysis 4.7 months. 29% of patients had 1st degree heart block, 12% had prolonged QRS (>100ms), 28% Sokolow-Lyon indexed LVH, 26% T-wave inversion, and 3% atrial fibrillation. 89 patients had follow up assessment with mean time between ECGs 18 months. New onset QRS ischemic changes in the absence of acute coronary events occurred at a rate of 50 per 1000 patient years. The rate of new T-wave abnormalities was 48 per 1000 patient years. QTc became prolonged only in female patients (female baseline mean 431ms vs. follow up 453ms p=0.000, male baseline 434ms vs. follow up 438ms p=0.486). The rates of new conduction defects were 50 and 36 per 1000 patient years for atrial fibrillation and 1st degree heart block, respectively.

Conclusions: The prevalent rate of conduction defects and rate of new onset changes was high. This may give insight into the source of the high rate of arrhythmic death suffered by dialysis patients. The rate of onset of new ischemic changes fits with previous reports of dialysis being associated with myocardial injury. This pilot study supports the need for further work to determine whether routine ECG can improve cardiovascular risk stratification for these patients.

FR-PO1634
Pre-Dialysis Factors and the Timing of Dialysis Initiation among Older Adults | Deidra C. Crews,1 Julia J. Siculla,1 Haifeng Guo,2 Hyamong Liu,2 Bernard G. Jaar,1,2 E. Ebony Boulware,1,2

Background: In recent years patients have been initiating dialysis at increasingly higher estimated glomerular filtration rates (eGFR), despite a lack of evidence of benefit and suggestions of harm in some studies. We examined pre-dialysis factors that might influence earlier initiation of dialysis by nephrologists treating older adults.

Methods: Using USRDS data, we identified patients initiating dialysis at age 67+ years from 2006-2008, with 2 years Medicare coverage prior to initiation, and at least one outpatient nephropathy visit in the 6 months preceding initiation. Medicare claims and the Medical Evidence form were used to ascertain comorbidities, and 6 months of Medicare claims were reviewed for the frequency of nephropathy visits and number of congestive heart failure (CHF) admissions immediately preceding dialysis initiation. Logistic regression assessed the relationship between pre-diagnosis patient characteristics (comorbidities and health care utilization patterns) and early (eGFR ≥10 ml/min/1.73 m²) versus late (<10) dialysis initiation. We controlled for demographics, ESRD cause, albumin, hemoglobin, and erythropoiesis stimulating agents and/or intravenous iron use.

Results: Among 70,662 patients, median age was 77 [interquartile range (IQR) 72-82] years and median eGFR at dialysis initiation was 11.0 (IQR 8.2-14.4) ml/min/1.73m². Early initiators comprised 58%. Greater burden of comorbidities, frequent nephropathy visits and CHF admissions were associated with early dialysis initiation.

FR-PO1635
Prospective Study on Clinical Effects of Dialysis in Treatment-Resistant Congestive Heart Failure | Frank Van de Sande,1 Trijntje T. Cnossen,1 Jeroen Meuwese,1,2 Abderrazak Laaroussi,1 Eberhard Visschers,1

Background: Dialysis patients have a high rate of cardiovascular disease. Dialysis is itself associated with arrhythmia and myocardial ischemia. Routine ECG would be of potential benefit in detecting early signs of new cardiovascular changes as it is low cost, non-invasive, and repeatable.

Methods: We performed a pilot study of incidental ECG abnormalities in a cross-section of dialysis patients from one centre. Patients were selected who had an elective ECG as part of transplant, pre-operative, or routine out-patient cardiology assessment. In patients with 2 such ECGs, comparison was made between tracings. Patients were excluded who had suffered acute troponin rises between ECGs, so as to assess changes that occur independent of distinct acute cardiac events.

Results: 176 patients were included in the cross-sectional study. The mean age was 68 years, with mean time on dialysis 4.7 months. 29% of patients had 1st degree heart block, 12% had prolonged QRS (>100ms), 28% Sokolow-Lyon indexed LVH, 26% T-wave inversion, and 3% atrial fibrillation. 89 patients had follow up assessment with mean time between ECGs 18 months. New onset QRS ischemic changes in the absence of acute coronary events occurred at a rate of 50 per 1000 patient years. The rate of new T-wave abnormalities was 48 per 1000 patient years. QTc became prolonged only in female patients (female baseline mean 431ms vs. follow up 453ms p=0.000, male baseline 434ms vs. follow up 438ms p=0.486). The rates of new conduction defects were 50 and 36 per 1000 patient years for atrial fibrillation and 1st degree heart block, respectively.

Conclusions: The prevalent rate of conduction defects and rate of new onset changes was high. This may give insight into the source of the high rate of arrhythmic death suffered by dialysis patients. The rate of onset of new ischemic changes fits with previous reports of dialysis being associated with myocardial injury. This pilot study supports the need for further work to determine whether routine ECG can improve cardiovascular risk stratification for these patients.

FR-PO1636
Impact of Baseline Levels and Trimestral Variation of Triiodothyronine and Thyroxine on Mortality in Maintenance Hemodialysis Patients | Christian I. Meuwese,1 Friedo W. Dekker,2 Bengt Lindholm,1 Abdul Rashid Tony Qureshi,1 Olof Heimberger,1 Peter F. Barany,1 Peter Stenwinkel,1 Juan J. Carrero,1 Renal Medicine and Baxter Novum, Karolinska Institutet, Sweden; 2Clinical Epidemiology, Leiden University Medical Center, Netherlands.

Background: Conflicting evidence exists with regards to the association of thyroid hormone levels and mortality risk in patients with end-stage renal disease (ESRD), which is limited to studies comprising single thyroid-hormone measurements. This study assesses the impact of basal and trimestral variation of thyroid stimulating hormone (TSH), triiodothyronine (TT) and thyroxine (T4) on cause-specific mortality in dialysis patients. Methods: In 210 prevalent hemodialysis patients serum T3, T4, TSH and Interleukin-6 were measured three months apart. Cardiovascular and non-cardiovascular deaths were registered during follow-up. Based on fluctuations along tertiles of distribution, four trimestral patterns were defined for each thyroid hormone: persistently low, decrease, increase and persistently high. By means of Kaplan Meier survival, Cox proportional hazard models, the impact of baseline levels and trimestral variation on mortality was investigated.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Results: TSH levels did not associate with mortality. At baseline, patients with low T3 (≤50th percentile) had higher hazards of dying than patients with high levels. Longitudinally, patients with persistently low levels of T3 or T4 during the 3-month observational period had higher mortality hazards than those having persistently high levels. These associations were mainly attributable to cardiovascular-related mortality. The association between T4 and mortality was not altered after adjustment for T3.

Conclusions: Hemodialysis patients with reduced T3 or T4 levels bear an increased mortality risk, especially due to cardiovascular causes. This was true when considering both baseline measurements and trimestral variation patterns. This longitudinal design allows us to observe causal evidence although non-decisive - that the link may underlie a causal effect. Thus, this study supports the hypothesis that restoration of thyroid hormone alterations in ESRD may improve patient’s outcome. Funding: Pharmaceutical Company Support, Private Foundation Support, Government Support - Non-U.S.

FR-PO1637
Biofeedback Dialysis for Intradialytic Hypotension and Extracellular Volume Excess: A Systematic Review and Meta-Analysis
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Background: Intradialytic hypotension (IDH) is associated with morbidity and mortality. Biofeedback (BF) devices which automate ultrafiltration and conductivity in response to changes in blood volume may reduce IDH and related complications. We conducted a systematic review to assess the benefits and harms of BF dialysis.

Methods: We adhered to a pre-specified protocol (PROSPERO ID: CRD4201101133). Data sources included CENTRAL (Issue 1,2011), MEDLINE (1966-2011), EMBASE (1980-2011), and ISI Web of Science (1976-2011). We included randomized parallel arm and crossover trials that randomized adults (<18 years) with symptomatic IDH or extracellular fluid volume expansion to receive HD with a BF device or usual care. All patients received 3 times weekly HD. Two authors assessed trial quality and independently extracted data in duplicate. We used a random effects model and expressed results as a risk ratio (RR) for dichotomous outcomes or mean difference (MD) for continuous data. We measured heterogeneity using the I² statistic.

Results: Seven studies met inclusion criteria. Two were parallel RCT's and 5 were cross-over studies. All studies were open-label. Studies generally included (median N = 20), and not powered to assess survival or hospitalization. BF devices significantly reduced IDH (RR 0.61, 95% CI 0.42 to 0.86; I²=0%), but not predialysis systolic BP (MD 3.9, 95% CI -2.8 to 8 mmHg; I²=49%). Quality of life (QoL) data was reported in two studies, but the results could not be pooled; neither study demonstrated improved QoL. Potential harms were not assessed in any study.

Conclusions: BF dialysis reduces the frequency of IDH. Whether BF devices improve survival, hospitalization or QoL requires further study in adequately powered randomized trials.

FR-PO1638
Role of Statins on C-Reactive Protein and the Kinetics of Erythrocyte Sodium Lithium Countertransport in Hemodialysis Patients
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Background: Cardiovascular disease as a result of accelerated atherogenesis is common in hemodialysis patients. Dyslipidemia may be a major contributor in this process and can be influenced by statins. Statins may exhibit additional inhibitory effects on the atherogenesis by improving the function of the sodium lithium countertransport (Na,LiCT). The activity of Na,LiCT is a sensitive membrane protein and has been reported to be abnormal (low Km) in hemodialysis patients. The activity of Na,LiCT has a positive correlation with the incidence of cardiovascular risk factors.

Methods: Twenty hemodialysed patients were divided into 2 groups. The study group (n=10) received Na,LiCT inhibitor (10 mg daily) for 4 months and the placebo group (n=10) has received placebo tablets for the same period as in the study group. We have measured serum CRP level, ESR, lipid profiles and the kinetics of Na,LiCT in all 20 patients before and after taking simvastatin.

After 4 months, the CRP level was decreased in the study group more than in the placebo group (18.6% reduction in the study group vs. 1.0% reduction in the placebo group). Before the treatment period, the Km for external sodium of erythrocyte of Na,LiCT was lower than that of normal controls in both groups of patients (56.3 ± 76.0 in group 1, P <0.01 and 59.2 ± 76.0 in group 2, P >0.05). After 4 months of simvastatin, the Km for external sodium was significantly improved (56.3 ± 69.5; P<0.01).

Conclusions: These results show that simvastatin exhibit comparable favorable effects on lipid profiles and the reduction of CRP level in hemodialysis patients. Moreover, the improvement of the kinetics of erythrocyte Na,LiCT as shown in this ESRD population, may indicate that these statins exhibit favorable effects on oxidative stress. Funding: Government Support - Non-U.S.

FR-PO1639
Predictors of Anti hypertensive Medication Exposure over Time for Dually Eligible Diabetes Patients
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Background: Renin angiotensin system antagonists (RASAs), beta-blockers (β-blockers), and calcium channel blockers (CCBs) are widely prescribed for their antihypertensive and cardioprotective benefits in patients on chronic dialysis, yet we do not know about chronic dialysis patients’ degree of exposure to these classes while on dialysis.

Methods: We examined exposure patterns for a retrospective cohort (2000-2005) to determine factors associated with varying levels of use. We created a Medicare-Medicaid eligible cohort of new dialysis patients and tracked their medication exposure until death, transplant, or end of observation. The proportion of days covered (PDC), adjusted for institutional stays, was computed for each drug class from Medicaid drug claims and UB04 core data. PDC was computed across the entire window of observation for each cohort member without regard to when treatment began, reflecting medication exposure rather than adherence.

Results: Of 45,127 subjects in the cohort, 61.6% used a RASA, 64.2% a CCB, and 33% a β-blocker. Among users in each class, PDCs were highest for CCBs (mean 0.76, SD 0.31) followed by RASAs (mean 0.52, SD 0.31) and β-blockers (mean 0.46, SD 0.32). Advancing age was associated with higher PDCs for all classes, and Caucasians had higher PDCs than other racial groups (p < 0.0001). Diabetes, hypertension, and CVA were associated with higher RASA PDC (p < 0.0001), β-blocker PDCs were higher in the presence of HF or CAD (p < 0.01). The absence of HF and CAD was associated with higher CCB PDCs (p < 0.01). The presence of hypertension and CVA was associated with higher CCB PDC (p < 0.0001).

Conclusions: Despite substantial cardiac comorbidity, just under 2/3 used a CCB or a RASA and 1/3 used a β-blocker. Their respective PDCs indicate that use was limited to approximately 50% of dialysis tenure time. While exposure levels were somewhat consistent with clinical indications, there was less use among non-Caucasians, a finding that deserves further investigation.

Funding: NIDDK Support

FR-PO1640
Predicting Mortality in Incident Hemodialysis Patients: Validation of a European Model in an International Cohort
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Background: The risk of death varies among patients on hemodialysis. We previously developed and internally validated a model to predict mortality in incident patients from the UK Renal Registry (UKRR). The model included a number of routinely collected variables and achieved adequate performance. Here, we present the external validation of the prediction model in a large international cohort of incident patients from the Dialysis Outcomes and Practice Patterns Study (DOPPS).

Methods: We included all patients initiating hemodialysis treatment in 2002-2004 (DOPPS), who survived the first 90 days on treatment. The variables in the prediction model comprised age, gender, race, primary renal disease; diabetes, CVD, smoking, albumin, hemoglobin, calcium, and creatinine. Discrimination was evaluated with a time-dependent c-statistic. Calibration was evaluated using the Nam and D’Agostino chi square statistic.

Results: The DOPPS dataset consisted of 3612 patients, of whom 355 died. The prediction model achieved adequate discrimination (c-statistic 0.74) and good calibration (observed vs. predicted risk, p-value 0.24) across different subpopulations.

Conclusions: Basic patient characteristics and laboratory variables are sufficient to accurately predict one-year mortality in patients incepting hemodialysis. Our model, developed in the UKRR, is now externally validated in an international cohort. Further research is needed to establish an easy-to-use clinical score that may provide useful information at the bedside.
**FR-PO1641**  
Factors Predicting Mortality of New Patients Commencing Dialysis Therapy after 5 and 10 Years Follow-Up  
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**Background:** The natural history of patients commencing dialysis is not well characterised and there is little evidence regarding the impact of potential pre-dialysis factors predicting mortality. This study examined pre-dialysis and co-morbid risk factors for 10 years mortality post start of dialysis therapy.

**Methods:** A prospective study: all new subjects commencing dialysis in 2001/02 in East Yorkshire were followed up for a mean of 11.6 years. Predictors of mortality (e.g. pre-dialysis factors - creatinine, albumin, haemoglobin etc) were determined by univariate, multi-variate analysis and survival via Kaplan-Meier analysis.

**Results:** 94 patients, mean age of 63±1y were analysed. Mortality rate at 106 months was 60%. 30% (29) of patients had been transplanted during the follow-up period. 20 transplant patients were still alive with a functioning transplant (67%), 7 experienced transplant failure and returned to dialysis. 2 transplants died (myocardial infarction and calciphylaxis). Low-eGFR and haemoglobin at dialysis commencement had a significant impact on early mortality on univariate analysis. At 5y vascular disease and sepsis accounted for 71% of mortality. This decreased to 46% by 10y for these causes. Cardiac disease was the commonest cause of death. In 17% of patients death was related to dialysis or its withdrawal. From Kaplan-Meier survival, patients with vascular disease had a cumulative survival of 14% vs. 33% of those without vascular disease (p<0.05). Diabetic patients had a cumulative survival of 18% vs. 27% without diabetes (p<0.02). Contrary to the 5 year data, calcium phosphate was no longer predictive of mortality.

**Conclusions:** Diabetes and vascular disease remain strong predictors of mortality. Cardiovascular disease (CVD) showed to be the most specific predictor of cardiac mortality. Low-eGFR and low haemoglobin at dialysis commencement had a significant impact on early mortality but were not predictive of mortality on survival analysis. Aggressive management of cardiac risk factors in addition to early transplantation is key to survival.

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**FR-PO1642**  
Clinical Characteristics and Coronary Plaque Morphology in Chronic Kidney Disease (CKD) Patients  
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**Background:** Coronary artery disease (CAD) is a major cause of death in patients with chronic kidney disease (CKD). However, the pathophysiology of CAD remains unclear. Virtual Histology - Intravascular ultrasound (VH-IVUS) can provide four major plaque components (fibrous, fibro-fatty, dense calcium and necrotic core) with high accuracy. The aim of this study is to evaluate the clinical characteristics and coronary plaque morphology by VH-IVUS analysis in CKD patients.

**Methods:** Seventy-eight patients (CKD stage 1-2, n=31; CKD stage 3, n=24; CKD stage 4-5, n=11; Hemodialysis (HD), n=12) with CAD were included in this study. They were divided into ACS group and non-ACS group. To the admission day, VH-IVUS analysis on culprit segments was performed for all the studied patients. From these profiles, we calculated Necrotic core/Dense calcium (NC/DC) ratio, and compared the ratio in patients with or without ACS in all the studied patients.

**Results:** The result of VH-IVUS analysis showed that the relative volume of dense calcium (% DC) and necrotic core (% NC) gradually increased with decreasing renal function. On the other hand, NC/DC ratio gradually decreased with the development of CKD (CKD stage 1-2: 2.5±1.4, CKD stage 3: 2.2±1.6, CKD stage 4-5: 1.6±0.9, HD: 1.4±0.7, p<0.06). Moreover, NC/DC ratio was significantly higher in patients with ACS compared with those without ACS (ACS: 2.3±1.5, non-ACS: 1.5±0.8, p<0.05). In multiple variant analysis, % DC was significantly correlated with diabetes mellitus (p=0.214, p<0.05) and estimated glomerular filtration rate (GFR) (p<0.313, p<0.05).

**Conclusions:** Our findings suggested that the compositional pattern of coronary plaque was transferred from necrotic core-rich plaque into calcium-rich plaque with the development of CKD, and NC/DC ratio was associated with incidence of ACS in CKD.
dialysis. A scanning capillary viscometer (Prometics Inc.) was used to measure WBV at multiple shear rates.

Results: For analysis, patients were divided into low-normal vs. high UF groups (cutoff 2700 mL). At baseline, patients in the high UF group were younger and had a greater proportion of diabetics. Mean hematocrit increased during dialysis in both groups. The intradialytic increase in hematocrit was significantly higher in the high versus the low UF group (3.2% vs. 1.28%, p = 0.01), with a significantly higher end-dialysis hematocrit in the high UF group (40.5% vs 38%, p = 0.02). At the end of dialysis both systolic (low shear rate) WBV (p = 0.01) and diastolic (high shear rate) WBV (p = 0.01) were significantly higher in the high UF group than the low UF group. There was an approximately twofold increase in systolic (p = 0.01) and diastolic (p = 0.01) WBV during dialysis in high versus low UF groups. The increase in systolic blood viscosity during dialysis was significantly correlated with an increase in Hct (R² = 0.6526, p < 0.01).

Conclusions: Hemodialysis results in hemocoagulation and increased WBV. Among patients requiring greater UF volumes, there are greater increases in Hct, systolic and diastolic WBV. Because of the potential harmful cardiovascular effects of increased WBV, patients with greater UF requirements may need lower Hgb targets during erythropoietin treatment.

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FR-PO1646

Serum Cystatin C as a Predictor for Cardiovascular Events in End-Stage Renal Disease Patients at the Initiation of Dialysis

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Background: Cystatin C has been known to predict cardiovascular outcomes in elderly persons and stage 3 or 4 CKD patients. However, there has been no study to investigate whether cystatin C could predict the cardiovascular events in ESRD patients. Furthermore, recent studies argue that cystatin C-based eGFR level (çysC ) is a better predictor of cardiovascular disease than eGFR, because the non-GFR determinants of cystatin C also reflect cardiovascular risk. Current study was performed to delineate the role of serum cystatin C and eGFR for prediction of the cardiovascular events and compare the other traditional variables with serum cystatin C in incident dialysis patients.

Methods: This study included 66 ESRD patients [mean age, 52.7±16.3 years; hemodialysis (HD), 46 pts; peritoneal dialysis (PD), 20 pts] who survived for more than 3 months after the start of dialysis, and serum cystatin C levels were measured at the point of dialysis initiation. We conducted a retrospective charts review and median follow-up period was 14.9 months.

Results: Serum cystatin C was correlated with BUN (r = 0.537, p = 0.001), serum creatinine (r = 0.480, p = 0.001) and smoking (r = 0.284, p = 0.021). Cystatin C was inversely correlated with age (r = -0.316, p = 0.01) and eGFR for by MDRD (r = -0.533, p = 0.001). The incidence of cardiovascular events was 16.7% (11/66). Kaplan-Meier analysis for cysC for prediction of the cardiovascular events and compare other traditional variables with serum cystatin C in incident dialysis patients.

Conclusions: Our study suggested that ÇysC independently predicted cardiovascular events in incident dialysis patients

FR-PO1647

Correlation between Extracellular Volume as Assessed by Calf Bioimpedance Spectroscopy (cBIS) and Blood Pressure Changes in Hemodialysis (HD) Patients

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Background: Chronic fluid overload is associated with hypertension in HD patients. cBIS is a noninvasive technique to determine volume and distribution of body fluids (F Zanedi et al 2008). The aim of the study is to investigate whether changes in blood volume as assessed by cBIS correlate with changes in systolic blood pressure (SBP).

Methods: Chronic HD patients who were enrolled as part of a sodium intervention trial were followed for one year. In each subject, cBIS was done monthly pre-HD and post-HD to assess normalized calf resistivity (ρ) and extracellular volume (cECV) using a Hydro 4200 device (Xenotron technologies, San Diego, CA). SBP and diastolic blood pressure (DBP) were recorded pre-HD and post-HD. Only patients with at least 6 month of follow-up were included. Temporal changes of pre-HD SBP, ncRho, and cECV were computed by simple linear regression.

Results: We studied 9 HD patients (6 men; age 45.8±16.8 yrs). Temporal changes of SBP were significantly correlated with those of ncRho (R² = 0.40, p = 0.031; Fig. 1A) and cECV (R² = 0.70; p = 0.005; Fig. 1B)

Conclusions: cBIS is a simple and non-invasive low cost means to objectively measure body fluid content in HD patients. Our study shows that cBIS derived measures (ncRho, cECV) are clinically meaningful. The use of cBIS for diagnosis and treatment guidance of fluid overload can facilitate blood pressure control.

Results of Follow-up Measurements

FR-PO1648

Mortality Due to Pulmonary Embolism, Myocardial Infarction, and Stroke among Patients During Dialysis

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Background: Dialysis patients have an increased cardiovascular mortality risk as compared to the general population. However, there is limited information on how specific cardiovascular causes contribute to this increased risk.

Methods: Age- and sex-standardized mortality rate ratios (SMRs) for cardiovascular causes were calculated for 130,439 adults starting dialysis and registered in the ERA-EDTA Registry as compared with the European general population. Furthermore, we calculated hazard ratios (HRs) with 95% confidence intervals (CIs) to investigate the association between potential risk factors and specific causes of cardiovascular death.

Results: The overall age- and sex-standardized mortality rate of cardiovascular causes was 8.9 (95% CI 8.7–9.1) times higher in dialysis patients than in the general population. The SMRs in dialysis patients as compared to the general population were 12.2 (95% CI 10.2–14.6) for pulmonary embolism, 11.0 (95% CI 10.6–11.4) for myocardial infarction, 8.4 (95% CI 8.0–8.8) for stroke, and 8.3 (95% CI 8.0–8.5) for other cardiovascular diseases. Primary kidney diseases due to diabetes and multi-system disease were associated with an increased mortality risk due to pulmonary embolism (HR 1.9; 95% CI 1.0–3.8 and HR 3.2; 95% CI 1.0–6.4, respectively), myocardial infarction (HR 4.1; 95% CI 3.4–4.9 and HR 2.2; 95% CI 1.7–2.7, respectively), stroke (HR 3.5; 95% CI 2.8–4.4 and HR 2.8; 95% CI 2.1–3.6, respectively), and other cardiovascular causes of death (HR 3.4; 95% CI 2.9–3.9 and HR 3.4; 95% CI 2.9–4.0, respectively) as compared to patients with polycystic kidney disease after adjustment for age, sex, calendar year, and country.

Conclusions: Compared to the general population, dialysis patients have an increased mortality risk due to myocardial infarction, stroke, and pulmonary embolism.

Funding: Government Support - Non-U.S.

FR-PO1649

Obesity Paradox in Japanese Hemodialysis (HD) Patients

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Background: Dialysis Outcome and Practice Patterns Study (DOPPS) had suggested that nutritional indicator, including higher body mass index (BMI), has an important factor on the survival of HD patients. On the other hand, obesity is considered to be independent risk factors for the development of cardiac risks in the general population (obesity paradox). We hereby assess the impact of BMI on cardiac function of Japanese HD patients in long term.

Methods: From April of 2005 to March of 2010, 94 HD patients in our facility with stable BMI and normal protein catabolic rate (nPCR) over 5 years were enrolled in this study. We adopted appropriate index (covariate adjustment) method (sodium fraction (EF) or left ventricle diameter (LVDD) were evaluated before and after the period of > 5 years. The objects were divided into three groups, one for the patients with BMI
FR-PO1650
Calcification of the Thoracic Aorta Determined by Three-Dimensional Computed Tomography Predicts Cardiovascular Complications in Hemodialysis Patients
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Objective: The most common cause of death in dialysis patients is cardiovascular disease, and this may be due in part to the presence of excessive vascular calcification.

Methods: Computed tomography using contrast medium was performed in 49 hemodialysis patients (29 males and 20 females; 17 diabetics, 32 nondiabetics; average age 68.9±11.0 years). Calcification score (CS) was defined as the ratio of the volume of the vascular calcification to the volume of the thoracic aorta, 10 cm caudal part from the bifurcation of the trachea.

Results: All patients were followed up for cardiovascular events: cerebral infarction and hemorrhage, myocardial infarction, ECG or echocardiographic abnormalities suggestive of myocardial ischemia, cardiac surgery, leg amputation, and death and hospitalization due to heart failure.

Results: After 3 years of follow-up, 12 patients reached the end point. Mann-Whitney U test showed that both high CS (P = 0.007) and male gender (P = 0.009) were significantly associated with cardiovascular events per patient-year. The increase in LVDd was observed in underweight group from 51.3±18.6 to 55.6±10.9, whereas there was no change in overweight or obese group. Nutritional factors including nPCR stayed the same in all groups.

Conclusions: In Japanese HD patients population where the average body weight is 53.5±6.0 kg in adults, higher BMI indicating obesity may increase cardiac risks presumably associated with various baseline health status including cardiac load.

Funding: Private Foundation Support

FR-PO1651
C-Reactive Protein Is a Strong and Independent Risk Factor for Cardiovascular Morbidity and Mortality in Hemodialysis Patients – Post Hoc Results from the AURORA Trial
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Objective: To compare clinical presentations and outcomes in patients with CIED access-related infections in HD and non-HD groups.

Methods: We reviewed all cases of CIED infections treated at our center between 1991 and 2008 and analyzed their pertinent clinical features.

Results: Among the 415 patients with CIED infection, mean age was 69±15 years and 75% were male. Seventeen (4%) had received HD therapy prior to CIED infection. HD patients were more likely to be female (59% vs 24%, p<0.001). Both localized signs at the pocket and systemic manifestations of infection were less frequent in HD as compared to the non-HD group. Although HD patients were more likely to be bacteremic (100% vs. 44%, p<0.001), endocarditis rates were similar (50% vs 34%, p=0.2). There were no differences in device removal complication rates between the two groups. However, HD patients were relatively less likely to undergo device removal (82% vs. 95%, p<0.02) or undergo implantation of a new device (43% vs 67%, p=0.04). Although mortality rates at 30- and 60-day were similar between HD and non-HD groups, 90-day mortality was significantly higher (24% vs 8%, p<0.02) among HD patients.
Conclusions: HD-dependent patients are more likely to present with bacteremia complicating CIED infection and have a higher 90-day mortality rate as compared to non-HD dependent patients.

FR-PO1654

Efficacy of Low-Dose Bisoprolol in Maintenance Hemodialysis Patients with Asymptomatic Left Ventricular Remodeling and Diastolic Dysfunction

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Background: Left ventricular hypertrophy and diastolic dysfunction are the most frequent cardiac alteration in ESRD. The aim of this study was to determine whether β-blockers, bisoprolol, had beneficial effects in maintenance hemodialysis patients with asymptomatic left ventricular diastolic dysfunction.

Methods: In this study we enrolled 30 patients with chronic kidney disease (CKD) undergoing maintenance hemodialysis accompanying left ventricular diastolic dysfunction more than six months. Bisoprolol was started with 1.25 mg once daily orally 30 minutes after breakfast and increased every week by 1.25 mg increments up to the maximum tolerated dose. Echocardiographic examination was used to measurement of left ventricular diastolic function.

Results: 27 patients finished 6 months study. After 6 months treatment, compared with the baseline, the cardiothoracic ratio was significant decreased. Echocardiographic examination showed that there was no significant change in EF, but LVEDd, LVEF, PWT were significant decreased. E/A ratio significantly increased.

Conclusions: Our study demonstrate bisoprolol efficacy in improving left ventricular remodeling and diastolic dysfunction in HD patient with normal blood pressure.

Funding: Government Support - Non-U.S.

FR-PO1655

Potassium-Binding Sodium-Based Resins: Associations with Serum Chemistries and Interdialytic Weight Gain

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Background: Na-based resins increase phosphoruric and plasma bicarbonate levels in non-uremic dogs, due to calcium binding in the gut, favoring phosphorus and bicarbonate absorption. Relevance to hemodiagnosis (HD) patients is unknown.

Methods: Data were from 10,487 HD patients in the Dialysis Outcomes and Practice Patterns Study (DOPPS) phases 2-4 (2002-2011) in countries with >5% Na-based K resin use: Belgium (12%), Canada (7%), France (49%), Italy (19%), and Sweden (25%). Linear mixed models were used to determine associations between baseline at K resin use and interdialytic weight gain (IDWG), serum concentrations of bicarb, P, K, Ca, and Na. Instrumental variable (IV) analyses were conducted because they can limit treatment-by-indication bias due to unmeasured confounders.

Results: Overall K resin use was 20%, with notable facility variation. The 95th percentile of facility % K resin use was 58%; 22% of facilities did not prescribe any K resin use. Patterns Study (DOPPS) phases 2-4 (2002-2011) in countries with >5% Na-based K resin use: Belgium (12%), Canada (7%), France (49%), Italy (19%), and Sweden (25%).

Conclusions: As hypothesized, Na-based K resin use in HD patients is associated with lower serum bicarbonate, phosphorus, and sodium concentrations and greater IDWG.

Funding: Pharmaceutical Company Support

FR-PO1656

Multiple Biomarkers Improve the Prediction of Cardiovascular Mortality in Patients on Chronic Hemodialysis

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Background: Patients on chronic hemodialysis (HD) are widely recognized to be at high risk for cardiovascular (CV) mortality, thus the more accurate prediction of CV mortality is clinically important. Three biomarkers, serum brain natriuretic peptide (BNP), Troponin T (TrnT) and C-reactive protein (CRP), are individually established to be predictive biomarkers for CV mortality in this population.

Methods: A total of 500 consecutive HD patients were examined by the measurement of three biomarkers for 10 years. The cut-off values were based on the tertiles of the individual biomarkers, and a multivariate Cox analysis including three biomarkers for CV mortality was performed. From the analyzed model, a simplified score was obtained by putting weight to individual biomarkers based on the adjusted hazard ratio (HR). Finally, the multi-marker score (MMS) was defined as the sum of these points, with higher points indicating a higher mortality risk.

Results: During follow-up period (80-413 months), 204 patients died including 95 CV deaths. Three biomarkers were individually independent predictors for CV mortality (<0.01 for all). However, by receiver operating characteristic (ROC) analysis, area under curve (AUC) for CV mortality was larger in the multi-marker score (0.76) compared to BNP (0.64), TrnT (0.70) and CRP (0.65) alone. Similarly, AUC for all-cause mortality was 0.80, 0.68, 0.74 and 0.73 in MMS. Among low-risk group (MMS≤3), middle-risk group (MMS=4-6) and high-risk group (MMS>7), 10-year survival rate was 93.5%, 73.3% and 54.0% for CV mortality and 83.1%, 52.6% and 26.6% for all-cause mortality (p<0.0001 for both), respectively. Even after adjustment, MMS had strongly predictive power.

Funding: Government Support - Non-U.S.

FR-PO1657

NT-ProBNP Has No Diagnostic Value in End Stage Renal Disease Patients Presenting with Dyspnea

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Background: NT-ProBNP/BNP although used to differentiate acute dyspnea states, has not been validated in End Stage Renal Disease (ESRD) patients. We examine hemodiagnosis (HD) patients presenting with dyspnea.

Methods: Retrospectively, 250 HD subjects admitted to Cooper Hospital from 07/2010 to 03/2011, with acute dyspnea were broken into a high group (NT-ProBNP>70.000) and a low group (NT-ProBNP<2.600) based on a rough cut of 2,500 reported for chronic kidney disease patients. Analysis used Chi-square to assess differences between low and high NT-ProBNP levels in relation to performing hemodiagnosis, number of cardiology consults and echocardiograms ordered corrected for the continuous data set. Standard t-test analysis evaluated the difference in creatinine, volume removed, weights, weight change, and ejection fraction (EF) between groups.

Results: Out of 250 subjects, 235 had NT-ProBNP levels performed. No statistically significant difference was found in the frequency of hemodiagnosis, obtaining cardiology consults and echocardiograms between ESRD patients with high and low NT-ProBNP. Chi-square analysis

Funding: Pharmaceutical Company Support

Table 1: Effect of Na-based K Resin Prescription (Estimate*, 95% CI)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Unadjusted</th>
<th>Adjusted</th>
<th>IV approach (adjusted)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Bicarbonate (mEq/L)</td>
<td>0.90 (0.70,2.08)</td>
<td>0.32 (0.14,0.51)</td>
<td>0.50 (1.26,1.46)</td>
</tr>
<tr>
<td>Serum Phosphorus (mg/dL)</td>
<td>0.37 (0.28,0.46)</td>
<td>0.20 (0.11,0.28)</td>
<td>0.32 (0.05,0.69)</td>
</tr>
<tr>
<td>Serum Sodium (mEq/L)</td>
<td>0.34 (0.30,0.38)</td>
<td>0.20 (0.16,0.24)</td>
<td>-0.16 (0.43,0.37)</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>0.38 (0.28,0.40)</td>
<td>0.35 (0.15,0.53)</td>
<td>0.96 (0.21,1.33)</td>
</tr>
<tr>
<td>IDWG (kg)</td>
<td>0.44 (0.36,0.51)</td>
<td>0.26 (0.10,0.33)</td>
<td>0.36 (0.07,0.75)</td>
</tr>
</tbody>
</table>

*Estimate is the difference in the outcome for patients with baseline K resin use compared to non-use

Adjustments: age, gender, black race, vintage, BMI, residual kidney function, vascular accesses, ferritin, hemoglobin, white blood cell count, serum albumin, serum creatinine, and 14 comorbid conditions

Note: All models adjusted for DOPPS phase and country and accounted for facility clustering effects

Funding: Pharmaceutical Company Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

498A
Conclusions: There is no difference in the initial management strategies of HD patients presenting with NT-ProBNP levels in both extremes. In ESRD, the NT-ProBNP has no clinical value for discriminating between primary pulmonary processes and volume overload states and, therefore, should not be ordered as part of an emergency room evaluation.

FR-PO1660

Pomegranate Juice Intake Attenuates Traditional Cardiovascular Risk Factors in Hemodialysis Patients: A Randomized Placebo Controlled Trial

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Background: The aim of the present study was to investigate the long term effects of Pomegranate juice (PJ) consumption, rich in polyphenols, on traditional cardiovascular (CV) risk factors: lipid profile, hypertension and on the progression of the atherosclerotic process.

Methods: 101 HD patients were randomized to receive 100 cc of PJ (0.7mM polyphenols) or matching placebo, three times a week for one year. The primary endpoints were systolic and diastolic blood pressure, number of antihypertensive drugs, level of triglycerides (TG), total cholesterol, HDL and LDL.

Results: After one year of intervention the change in the number of antihypertensive drugs was significantly different between the two groups (PY = 0.05). The number of antihypertensive drugs decreased in 22% of patients in the PJ group compared to 7.7% in the placebo group, while an increase was documented in 12.2% of patients in the PJ compared to 34.6% in the placebo group. Furthermore, a significant time response improvement in systolic blood pressure, triglycerides and HDL was observed in the PJ group.

Conclusions: PJ consumption attenuates traditional CV risk factors. Hence, it may favor the high incidence of morbidity and mortality in HD patients. Funding: Government Support - Non-US.

FR-PO1661

Treatment of Chronic Low Back Pain in Hemodialysis Patients: A Randomized Controlled Trial

Ricardo Sesso, Tatiana Cristofolini, Jamil Natour. Nephrology, Federal University of São Paulo, Brazil.

Background: Low back pain is a very common and disabling symptom that has not been properly studied in hemodialysis patients. The aim of this study was to evaluate the effectiveness of a physiotherapy approach to chronic low back pain.

Methods: 104 patients with chronic low back pain (lasting more than 3 months) not related to infection, tumor or fractures, and undergoing chronic hemodialysis in two hemodialysis units were prospectively randomized to one of two intervention groups: 1. Physiotherapy [N=53] with the McKenzie method consisting of repetitive and sustained flexion/extension exercises of the lumbar spine. Four movements were selected: flexion in standing, extension in standing, flexion in lying and extension in lying position; repeated twenty times per session (30 min. total) three times a week for eight weeks. Group 2 [N=51]: received transcutaneous electrical nerve stimulation (TENS) for 20 min at 50-100 Hz frequencies for the same time frame. Outcome measures: lumbar pain visual analogue scale (VAS, ranging from 0-10[worse]), and the functional lumbar spine status measured by the Roland Morris (RM) disability questionnaire (with 24 items and scores ranging from 0 to 4[worse]) reported by the patients 1 week after the end of treatment.

Results: Mean±SD scores of the VAS pre- and post-treatment were 6.9±1.6 and 4.4±1.4, respectively, in group 1, and 7.1±1.6 and 6.4±1.6, respectively in group 2. Mean change from baseline to after treatment was 5.5±1.8 and 0.8±0.6 for groups 1 and 2, respectively, P<0.001. Baseline RM scores were 20.3±7.2 and 20.9±7.2, for groups 1 and 2, respectively. Mean change from baseline to after treatment was 16.0±2.6 and 1.1±1.8 for groups 1 and 2, respectively, P<0.001. The effectiveness of the physiotherapy intervention was observed in all strata of the sociodemographic, laboratory and clinical variables.

Among patients in the PJ group attenuation and aggravation in the atherosclerotic process was detected in 25% and 5%, respectively, while more than 50% of patients in the Placebo showed progression and none showed improvement. Conclusions: PJ consumption attenuates traditional CV risk factors. Hence, it may favor the high incidence of morbidity and mortality in HD patients. Funding: Government Support - Non-U.S.
Conclusions: Physiotherapy with the McKenzie method is effective in decreasing chronic low back pain and improving disability in chronic hemodialysis patients.

Funding: Government Support - Non-U.S.

FR-PO1662

Hemodialysis Time of Day and Restless Legs Complaint: USRD Special Study Data

Nancy G. Kutter, Donald L. Bliwise, Rebecca H. Zhang, Kirsten L. Johansen, Lynda A. Szczeczen.

Methods: The CDS surveyed incident dialysis patients aged >18 from 296 randomly selected clinics throughout the US. Participants included 1,174 patients whose HD started before 2 pm (early shift) and 270 patients whose HD started 2 pm or later (late shift). RLS was defined by positive response to the three NIH workshop criteria: unpleasant sensations plus urge to move legs, sensations occur mainly at rest and improve with movement, and symptoms worse in evening/night than in morning. Patient characteristics potentially associated with RLS were compared by HD shift using t-test and chi-square analysis, and logistic regression was used to predict RLS.

Results: RLS was reported more often by late shift patients than by early shift patients (35% vs 28%; p = 0.008). Race/ethnicity, gender, educational level, and mean hemoglobin level were similar for early and late shift patients, while early shift patients were more likely to have diabetes and to be older. With adjustment for diabetes and age, the odds ratio for RLS among late shift patients compared to early shift patients was 1.37 (CI 1.05-1.79); p = 0.02.

Conclusions: RLS is time-of-day and activity dependent. For patients at risk for RLS, undergoing HD later in the day may increase distress and could contribute to patients’ shortening treatment; it has been shown that RLS has negative implications for patient survival. CDS data suggest the value in clinical practice of early screening for restless legs complaint and avoiding late shift HD in at-risk patients.

Funding: NIDDK Support

FR-PO1663

A Prospective Trial Assessing the Longitudinal Stability of CKD-MBD Parameters in Hemodialysis Patients

Theresa Gross, Thilo Krueger, Markus Ketteler, Vincent Brandenburg, Nephrology, RWTH Aachen, Aachen, Germany; Nephrology, Klinikum Coburg, Coburg, Germany; Cardiology, RWTH Aachen, Aachen, Germany.

Methods: In this prospective, monocentric study over 32 weeks 56 chronic HD pts. (63% male, mean age 65±13 yrs, 35% diabetics) were investigated. 49 pts. underwent three to six serial measurements of FGF23 and other routine CKD-MBD parameters. We calculated the intraclass correlation (ICC) from estimates of between-subject variability (σ2b) and within-subject variance (σ2w) derived from mixed linear models. We applied the following formula: σ2b / (σ2b + σ2w). FGF23 was measured by c-t assay.

Results: During the study period the within-subject variability of FGF23 accounted for 15% of the total variability compared to 30% inPTH, 50% in Ca, 27% in PO4 and 5.5% in bone alkaline phosphatase (BAP).

According to serial serum FGF23 measurements HD pts could be grouped into categories: group 1 (n=13): constantly low values <2000 RU/ml, group 2 (n=14): medium values 2000-10 000 RU/ml, group 3 (n=10): constantly high values >10 000 RU/ml and group 4 (n=12): changing values. These groups were sign. different in classical CKD-MBD parameters. Pts. with high FGF23 (group 3) had sign. higher time averaged values for Ca [2.5±0.12 vs 2.36±0.08 mmol/l], ion.Ca [1.32±0.07 vs 1.25±0.04 mmol/l], P04 [2.41±0.38 vs 1.65±0.27 mmol/l] and CaPO4 [6.08±0.85 vs 3.89±0.6 mmol²/l] then pts. in group 1 (all p<0.01).

Conclusions: FGF23 and BAP were characterized by lower within-subject variability compared to PTH, Ca and PO4 in HD pts.. About 75% of the pts. showed stable values in different ranges in this cohort. These data may help establishing FGF23 as diagnostic marker in HD pts.

Funding: Pharmaceutical Company Support

FR-PO1664

IMPACT-SHPT Study: Comparative Quality of Life Analysis of the Treatment of Secondary Hyperparathyroidism

Donald L. Goldsmith, Markus Ketteler, Kevin J. Martin, Mario Coszoilino, Myles S. Wolf, Amit Sharma, Michael Andahl, Samina Khan, Steven E. Marx, Gay’ Hosp; Klinikum Coburg; Saint Louis U; University of Milan; U of Miami; Boise Kidney and Hypertension Inst; Abbott.

Background: The IMPACT-SHPT is a randomized, open-label, 28-week, multicenter trial to compare paricalcitol and cinacalcet to determine the most effective therapy for the treatment of SHPT in subjects undergoing hemodialysis. The objective of this analysis is to assess patient reported outcomes using the KDQOL-SF in subjects receiving paricalcitol (IV Stratum-US) compared with cinacalcet plus low dose vitamin D.

Methods: 268 Subjects were randomly assigned to IV paricalcitol or cinacalcet treatment groups and received at least one dose for up to 28 weeks. The cinacalcet group received a fixed dose of doxercalciferol. If serum calcium was >10.5 mg/dL on two consecutive levels cinacalcet was administered in the paricalcitol arm. The treatment goal was to achieve a iPTH between 50 to 300 pg/mL during weeks 21–28. KDQOL-SF questionnaire was self administered. The baseline and final KDQOL-SF measurement were compared between US treatment arms.

Results: KDQOL Mean Change From Baseline to Final Measurement

Conclusions: Paricalcitol treated patients experienced a 5 point mean difference in change from baseline to final measurement in cognitive function, sensory function, work status, and physical function compared with cinacalcet plus low dose vitamin D. Paricalcitol treated patient’s mean difference in change from baseline to final measurement were higher in15 out 20 domains compared with cinacalcet plus low dose vitamin D. Higher scores represent a better health state. Clinicians and healthcare decision makers may consider these findings when evaluating treatment options for secondary hyperparathyroidism.

Funding: Pharmaceutical Company Support

FR-PO1665

Achievement of KDQOL Guidelines for Bone Metabolism and Mortality in Incident Hemodialysis Patients in Relationship to Age

Len A. Usvyat, Rakesh Malhotra, Stephan Thijsesen, Nathan W. Levin, Peter Kotanko, Renal Research Institute, New York; Beth Israel Medical Center, New York.

Background: The National Kidney Foundation Kidney Disease Outcomes Quality Initiative (NKF-KDQOL) guidelines for bone and mineral metabolism (BMM) do not take age into account. This study aims to investigate the effect of age on the achievement of BMM NKF-KDQOL goals during the first three months in HD and the impact of this achievement on all-cause mortality at 24 months.

Methods: The study included all incident patients aged ≥18 years that received HD at the facilities of the Renal Research Institute from 2001 to 2008. Patients were divided into four age groups at start of dialysis: 1: 18-60, 2: 61-70, 3: 71-80, and 4: >80 years old. Demographic data, etiology of ESRD, the average levels during the first 3 months on HD of total calcium (Ca), phosphorus (P) and parathyroid hormone (PTH) were collected by chart review. Multivariate Cox regression models were used to calculate HR and 95% CI.

Results: We included 3456 patients, 56.6% male, 51% Diabetics and 48.1% whites. HR for all cause Mortality. At target groups were used as reference.

Funding: NIDDK Support
Conclusions: Hypocalcemia increased all cause mortality risk in patients below 80 year old. The reasons for this association are unclear, but may be related to arrhythmias. Hypophosphatemia, a likely reflection of poor nutrition, was associated with increased all cause mortality risk in the age group 61-70 years.

Funding: Private Foundation Support

FR-PO1666

Influence of Age on Bone Mineral Metabolism Parameters: Differences and Commonalities between Two Countries

Olyvka Vega,1,2 Gero D. von Gersdorff,2 Mathias Schaller,1 Len A. Usvyat,2 Nathan W. Levin,2 Peter Kotanko,1,3 Claudia Barth,1 Renal Research Institute, New York; 2Beth Israel Medical Center, New York; 3Nephrology, University of Cologne Medical Center, Germany; 4Karatorium für Heimdialyse, Neu-Isenburg, Germany.

Background: The KDOQI guidelines for bone and mineral metabolism (BMM) do not take patient’s age into account. This study aims to investigate the effect of age on the achievement of BMM KDOQI goals using two cohorts of HD patients from the US and Germany.

Methods: We included all patients aged >18 years who received in 2009 HD at the facilities of the German Kuratorium für Heimdialyse (KfH), and the Renal Research Institute (RRI), USA. Patients were stratified by age: 18-59; 60-69; 70-75; 75-80, and >80 years. Etiology of ESRD, levels of total calcium (Ca), phosphorus (P), and parathyroid hormone (PTH) were collected from the databases. ANOVA was used.

Results: There were 8638 patients in the KfH-cohort and 2446 in the RRI-Cohort. Diabetes was the most common cause of ESRD in both cohorts (KfH=26% and RRI 33%).

Conclusion: Bixalomer was able to control serum P levels for a long period and would not raise any major safety concerns when administered for a long period. Therefore, bixalomer could be a clinically effective drug that can be administered for a long period to treat hyperphosphatemia.

Funding: Pharmaceutical Company Support

FR-PO1668

IMPACT-SHPT Study: Comparative Economic Analysis of the Treatment of Secondary Hyperparathyroidism

Amit Sharma,1 Markus Ketteler,2 Kevin J. Martin,3 Myles S. Wolf,4 Mario Cozzolino,5 David J. Goldsmith,6 Michael Amadahl,7 Samina Khan,8 Steven E. Marx,9 Boise Kidney and Hypertension Inst; 2Klinikum Coburg; 3Saint Louis U; 4U of Miami; 5University of Milan; 6Guy’s Hosp; 7Abbott.

Background: The IMPACT-SHPT is a randomized, open-label, 28-week, multicenter trial that compared paricalcitol and cinacalcet to determine the most effective therapy for the treatment of SHPT in subjects undergoing hemodialysis. The objective of this analysis is to assess the cumulative dose and cost of paricalcitol and cinacalcet treatment during the study.

Methods: Subjects were randomly assigned to receive paricalcitol or cinacalcet and fixed IV doxercalciferol or oral alfacalcidol for 28 weeks. Cinacalcet was administered if serum calcium is ≥10.5 mg/dL on two consecutive levels in the paricalcitol arm. The treatment goal was to achieve a mean iPTH value between 50 to 300 pg/mL during weeks 21–28. Data were collected from 268 subjects who received at least one dose of randomized study drug. Using a US perspective, costs were estimated by utilizing average wholesale price minus fifteen percent.

Conclusions: The 28 week treatment dose in the paricalcitol arm was: paricalcitol 56,668 mcg, and cinacalcet 20,850 mcg, compared to the cinacalcet arm: cinacalcet 895,200 mcg, doxercalciferol 4,087 mcg, and alfacalcidol 2,780 mcg. The treatment cost was $184,279 in the paricalcitol arm compared to $490,611 in the cinacalcet arm over 28 weeks.

Funding: Pharmaceutical Company Support

FR-PO1669

Long-Term Treatment of Bixalomer in Chronic Kidney Disease Patients on Hemodialysis with Hyperparathyrommia

Tadao Akizawa,1 Hidemi Origasa,2 Chisato Kameoka.2 1Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; 2Division of Biostatistics and Clinical Epidemiology, University of Toyama School of Medicine, Japan; 3Astellas Pharma Inc., Tokyo, Japan.

Background: Bixalomer is a new phosphate-binding polymer in development for the treatment of hyperphosphatemia in Chronic Kidney Disease(HD) patients in Japan. In this study, the non-inferiority of bixalomer to Sevelamer HCI(SH) in efficacy was assessed and the safety of bixalomer was also compared with that of SH.

Methods: This was a multicenter, open-label study in CKD-HD patients with hyperparathyrommia. Primary endpoint was serum phosphorus(Pi) levels at the end of treatment. The starting dose was 1.5g/day(bixalomer) and 3g/day or 6g/day(SH, based on the serum Pi level before treatment period). Depending on serum Pi levels, the dose of bixalomer or SH was titrated up to 7.5g/day and 9g/day, respectively. Subjects received the study drug for 12 weeks.

Results: Overall 110 subjects were randomized to either bixalomer group or SH group (55 of each). The adjusted mean serum Pi level at the end of treatment was 7.71±0.57mg/ dl and 7.55±0.62mg/dl, respectively. The upper limit of the 95%CI for the difference between the adjusted mean for bixalomer group and SH group (bixalomer-SH) was below 1.0mg/ dl(non-inferiority margin in this study). Thus the non-inferiority of bixalomer to SH was confirmed. The incidence of common gastrointestinal adverse drug reactions(GI ADRs) which were assessed by investigators as related to the study drug was summarized in the following table with the final mean daily dose. No serious or severe GI AEs were reported. In addition, bixalomer did not decrease HCO3 levels, which were measured to assess the metabolic acidosis risk.

Conclusions: Irrespective of country, BMM KDOQI goals are achieved more frequently with increasing age. Older patients may increase susceptibility to low PTH levels

FR-PO1667

A Phase III, Sevelamer HCl-Controlled Study of Bixalomer in Chronic Kidney Disease Patients on Hemodialysis with Hyperparathyrommia

Tadao Akizawa,1 Hidemi Origasa,2 Chisato Kameoka.2 1Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; 2Division of Biostatistics and Clinical Epidemiology, University of Toyama School of Medicine, Japan; 3Astellas Pharma Inc., Tokyo, Japan.

Background: Bixalomer is a new phosphate-binding polymer in development for the treatment of hyperphosphatemia in Chronic Kidney Disease(CKD)-Hemodialysis(HD) patients in Japan. In this study, the non-inferiority of bixalomer to Sevelamer HCI(SH) in efficacy was assessed and the safety of bixalomer was also compared with that of SH.

Methods: This was a multicenter, randomized, SH-controlled, open-label study in CKD-HD patients with hyperparathyrommia. Primary endpoint was serum phosphorus(Pi) levels at the end of treatment. The starting dose was 1.5g/day(bixalomer) and 3g/day or 6g/day(SH, based on the serum Pi level before treatment period). Depending on serum Pi levels, the dose of bixalomer or SH was titrated up to 7.5g/day and 9g/day, respectively. Subjects received the study drug for 12 weeks.

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FR-PO1667

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Tadao Akizawa,1 Hidemi Origasa,2 Chisato Kameoka.2 1Department of Nephrology, Showa University School of Medicine, Tokyo, Japan; 2Division of Biostatistics and Clinical Epidemiology, University of Toyama School of Medicine, Japan; 3Astellas Pharma Inc., Tokyo, Japan.

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GLA-DRZ 29.1% 17.3%
-Constipation 18.2% 29.1%
-Abdominal distension 1.8% 12.7%
Mean Drug Dosing (Week 1 to 12) 3.5 g/day 0.6 g/day

Conclusions: Bixalomer was as effective as SH in decreasing serum Pi level. Bixalomer caused fewer GI ADRs and showed no deterioration of metabolic acidosis. These results indicated the clinical benefits of bixalomer as a treatment for hyperphosphatemia.

Funding: Pharmaceutical Company Support

FR-PO1670

N-DEPTH – Nephrology DVT and Pulmonary Embolism Prophylaxis Study in Hospitalized Patients Christine M. Ribe, Andrew M. Burke, Sarah Karkhaneci, Catherine M. Clase, Azim S. Gangji. Medicine, McMaster University, Hamilton, ON, Canada.

Background: In hospitalized patients, the risk of venous thromboembolism (VTE) has been reported to be higher in patients with chronic kidney disease. Prophylaxis prescription practices, and the risk of VTE and bleeding in hospitalized patients treated with dialysis are unknown.

Methods: This single centre, retrospective chart review was designed to determine the rate of VTE pharmacological prophylaxis in adult patients treated with chronic dialysis (≥ 3 months) on index admission during Sept 2008 to Sept 2009. Exclusion criteria included admission ≤ 24 hours, for suspected VTE, bleed or renal transplant. We defined VTE prophylaxis as administration of prophylactic doses of heparin within 48 hours of admission. We collected data on VTE risk factors as per the ACCP guidelines, VTE events defined objectively with imaging and major bleeding events. We screened 329 patient admissions: 143 met eligibility criteria. Of these, 23 (16.1%) were in full dose anticoagulation (coumadin with INR ≥ 2.0, or heparin) and were ineligible for VTE prophylaxis. Of the 120 eligible patients, 31 (25.8%) received VTE prophylaxis. The number of baseline VTE risk factors (range 1 to 7) and anticoagulant or antiplatelet use did not predict the use of VTE prophylaxis (p=0.86). There was a trend towards less use of prophylaxis in patients receiving clopidogrel at baseline (p=0.05).

VTE events (1 pulmonary embolism; 1 deep vein thrombosis) occurred in 2 (2.2%) eligible patients not receiving prophylaxis and no events occurred in those receiving prophylaxis (p=0.55). There was no difference in minor or major bleeds between those that did (n=3) and did not receive prophylaxis (n=6; p=0.66). The majority of bleeding events were gastrointestinal (n=7).

Conclusions: To our knowledge this is the first account of use of prophylaxis in this population. Three-quarters of apparently eligible patients on dialysis admitted to a tertiary centre under 13 different nephrologists were not prescribed prophylaxis, and baseline VTE risk factors did not predict for whom it was prescribed. Bleeding risk was not increased in patients receiving prophylaxis. This issue deserves wider study.

FR-PO1671

Retrospective Analysis of Etiology and Management of Pneumonia in End Stage Renal Disease Rupan Ruchi1, Matthew Whitbeck1, Andrew M. Burke, Johann D. Hummel1, William E. Wollheim1, Candace Bednarek1, Giovanni F.M. Strippoli1. Medicine, McMaster University, Hamilton, ON, Canada.

Background: The American Thoracic Society guidelines for management of community-acquired pneumonia in ESRD should be empirically be treated as low risk and considered care associated pneumonia (HCAP). We conducted this study to investigate if patients who are initially treated as HCAP do better vs patients who are empirically treated as community acquired pneumonia(CAP).

Methods: It is a retrospective cohort study including patients 18-89 yrs with ESRD on hemodialysis for ≥3 months, admitted with pneumonia. According to initial antibiotic used, patients were divided into group A (treated empirically as HCAP) and group B (treated empirically as CAP). Data was collected regarding demographic profile, severity of pneumonia (as assessed by PSI and CURB-65), co-morbidities, microbiology, length of hospital stay, in-hospital mortality. The chi-square statistics & multiple linear regression were used.

Results: Of 1744 hemodialysis patients in the participating clinics, 1308 (75%) received a pneumococcal vaccine (mean age 66.61 ± 13.55 years). 323 (27%) had received a pneumococcal vaccination in ESRD should be empirically be treated as low risk and considered care associated pneumonia (HCAP). We conducted this study to investigate if patients who are initially treated as HCAP do better vs patients who are empirically treated as community acquired pneumonia (CAP).

Conclusions: In conclusion, we found oral lesions to be highly prevalent in people receiving hemodialysis. This ongoing study will be completed in 2012 and prospectively analyzed the relationship between exposure to any oral lesions and the risk of major patient level endpoints including mortality and cardiovascular events. Focus on oral health could be an essential component of managing people with end stage kidney disease.


FR-PO1674


Background: Oral diseases are common in the general population and particularly in underprivileged portions of society. It is plausible that prevalence would be high in people with end stage kidney disease receiving hemodialysis but this has not been formally established. We globally surveyed the prevalence of any oral lesion in people on hemodialysis.

Methods: In this ongoing multinational cross-sectional and prospective cohort study, we enrolled people receiving hemodialysis in 30 outpatient clinics selected randomly from a self-nominated network. Prevalence of dental, periodontal, oral, and salivary lesions was assessed based upon standard dental practice methodology. Analysis was with descriptive statistics.

Results: Of 1744 hemodialysis patients in the participating clinics, 1308 (75%) received a pneumococcal vaccine (mean age 66.61 ± 13.55 years). 323 (27%) had received a pneumococcal vaccination in ESRD should be empirically be treated as low risk and considered care associated pneumonia (HCAP). We conducted this study to investigate if patients who are initially treated as HCAP do better vs patients who are empirically treated as community acquired pneumonia (CAP).

Conclusions: In conclusion, we found oral lesions to be highly prevalent in people receiving hemodialysis. This ongoing study will be completed in 2012 and prospectively analyzed the relationship between exposure to any oral lesions and the risk of major patient level endpoints including mortality and cardiovascular events. Focus on oral health could be an essential component of managing people with end stage kidney disease.


FR-PO1676


Background: Thirst and xerostomia, the subjective complaint of dry mouth due to a lack of saliva, are common side-effects of various medications, and it is plausible that their prevalence would be high in people with end stage kidney disease receiving hemodialysis as well as dialysis treatment itself being a major determinant. In this cohort study, we globally survey the prevalence of any oral symptoms in hemodialysis.

Methods: In this ongoing multinational cross-sectional and prospective cohort study, we enrolled consenting people receiving hemodialysis in 30 outpatient clinics selected
randomly from a collaborative dialysis network. Xerostomia inventory and dialysis thirst inventory were assessed based upon validated methodology. Analysis was with descriptive statistics.

Results: Of 1733 hemodialysis patients in the participating clinics, 1308 (75%) received a GBCA. Most patients received a macrocyclic contrast agent (80.4%) and 14.8% received a non-macrocyclic agent. Dermatological diagnoses did not report any evidence of NSF. As shown in the table, there was significant difference between CC and control groups in terms of mean skin thickness at all time points. In addition, a higher skin thickness was observed in control group compared to CC group over time.

Skin thickness at 4 weeks:

<table>
<thead>
<tr>
<th>Period</th>
<th>CC</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 weeks</td>
<td>56.78±2.06</td>
<td>0.044*</td>
</tr>
</tbody>
</table>

Adverse effects including skin rash or erosion were not observed.

Conclusions: Our results strongly indicate the potential effects of CC on protecting the formation of keloids and hypertrophic scars, and eventually improving QOL of HD patients.

Funding: Private Foundation Support

FR-PO1677

Prevalence of Nephrogenic Systemic Fibrosis (NSF) in Dialysis Patients: The Pro-FINEST Study

Sabine Amet,1 Vincent Launay-Vacher,1 Benedicte Stengel,2 Anne Castot,1 Camille Frances,1 Nicolas Grenier,1 Jean-Yves Gauvrit,1 Genevieve M. Reinhardt,1 Olivier Clement,1 Nicolas Janus,1 Carmen Kreft-Jaix,6 Genevieve M. Reinhardt,1 Olivier Clement,1 Nicolas Janus,1 Carmen Kreft-Jaix,6

Stefan Walter, Andreas Kriben, Oliver Witzke, Thorstien Feldkamp, Stefan Becker. Department of Nephrology, University Hospital Essen, Essen, NRW, Germany.

Background: Pathologies and symptoms of the skin are frequently encountered in end-stage renal disease patients undergoing chronic hemodialysis therapy. Uremic pruritus, pigmentary disorders or further skin-diseases like calciphylaxia are often described in the literature.

Aim of the study was to detect skin pathologies of such patients, to identify risk factors and to test for associations with mortality.

Methods: 508 patients undergoing chronic hemodialysis therapy (8 centres in North Rhine-Westphalia, Germany) were included into the study. Patients were interviewed and further data was taken from their medical records. Physical examination included upper and lower extremities and focused on xerosis, edema, papules, pustula, hardening, hyperpigmentation, and tethering of the skin.

To identify risk factors, we performed a multivariate analysis. 6 month after the visit the mortality-status was checked.

Results: 86.8 % (441/508) of the patients presented at least one of the respected skin pathologies. Frequently were found: xeroderma (71.9%, 365/508), itching (39.0%, 198/508) and edematous (17.5%, 89/508) skin, as well as hyperpigmentation (14.8%, 73/508), induration (1.6%, 8/508) and tethering of the skin (0.4%, 2/508).

Risk factors for hyperpigmentation, induration or tethering of the skin were simutaneous presence of coronary heart disease (p<0.005), warfarin therapy (p=0.001), peripheral arterial disease (p=0.002) or a previous thrombosis (p<0.003).

Patients with this skin lesions had a significantly higher mortality (p<0.002, OR=2.6) than patients without these lesions.

Conclusions: Skin lesions are a frequent finding in end-stage renal disease patients undergoing chronic hemodialysis therapy. One may assume that certain findings are associated with certain comorbidities and hence increased mortality.

Funding: Private Foundation Support

FR-PO1678

Skin Pathologies and Associated Mortality in a Cohort of End-Stage Renal Disease Undergoing Chronic Hemodialysis Therapy

Stefan Walter, Andreas Kriben, Oliver Witzke, Thorstien Feldkamp, Stefan Becker. Department of Nephrology, University Hospital Essen, Essen, NRW, Germany.

Background: Pathologies and symptoms of the skin are frequently encountered in end-stage renal disease patients undergoing chronic hemodialysis therapy. Uremic pruritus, pigmentary disorders or further skin-diseases like calciphylaxia are often described in the literature.

Aim of the study was to detect skin pathologies of such patients, to identify risk factors and to test for associations with mortality.

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Patients with this skin lesions had a significantly higher mortality (p<0.002, OR=2.6) than patients without these lesions.

Conclusions: Skin lesions are a frequent finding in end-stage renal disease patients undergoing chronic hemodialysis therapy. One may assume that certain findings are associated with certain comorbidities and hence increased mortality.

Funding: Private Foundation Support

FR-PO1676

The Use of External Carotene Cream (CC) Can Inhibit Hypertrophic Scars Caused by Needle Injury in Hemodialysis (HD) Patients

Satoshi Funakoshi,1 Jyunichiro Hashiguchi,1 Rica Etoh,1 Junko Kubo,1 Yoshiaki Lee,2 Takashi Harada,1 Kazunori Utsunomiya,1 Mineaki Kitamura,2 Tomoya Nishino,1 Shigeru Kohno,2 "Division of Blood Purification, Nagasaki Renal Center, Nagasaki, Japan; Department of Internal Medicine, Nagasaki University Graduate School of Medicine, Nagasaki, Japan; Department of Diabetes, Jikei University, Tokyo, Japan.

Background: Hypertrophic scars and keloids both represent fibrotic skin conditions which is difficult to treat with a high recurrence rate. Since upper arms are most susceptible to developing these types of conditions, HD patients are under high risks as they receive needle injuries in scar area were monitored using photometer (Spectrophotometer NF333, SOUKEN, Tokyo) and superficial ultrasound study (LOGIQ E9, GE Yokokawa, Tokyo) for 4 weeks (the period of skin turnover).

Results: As shown in the table, there was significant difference between CC and control groups in terms of mean skin thickness at all time points. In addition, a higher skin thickness was observed in control group compared to CC group over time.

Skin thickness at 4 weeks:

<table>
<thead>
<tr>
<th>Period</th>
<th>CC</th>
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Adverse effects including skin rash or erosion were not observed.

Conclusions: We found the formation of keloids and hypertrophic scars, and eventually improving QOL of HD patients.

Funding: Private Foundation Support
FR-PO1679

Efficacy of Glycerol-Paraffin in Uremic Xerosis: A Randomized, Double-Blind, Comparative Study

Suetonia Palmer,1 Giovanni F.M. Strippoli.2,3

1Department of Dermatology, Martin Luther University, Halle, Germany. 2Division of Dermatology, Hospital Saint Joseph, Thessaloniki, Greece; 3Department of Dermatology, Hospital Saint Vincent, Paris, France.

Background: Uremic xerosis is a bothersome condition that is poorly responsive to moisturizing and emollient therapy. It is associated with cutaneous deficiency in glycerol and exacerbated pruritus (uremic pruritus).

Methods: A randomized, double-blind, intra-individual (left vs right comparison), multicentric clinical study was performed on 100 patients with moderate to severe uremic xerosis for 7 days, during which the patients applied twice daily an emulsion combining glycerol and paraffin (test product) on one allocated lower leg, and the emulsion alone (comparator) on the other lower leg. This was followed by an open-labeled use of the test product on all the xerotic areas for 49 days. The main efficacy parameter was treatment response on each lower leg, as defined by a reduction from baseline of at least 2 grades in a pre-defined 5-point clinical score, on day 7.

Results: Among the ninety-nine (99) patients analyzed, the test product was highly effective with a treatment response in 72 patients (73%), whereas 44 patients (44%) responded to the comparator (p < 0.0001, inter-group analysis). This was associated with an objective reduction in the thickness and density of the scales using D-squares on day 7 (p < 0.0001 compared to the comparator), and a substantial improvement of the uric pruritus intensity (-2.5%) and quality of life at study end (p < 0.0001, inter-group analysis). The test product was very well tolerated, product-related local intolerance (exacerbated pruritus, local burning, erythema) occurring in only 5 patients (5%).

Conclusions: In conclusion, uremic xerosis and pruritus can be managed successfully when an appropriate skin protectant is used.

Funding: Pharmaceutical Company Support

FR-PO1680

Quality of Life in Patients with Uremic Xerosis

Patrick Dupuy,1 Jacek Szepliewtowski,2 Elias V. Balasaks,3 K.M. Taube.4 1Division of Research and Development, Orfagen, of Otago, Christchurch, New Zealand; 2Division of Dermatology, Hospital Saint Joseph, Thessaloniki, Greece; 3Department of Dermatology, Hospital Saint Vincent, Paris, France.

Background: Xerotic skin is a common and uncomfortable dermatological condition that is particularly prevalent in patients with renal failure. Xerosis is a significant marker of poor general health and quality of life. Patients with xerosis have increased levels of anxiety and depression, interrupted sleep, impaired physical performance, and decreased daily functioning and global health-related QoL.

Methods: A cross-sectional study was performed on 100 patients with moderate to severe uremic xerosis, aged 18–70 years. The study included a standardized questionnaire assessing sleep quality, the dermatological quality of life (DLQI), and the quality of health-related QoL (SF-12). The results were compared with 80 healthy control subjects.

Results: The test product was highly effective with a treatment response in 72 patients (73%), whereas 44 patients (44%) responded to the comparator (p < 0.0001, inter-group analysis). This was associated with an objective reduction in the thickness and density of the scales using D-squares on day 7 (p < 0.0001 compared to the comparator), and a substantial improvement of the uric pruritus intensity (-2.5%) and quality of life at study end (p < 0.0001, inter-group analysis). The test product was very well tolerated, product-related local intolerance (exacerbated pruritus, local burning, erythema) occurring in only 5 patients (5%).

Conclusions: In conclusion, uremic xerosis and pruritus can be managed successfully when an appropriate skin protectant is used.

Funding: Pharmaceutical Company Support

FR-PO1681

Sexual Dysfunction in Women Receiving Hemodialysis: A Multinational Cross-Sectional Study

Suptonia Palmer, Giovanni F.M. Strippoli.1 University of Otago, Christchurch, New Zealand; 2Diaverum Medical Scientific Office, Lund, Sweden; 3Mario Negri Sud Consortium, S Maria Imbaro, CH, Italy.

Background: Existing data for the prevalence and correlates of sexual dysfunction, including depression, in women on hemodialysis are limited by suboptimal study design. We aimed to estimate the prevalence and correlates of sexual dysfunction in women on hemodialysis between January and June 2008 within a collaborative network in Europe and South America. Sexual dysfunction (SD) was identified using the 19-item Female Sexual Function Index questionnaire based on self-reported sexual experiences in the four weeks before questionnaire. Depression was measured using the Center for Epidemiologic Studies-Depression (CES-D) questionnaire. Correlates of self-reported SD were identified by multiple regression analysis with stepwise partitioning and amalgamation analysis to group clinical characteristics associated with SD.

Results: 659 of the 1472 eligible women (45%) completed the questionnaires. Over half (386 [56%]) lived with a partner and 232 (35%) were sexually active. Overall, 555 (84%) of respondents reported SD. Women with a partner were less likely to experience SD (78% versus 92% without a partner). In multivariate analysis, age was a strong correlate of SD; for each 1 year increase in age, sexual dysfunction increased by 8% (adjusted odds ratio [AOR] 1.08 [95% CI, 1.06-1.11]). SD was also independently associated by depressive symptoms, lower sexual desire, marital separation, depression, and exacerbated pruritus. Nearly all women who were not wait-listed for a kidney transplant and not living with a partner (249/260 [96%]) reported SD. Over half (55%) of sexually active women reported SD which was associated with age, depressive symptoms, menopause, low serum albumin, and diuretic treatment. T-tests were limited due to the response rate and residual confounding. SD was highly prevalent among women with depressive symptoms; 71% versus 32% of controls (p < 0.0001).

Conclusions: This descriptive study suggests most women on hemodialysis may experience sexual problems. Additional research on the relevance of SD to the quality of life, well-being, and outcomes in these women is now required.

Funding: NIDDDK Support

FR-PO1682

Effect of Hypnosis on Anxiety, Depression, Fatigue and Sleepiness in People Undergoing Hemodialysis

Philippe Chaureau,1 Aurelie Untas,2 Catherine Dupre,3 Anne Kolko-Labaden,4 Nicolas Cazenave,4 Aurad-Aquitaine, Bordeaux, France; 1Universite Paris Descartes, Paris, France; 2Hopital Foch, Paris, France; 3CHU de Toulouse, Toulouse, France.

Background: Hypnosis has shown positive effects in stress related disorders (e.g., cancer, surgery, burns), but has not been studied in chronic renal disease. We investigated the effects of a hypnotic session on anxiety, depression, fatigue and sleepiness in patients undergoing hemodialysis.

Methods: The sample consisted in 29 patients (mean age 62.6 years, SD=16.8, 52% of men). Patients assigned to take part to a hypnotic session during a hemodialysis session. Anxiety, depression, fatigue and sleepiness were measured weekly with validated scales (Hospital Anxiety and Depression Scale, Multidimensional Fatigue Inventory, Epworth Sleepiness Scale). Fatigue was also measured daily using a numeric scale. Study participation lasted fifteen days. Hypnosis session took place on the eighth day.

Results: Depression was significantly associated to anxiety, fatigue and sleepiness. Fatigue was strongly correlated to sleepiness. Anxiety, depression and sleepiness significantly decreased after hypnotic, whereas fatigue remained constant.

Conclusions: This preliminary study shows encouraging results which suggest that hypnosis is an effective intervention to help hemodialysis patients face treatment consequences, such as feelings of negative emotions. Future studies should investigate the effect of a longer intervention getting people to do self-hypnosis.

FR-PO1683

Subjective Sleep Quality in Kidney Disease Patients

Sameer Shaker, Gabrielle R. Paolotti, Elizabeth K. Lee, Dahlia Raymon, Brett A. Tomlin, Sarah Ramer, Mark L. Unruh. University of Pittsburgh School of Medicine, PA.

Background: Sleep affects one’s quality of life. Patients’ subjective ratings of their sleep, however, do not correlate well with objective measures. This study examined predictors of subjective sleep quality in patients with CKD and ESRD compared to controls.

Methods: The cross-sectional sample consisted of 75 CKD and 77 ESRD patients as well as 224 controls from the Sleep-Strategies Concentrating on Risk Evaluations study. The outcome measure, the Pittsburgh Sleep Quality Index (PSQI), is a 19-item scale with a total score of 0-21. Lower scores indicate higher sleep quality. Patients underwent home polysomnography (PSG), filled out the Perceived Stress Scale-4 (PSS-4), a measure of life stress, and the Life Orientation Test-Revised (LOT-R), a scale of optimism. Other measured variables included demographics; anthropometrics; hypertension, diabetes, depression status; and medications.

Results: Mean age was 56.8±11.5 years. Males made up 56.3% and non-African-Americans 62.5% of the sample. The mean PSQI score for CKD patients was 6.5±3.7; for ESRD patients, 5.0±4.0; and for controls, 4.8±2.5 (p =0.06). No other differences were noted between the three groups. In univariate analysis, age was a strong correlate of SD; for each 1 year increase in age, sleep quality decreased by 1.4 (95% CI, 1.2-1.6) (p < 0.0001). Female sex (1.40, 95% CI 0.91, 2.17), ESRD (vs. control) (1.81, 95% CI 1.08, 2.81), and sleep efficiency (-0.03, 95% CI -0.06, -0.005) significantly predicted higher PSQI scores. In multivariate analysis, age, sex, race, and presence of diabetes were strongly associated with PSQI score. After adjusting for demographic variables, PSQI score was higher in ESRD patients (1.3, 95% CI 0.7-2.0). In multivariate regression analysis, age, sex, and ESRD were strongly associated with PSQI score.

Conclusions: ESRD patients experience significantly worse subjective sleep quality, as measured by the PSQI, than controls, but female sex and higher stress level are stronger predictors of worse sleep quality than any objective measure obtained from PSG. A full assessment of sleep quality must therefore include subjective and objective ratings.

Funding: NIDDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
FR-PO1684
The Prevalence of Sleep Disturbances (SD) in Pediatric Chronic Kidney Disease (CKD): A Report of the Midwest Pediatric Nephrology Consortium
Ira D. Davis,1 Laurence A. Greenbaum,2 Debbie S. Gipson,3 Lieling Wu,1 John D. Mahan.4* Medical Products, Baxter Healthcare Corporation, McGaw Park, IL;1,2Pediatrics, Emory University and Children’s Healthcare of Atlanta, Atlanta, GA;3Nephrology, University of Michigan, Ann Arbor, MI;4Pediatrics, Ohio State University, Columbus, OH.

Background: Although SD are common in adults with CKD, little is known about the prevalence of SD in pediatric CKD.

Methods: Between May 1, 2006 and July 1, 2008, a clinic-based survey of sleep habits and common symptoms (SD) of CKD was conducted in 159 school-aged CKD patients (pts) and or parent-proxy. Three sleep study groups of CKD were conducted: 1) no on dialysis, not transplanted (n=68), 2) dialysis (n=30), 3) functioning transplant (n=41). Four SD domains for SD were assessed: 1) Excessive daytime sleepiness (EDS) using pediatric modification of the Epworth Sleepiness Scale; 2) Sleep disordered breathing (SDB) sx using Pediatric Sleep Questionnaire; 3) Restless Legs Syndrome sx using a standard questionnaire; and 4) Insufficient Sleep duration based on normative data for age, gender. Pts and parent-proxy completed the Pediatric Quality of Life Inventory Version 4.0. 24 Core Scales questionnaire (PedsQL) to assess health-related quality of life (HRQOL).

Results: The overall characteristics of these pts were: mean (SD) age=13.8 (3.6) years, 59% male, 30.2% non-Caucasian, 50.9% congenital CKD; no significant difference between CKD study groups were noted (p > 0.05). Overall, 93 pts (58.5%) had sx of SD in at least one sleep domain, the most common being EDS (n=73) and SDB (n=37). Multivariate-adjusted logistic regression of associations (age, gender, CKD study group, CKD diagnosis, BMI z-score, PedsQL Total Score, and steroid use) with SD revealed that lower PedsQL Total Scores were associated with a higher likelihood of any SD (p < 0.001), SDB (p< 0.001), or EDS (p = 0.003). The association of a SD and a decrease in HRQOL scores was independent of the CKD pt study group.

Conclusions: SD are common throughout the spectrum of pediatric CKD and are associated with diminished HRQOL scores independent of the CKD pt study group.

Funding: Private Foundation Support

FR-PO1685
Sudden Discontinuance of On-Line HDF Clearly Proved the Clinical Advantage of Itself in Relieving Dialysis Related Symptoms Kunto Masakane, Dialysis Center, Yabuki Shima Clinic, Yamagata, Japan.

Background: On 11th march 2011, a terrible disaster smashed the north east part of Japan. Our facility is just next to the smashed area and we experienced 3 days of electric power failure. We had been treated many dialysis patients by on-line HDF; however, just after the disaster we were not able to continue on-line HDF for 18 days because we could not validate the quality of dialysis fluid as safe as enough for on-line HDF. Future, in current study we reviewed the changes in dialysis related symptoms through the discontinuance of on-line HDF.

Methods: In our facility 94 out of 159 chronic dialysis patients (59%) had been treated by on-line HDF for relieving their itchiness, restless leg syndrome and insomnia and so on. On-line HDF was stopped on 11th March 2011 and restarted on 28th March. The changes in their subjective symptoms were retrospectively monitored 2 or 3 weeks after the disaster and followed up 1 and 2 months later. Ninety one out of 94 patients admitted to be monitored about their symptoms.

Results: Forty-three out of 91 patients (47%) recognized recurrence of their symptoms or new onset dialysis related symptoms. The recurrence of the same symptom for that on-line HDF was introduced was observed in 37% of the patients. The frequent symptoms were itchiness (29%), irritable state (15%), fatigue (15%) and insomnia (7%), and its degree was different in each patient. These symptoms gradually disappeared as at 51% by 2 weeks later and the restart of on-line HDF, at 68% by 1 month, and 99% by 2months.

Conclusions: We have proposed that on-line pre-dilution HDF is effective to relieve the patients' dialysis related symptoms such as itchiness, irritability, insomnia and skin pigmentation. (NDT plus 3 suppl[28-35], 2010) However, this proposal is not based dialysis patients of their dialysis related symptoms such as itchiness, irritability, insomnia, (NDT plus 3 suppl)28-35, 2010) However, this proposal is not based

Funding: None

FR-PO1686
Trends in the Occurrence and Outcomes of Acute Non-Variceal Upper Gastrointestinal Bleeding in U.S. Patients with End-Stage Renal Disease (1998-2007) Juehe Yang,1,2 Tsung-Chun Lee,3 Maria E. Montez-Rath,1 Manisha Desai,4 Jane Paik,5 Glenn M. Chertow,6 Wolfgang C. Winkelmaier.7* Divisions of Nephrology, Stanford University School of Medicine, Palo Alto, CA;1 Division of Gastroenterology, Far Eastern Memorial Hospital, New Taipei, Taiwan;2,3General Medical Diseases, Stanford University School of Medicine, Palo Alto, CA;4Department of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan.

Background: Bleeding is a risk factor for acute nonvariceal upper gastrointestinal bleeding (ANVUGIB) and associated with poor outcomes. We examined the burden of ANVUGIB and its outcomes on dialysis patients.

Methods: Using the United States Renal Data System, we quantified the occurrence rate of 30-day mortality of ANVUGIB in 2 criteria in dialysis patients. We used Poisson and logistic regression to estimate occurrence rates of ANVUGIB and 30-day mortality from 1998-2007, respectively.

Results: 938533 patients contributed 2403545 episodes to 1000 person-years for stringent and lenient criterion respectively. Crude occurrence rates remained flat (stringent) or increased (lenient) over the decade; only adjustment for socio-demographics and comorbidities resulted in significant declining trends. Patients had higher hematocrits prior to and were more likely to receive blood transfusions during their ANVUGIB episodes in later years. Overall 30-day mortality was 11.7% and declined over time.

Annual changes in the occurrence of and 30-day mortality rate after ANVUGIB

Method:

Funding: None

FR-PO1687
Exercise Interventions in Chronic Kidney Disease: A Systematic Review and Meta-Analysis Sankar D. Navaneethan,1 George Thomas,1 Edgard I. Weihe,3 John P. Kirwan.2* Nephrology & Hypertension, Cleveland Clinic, Cleveland, OH;1Pathobiology, Lerner College of Medicine of Cleveland Clinic, Cleveland, OH.

Background: Exercise interventions improve physical performance and cardiovascular risk factors in the general population. Their beneficial effects are unclear in various stages of chronic kidney disease (CKD). We systematically reviewed the effects of exercise interventions in CKD.

Methods: We searched MEDLINE (1966- September 2010) and SCOPUS (September 2010) for relevant randomized trials comparing exercise interventions (aerobic, resistance or combination of aerobic and resistance regimen) in non-dialysis dependent CKD, dialysis and renal transplant recipients. Two reviewers independently extracted data on relevant outcomes from included studies. Results were summarized as mean difference (MD) with 95% confidence intervals (CI) using a random effects model.

Results: Thirty-three trials (3 non-dialysis dependent CKD, 25 dialysis and 5 renal transplant studies) were included; most were small and of short duration. When compared with control group, exercise interventions significantly improved peak oxygen consumption (11 trials, 467 patients; MD 4.79 ml/kg/min; 95% CI 3.31 to 6.26) and lowered systolic blood pressure (MD -4.76 mmHg; 95% CI -8.89 to -0.62), and diastolic blood pressure (MD -3.43 mmHg; 95% CI -5.76 to -1.10) at the end of treatment period among dialysis patients. There were no significant differences in the 6 minute walk time between exercise and control groups in the dialysis population. No significant heterogeneity was noted in these analyses. Few studies reported better health related quality of life with exercise use compared to the control groups.

Conclusions: Short-term exercise interventions improve physical performance and blood pressure and with possible beneficial effects on health-related quality of life in dialysis patients. Data among non-dialysis dependent CKD and renal transplant recipients are sparse. Larger, long-term and better quality studies exploring various exercise interventions are warranted.

Funding: Other NIH Support - National Center for Research Resources, Multidisciplinary Clinical Research Career Development Program Grant #: R24DK055211

FR-PO1688
Open Dissection Versus Laparoscopic Peritoneal Dialysis Catheter Insertion: A Randomized Prospective Comparison on Outcome and Economical Evaluation ZL Li,1 Ping Fu.2 Department of Medicine-Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.

Background: Peritoneal dialysis (PD) catheter malfunction owing to displacement is another important complication lead to technique failure besides peritonitis. Previous study indicated the success of PD depended more on placement technique than on catheter design. Currently available methods for catheter placement are principally including open dissection and laparoscopic insertion. The best method is still controversial while the laparoscopy is much more expensive. In this study, we compared the outcome and economical expense between the two methods.

Methods: We conducted a prospective randomized study in which patients underwent PD catheter placement by either the open dissection or the laparoscopic technique. Open dissection was performed under local anesthesia and laparoscopic insertion was performed by one general surgeon under general anesthesia. Tenclokhoff direct PD catheters were placed

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represents presenting author.

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in all patients and continuous ambulatory peritoneal dialysis was started at the third day of hospital stay. 

Results: Seventy-four patients were enrolled from January 2011 to April 2011. The mean hospital stay time was 13.2 days and 8.66 days in open dissection group and laparoscopic group respectively (P > 0.05). Fluid leakage was observed in 1 patient in open dissection group, but in no patients in laparoscopic group (P > 0.05). Bleeding occurred in 2 patients in open dissection group and in 1 patient in laparoscopic group (P > 0.05). Catheter displacement occurred in 5 patients in open dissection group and in 3 patients in laparoscopic group (P > 0.05). Neither group had exit site infection, peritonitis or death during the follow-up.

Conclusions: Compared to open dissection, although the operative cost is higher, laparoscopic placement does not increase the total hospital expense maybe due to the shortened hospital stay. Current study did not find which method could lower the perioperative complications and catheter displacement.

FR-PO1689

Sarpogrelate Hydrochloride Improves Skin Perfusion Pressure of Lower-Extremity in the Hemodialysis Patients with Peripheral Arterial Disease

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Background: Peripheral arterial disease (PAD) is more prevalent in patients on hemodialysis (HD), however, little is known about the effectiveness of drugs in HD patients. This study aimed to clarify the effects of sarpogrelate hydrochloride, a selective 5-IT antagonist, on HD patients with PAD comparing with clopiogrel.

Methods: We conducted prospective, randomized, open-label, multicenter trial for 6 months on HD patients with PAD. PAD was diagnosed by the clinical symptoms (Fortune classification: 1 or II) and positive sign of the following test: 1) ultrasonography,2) multidetector-row CT or angiography for arteries of lower legs, 3) ankle-brachial pressure index of either foot below 1.0 or skin perfusion pressure (SPP) below 50mmHg. All eligible patients (n=35) were divided into age-, and HD duration-matched two groups, with medication given sarpogrelate of 300mg/day (n=17) or clopiogrel of 200mg/day (n=18) followed by 6 months. We measured SPP before and 6 months after with serum levels of hsCRP, MDA-LDL, plasma levels of fibrinogen and pentosidine.

Results: There was no significant difference in the patient’s characteristics. At 6 months, SPP was increased in both groups (sarpogrelate: 43±17 to 55±15 mmHg, clopiogrel: 49±21 to 66±29 mmHg, p>0.05) and there was no difference in both groups before and after 6 months. The levels of hsCRP and fibrinogen showed no differences between both groups before and after 6 months. Plasma pentosidine levels were decreased in both groups (0.65±0.24 to 0.84±0.07 µmol/L, p<0.05) and there was no difference in both groups. Serum MDA-LDL level was not increased in sarpogrelate group, while significantly increased from 60.5±25.2 to 87.0±56.5 (P<0.05) in clopiogrel group (P<0.05). Heart rate was significantly increased in clopiogrel group from 77.1±3 to 83±16 bpm (p<0.05), but not in sarpogrelate group.

Conclusions: Sarpogrelate clearly improved SPP without increases in heart rate and serum MDA-LDL level in HD patients with PAD.

FR-PO1690

Predictors of Intradialytic Blood Pressure Drops in Hospitalized Hemodialysis Patients

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Background: Estimation of the hydration status of hospitalized and multimorbid hemodialysis (HD) patients by clinical examination only is often imprecise and can result in severe drops in blood pressure (BP). We analyzed whether a combination of bioimpedance spectroscopy (BIS), blood gas analysis (BGA), electrocardiogram (ECG) and clinical evaluation could predict BP instability during the course of HD treatments.

Methods: Prospective, non-interventional study in 72 hospitalized HD patients (38% female, age 65±15 yrs, BMI 25.5 ± 5.2; 38% diabetics), examined by BIS (hand-to-foot), ECG, BGA, BP and clinical evaluation every 30 min during a 4 hrs HD; 3 groups were separated: A: n=30, stable BP, no hypotensive symptoms (symptoms defined as dizziness, vomiting, nausea, numbness or an intervention like: Trendelenburg maneuver, stop blood flow of the procedure). The three groups differed significantly in the BP drop in BP > 25mmHg plus symptoms.

Results: Based on the clinical evaluation 40% of group C patients were considered to be hypervolemic and 50% to be euvolemic prior to the dialysis session. Clinical evaluation did not correlate with BIS measurements. The three groups differed significantly in the measured parameters:

<table>
<thead>
<tr>
<th>groups</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular resistance (Rex)</td>
<td>534±79¹</td>
<td>535±101²</td>
<td>625±95</td>
</tr>
<tr>
<td>Extracellular water (ECW) (ml)</td>
<td>19.4±2.6³</td>
<td>19.5±2.8³</td>
<td>65.3±3.3</td>
</tr>
<tr>
<td>Intracellular water (ICW) (ml)</td>
<td>24.5±4.5³</td>
<td>26.6±4.8³</td>
<td>21.7±6.7</td>
</tr>
<tr>
<td>Potassium [mmol/L]</td>
<td>3.9±0.04³</td>
<td>4.0±0.05</td>
<td>4.1±0.06</td>
</tr>
<tr>
<td>Hematocrit [%]</td>
<td>31±0.5²</td>
<td>31±0.5³</td>
<td>34±0.7</td>
</tr>
<tr>
<td>pH</td>
<td>7.34±0.05²</td>
<td>7.42±0.05</td>
<td>7.40±0.07</td>
</tr>
</tbody>
</table>

¹ p<0.05 between group A and C; ² p<0.05 between group A and B; ³ p<0.05 between group B and C

A measured ECW <18ml early in the course of the HD session identified patients with subsequent symptomatic BP drops (and so belonging to group C) with a sensitivity of 67% and a specificity of 66%.

Conclusions: HD patients who develop symptomatic intradialytic BP drops reveal different BIS and BGA profiles prior to the BP drops. BIS and BGA monitoring might help preventing BP drops in hospitalized HD patients.

FR-PO1691

Comparison of the Efficacy of Senna Glycoside Versus Lactulose in the Treatment of Chronic Constipation in Maintenance Hemodialysis Patients: Naetirat Kittiyanpanya,1 Bussabong Noolla,2 Bancho Sirapatip,3 Wanich Pyramun,4 Ouppatham Supasyndh.1 1Nephrology, Phramongkutklao Hospital; 2Radiology, Phramongkutklao Hospital; 3Gastroenterology, Phramongkutklao Hospital, Bangkok, Thailand.

Background: Chronic constipation is one of the frequent gastrointestinal symptoms in patients on both maintenance hemodialysis (MHD). There are several inevitable factors contributed to the symptom and often the patients are more likely to use laxatives. We aimed to compare the efficacy of senna glycoside and lactulose in treating chronic constipation in MHD patients and to demonstrate the colonic transit time among those.

Methods: The randomized, double blind, cross-over study in MHD patients at Phramongkutklao Hospital was conducted during July to December 2010. The MHD patients with chronic constipation matched to ROME III criteria were eligible and randomized to 2 arms; the senna glycoside (S) and the lactulose (L). The colonic transit time was performed by using radio-opaque marker before and after the study periods. The stool daily charts defined by ROME III criteria were evaluated as the tool during the study.

Results: Twenty-nine of patients per protocol were studied. Sixteen were male (55%) with average age 58.17±12.26 years. Median colonic transit time was 38.4±16.8±52.8 hours. Only 3 patients were reported having prolonged colonic transit time over 72 hours. There was no difference between the cathartic effect of senna glycoside and lactulose (58.6% vs. 75.9%, p=0.403). The S-group had higher abdominal cramp than the L-group (65.5% vs. 13.8%, p<0.001), while the L-group had higher abdominal bloating than the S-group (93.1% vs. 6.9%, p<0.001). The average doses related with adverse effect was 4.00±1.76 tablets for senna glycoside and 22.8±12 mL for lactulose. No serious drug adverse events were reported.

Conclusions: The present study shows that both laxative agents are effective in treatment of constipation in MHD patient. However, each of them may have different gastrointestinal adverse effects.

FR-PO1692

Dialyzability and Pharmacokinetics of Oral Levofloxacin in Infected Hemodialysis Patients

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Background: Pharmacokinetic parameters of the drugs are altered by infection, because increase of systemic cytokines during the infection changes metabolism and excretion of the drug in the body. This study was performed to evaluate the dialyzability and pharmacokinetics of oral levofloxacin simultaneously in infected hemodialysis patients, which has not been reported previously.

Methods: Seven infected maintenance hemodialysis patients due to pyelonephritis lacking residual renal function were enrolled. Levofloxacin (500 mg after hemodialysis session for the first day and 250 mg for the 4 hours before scheduled hemodialysis session on the 3rd day) was orally administered. On the 3rd day, blood was taken from arterial and venous sides before and 2 and 4 hours after session initiation. Another sampling was performed on the 5th day of the study. Drug concentration, hematocrit, and urea nitrogen concentration were measured. Dialyzability and pharmacokinetic parameters of levofloxacin were evaluated by arterio-venous difference method.

Results: All patients exhibited improved symptoms without major problems. Drug concentrations in all arterial samples were above MIC of targeted bacteria. Dialyzer clearance and elimination fraction were 57.8±3.9 ml/min/m2 and 64.1±1.6%, respectively. Apparent half-lives during and after dialysis session were 4.66±0.5 and 27.1±3.8 hours, respectively. Dialyzer clearance was positively correlated with urea reduction ratio and negatively correlated with serum albumin concentration. About 1/3 of the drug was removed by dialysis when administered 4 hours before the session.

Conclusions: Oral dosing of this drug at 500 mg on the 1st day, followed by 250 mg on the 3rd day, in infected maintenance hemodialysis patients provides safe drug concentration compatible with that of healthy subjects orally receiving 500 mg daily. Because a significant amount of the drug was removed, administration might be undertaken after the dialysis session.

Funding: Government Support - Non-U.S.
FR-PO1693

An Efficacy Study of Combined Hepatitis A and B Vaccine (TWINRIX®) in Non-Responders to Double Dose Hepatitis B Vaccination in Dialysis Patients Narotahma Reddy Aeddula,1 Robert Mark Black,1,2,4 George M. Abraham,1,5,7 Elizabeth Ann Normand,2 1Dept. of Medicine, St Vincent Hospital, Worcester, MA; 2Division of Renal Medicine, St Vincent Hospital, Worcester, MA; 3Division of Infectious Diseases and Geographic Medicine, St Vincent Hospital, Worcester, MA; 4Dept of Medicine, Univ. of Massachusetts Medical School, Worcester, MA.

Background: Dialysis is an established route of transmission for Hepatitis B virus (HBV). The Centers for Disease Control and Prevention recommends routine immunization in dialysis patients against HBV. An average of 64% of dialysis patients develops seroprotective antibodies with immunization when compared to 90-95% of healthy, immunocompetent adults. Recent reports indicate that combined hepatitis B and hepatitis A vaccine(Twinrix®, GSK, UK) may improve immunogenicity in healthy non-responders to hepatitis B vaccine. The purpose of our study is to determine whether Twinrix® results in increased hepatitis B surface antibody titers (anti-HBs) in dialysis patients who are non-responders to a primary course of double dose monovalent hepatitis B vaccine.

Methods: This pilot study was designed as a randomized controlled trial. Twenty three subjects (15 male) who were non-responders to an initial double dose monovalent hepatitis-B vaccine were recruited. Twelve patients were randomized to receive a double dose of Twinrix® at 0, 1 and 6 months (treatment arm) and 11 patients received repeat double dose monovalent hepatitis B vaccine at 0.1, and 6 months (control arm). The anti-HBs titer were determined before vaccination and 4 weeks after each dose.

Results: Of the 18 patients who completed the study, 7 of 9 (77.8%) in treatment arm and 5 of 9 (55.5%) in control arm had antibody titers >10 mIU/ml (Relative Risk [RR]=1.4 (95% CI 0.71-2.77). There was no difference in adverse events in either group.

Conclusions: Vaccination of non-responders to monovalent hepatitis B vaccine with combined hepatitis B and hepatitis A vaccine produced improved hepatitis B seroconversion rates in dialysis patients compared to monovalent hepatitis B vaccine. This is the first evaluation in a US population and merits larger studies to better evaluate this potential benefit.

FR-PO1694

Proteinase-Activated Receptor-2, a Novel Mechanism of Uremic Pruritus: A Pilot Study Sung Jin Moon,1 Sang Cheol Lee,1 Soo Young Yoon,1 Sung-Kyu Ha.1 1Department of Internal Medicine, Myongji Hospital, Kwandong University College of Medicine, Goyang, Korea; 2Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea.

Background: Pruritus is a common problem in ESRD patients. The underlying mechanisms are not yet fully understood. We examined the relationship of the proteolytic activity, expression of PAR2, and pruritus in the skin of ESRD patients.

Methods: The Skin of ESRD patients with pruritus (n=12) and without pruritus (n=4) were compared about activity of serine protease and expression of PAR2. The skin biopsy was conducted on the abdomen. In addition, the effect of soybean extracts treatment for 4 weeks containing protease inhibitor was examined in ESRD patients with pruritus (n=6). The status of pruritus was examined by pruritus score and VAS scale.

Results: Proteolytic activity and PAR2 expression was increased in ESRD patients with pruritus compared with those without pruritus. Soybean extracts treatment decreased the activity of proteolytic activity and expression of PAR2, and the severity of pruritus.

Conclusions: We suggested that the soybean extracts containing protease inhibitor is a new candidate for the treatment of uremic pruritus and the new pathogenesis based on the relationship of PAR2 and uremic pruritus.

FR-PO1695

Clinicopathological Study on 11 Patients with End-Stage Renal Disease Showing Intestinal Perforation Due to Cation-Exchange Resin Against Hyperkalemia Akira Kurosu,1 Keiko Kanemoto,2 Kensuke Joh.1 1Department of Legal Medicine, Dokkyo Medical University, Japan; 2Department of Nephrology, Kanto Rosai Hospital, Japan; 3Division of Pathology, Sendai Shukakuboken Hospital, Japan.

Background: The administration of cation-exchange resin (CER) of potassium absorbing agent such as sodium polystyrene sulfonate (Kayexalate) or calcium polystyrene sulfonate (Sevelamer HCL) is used extensively for treatment against hyperkalemia, especially in the patients with end-stage renal disease (ESRD). The purpose of this study to survey a rare side effect of CER.

Methods: The 11 patients with ESRD, who experienced intestinal perforation after oral administration of CER, were selected. Evidence of CER administration was documented by identifying basophilic polygonal crystals, which are characteristic of both Kayexalate and Sevelamer.

Results: Median age of the patients (male 7, female 4) was 72.8 years old (43-90 years). The site of perforation was sigmoid colon in 9 patients and rectum in 2 patients. Underlining diseases consisted of CGN in 8 patients and diabetic glomerulosclerosis in 3 patients. Regardless of underlying diseases, all patients showed the event of intestinal perforation during CKD stage 4 to 5D. The 8 patients and 3 patients were orally administrated with Kayexalate (without sorbitol) and with Sevelamer, respectively. Total dose of both CER was 30g/day. After emergent events, all patients were treated by Hartmann’s operation. Resected intestine was examined. Microscopically, all patients revealed scattered basophilic and polygonal crystals at necrotic perforating site. Diverticulitis was confirmed in all patients. Besides necrotic lesion, remaining intact mucosa penetrated between discontinuous muscularis propria suggesting underlying diverticulum.

Conclusions: Patients with ESRD have a common physiological background to generate a diverticulum. Even though the patients were treated only with CER without sorbitol, which can be one of the causes of colonic necrosis, an intestinal perforation occurred on the basis of diverticulum. The present study proposes that the clinicians can decrease the risk of intestinal perforation by avoiding administration of CER in the ESRD patients with diverticulum.

Funding: Government Support - Non-U.S.

FR-PO1696

The Incidence of Intestinal Perforation in U.S. Dialysis Patients Remained Unchanged after Approval and Subsequent Widespread Adoption of Sevelamer Hydrochloride Juyeh Yang,1,2 Tsung-Chun Lee,1,3 Maria E. Montez-Rath,2 Manisha Desai,4 Wolfgang C. Winkelmaier.5 1Division of Nephrology, Far Eastern Memorial Hospital, New Taipei, Taiwan; 2Division of Nephrology, Stanford University School of Medicine, Palo Alto, CA; 3Division of Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 4General Medical Disciplines, Stanford University School of Medicine, Palo Alto, CA.

Background: Concerns have been raised that sevelamer hydrochloride may increase the risk of intestinal perforation. We examined temporal trends of the incidence of intestinal perforation among U.S. dialysis patients between 1992 and 2005.

Methods: In the United States Renal Data System database, we studied dialysis patients between 1992 and 2005. We used ICD-9 diagnosis code 569.83 to ascertain events of intestinal perforation. We defined as spontaneous perforations those events that had no identifiable leading disease and no potential iatrogenic procedures. We used Poisson regression to model the annual number of intestinal perforations, and interrupted time-series analysis was used to test for any changes of incidence rates before versus after 1999.

Results: Overall, 1068594 patients contributed 2.85 Million patient-years. We observed 12518 events of intestinal perforation of which 7928 were considered spontaneous perforations. Annual incidence rates before and after adjustment for demographic and comorbid factors over time are shown in the figure.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Venous Needle Dislodgement Prevention in Hospital Based Hemodialysis

**FR-PO1697**

**Venous Needle Dislodgement Prevention in Hospital Based Hemodialysis**

*Martin F. Lascaro, Michael Bradley Andersen. Nephrology & Hypertension, Cleveland Clinic, Cleveland, OH.*

**Background:** Venous needle dislodgement (VND) is a serious complication of hemodialysis (HD) and, if undetected, can cause serious morbidity. VND is not a reportable statistic so precise incidence is unavailable. Prior reports of ~4 to 14 episodes/year in USA are likely an underestimate. Our hospital HD unit cares for high-acuity patients, frequently with altered mental status, with a higher than average risk for VND.

In February 2010, we introduced a quality improvement project aiming to reduce VND rates, with a goal of zero undetected VND episodes.

Internal data showed 3 undetected VND episodes during the prior 3 months (2 class IV hemorrhages), incidence of 1 VND per 538 HD treatments.

**Methods:** Components of the project were: 1) standardizing cleaning and disinfection procedure of the arteriovenous (AV) access and surrounding skin; 2) implementing a unique single-use sensor patch placed over the venous needle site where it will absorb blood if VND occurs and emitting an audible alarm; 6) modifying staffing levels with the addition of a quality control nurse, and ensuring adequate staff-to-patient ratios to allow routine AV access monitoring during HD.

**Results:** All staff were trained in a 1-month time frame and required to demonstrate proficiency in the above. These included physicians, nurses, HD technicians, risk management personnel, development of educational materials and technical support.

Between 2/15 and 12/31/2010 there have been zero undetected VND episodes and the overall occurrence dropped from 13 in 2009 to 4 in 2010 (incidence 1 VND per 1750 treatments).

**Conclusions:** In conclusion, prevention of VND in high-risk hospitalized HD patients is achievable with effective education, protocol standardization and ongoing monitoring. Minimization of undetected VND episodes can be aided by blood loss detection devices use.

**FR-PO1698**

**Lymphangiogenesis Develops during Peritoneal Fibrosis in Peritoneal Dialysis Patients and Rat Peritonitis Model**

*Hiroshi Kinashi,1 Yasuhiko Ito,1 Masashi Mizuno,1 Yasuhiro Suzuki,1 Fumiko Nagura,1 Taichi Sato,1 Naotake Tsuboi,1 Shoichi Maruyama,1 Eyu Imai,1 Yoshihumi Takei,1 Seichi Matsuo,1 Masahito Tamura,1 Junichi Nakamura,2 Nana Ishimatsu,2 Akihiro Kuma,2 Yutaka Otsuji.2 Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan; 3Department of Cardiology and Nephrology, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** Peritoneal fibrosis/sclerosis (PF) causes ultrafiltration failure (UFF) and is an important complication in long-term peritoneal dialysis (PD). We studied the roles of lymphangiogenesis and vascular endothelial growth factor-C (VEGF-C), which is a potentially important mediator of lymphangiogenesis, in the relationship between PF and UFF.

**Methods:** VEGF-C contents in human dialysate effluent (n=124) and VEGF-C tissue expression (n=69) were investigated by ELISA, real-time PCR and immunohistochemistry. VEGF-C production with TGF-β1 in Met-5A mesothelial cells and human mesothelial cells (HPMC) from the spent patient peritoneal dialysate (n=28) were studied. Expression of lymphatic vessels was examined by immunohistochemistry. We developed a rat model of PF induced by intraperitoneal injection of chlorhexidine gluconate (CG) every other day. Rats were treated with TGF-β1 Type 1 receptor inhibitor (TGFβRI). Lymphatic vessels and VEGF-C were evaluated by immunohistochemistry and real-time PCR.

**Results:** The dialysate-to-plasma ratio for creatinine (D/P Cr) was positively correlated with dialysate VEGF-C concentration. VEGF-C mRNA expression was 4.3-fold higher in peritoneal membranes with UFF than in pre-PD renal failure peritoneum. Lymphatic vessels and VEGF-C, which was detected in the mesothelial cells and some macrophages, were higher in the advanced fibrotic peritoneum. VEGF-C expression was upregulated by TGF-β1 in cultured Met-5A cells, which was specifically suppressed by TGFβRII. In cultured HPMC, TGF-β1 upregulated VEGF-C mRNA expression at 12 hours, and was correlated with D/P Cr (R=0.64, p<0.001). In the rat CG model, lymphatic vessels and VEGF-C expression were high, and significantly suppressed by TGFβRII.

**Conclusions:** Our results suggest that high peritoneal transport is associated with lymphangiogenesis and fibrosis via the TGF-β1/VEGF-C pathway.

**FR-PO1699**

**Effects of Icodextrin on Inter-Cellular Adhesion Molecule-1 Expression Via Protein Kinase C in Human Endothelial Cells**

*Masahito Tamura,1 Narutoshi Kabashima,1 Ryota Serino,2 Tetsu Miyamoto,3 Kaori Kanegae,1 Yumi Furuno,2 Junichi Nakamata,2 Nana Ishimatsu,2 Akihiro Kuma,2 Yutaka Otsuji.2 Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Fukuoka, Japan.*

**Background:** Intracellular adhesion molecule (ICAM)-1, which is one of the adhesion molecules, plays an important role in inflammatory processes of peritoneal damages in patients on peritoneal dialysis (PD) by mediating leukocyte-endothelial cell adhesion, leukocyte migration, and T cell-antigen-presenting cell interactions. We compared the effects of glucose and icodextrin on ICAM-1 expression in human endothelial cells.

**Methods:** We used icodextrin powder-dissolved culture medium to exclude the effects of ingredients of the PD fluid. Quiescent cultured human umbilical vein endothelial cells (HUVECs) were exposed to either glucose or icodextrin, and the expression levels of ICAM-1 were analyzed by flow cytometry using FACScan. Phosphorylation of protein kinase C (PKC) was analyzed by western blotting using antibodies against phosphorylated forms of PKC.

**Results:** High glucose levels induced ICAM-1 expression on cell surface of HUVEC in time- and concentration-dependent manners, while icodextrin did not influence the expression levels of ICAM-1. Glucose increased phosphorylation levels of PKC, and calphostin C, which is a specific inhibitor of PKC, completely suppressed glucose-induced PKC phosphorylation and ICAM-1 expression. Icodextrin had no effects on phosphorylation levels of PKC.

**Conclusions:** Our results indicate that glucose induces ICAM-1 by activating PKC, while icodextrin has no effects on PKC activation and ICAM-1 expression; hence, icodextrin-containing PDF has superior biocompatibility and does not influence leukocyte-endothelial cell adhesion in the peritoneum of patients on PD.
FR-PO1700
Effects of Short Dwell Versus Conventional Hemodialysis on Serum FGF-23 Levels
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Increased: FGF-23 levels have been associated with cardiac abnormalities, vascular calcifications and mortality in hemodialysis (HD) patients. However, it remains unknown whether the frequency and type of HD influence FGF-23 levels. 

Methods: We therefore compared FGF-23 levels as well as other biochemical variables between 15 patients (pts) undergoing short daily home HD (DHD) using the NxStage System® and 48 pts undergoing conventional in-center HD (CHD) (table). FGF-23 levels were measured using the 2nd generation Immunospect®-C-terminal assay.

Results: DHD pts were younger but there were no differences in the duration of end stage renal disease (ESRD). A greater number of DHD patients received vitamin D sterol therapy than CHD patients while there was no difference in calcimimetic therapy between groups. Overall serum calcium, phosphorus and intact parathyroid hormone (iPTH) levels were similar and phosphorus levels correlated with FGF-23 in both groups as expected; r=0.67 (p<0.005) and r=0.42 (p<0.003) in DHD and CHD respectively (figure). However, FGF-23 levels were lower in the CHD group despite greater use of vitamin D sterols.

Conclusions: These findings suggest that FGF-23 levels may be a more sensitive biomarker of cumulative phosphate burden than single phosphorus determinations. Future trials are needed to assess whether targeted reduction of FGF-23 by DHD will result in improved cardiovascular outcomes.

Funding: NIDDK Support

FR-PO1701
Effects of Toll-Like Receptor 2 on the Inflammatory Response and Cytokine Production from Intraperitoneal Polymer Catheters
Michael F. Flessner,1 Elise Peery Gomez-Sanchez,2 Xiaorong Li,2 1KUH, NIDDK, Bethesda, MD; 2Medicine, GV Montgomery VA Medical Center, Jackson, MS.

Background: To test the hypothesis that inflammation from a sterile intraperitoneal (ip) foreign body is mediated by Toll-Like Receptor 2 (TLR2), we compared the responses of normal C57Bl mice (C, n=12) and TLR2-Knock-out mice (TKO, n=20) to polyethylene catheter rings in solutions of saline (S), saline + 1.5 mg/ml fibrinogen (F), or saline + 5 mg/ml albumin (A) for 4 hours and placed 5 rings each into the peritoneal cavity of 18 C57Bl mice. After 1 week, the rings were aseptically recovered, and cells adhering to the catheters were separated using ultrasound and stained for immunocytochemical cell markers (ICC). Cells, catheter, and a swab from the abdomen were cultured for 96 hours to insure sterility. Abdominal wall tissues were collected after sacrifice and processed for CD31 (angiogenesis marker), Trichrome (peritoneal thickness), and immunohistochemistry for cytokines (IHC, 1=no stain to 4=heavy stain).

Results: Cell densities on the catheter material (10⁴/cells/cm², mean±SE) were: (A), 138.2±21.0 (S), 177.2±23.0; (F), 273.3±21.0 (p<0.002, 1-way ANOVA). ICC for macrophages (MacB F4/80) was lowest with (F), 2.0±3.3; (S), 2.7±3.5, and highest for (A), 3.7±2.3; (C), 2.9±1.9; and (F) (CD3, CD68, cytokeratin, vimentin) were similar for adherent cells. Tissue CD31 staining, peritoneal thickness, and IHC for Fibroblast Growth Factor and Transforming Growth Factor β were not different among the treatments (1-way ANOVA). IHC for Vascular Endothelial Growth Factor (VEGF), 2.5±2.5; 2.6±2.6; 1.9±2.6; p<0.004 and for α-Smooth Muscle Actin (αSMA), 3.4±2.8;3.5±2.2; F, 2.8±2.5; p<0.05 were significantly less for F than for S for αSMA and for S for F for VEGF. These findings suggest that Toll-Like Receptor 2 of PM cells, and indirectly, fibrinogen enhances overall cell adhesion, while decreasing the relative macrophage number when compared to saline or albumin solution; this also leads to decreased appearance of VEGF and αSMA in the peritoneal response at 7 days. We conclude that the addition of fibrinogen affects the foreign body response to polyethylene in mice.

Funding: Private Foundation Support

FR-PO1703
Interleukin-10 Prevents the Progression of Peritoneal Fibrosis
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Background: Peritoneal fibrosis (PF) is an intractable complication of peritoneal dialysis (PD). Interleukin-10 (IL-10) was reported to have anti-fibrotic effects; however, little is known about its effects on PF. In the present study, we examined the effects of IL-10 on PF.

Methods: Rat peritoneal mesothelial cells (RPMCs) purified from Sprague-Dawley (SD) rats were incubated with IL-10 for 48 h. Then, 50 nM methyglyoxal (MGO); an inciting agent of PF was added. After 24 h, the phenotype and signal transduction in RPMCs were analyzed by qRT-PCR. In vivo study: Male SD rats aged 6 weeks were injected intramuscularly with an AA type 1-based vector encoding IL-10 (AA-IL-10). The rats were injected with the vector encoding GFP (AA-GFP) or PBS served as controls. From the 28th day after the injection, PF fluid containing 10 mM MGO was injected intraperitoneally every day for 3 weeks. Then, the parietal peritoneum was sampled for analyses.

Results: In vitro study: IL-10 mitigated a decrease in epithelial markers (E-cadherin and occludin), an increase in mesenchymal markers (fibronectin and vimentin) and up-regulation of TGF-β1 and Snail induced by MGO in RPMCs. In vivo study: Histological analysis revealed marked fibrous thickening of the peritoneum in AA-GFP and PBS groups. In AA-IL-10 group, the thickness of the peritoneum was reduced to less than one-half of AA-GFP. Immunohistochemistry showed that epithelial markers and increased mesenchymal markers of the peritoneal cells were mitigated in AA-IL-10 group compared to AA-GFP or PBS group. qR-PCR analysis revealed that TGF-β1 and Snail expression levels in the peritoneum in AA-IL-10 group was less than 0.3-fold compared to those in AA-GFP or PBS group.

Conclusions: The results of present study suggested that IL-10 inhibited the progression of PF through the suppression of TGF-β1 signaling. AA-GFP mediated systemic IL-10 delivery would be a potentially powerful option for treatment of PF.

FR-PO1704
Protective Effect of Icordextrin on Mesothelia-to-Mesenchymal Transition (MMT) of Mesothelial Cells (MCs) Induced by Peritoneal Dialysis (PD) Liquids
Abelardo I. Aguilera,1 Pilar Sandoval-Correa,2 Rafael Selgas,1 Maria Luisa Perez Lozano,1 Manuel Lopez-Cabrera,1 1Biologia Molecular, Centro de Biologia Molecular Severo Ochoa, Madrid, Spain; 2Servicio de Nefrologia, Hospital Universitario de Madrid, Madrid, Spain; 3Servicio de Nefrologia, Hospital Universitario de Madrid, Madrid, Spain.

Background: MMT of MCs is a key process in the initiation of peritoneum membrane (PM) damage in PD. MCs exposed to glucose degradation products (GDPs), low pH and pro-inflammatory cytokines undergo on MMT. Some clinical studies suggest that Icordextrine (Ico) can show better biocompatibility. This study analyzes the effects of Ico on MMT of MCs as triggering of PM damage.

Methods: In the last 10 years the periodically cultured MCs from PD effluents. In MCs lysate we determined E and N-cadherin, Snail and α-SMA as MMT markers. Fibronectin and collagen-I as extracellular matrix-component (ECM) and VEGF as pro-angiogenic factor. These values were compared between the different PD liquids and clinical events. MCs from omentum (HPMO) were used to in-vitro experiments. In our PD model we are studying the effects of Ico on PM

Results: We included 157 PD patients of whom 137 were extracted. 63 cultures were from 23 patients using ico, 23 were from 12 using Dianel. 26 and 35 cultures

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
were from 9 and 13 patients using Physioneal 35 and 40, respectively. 66 cultures were classified as fibroblastoid phenotype, 59 as cobblestone and 12 as mixed or not grown. Transdifferentiated MCs showed higher levels of snail PCR, fibronectin, VEGF, TGF-β and IL-8. High Cr-MTC and lower UF capacity were also found in patients who drained these cells. Although there were 2 more cases of peritonitis in the Ico group, the frequency of MTAC was 50% compared to 75% of Dialose. As expected, Ico group showed lower values of EMC, VEGF and TGF-β and similar frequency of MMT than Physioneal. In HPMO, Ico partially inhibited the MMT induced by TGF-β and PD liquids but decreased ECM. Animal experiments are underway.

Conclusions: In PD Ico showed a protective effect on PM through a partial inhibition of MMT of MCs.

**FR-PO1705**

Are Matrix Metalloproteinase-2 and Plasminogen Activator Inhibitor-1 Determined by Peritoneal Transport or Local Production? Deirisia Lopes Barreto, Dirk Gijsbert Struijk, Raymond T. Krediet. Department of Internal Medicine, Division of Nephrology, Academic Medical Center, University of Amsterdam, Amsterdam, Netherlands.

**Background:** Solute transport in peritoneal dialysis occurs from the peritoneal microcirculation to the peritoneal cavity, and visa versa by diffusion and by hydrostatic, and osmotic pressure gradients. Peritoneal effluent contains clinical relevant substances derived from intraperitoneal production or transperitoneal transport or both. Matrix metalloproteinase-2 (MMP-2) is a glycoproteinase that cleaves denatured collagen, and complement other collagenases in the degradation of fibrillair collagens. Elevated intraperitoneal levels of plasminogen activator inhibitor-1 (PAI-1) have been demonstrated to be present in patients with intra-abdominal adhesions. Therefore, the aim of this study was to investigate the potential use of MMP-2 and PAI-1 in effluent as markers in the development of peritoneal alterations.

**Methods:** For this purpose the roles of peritoneal transport and local peritoneal production of these parameters were studied. This single center cohort study included 86 incident PD patients. All patients were treated with biocompatible dialysis solutions and underwent a standard peritoneal permeability analysis (SPA). The presence of local production as well as correlations between MMP-2, PAI-1 and peritoneal transport parameters were studied.

**Results:** Median effluent levels of 22.1ng/mL for MMP-2 and 0.95ng/mL for PAI-1 were found. Local peritoneal production averaged 93% of effluent MMP-2 concentration and 77% for PAI-1. Also, when expressed as ratio, D/P_MMP-2 over D/P_PA1 exceeded 1 and therefore local production could be established on top of transport. Furthermore, correlations between MTAC_creatine and MMP-2 (r=0.38, p<0.001) or PAI-1 (r=0.42, p<0.001) were present.

**Conclusions:** In this conclusion this study demonstrates that MMP-2 and PAI-1 pass the peritoneal membrane via peritoneal transport. However, the presence of local production was much more important. This data illustrates the potential of MMP-2 and PAI-1 in effluent markers in the development of peritoneal alterations.

**FR-PO1706**

**Indoxyl Sulfate and p-cresyl Sulfate Concentrations Increase in Incident Peritoneal Dialysis Patients along the Loss of Residual Renal Function Liesbeth Vlajner, Bjorn K. Meijers, Bert Bammens, Pieter Evenepoel. Nephrology, University Hospital, Leuven, Belgium.

**Background:** Residual renal function (RRF) is of critical importance for the clearances of p-cresyl sulphate (PCS) and indoxyl sulphate (Inds) in peritoneal dialysis (PD) patients. Besides other mechanisms, high serum PCS and Inds concentrations may contribute to the association between poor RRF and increased cardiovascular (CV) morbidity in PD patients. Studies evaluating the impact of RRF on serum levels of PCS and Inds have so far yielded conflicting results, probably related to small sample size and retrospective design.

**Methods:** We performed a prospective, observational cohort study in incident PD patients. Serum concentrations, mass removal and total, renal and dialytic clearances of creatinine, urea nitrogen, PCS, and Inds were assessed 1, 6, 12, and 24 months after start of PD. Data from 35 patients (19 male, age 55±17 year) with a technique survival exceeding 2 years were analyzed.

**Results:** Serum concentrations of PCS and Inds increased with dialysis vintage, along the decline in RRF. Conversely, mass removal of both toxins remained stable.

**FR-PO1707**

**Baroreceptor Function Is Similar in Chronic Peritoneal Dialysis and Hemodialysis Patients**

Dvora Rubinger, Ilana Harel, Dan Sapoznikov. Nephrology and Hypertension Services, Hadassah University Medical Center, Jerusalem, Israel.

**Background:** The baroreflex function (BRS), a measure of the autonomic nervous system activity was not well characterized in patients with end-stage renal disease on chronic peritoneal dialysis treatment (CCPD/CAPD).

**Methods:** To assess BRS indices, continuous beat-to-beat intervals (IBI) and systolic blood pressure (SBP) were monitored using the Finometer equipment in a group of patients on chronic peritoneal dialysis treatment (CCPD/CAPD, n=10) as compared with age matched patients on chronic hemodialysis (HD, n=91), and control subjects (C, n=27).

**Results:** Mean SBP and IBI variability, BRS indices and BEI (baroreflex effectiveness index) were (median and interquartile range) Table 1.

**FR-PO1708**

Is the Sodium Restriction Harmful or Beneficial in the Long-Term Peritoneal Dialysis? Jie Dong, Yanjun Li, Rong Xu. Institute of Nephrology, Peking University, Beijing, China.

**Background:** Sodium restriction is routinely recommended for patients on peritoneal dialysis (PD). However, a very low sodium intake was shown to be associated with protein-energy wasting and high mortality in patients on PD most recently. We aimed to determine whether a reduction in sodium intake in the early stage of PD is associated with a decline in dietary and nutritional parameters, and with a high risk for mortality.
Methods: A total of 305 incident patients were enrolled in our single-center cohort study. All patients were followed until death or censored. Demographic data was collected at baseline. Biochemical, dietary and nutrition data were examined at baseline and thereafter at regular intervals. Three groups of patients were defined according to the change of sodium intake over the first two quarters: one in which sodium intake decreased, one in which sodium intake remained stable, and a third group in which sodium intake increased.

Results: Participants with decreased sodium intake tended to be younger, non-diabetic and less inflammatory status. There were no significant differences in the longitudinal change of dietary protein and energy intake, serum albumin and lean body mass during the long-term follow-up between groups. The decreased sodium intake significantly predicted a lower risk for cardiovascular mortality with HR of 0.57 (0.38–0.86) and for first cardiovascular event with HR of 0.68 (0.52–0.88) after adjusting for recognized confounders.

Conclusions: Our study revealed that decreased sodium intake in the early stage of PD was not associated with declined dietary and nutritional status in the long term. The decreased sodium intake independently predicts a lower risk for cardiovascular death and first cardiovascular event.

FR-PO1709

Sleep-Disordered Breathing, Restless Legs Syndrome and Daytime Sleepiness in Automated Peritoneal Dialysis Maria-Eleni Roumelioti, Christos Argyropoulos, Filitsa H. Bender, Beth M. Piraino, Mark L. Unruh. Renal and Electrolyte, University of Pennsylvania.

Background: Sleep-Disordered Breathing (SDB), Restless Legs Syndrome (RLS), and excessive daytime sleepiness (EDS) are highly prevalent among hemodialysis (HD) patients (pts). The burden of these conditions remains poorly defined among Automated Peritoneal Dialysis (APD) pts.

Methods: APD pts were matched to pts with CKD (MDRD eGFR<40 ml/min) on HD and healthy control participants from the Sleep-SCORE Study of sleep and cardiovascular risk with respect to age, gender, and BMI. We used in-humane unattended polysomnography to measure total sleep time (TST), sleep efficiency (SE) (TST as a proportion of the time spent in bed), Apnea/Hypopnea Index (AHI, apneas and hypopneas/hour) and Arousal Index (AI, microarousals/hour). EDS was defined by a score≥10 on the Epworth Sleepiness Scale (ESS). Presence of RLS was examined with the Hopkins RLS Diagnostics Questionnaire. Results: A total of 88 pts were studied (22 in each group): Patient Characteristics

<table>
<thead>
<tr>
<th>Group</th>
<th>APD</th>
<th>CKD</th>
<th>HD</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>42±14</td>
<td>47±12</td>
<td>50±6</td>
<td>52±6</td>
</tr>
<tr>
<td>Women</td>
<td>16(73%)</td>
<td>15(68%)</td>
<td>16(73%)</td>
<td>16(73%)</td>
</tr>
<tr>
<td>African Americans</td>
<td>11(50%)</td>
<td>9(46%)</td>
<td>11(50%)</td>
<td>11(50%)</td>
</tr>
<tr>
<td>BMI</td>
<td>26±6.6</td>
<td>30±7</td>
<td>28.8±6</td>
<td>31±7</td>
</tr>
<tr>
<td>Waist</td>
<td>98±17</td>
<td>107±21</td>
<td>104±18</td>
<td>96±18</td>
</tr>
<tr>
<td>Circumference (cm)</td>
<td>36±6</td>
<td>37±6</td>
<td>40±6</td>
<td>37±3</td>
</tr>
<tr>
<td>Neck</td>
<td>34±15</td>
<td>14±22</td>
<td>14±32</td>
<td>12±15</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134±15</td>
<td>147±22</td>
<td>148±32</td>
<td>127±15</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>85±16</td>
<td>82±14</td>
<td>80±11</td>
<td></td>
</tr>
</tbody>
</table>

HD pts had the lowest TST (302±114 min) and SE (69±17%) among the 4 groups. APD pts were similar to controls (TST: 355±113 min vs 366±102 min p=0.78, SE: 72±19% vs 74±13% p=1). The prevalence of EDS was 45% and did not differ among the 3 groups ($\chi^2=0.32$). AHI and AI (SBDB) scores were not different among the groups.

FR-PO1710

Efferent Markers and Epithelial Mesenchymal Transition in Transition in PD Sonoo Mizuiri,1 Ken Sakai,2 Yasushi Ohashi,2 Yoshihide Tanaka,2 Yasunori Suzuki,2 Yoshinari Hattori,2 Atsuhiko Mutou,2 Yoshiko Nishizawa,1 Kenichiro Shimamoto,1 Atsushi Aikawa.2 Division of Nephrology, Ichiyokai Harada Hospital, Hiroshima, Japan; 2Nephrology, Toho University School of Medicine, Tokyo, Japan.

Background: Epithelial mesenchymal transition (EMT) of peritoni cellular cells is important for peritoneal deterioration in PD. It is reported that hepatocyte growth factor (HGF) and bone morphogenic protein-7 (BMP-7) ameliorate EMT, but the clinical significance of effluent HGF and BMP-7 levels remain unclear. It has also been reported that diacylate growth factor levels should be measured relative to the level of cancer antigen 125 (CA125).

Methods: We examined the relationship between peritoneal solute transport rate (PSTR) and effluent markers related to EMT with adjusted values for efferent cancer antigen 125 (CA125). One hundred five incident peritoneal dialysis (PD) patients on PD for 25 (12-68) months with biocompatible solutions were included in the study. Fast peritoneal equilibration test was used to evaluate PSTR. Effluent hepatocyte growth factor (HGF), bone morphogenic protein-7 (BMP-7), vascular endothelial growth factor (VEGF), interleukin-6 (IL-6) and CA125 at 4h were measured.

Results: Clinical data are shown in the table.

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients with high transport rate</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td>14</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td>58 (44-68)</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87±28</td>
<td>82±14</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134±15</td>
<td>147±22</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>167±22</td>
<td>167±22</td>
</tr>
<tr>
<td>Neck (cm)</td>
<td>34±15</td>
<td>14±22</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>85±16</td>
<td>82±14</td>
</tr>
<tr>
<td>CA125 (ng/ml)</td>
<td>31±7</td>
<td>31±7</td>
</tr>
</tbody>
</table>

Conclusions: Effluent HGF levels as a compensatory mechanism is a marker of peritoneal deterioration, but controversy remains regarding the adjustment of markers for CA125. Funding: Private Foundation Support

FR-PO1711

Biological Effects Research of Maltese Peritoneal Dialysis Solution Zhanjun Shu,1 You-Ming Peng,1 Lin Sun,2 Li Xiao,3 Guanghui Ling,1 Fu-You Liu,1 1Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha; 2Departments of Pathology and Medicine, Northwestern University, Chicago, IL.

Background: Over the past 15 years, peritoneal dialysis (PD) has undergone considerable development from a technological point of view, and osmotic agent has played the essential role in peritoneal dialysis fluid.

Methods: We set up a method to quantitative the maltose by HPLC. Rabbits were enrolled as acute renal failure model to study transport capability in small molecule solute. Aneu non-uremic rat model of long-term peritoneal dialysis were enrolled to research the influence of peritoneum structure in long-term peritoneal dialysis.

Results: The glucose and maltose concentrations can be determined with a recently established HPLC methods . The elution peak retention time of glucose and maltose is 4.85min and 4.112min respectively. In our study we select 2.5% and 4.25%maltose peritoneal dialysis solution as subject investigated. The osmotic pressure of maltose is lower than that of glucose but higher than that of Icodextrin. The PH of maltose is higher than that of glucose but lower than that of Icodextrin.

Conclusions: The net ultrafiltration of maltose peritoneal dialysis solution excelled equal concentration glucose peritoneal dialysis solution in 4h. Meanwhile the net ultrafiltration of 4.25%maltose peritoneal dialysis solution excelled 7.5%Icodextrin. In 8h the net ultra filtration of maltose peritoneal dialysis solution still excelled equal concentration glucose peritoneal dialysis solution, but lower than that of Icodextrin.

Funding: Government Support - Non-U.S.
The following data was documented from the patients' record: age at the initiation of PD, among them, patients with < 2 years of follow-up were excluded. BMD measurement was performed to confirm the independent association of obesity indices with CA. In addition, the following histological characteristics: mesothelial denudation, fibrosis, acute inflammation, and podoplanin.

The following findings were significantly more common in EPS: FLC deposits (p=0.04), acute and chronic inflammation (p=0.03), fibrin deposits (0.04) and cellularity (p=0.02) as significant histological parameters. Inclusion of podoplanin varied in different parameters according to binary logistic regression: podoplanin vascular (p=0.008), calcium deposits (p=0.02), cellularity (p=0.02) and chronic inflammation (p=0.006).

The following histological characteristics were significantly more common in EPS: FLC deposits (p=0.04), acute and chronic inflammation (p=0.03), fibrin deposits (p=0.04), and cellularity (p=0.02) as significant histological parameters. Inclusion of podoplanin (p<0.03), positive iron staining (p=0.05) and IHC podoplanin vascular (p<0.001), podoplanin (p<0.001), meothelial denudation (p<0.001), decreased cellularity (p=0.008), fibrin deposits (p=0.02), cellularity (p=0.02) and chronic inflammation (p=0.006).

Visceral Fat is known to be more metabolically active and to be associated with atherosclerosis, inflammation, and insulin resistance. However, the impact of visceral fat on cardiovascular disease in patients on peritoneal dialysis (PD) has never been elucidated. This study was conducted to investigate whether visceral fat thickness (VFT) has a predictive role in carotid atherosclerosis (CA) determined by carotid intima-media thickness (cIMT) in PD patients.

Methods: A cross-sectional study was undertaken in 88 prevalent PD patients. Body mass index (BMI) and waist circumference (WC) were measured as anthropometric indices of obesity. VFT and subcutaneous fat thickness (SFT) were determined by sonographic measurement of abdominal fat. CA was defined as increased cIMT (>1.0 mm) or presence of plaque. Logistic regression analysis (age and sex-adjusted, and multivariate-adjusted) was performed to confirm the independent association of obesity indices with CA. In addition, various biochemical markers for inflammation and insulin resistance according to VFT were compared by ANOVA.

Results: Thirty-two patients (36.3%) had CA. Patients with CA had significantly higher VFT, BMI, and WC compared to patients without CA. In univariate logistic analysis, BMI, WC, and VFT, but not SFT, were significant risk factors for CA. However, multivariate analysis revealed VFT was an independent factor associated with CA after adjusting for demographic, biochemical parameters, and anthropometric indices (per 1 mm increase; OR, 2.294; 95% CI, 1.048-5.021; P=0.038). When the patients were divided into three groups according to VFT, log high-sensitivity C-reactive protein levels, fibrinogen concentrations, and HOMA-IR were significantly higher in the 3rd quartile compared to the other tertiles.

Conclusions: VFT, not SFT, is independently associated with CA in PD patients. Therefore, sonographic measurement of VFT could be useful to stratify the risk of cardiovascular disease in these patients.

Low Calcium Dialysate as a Risk Factor for Decline in Bone Mineral Density in Female Patients on Peritoneal Dialysis: A Single-Centre Retrospective Observational Study
Seokhui Kang, Jun-Young Do, Kyu-Hyang Cho, Jong-Won Park, Kyung-Woo Yoon. Division of Nephrology, Department of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.

Background: Previous studies have showed that low calcium dialysate (LCD) induce an increase of serum intact-parathyroid hormone (i-PTH) in PD patients. There are few reports on the effects of LCD for decline in bone mineral density (BMD) in PD patients. Methods: We reviewed the medical records at Yeungnam University Hospital in Korea and identified all the female patients who received LCD and had been treated with peritoneal dialysis. Among them, patients with < 2 years of follow-up were excluded. BMD measurement was performed yearly by the Hologic (Discovery Wi). One hundred ten patients were enrolled. The following data was documented from the patients' record: age at the initiation of PD, underlying disease, the time of started dialysis, and sex. Results: Two forty two patients were enrolled in LCD and 86 patients were underdialyzed with standard calcium dialysate (SCD). The mean age was 49.3 ± 11.4 years old in low calcium dialysate group and 49.5 ± 13.7 years old in standard calcium dialysate group. Total BMD (g/cm²) was 1.02 ± 0.13, 1.02 ± 0.13 and 1.00 ± 0.12 at baseline, 1 year and 2 years after the initiation of PD. There was a significant decrease in the BMD between 1 and 2 years after the initiation of PD. There was a significant decrease in the BMD between 1 and 2 years after the initiation of PD. Time averaged intact-PTH was 249.4 ± 161.3 in LCD group and 141.3 ± 118.9 in SCD group (p=0.009). Six episodes resulted in relapse peritonitis and 10 (18%) proved refractory to treatment. In 5 cases (9%) no pathogen could be identified on culture. Because PMN ratio was <50% in these 5 patients, they were treated with antibiotics. All five recovered readily.

Conclusions: Immediate availability of differential WBC counts in PD effluents, using automated hemocytometric analysis, prevents unnecessary treatment of PD associated peritonitis with antibiotics in 10% of presenting cases.
Conclusions: Low calcium dialysate is a risk factor for decline in BMD. LCD may be associated with increment of ALP and intact-PTH. Therefore, LCD should be carefully used for female PD patients with risk of decline in BMD.

FR-PO1717
Neutrophil Gelatinase-Associated Lipocalin in Peritoneal Dialysate Effluent as a Marker of Acute Episode of Peritonitis Francesca K. Martino,1 Pierluigi Di Loreto,1 Illenia Filippi,2 Maria Pia Rodighiero,1 Claudio Ronco.1,2 Nephrology, Dialysis and Kidney Transplant, San Bortolo Hospital, Vicenza, Italy; 1IRRI, Vicenza, Italy.

Background: The major infective complication of peritoneal dialysis (PD) is the development of peritonitis. Serum neutrophil gelatinase-associated lipocalin (NGAL) was reported as biomarker of bacterial infection and a previous study of Leung showed its increase during the first days of peritonitis. The aim of study is to access the utility of NGAL in peritoneal dialysate effluent (dNGAL) for the diagnosis of peritonitis in PD patients.

Methods: In this cohort study dNGAL concentration (evaluated by Archicet NGAL assay Abbott) was measured in heterogeneous group of PD patients (n=57) with or without any suspect of peritonitis. Moreover we evaluated CRP, Procalcitonin in the blood and lesocytes in the blood and in PD effluent. The episodes of peritonitis are defined in agreement with the guidelines of the International Peritoneal Dialysis Society.

Continuous variables were presented as the median values and interquartile range (IQR). The Mann-Whitney U tests was used to compare continuous variables. Binary regression analysis was performed to study the ability of variables to predict peritonitis and ROC analysis was used to calculate area under curve (AUC) for biomarkers. All statistical analysis were performed with SPSS version 17.0.

Results: During 8 months of observation, we had 38 peritonitis. In univariable analysis CRP and dNGAL were significantly associated with peritonitis, OR of 1.5 (p=0.02) and 1,008 (p=0.01), respectively. In multivariable analysis, only NGAL (p=0.042) in PD effluent was independent predictor of peritonitis (HR, 1,007). AUC for dNGAL was 0,988 while AUC for CRP was 0,792.

Conclusions: In our analysis, dNGAL could be used as a potential biomarker of peritonitis in PD patients.

FR-PO1718
Non-Infectious Complications of Fistulas in Patients Receiving Frequent Hemodialysis Deborah Lynn Zimmerman,1 Sarah Daisy Kosa,2 Christopher T. Chan,1 Charmaine E. Lok.1 Ottawa General Hospital, Ottawa, ON, Canada; 2Toronto General Hospital, Toronto, ON, Canada.

Background: Frequent hemodialysis includes short daily dialysis (SDD), frequent conventional hemodialysis (FHD) and nocturnal dialysis (NHD). FHD is associated with many beneficial clinical outcomes. There is a paucity of data directly comparing these modalities in terms of vascular access complications, interventions, and fistulas (AVF) survival.

Methods: Patients who received SDH (>5x/wk), FHD (4 days/wk; <4 hrs/session) and NHD (>3 x/week; >5 hrs/session) who were dialyzed with a catheter (CVC), AVF, or graft (AVG) were prospectively followed between Jan 2001-Dec 2010. The intervention rate for each type of vascular access between FHD, SD, and NHD were compared. AVF survival in SDH vs. NHD were compared by Kaplan Meier analysis (log rank test) using SAS, v.9.2.

Results: 193 accesses were studied.

<table>
<thead>
<tr>
<th>Fistula</th>
<th>FHD (n=19)</th>
<th>SDH (n=46)</th>
<th>NHD (n=128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graft</td>
<td>2 (11%)</td>
<td>1 (2%)</td>
<td>6 (5%)</td>
</tr>
<tr>
<td>Catheter</td>
<td>7 (36%)</td>
<td>13 (28%)</td>
<td>54 (42%)</td>
</tr>
</tbody>
</table>

AVF interventions (avg,pet) 1.28 0.93 1.45

On average, more AVF are attempted and used in NHD (0.51 pet) compared with SDH (0.20 pet); p<0.0001. Patients cannulating AVF with buttonhole technique needed more retraining than rotating site cannulation. On average there were more AVG interventions (for stenosis and thrombosis) in NHD than in SDH (p=0.04). However, the overall intervention rates and complication rates did not differ between NHD and SDH. There was longer AVF survival for NHD patients compared with both frequent FHD and SDH (p=0.004)

Conclusions: Patients on frequent dialysis have similar vascular access interventions. AVF in patients on NHD have greater patency compared with SDH and FHD. Further evaluation of the impact of patient characteristics and prior dialysis history on AVF outcomes in frequent dialysis is required.

Funding: Clinical Revenue Support

FR-PO1719
Impact of Early Decline in Residual Renal Function on the Body Composition Changes and Clinical Outcomes during the First Year in the Continuous Ambulatory Peritoneal Dialysis Patients Kyu-Hyang Cho,1 Jun-Young Do,1 Seokhui Kang,1 Jong-Won Park,1 Kyung-Woo Yoon,1 Tae Woo Kim,2 Internal Medicine, Yeungnam University Hospital, Daegu, Republic of Korea; 2Internal Medicine, Soonchunhyang University Gumi Hospital, Gumi, Republic of Korea.

Background: Preservation of residual renal function in peritoneal dialysis patient is essential to improve clinical outcomes. Therefore, the authors investigated the effect of rapid decline of residual renal function in early period on clinical outcomes and body composition changes in CAPD patients.

Methods: Among new incident CAPD patients from May 2001 to December 2009 in our hospital, 200 patients who finished 12 month protocol (male: 103, mean age: 50.0 ± 13.2 years, DM: 94) were analyzed. Patients were assigned to high GDP group (n=103, Dianeal® and Stay-safe®) and low GDP group (n=97, Physioneal® and Balance®). We defined early RRF decline group (n=68) as more than 5 ml/min decline in RRF at the first month after initiation of PD from GFR just before initiation of PD. Clinical indices and UFV during the PET were measured at the first, 6th and 12th month. Body composition including LBW and fat mass were measured using BIA at the first and 12th month.

Results: 1) Baseline characteristics between early RRF decline group and non-early RRF decline group were not significantly different. 2) Incidence of early RRF decline group was 34%. There is significant positive correlation between RRF at the first month and RRF at the 12th month .3) Early RRF decline group showed significant lower RRF at the 6th and 12th month, and also significant higher CRP and lower serum albumin at the 12th month than non-early RRF decline group. Early RRF decline group showed significant lower BW, TBW and LBW at the 12th month than non-early RRF decline group. There were no significant differences in RRF at the 12th month between the high GDP and the low GDP group.

Conclusions: Early decline of RRF in the first month could be associated with body composition changes and clinical outcomes during the first year in CAPD patients. It is suggested that preservation of RRF in early period of peritoneal dialysis is important to improve clinical outcomes and nutritional status.

FR-PO1720
Adequate Kt/V and Its Practical Data in Infants Receiving Peritoneal Dialysis Shojoiro Okamoto, Tomoyuki Sakai, Riku Hamada, Yuko Hamasaki, Kenji Ishikura, Hiroshi Hataya, Masataka Honda. Department of Nephrology, Tokyo Metropolitan Children’s Medical Center.

Background: In children who are receiving peritoneal dialysis (PD), the adequate Kt/V urea/week remains controversial. The KDOQI Guidelines recommendation that an adequate Kt/V urea/week is above 1.8/week. The Japanese guidelines recommend a value above 2.5/week for children and above 3.0/week for infants.

Key: TH- Thursday; FR- Friday; SA- Saturday; OR- Oral Abstract; PO- Poster; PUB- Publication Only

Underline represents presenting author.
Methods: In our institution, the target BUN level is below 70 mg/dL for children who are receiving PD and adequate nutrition. We studied infants who were receiving ambulatory PD treatment during the 1-year period from January 2009. In these patients, we analyzed the Kt/V urea/week and n-PNA (neutralized-protein equivalent of nitrogen appearance). Residual kidney function (RFK) was also evaluated.

Results: Nine patients were studied, including 4 with RFK. The median age was 5.0 years. Average Kt/V urea/week was 2.83 (2.66 in patients with RFK, 2.97 in anuric patients). Average n-PNA was 1.31 g/kg/day (1.34 g/kg/day in patients with RFK, 1.29 g/kg/day in anuric patients). Five patients received nightly intermittent PD (4 had RFK), 2 received continuous PD (both were anuric), and 2 received continuous ambulatory PD (both were anuric). The average BUN level in patients with RFK was 64.05 mg/dL, which was higher than that in anuric patients (61.4 mg/dL). The average ultrafiltration volume of PD was higher in anuric patients. The average rate of increase in the height standard deviation was 0.11. The average intraperitoneal volume was 895 ml/m². Only 1 patient had a complication of inguinal hernia.

Conclusions: In our series, the Kt/V urea/week of infants who were receiving PD was higher than the value recommended by the K/DOQI guidelines. Growth was satisfactory under appropriate nutrition, without complications. In anuric patients, the elevated level of Kt/V was consistent with the lower level of BUN, suggesting that the Kt/V resulted from other determinants of the PD prescription, such as ultrafiltration volume. To perform PD appropriately in infants, not only the Kt/V, but also growth, nutrition, membrane permeability, and ultrafiltration volume should be comprehensively considered.

FR-PO1721


Background: Extracorporeal removal of B2M using high-flux hemodialyzers is largely hindered by the compartmentalization of its distribution volume. While the CONTRAST study has recently demonstrated that therapies using enhanced convective transport can reduce serum B2M levels in patients with and without RRF (Penne et al, CJASN 2010), an equivalent analysis is not yet available for frequent HD therapies.

Methods: We used a variable-volume, two-compartment kinetic model (Ward et al, K1 2006) to calculate weekly mean pre-treatment serum B2M concentrations (MPC) with in-center (ICHD), short-daily (SDHD), and nocturnal (NHD) HD therapies in patients with increasing degrees of residual renal B2M clearance (RRC-B2M). The modeled B2M parameters were the intercompartmental mass transfer coefficient (40 ml/min), generation rate (0.17 mg/min), non-renal clearance (3 ml/min), and distribution volume (13.3 L) (Clark et al, JASN 1999). The RRC-B2M considered ranged from 0 to 4 ml/min. Dialysis frequency and duration were: 3 times/week and 4 hours of treatment (3X/4hr) (ICHD), 6X/3hr (SDHD), and 6X/8hr (NHD). A dialyzer clearance rate of 50 ml/min was assumed.

Results: Considerable reductions in MPC were achieved with both frequent therapies compared with ICHD. Similar, less pronounced, reductions were also found when RRC-B2M was gradually increased from 0 to 4 ml/min. NHD in patients without RRF and ICHD in patients with RRC-B2M of 4 ml/min resulted in comparable reductions.

Conclusions: These simulations show that frequent HD therapies may help achieve considerable reductions in serum B2M levels in patients with and without RRF.

Funding: Pharmaceutical Company Support

FR-PO1722

PD-Associated Peritonitis as a Risk Factor for the Development of Peritoneal Dialysis: Peritoneal Dialysis - Hideki Nosaka1 Michiko Bessho,2 Toshiya Takeda,3 Fumiaki Nogaki,1 Yoshiaki Iiogawa,1 Tomomi Takeda,4 Keiko Nomura,5 Yoko Matsuo,6 Noriko Morik,7 Takahiko Ono.1 1Shimada Municipal Hospital, Shimada, Japan; 2University of Shizuoka, Japan; 3Kyoto Takeda Hospital, Kyoto, Japan; 4Shizuoka General Hospital, Shizuoka, Japan.

Background: Fibrin deposition was frequently observed in peritoneal fibrosis induced with long-term peritoneal dialysis (PD). Factor V in its active form (Va) serves as a membrane-bound coagulant to factor Xa, which facilitates activation of prothrombin to thrombin. Consequently, we have hypothesized that factor Va is the major contributor to factor X depletion, while factor X does not play a significant role in consideration of the peritoneum.

Methods: We used a variable-volume, two-compartment kinetic model (Ward et al, K1 2006) to calculate weekly mean pre-treatment serum B2M concentrations (MPC) with in-center (ICHD), short-daily (SDHD), and nocturnal (NHD) HD therapies in patients with increasing degrees of residual renal B2M clearance (RRC-B2M). The modeled B2M parameters were the intercompartmental mass transfer coefficient (40 ml/min), generation rate (0.17 mg/min), non-renal clearance (3 ml/min), and distribution volume (13.3 L) (Clark et al, JASN 1999). The RRC-B2M considered ranged from 0 to 4 ml/min. Dialysis frequency and duration were: 3 times/week and 4 hours of treatment (3X/4hr) (ICHD), 6X/3hr (SDHD), and 6X/8hr (NHD). A dialyzer clearance rate of 50 ml/min was assumed.

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Conclusions: These simulations show that frequent HD therapies may help achieve considerable reductions in serum B2M levels in patients with and without RRF.

Funding: Pharmaceutical Company Support

FR-PO1723

Macroglial Infiltration and Factor V Expression in the Peritoneum through Peritoneal Dialysis - Hideki Nosaka1 Michiko Bessho,2 Toshiya Takeda,3 Fumiaki Nogaki,1 Yoshiaki Iiogawa,1 Tomomi Takeda,4 Keiko Nomura,5 Yoko Matsuo,6 Noriko Morik,7 Takahiko Ono.1 1Shimada Municipal Hospital, Shimada, Japan; 2University of Shizuoka, Japan; 3Kyoto Takeda Hospital, Kyoto, Japan; 4Shizuoka General Hospital, Shizuoka, Japan.

Background: Fibrin deposition was frequently observed in peritoneal fibrosis induced with long-term peritoneal dialysis (PD). Factor V in its active form (Va) serves as a membrane-bound coagulant to factor Xa, which facilitates activation of prothrombin to thrombin. Consequently, we have hypothesized that factor Va is the major contributor to factor X depletion, while factor X does not play a significant role in consideration of the peritoneum.

Methods: We used a variable-volume, two-compartment kinetic model (Ward et al, K1 2006) to calculate weekly mean pre-treatment serum B2M concentrations (MPC) with in-center (ICHD), short-daily (SDHD), and nocturnal (NHD) HD therapies in patients with increasing degrees of residual renal B2M clearance (RRC-B2M). The modeled B2M parameters were the intercompartmental mass transfer coefficient (40 ml/min), generation rate (0.17 mg/min), non-renal clearance (3 ml/min), and distribution volume (13.3 L) (Clark et al, JASN 1999). The RRC-B2M considered ranged from 0 to 4 ml/min. Dialysis frequency and duration were: 3 times/week and 4 hours of treatment (3X/4hr) (ICHD), 6X/3hr (SDHD), and 6X/8hr (NHD). A dialyzer clearance rate of 50 ml/min was assumed.

Results: Considerable reductions in MPC were achieved with both frequent therapies compared with ICHD. Similar, less pronounced, reductions were also found when RRC-B2M was gradually increased from 0 to 4 ml/min. NHD in patients without RRF and ICHD in patients with RRC-B2M of 4 ml/min resulted in comparable reductions.

Conclusions: These simulations show that frequent HD therapies may help achieve considerable reductions in serum B2M levels in patients with and without RRF.

Funding: Pharmaceutical Company Support

FR-PO1724

Reduced Sodium Sieving on Chronic Peritoneal Dialysis: Clinical and Physiologic Correlates - Javier De Arteaga1,2 Carlos R. Churchur1,2 Walter Guillermo Douthat,1 Jorge Luis De la Fuente.1 1Nephrology, Hospital Privado, Cordoba, Argentina; 2Postgraduate Nephrology, Catholic University, Cordoba, Argentina.

Background: A reduced sodium sieving (RNAs) during a hypertonic glucose dwell can be the consequence of aquaporin dysfunction or a reduced osmotic conductance to glucose. Also has been claimed that a high transport status associates to RNAs. Other clinical correlates that could be associated to this situation like pts age, gender, previous transplants or total dialysis time have not been extensively evaluated.

Methods: To study these clinical and physiologic variables that could be linked to RNAs in our chronic PD pts.

Results: Since 2001, modified PET (4.25%) tests have been done yearly with ultrasound measured every hour mannualy in 46 chronic PD pts. In PET, initial plasma and hourly dialysate NA were analysed by ISE ( indirect ion selective electrode). A High transport state was defined as a D/P creat > 0.81 at 4hs and RNAs was defined as a delta initial plasmatic NA - initial dialysate NA < 10 ml/l. In RNAs, the number of peritonitis episodes was significantly higher (1.80 ± 2.19 vs. 0.75 ± 1.07, P = 0.0019) in the EPS group. Moreover, the duration of treatment of peritonitis was significantly longer in the EPS group than in the non-EPS group (18.11 ± 15.3 vs. 10.2 ± 4.90, P = 0.002). On the other hand, Macroglial infiltration was more common in patients with EPS episodes than in the EPS group. Oppositely Staphylococcus aureus was more common causative organisms in the EPS group. These results might indicate that the prevalence of antibiotic-resistant peritonitis might be higher in the EPS group.

Conclusions: The number of peritonitis episodes and the prevalence of antibiotic-resistant peritonitis might be associated with the development of EPS.

Funding: Government Support - Non-U.S.
Conclusions: 11 patients (23.9%) have a reduced sodium sieving (RNAS). There is a strong association with a high transport state (p < 0.0006). A reduced osmotic conductance for glucose was not associated to RNAS in the only 3 patients evaluated (unpaired). Although not significant (p < 0.10), there seems to be a trend towards a male gender in the RNAS group of our population.

**FR-PO1725**


Division of Nephrology, Department of Internal Medicine, Yeungnam University Hospital, Daegu, Korea.

**Background:** Peritoneal dialysis (PD) is an established treatment modality for patients with end stage renal disease (ESRD). The mortality rates of ESRD patients have significantly declined over the past decade. However, there are few reports on the risk factors for mortality in stable PD patients who survive for a considerable time.

**Methods:** We reviewed the medical records and we identified all the adult patients who received PD. Among these patients, those with <2 years of follow-up were excluded. Two hundred and sixty-six patients were enrolled. The following data was documented from the patients’ record: age at the initiation of PD, gender, the laboratory findings, the comorbidities (Davies index) and survival.

**Results:** The mean follow-up was 62.4 ± 24 months. The cumulative survival was 95.5% at 3 years and 80.3% at 5 years. On the univariate regression analysis, old age (>60-years-of-age), hypoalbuminemia, low residual renal function (RRF) (<0.95 ml/min) and a high Davies index were associated with increased mortality for the stable PD patients. On the multivariate analysis, old age, low RRF and a high Davies index were proved to be the independent risk factors for mortality.

**Table 1. Predictive factors affecting the mortality of long-term survivors on PD**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at the initiation of PD (≥60-years)</td>
<td>4.416 (2.350-7.591) 0.000</td>
<td>4.542 (2.446-8.436) 0.000</td>
</tr>
<tr>
<td>Gender (Female)</td>
<td>0.850 (0.705-1.429) 0.540</td>
<td></td>
</tr>
<tr>
<td>TA-albumin (≤55g/L)</td>
<td>2.015 (1.983-3.931) 0.008</td>
<td>1.123 (0.627-2.010) 0.697</td>
</tr>
<tr>
<td>TD-CRP (≥2.5mg/L)</td>
<td>1.672 (0.975-2.683) 0.062</td>
<td>1.296 (0.725-2.318) 0.381</td>
</tr>
<tr>
<td>RRF (≤4ml/min) at 24 months</td>
<td>2.380 (1.021-5.548) 0.045</td>
<td>2.876 (1.178-7.021) 0.020</td>
</tr>
</tbody>
</table>

**Conclusions:** The preservation of the RRF and proper management of the comorbidities may help to improve the survival of stable PD patients.

**FR-PO1726**

Efficacy of Lanthanum Carbonate in Patients on Peritoneal Dialysis: An Opportunity To Improve Nutrition? Rosamund Wilson, 1 Maggie Gill, 2 John Brian Copley. 1 Spica Consultants, Marlborough, United Kingdom; 1 Shire Pharmaceuticals, Basingstoke, United Kingdom; 1 Shire Pharmaceuticals, Wayne.

**Background:** There may be an increase in the use of peritoneal dialysis (PD) in patients with chronic kidney disease (CKD) in the USA as a result of recent changes in dialysis reimbursement. Both PD and dietary phosphate restriction may have a negative effect on nutritional status so the use of an effective phosphate binder to control hyperphosphataemia may allow higher protein intake. This analysis assesses the efficacy of the non-calcium, non-resin phosphate binder lanthanum carbonate (LC) in reducing serum phosphorus (P) in patients on continuous ambulatory PD (CAPD).

**Methods:** This was a double-blind, placebo-controlled, parallel-group study, conducted in two parts. Part 1 involved dose titration up to 2250mg/day of LC over a 4-week period to achieve P ≤3.5mg/dL. In part 2 (a double-blind period), patients were randomized to receive their maintenance dose of LC or matching placebo for 4 weeks.

**Results:** Patients enrolled in this study had albumin levels of approximately 3.8g/dL (normal range 3.4-5.4g/dL) and these levels were maintained during LC treatment. Twenty-one patients receiving CAPD entered the double-blind phase; 10 were randomized to LC and 11 to placebo. At the end of treatment, 60% of patients treated with LC had controlled P (4.03–5.57mg/dL) vs. 10% in the placebo group. There was no difference in P levels between treatment groups at the start of the double blind phase (LC=4.87mg/dL, placebo=4.89mg/dL; P=0.96), but there was a significant difference at last visit (LC=4.83 mg/dL, placebo=4.96 mg/dL; P=0.0015). The most common adverse events during LC treatment were vomiting (26%) and nausea (23%) in part 1 and localized infection (20% not considered related to LC) in part 2.

**Conclusions:** Patients on PD may require higher protein intake than those on hemodialysis, so it is important not to impair nutritional status while controlling serum P. Treatment with LC resulted in significantly reduced P levels in patients receiving CAPD at doses up to 2250mg. The more commonly used dose of 3000mg may be expected to give further reductions, possibly allowing greater protein intake.

**Funding:** Pharmaceutical Company Support

**FR-PO1727**

Baseline Residual Renal Function and Female Sex Are Associated with Technique Survival in Incident Peritoneal Dialysis Patients Kai Li, 1 Jason Jones, 2 David C. Slevan, 3 Peggy Balcius, 2 Victoria A. Kumar. 1 Nephrology, SCPMG, Los Angeles, CA; 2Research and Evaluation, SCPMG, Pasadena, CA; 1Renal Business Group, SCPMG, Pasadena, CA.

**Background:** Previous studies have reported that technique survival for peritoneal dialysis (PD) patients is highest in our region (Network 18) compared to the rest of the nation. We therefore sought to examine predictors of technique survival in a cohort of PD patients at a large healthcare maintenance organization.

**Methods:** We identified all adult patients in our database who initiated PD at our institution between January 1, 2001 and December 31, 2010. We included only patients with a renal creatinine clearance (pCrCl), and a total creatinine clearance (totCrCl) at initiation of PD. Baseline peritoneal creatinine clearance (pCrCl) was obtained by subtraction of rCrCl from totCrCl. Patients who received a renal transplant during the study period were censored from the analysis. A Cox proportional hazards model was used to individually evaluate the effect of pCrCl, pCrCl, and totCrCl, on technique survival after adjusting for age, sex and Charleson Comorbidity Index (CCI).

**Results:** Baseline patient demographics are shown in Table.

<table>
<thead>
<tr>
<th>Patient demographics</th>
<th>Number of patients</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>649</td>
<td></td>
</tr>
<tr>
<td>Median age in years</td>
<td>56.8 (47.5-65.6)</td>
<td></td>
</tr>
<tr>
<td>Number of females</td>
<td>296 (45.7)</td>
<td></td>
</tr>
<tr>
<td>CCI</td>
<td>6 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Number of diabetes</td>
<td>399 (61.7)</td>
<td></td>
</tr>
<tr>
<td>PD as first modality</td>
<td>402 (62.1)</td>
<td></td>
</tr>
</tbody>
</table>

**95% Confidence Interval (CI)**

**Conclusions:** rCrCl and totCrCl are associated with PD technique survival, but pCrCl is not. Baseline renal function and female sex appear to be the strongest predictors of technique survival in our patients.

**Funding:** Pharmaceutical Company Support

**FR-PO1728**

Vascular Access Use in Patients Who Transfer from Peritoneal Dialysis to Hemodialysis Leslie P. Wong, Suni J, Sun, David Francis Nash. Medical and Clinical Affairs, Satellite Healthcare, San Jose, CA.

**Background:** PD dropout is common, with 45% of incident patients leaving PD by 2 years. Many patients transfer to HD and require vascular access. Unfortunately, there is little data describing access use and outcomes in these patients.

**Methods:** We analyzed the Satellite Healthcare database between January 1993 and May 2010 for patients who transferred from PD to HD. After excluding 432 records with insufficient data or multiple transfers, 439 patients were studied. One-year outcomes included probability of central venous catheter (CVC) use over time, rate of access or bacteremia-related hospitalization and subsequent modality status.

**Results:** Demographics were: mean age 63, 56% white, 55% male, 49% DM, 13% CHF, 96% Kt/V > 1.7, 85% high transporters, 31% with residual kidney function and 75% with albumin < 3.5. Median duration on PD was 358 days and 48% were incident PD patients. Leading reasons for transfer were peritonitis 15% and psychosocial 11% but only recorded for 42% of patients. Sixty-six percent of patients transferred to HD with a CVC. More incident patients started with a CVC (73%) compared to patients with prior HD (59%) p<0.003. Median number of CVC days was 215, with 84% of patients still using a CVC after 90 days (see figure). Forty percent of CVC patients switched to an AVF/AVG within 1 year. Access or bacteremia-related hospitalization rate was 49 admissions/1000 patient years and did not differ between groups. At 1 year 53% of patients remained on HD, 15% died, 21% transferred back to PD, 3% were transplanted and 8% moved.

**Funding:** Medical and Clinical Affairs, Satellite Healthcare, San Jose, CA.
Conclusions: CVC use predominates in patients who transferred from PD to HD. Though most patients stayed on HD, conversion to AVF/AVG was suboptimal. Hospitalization rate was not higher for CVC patients but may reflect underreporting. Vascular access planning for PD patients warrants further study.

FR-PO1729
Modeling Intraperitoneal Cefazolin and Cefepime for Nocturnal Intermittent Peritoneal Dialysis
Pisut Katavetin, Talerngsak Kanjanabuch, Kriang Tungsanga, Somchay Eiam-Ong. Division of Nephrology, Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand.

Background: Nocturnal intermittent peritoneal dialysis (NIPD) has been increasingly used. Cefazolin and cefepime are the principal drugs for treatment of peritoneal dialysis (PD)-related peritonitis. Current literature suggested daily intraperitoneal (IP) dosing of cefazolin as a drug in the additional long day dwell which is not always practical. We, therefore, developed a mathematical model to test whether intermittent IP drug delivery into the first bag (5 liter) of NIPD could yield an optimal antibiotics level both in serum and in peritoneal fluid in patients undergoing 10 liters (L) NIPD in 5 cycles over 12 hours.

Methods: Two-compartment, fixed volume model for pharmacokinetics of cefazolin and cefepime in PD patients were developed. Level of cefazolin and cefepime in serum and in dialysate were then calculated according to the model using the pharmacokinetic parameters from the literature. The concentration of antibiotics in the PD fluid in the second to fifth cycle were assumed to be approximately 0.6 times of that in the previous cycle due to the dilution of antibiotics by adding 2L of fresh PD fluid from the second bag into the remaining 3L first antibiotics containing bag.

Results: In a 70 kilogram (kg) NIPD patient, adding 1.5 gram (g) of cefazolin in the first 5L bag of NIPD could yield optimal serum and peritoneal antibiotics concentration during the on-cycler period but might yield marginal concentration in peritoneal fluid (6.7 μg/ml) despite adequate serum concentration during the off-cycler period. For cefepime, 1.5g of cefepime in 70kg patient could yield optimal serum and peritoneal antibiotics concentrations during both on-cycler and off-cycler period although there was a considerably delay in time to reach optimal serum concentration in the first day. Increasing dose of cefepime to 1.5g could ameliorate this problem.

Conclusions: Intraperitoneal administration of cefazolin and cefepime in the first bag of NIPD is a reasonable option for treatment of NIPD-related peritonitis with favorably increasing dose by 1.5 time.

FR-PO1730
Periostin- A Matricellular Protein Involved in Peritoneal Injury during Peritoneal Dialysis
Niko Braun,1 Kontheerai Sen,2 Peter Fritz,3 Martin Kimmel,4 Achin Joerres,5 Clemens D. Cohen,6 Stephan Segerer.1 1Department of Internal Medicine, Division of Nephrology, Robert-Bosch Hospital, Stuttgart, Germany; 2Division of Nephrology, University Hospital Zurich, Switzerland; 3Department of Diagnostic Medicine, Division of Nephrology, University of Erlangen, Germany; 4Department of Nephrology and Medical Intensive Care, Charité-Universitätsmedizin, Campus Virchow-Klinikum, Berlin, Germany.

Background: Periostin is a matricellular protein involved in tissue remodeling. We hypothesized that this protein might be expressed in the peritoneal cavity of patients on peritoneal dialysis (PD) and patients with signs of encapsulating peritoneal sclerosis (EPS).

Methods: We localized periostin in peritoneal biopsies from patients on PD with EPS (n=7), on PD without signs of EPS (n=10), and compared it with biopsies taken during hernia repair as controls (n=11).

Results: Periostin was found in the wall of larger arteries and focally in the submesothelial zone in control biopsies. Patients on PD demonstrated intestinal periostin in variable amounts depending on the severity of submesothelial fibrosis. In EPS there was a very prominent expression in the sclerosis layer. Commonly the superficial layer was periostin negative. A semiquantitative score was most prominently associated with the diagnosis of EPS, as well as with the thickness of the submesothelial fibrosis zone.

Conclusions: Periostin is expressed in EPS and to a lesser extend in simple peritoneal sclerosis. It might play a role in the progression of peritoneal injury.

FR-PO1731
Bone Mineral Density and Abdominal Aortic Calcifications Detected by Computed Tomography in Peritoneal Dialysis Patients
Pierluigi Di Loreto, Francesca K. Martino, Claudio Ronco. Nephrology Dialysis Transplantation, San Bortolo Hospital, Vicenza, Italy.

Background: Bone mineral density (BMD) is negatively correlated with vascular calcification and cardiovascular risk in CKD pts. Aim of this study is to confirm these observations in Peritoneal Dialysis (PD) patients.

Methods: We studied 45 PD patients (mean age 61±14 years, 41.3% Women and 58.7% Man, mean age Man 65±7 vs. Women 65±17 vs. 50±11 for age) mean duration of PD was 21.9±25.4 months. This patients underwent an abdominal computerized tomography scan. The severity score for Abdominal Aortic Calcifications (AOA) was: 1=mild, 2=mild, 3=moderate, 4=severe. For each patient we collected the following laboratory data: calcium corrected for albumin, PTH, phosphorus, alkaline phosphate, hemoglobin, BMI, and diabetes mellitus on admission. In addition, the incidence of atherosclerotic vascular events was assessed: MI, diuresis, warfarin, Quantitative CT measurements of BMD (mg/ml) using fully automated software were obtained at the first, second, third and fourth lumbar vertebrae. Statistical analysis was performed with SPSS.

Results: We found a negative correlation between BMD and age (M r=0.006, r=0.516, W r=0.001, r=−0.763) and between BMD and calcium in women (p=0.037, r=0.481). 25 pts (55.6% Man and 42.1% Women with no statistically significant difference between sexes) were osteoporotic (BMD <160 mg/ml), and respectively 14 and 11 of these were osteoporotic (BMD <160 mg/ml) and severe and mild AOA. After 2 years the overall prevalence of fractures was 15.5% (7 pts). 12 pts (26%) died. All the pts who died and those with fracture showed low BMD and a severe AOC. No correlations was found between laboratory data, BMI, diuresis, Kt/V, use of medications, length of dialysis and BMD-AOC.

Conclusions: Our data confirm that low BMD is correlated with age, vascular calcification and cardiovascular risk as in CKD pts. We could not confirm the findings of other Authors who suggested that low body weight and low Kt/V were the most important risk factors for low BMD in PD pts.

FR-PO1732
Effects of Spironolactone on Residual Renal Function in Patients Receiving Peritoneal Dialysis
Berna Yelken,1 Numan Gorgulu,2 Metem Gursu,3 Yasar Caliskan,4 Halil Yazici,4 Aysegul Telci,4 Rumeysa Kazancioglu,4 Tefek Ecder,4 Semra Bozkaktolu.4 1Division of Nephrology, Department of Internal Medicine, Gaziosmanpasa Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey; 2Division of Nephrology, Department of Internal Medicine, Istanbul, Turkey; 3Division of Nephrology, Department of Internal Medicine, Haseki Education and Research Hospital, Istanbul, Turkey; 4Division of Nephrology, Department of Internal Medicine, Istanbul Faculty of Medicine, Istanbul University, Istanbul, Turkey; 5Department of Biochemistry, Istanbul Faculty of Medicine, Istanbul University, Turkey; 6Faculty of Medicine, Bezmialem vakfi university, Istanbul, Turkey.

Background: There is increasing evidence that long-term peritoneal dialysis (PD) is associated with structural changes in the peritoneal membrane. It is unknown whether spironolactone affects RRF in addition to the structural changes in the peritoneal membrane.

Methods: 23 (13 female) patients with RRF (>400 ml/day) receiving PD were evaluated. After measuring baseline serum high sensitive C-reactive protein (hs-CRP), vascular endothelial growth factor (VEGF), transforming growth factor beta (TGF-β), connective tissue growth factor (CTGF) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) level, ultrafiltration (ml/day), RRF, creatinine clearance (CrCL), Kt/V, normalized protein catabolic rate (nPCR), peritoneal transport status and dialysate CA125 level and VEGF, spironolactone (25 mg daily) therapy was given for six months. At the end of six months all measurements were repeated.

Results: The mean age of the patients was 46±13 years. Duration of PD was 15±21 months (range; 2-88). After spironolactone therapy, the mean dialysate CA125 level significantly increased as compared to baseline (20.52±12.06 U/ml vs. 24.44±13.97 U/ml, p=0.028).Serum hs-CRP, VEGF, TGF-β, CTGF and NT-proBNP levels, dialysate VEGF levels, daily ultrafiltration, Kt/V, nPCR and peritoneal transport status of patients were similar between two study periods. There was no decrease in RRF and CrCL at the end of six months.

Conclusions: Spironolactone therapy for six months appears to preserve RRF and peritoneal structure in patients with PD.

FR-PO1733
Agreement between Peripheral Venous and Arterial Blood Gas Measurements in the Intensive Care Unit
Ho Sik Shin, Jin Hee Park, Sung Bin Kim, Yeon Soon Jung, Hark Rim. Internal Medicine, Kosin University College of Medicine, Busan, Korea.

Background: Venous blood gas (VBG) analysis is safer than arterial blood gas (ABG) analysis and may be a suitable alternative for determining acid-base status. The objective of this study was to examine the agreement between ABG and peripheral VBG samples for all commonly used parameters in medical intensive care unit (ICU) patients.

Methods: We performed a single-center, prospective trial to assess the agreement between arterial and peripheral VBG measurements in a medical ICU. When an ABG was deemed necessary as part of ICU management, a peripheral venous sample was also taken. All commonly used parameters in medical intensive care unit (ICU) patients.

Results: Regression equations were derived to predict arterial values from venous values: for arterial pH: Arterial pH = 0.756 X venous pH + 1.786, arterial bicarbonate = 0.822 X Venous bicarbonate + 2.815 and arterial HCO3⁻ = 0.639 X Venous total CO2 + 5.360. The mean ABG minus peripheral VBG differences for pH, PCO2, and bicarbonate were not clinically important.
FR-PO1735

Aggressive and Unnecessary Treatment of Mild Hyperkalemia: Inappropriate Use of Kayexalate

Gabriel El-kass, Farhanah Yousaf, Emily Yan, Bruce S. Spinowitz, Chaim Charytan. Department of Nephrology, New York Hospital Queens, Flushing, NY.

Background: Although hyperkalemia is potentially a lethal metabolic complication which may require emergent treatment, there is no consensus defining clinically significant hyperkalemia, nor the settings which require K+ lowering therapy. The literature suggests that clinically significant hyperkalemia occurs at K+ levels ≥6.0 mEq/L with EKG changes or at K+ >6.5 mEq/L [Levinsky, 1966; Greenberg, 1998; AHA 2005]. A recent study suggested that even more severe hyperkalemia may not require aggressive therapy nor hospitalization [Charytan D, 2000]. By extrapolation, it may be assumed that lower levels of hyperkalemia might not require aggressive intervention. This becomes particularly important in view of concerns raised about the efficacy and safety of Kayexalate (SPS) [Stem, 2010]. During an investigation on the efficacy and safety of SPS at our institution, we decided to explore how frequently clinically mild hyperkalemia, a serum K+ ≤5.6 mEq/L, was treated with SPS with or without additional interventions.

Methods: Medical records of patients ≥18 years receiving SPS in emergency department or as in-patients between June 2010 and August 2010 were reviewed.

Results: 154 patients were prescribed SPS 249 times. Of these, 92 patients, 45 males and 47 females, aged 76.0±13.3 yrs, received 106 doses of SPS for a serum K+ ≤5.6 mEq/L. SPS was administered as a 30 g dose in 99 cases and as a 15 g dose in 7 cases. 103 doses were given orally, 2 doses rectally, and 1 dose via PEG.

Conclusions: Peripheral venous pH, PCO2, bicarbonate and total CO2 can replace their arterial equivalents in many clinical contexts encountered in the ICU.

FR-PO1736

Hospital Pharmacy Guidelines for the Administration of 3% Sodium Chloride in Children

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Background: Hyponatremic encephalopathy is a medical emergency with the treatment of choice being 3% sodium chloride (NaCl). A 100 cc or 2 cc/kg bolus of 3% NaCl is the preferred therapy (NEJM 2005:353) that has now been accepted as the standard of care (NephSAP 2011:10). We suspect that children’s hospital pharmacies may have policies that would restrict the administration of a 3% NaCl bolus due to concerns for overcorrection of hyponatremia.

Methods: An internet survey was distributed to the pharmacy directors of 43 children’s hospitals participating in the Child Health Care Corporation of America (CHCA) network in order to assess their policies for administering 3% NaCl.

Results: The response rate was 65%. 71.4% of respondents had policies for the administration of 3% NaCl. The majority of respondents had policies restricting the volume dispensed (50-60 mL) and rate of administration allowed (30-40 mL/min) in hospital inpatients. The majority of pharmacies (57.1%) also would not allow 3% NaCl to be used in a non-ICU setting or via a peripheral IV. Of those who allowed 3% NaCl through a peripheral IV, 57.1% had restrictions on its use. Only 24% of respondents had similar policies for the administration of hypertonic sodium bicarbonate or mannitol.

Conclusions: The majority of children’s hospital pharmacies have restrictive policies for the rate, volume, route and setting of administration of 3% NaCl. These restrictive policies could prevent the timely use of a 3% NaCl bolus for the treatment hyponatremic encephalopathy in children.

FR-PO1737

Urine of Patients with Cerebral/Renal Salt-Wasting Syndrome Contains a Substance That Inhibits Reabsorptive Sodium Flux in LLC-PK1 Cells

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Background: When cerebral and renal salt-wasting syndrome is present, there is a decreased ability to secrete sodium and urine volume is increased.

Method: We studied the urine of patients with cerebral and renal salt-wasting syndrome, and normal controls.

Results: The urine of patients with cerebral and renal salt-wasting syndrome, and normal controls.

Conclusions: The urine of patients with cerebral and renal salt-wasting syndrome, and normal controls.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
at the same concentrations. (Mean ± SEM, P<.002 and P<.003 at 5 and 10 µg protein/ml respectively; 3-5 repetitions per RSW and 2-4 per Control patient.) It was noted that means for Controls showed some stimulation of Na absorption versus vehicle, which did not reach significance (P=.18 and P=.14). In paired experiments in which different concentrations of RSW ppt were tested in the same run, a dose-response was seen, inhibition being greater for a change from 10 to 20 µg/ml than for a change from 5 to 10 µg/ml (-23.0±3.8% vs. -11.6±4.4% respectively; P=.045).

Conclusions: The results suggest that ammonium precipitable substance(s) in urine from NS patients with increased FEurate and normonatremia inhibits transcellular transport of Na as compared to ppts from normonatmic NS patients with normal FEurate and SIADH. The natriuretic substance may contribute to the renal Na loss seen in RSW. These results support our proposal that normonatremia with increased FEurate might identify patients with RSW and that RSW is common in NS patients.

FR-PO1738
Hyponatremia and Acute Kidney Injury (AKI) Are Associated with Mortality in HIV Patients with Neurotoxoplasmosis
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Background: Toxoplasmosis is a main cause of neurologic symptoms among HIV-infected patients. AKI has not been studied in this population. Hyponatremia is a potential complication of neurologic-related disorders. The objective of this study is to describe the occurrence of hyponatremia and its relationship with AKI and mortality in HIV-associated neurotoxoplasmosis.

Methods: Retrospective cohort of patients with HIV-related neurotoxoplasmosis. AKI was considered only if RIFLE criteria were achieved after hospital admission. Serum creatinine (Scr) at admission was considered baseline Scr.

Results: A total of 92 patients were included, with an average age of 36.9 ± 9 years old. 73.9% were male. Hyponatremia at admission was observed in 40 (43.4%) patients and AKI developed in 25 (27.2%) during hospital-stay. Sulfadiazine was the choice treatment for Controls showed some stimulation of Na absorption versus vehicle, which did not reach significance (P=.18 and P=.14). In paired experiments in which different concentrations of RSW ppt were tested in the same run, a dose-response was seen, inhibition being greater for a change from 10 to 20 µg/ml than for a change from 5 to 10 µg/ml (-23.0±3.8% vs. -11.6±4.4% respectively; P=.045).

Conclusions: The results suggest that ammonium precipitable substance(s) in urine from NS patients with increased FEurate and normonatremia inhibits transcellular transport of Na as compared to ppts from normonatmic NS patients with normal FEurate and SIADH. The natriuretic substance may contribute to the renal Na loss seen in RSW. These results support our proposal that normonatremia with increased FEurate might identify patients with RSW and that RSW is common in NS patients.

FR-PO1740
The Cost and Clinical Outcomes of Hyponatremia in Hospitalized Cancer Patients
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Background: Disorders of sodium balance are the most common of the electrolyte abnormalities seen in clinical practice. However, unlike hyponatremia, little is known about hypernatremia, especially related to outcomes. The objective was to evaluate the frequency and severity of hypernatremia in patients admitted to the hospital and its associations with clinical outcomes and hospital costs.

Methods: We analyzed prospectively collected data on patients with cancer admitted to the UT MD Anderson Cancer Center over a 3-month period in 2006. Clinical outcomes and costs were compared among hypernatremics, eunatremics and hyponatremics (serum Na >147 mEq/L, 135-147 mEq/L and <135 mEq/L, respectively).

Results: Of 3886 patients admitted, 3%, 46% and 51% were respectively hypernatremic, eunatremic and hyponatremic. The length of hospital stay in hypernatremics was 4-fold higher than eunatremics (e.g., 27 ± 22 days vs. 6 ± 5 days, p < 0.001) and 2-fold higher than hyponatremics. Moreover, the multivariate hazard ratios (HR) for mortality of hypernatremics were significantly higher than eunatremics (in-hospital HR: 3.83, 95% CI: 2.10 - 6.95; p<0.001 and 90-day HR: 10.20, 95% CI: 6.50-15.99; p<0.001) or hypernatremic (in-hospital HR: 1.73, 95% CI: 1.20-2.50; p=0.005 and 90-day HR: 2.99, 95% CI: 2.14-4.20; p<0.001) (see figure: survival curve). The cost was higher for hyponatremics compared to rest of the groups (46% higher than eunatremics, 37% higher than hypernatremics; p<0.001 for both).

Conclusions: We report for the first time to our knowledge that hypernatremia is associated with substantially worse clinical outcomes and higher costs than is hyponatremia and eunatremia. These findings grant similar studies in non-cancer patients.

FR-PO1739
Association of Hyponatremia and Hypernatremia with Mortality in Burn Patients
Molly A. Tilley,1 Ian J. Stewart,1 Benjamin D. Morrow,1 Keith W. Kramer,1 Chris A. Gisler,2 James K. Aden,3 Kevin Chung,3 Sko Antonio Military Medical Center; 2University Texas Health Sciences Center San Antonio; 3Burn Center, US Army Institute of Surgical Research.

Background: Dysnatremias are associated with mortality in many groups. However, this relationship has not been well described in burn patients. Our study seeks to determine if moderate to severe hyponatremia or hypernatremia are independently associated with mortality in this population.

Methods: We examined all admissions to our institution’s burn center from January 2003 to December 2008. Patients were included if they were at least 18 years old and had a serum sodium obtained during their hospitalization. Exclusion criteria included end stage renal disease and death within 24 hours of admission. Independent variables included age, gender, percentage total body surface area burned (%TBSA), percentage of third degree burn, inhalation injury, injury severity score (ISS), Acute Kidney Injury Network (AKIN) stage, hypernatremia, and hyponatremia. These variables were examined via multiple logistic regression analysis against death. Moderate to severe hyponatremia and hypernatremia were defined as serum sodium less than 130mmol/l and greater than 150mmol/l, respectively.

Results: In 1973 subjects with a mean age of 36±16, average %TBSA of 16±18, and average ISS of 10±12, hypernatremia occurred in 9.8% (n=194) while hyponatremia occurred in 7.0% (n=138) during their admission. Overall in-hospital mortality was 7.6% (n=150). Among those with hypernatremia and hyponatremia, mortality was 33.5% and 13.5% respectively which were both significantly higher than the mortality rate (4.5% and 6.8%) among those without these abnormalities (p<0.0001 and p=0.0027, respectively).

On multiple logistic regression, however, only age, %TBSA, ISS, and AKIN stage were found to be significant predictors of mortality. Hypernatremia (OR 0.66, 95% CI 0.35-1.25, p=0.20) and hyponatremia (OR 0.46, 95% CI 0.21-1.03, p=0.06) were not.

Conclusions: In contradistinction to other groups, neither hypernatremia nor hyponatremia are independent predictors of mortality in the burn population.

Funding: Other U.S. Government Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
In this retrospective case study based on chart review, 2,027 children (1,150 males and 877 females, 1,418 febrile and 609 non-febrile patients) presenting with acute illnesses were enrolled. The height of admission body temperature was recorded, and serum sodium levels were determined. The study compared temperature range of fever patients to that of non-fever patients, and examined associations between body temperature and serum sodium levels.

**Methods:** A total of 610 Korean patients over age 18 who were hospitalized with a diagnosis of severe hyperkalemia from August 2007 to July 2010 became the subject of this study. The relationships between body temperature and serum sodium levels were analyzed.

**Results:** There were no differences in blood or urinary electrolytes when comparing mutant and control animals, nor was there a difference in urinary aldosterone levels.

**Conclusions:** We conclude that high dietary potassium intake does not increase the risk of potassium toxicity.

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**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

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**FR-PO1741**

Involuntary Change of Serum Sodium Level with Body Temperature in Children with Common Febrile Diseases

**Authors:** Hideki Matsuguma, Akira Ashida, Akihiko Shirasu, Hyogo Nakamura, Motoshi Hattori, Hiroshi Tamaki, Hideki Hata, Hiroshi Arimura, Noriko Hata, Kyoko Nakamura, and Toshihiro Hattori

**Background:** Hyponatremia is a common electrolyte abnormality in hospitalized patients. Routine use of hypotonic maintenance fluids can lead to potentially fatal hyponatremia in cases of excess antiuretic hormone (ADH) production. Several studies have demonstrated that non-osmotic ADH activity contributes to the development or persistence of hyponatremia in children with common febrile diseases. However, the relationship between hyperkalemia and body temperature has remained unclear; we examined this relationship in children with common febrile diseases.

**Methods:** In this retrospective case study based on chart review, 2,027 children (1,150 males and 877 females, 1,418 febrile and 609 non-febrile patients) presenting with acute illnesses were enrolled. The height of admission body temperature was recorded, and serum sodium levels were determined. The study compared temperature range of fever patients to that of non-fever patients, and examined associations between body temperature and serum sodium levels.

**Results:** The mean sodium level in severely ill patients was significantly lower than that of non-severely ill patients. The serum sodium levels in the temperature groups were in ascending order, respectively. The serum sodium concentration was significantly higher in patients with underlying disease such as hypertension, AKI on October 2009. The serum sodium concentration was significantly lower in patients with underlying disease such as malignancy and severe medical conditions including infection and bleeding. In addition, AKI in patients with normal baseline renal function and metabolic acidosis were definite predictors of mortality. Whereas, mortality rate had increased the apical abundance of ROMK in AKP2-positive tubules in WT mice, but had a much less effect on ROMK abundance in KS-WNK1 mice.

**Conclusions:** These results support the hypothesis that increased expression of KS-WNK1 contributes to the downregulation of NCC and upregulation of ROMK by dietary K+ loading.

Funding: NIDDK Support

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**FR-PO1742**

Severe Hyperkalemia Requiring Hospitalization: Predictors of Mortality and Improvement

**Authors:** Jung Nam An, Jung Pyo Lee, Yun Kyu Oh, Chun Soo Lim

**Background:** Severe hyperkalemia (K≥6.5mEq/L) is a potentially life-threatening electrolyte disorder. To treat it promptly and effectively, it is essential to know its causes, risk factors, clinical manifestations, and predictors of mortality and improvement.

**Methods:** A total of 610 Korean patients over age 18 who were hospitalized with a diagnosis of severe hyperkalemia from August 2007 to July 2010 became the subject of this retrospective study. The medical records were reviewed.

**Results:** Hypertension was the most common underlying medical condition, followed by diabetes and chronic kidney disease (CKD). The most common precipitating factor was metabolic acidosis, followed by acute kidney injury (AKI), drugs such as angiotensin receptor blocker, K-sparing diuretics, beta blocker, and non-steroidal anti-inflammatory drug. The serum potassium levels were significantly associated with the use of NSAIDs, metabolic acidosis, and the history of recurrent severe hyperkalemia. Also, mortality was strongly correlated with the severity of hyperkalemia. Changes in electrocardiogram findings were associated with higher potassium levels, but didn’t increase the mortality rate themselves. The mortality rate was higher in patients with underlying disease such as malignancy and severe medical conditions including infection and bleeding. In addition, AKI in patients with normal baseline renal function and metabolic acidosis were definite predictors of mortality. Whereas, mortality rate had a lowering tendency in patients with underlying disease such as hypertension, AKI on underlying CKD, and drug-induced hyperkalemia.

**Conclusions:** Severe hyperkalemia requiring hospitalization and prompt treatment occurred in various medical conditions. The precipitating factors were also diverse. The mortality rate was especially higher in patients with severe underlying diseases and with new-onset AKI rather than AKI on underlying CKD.

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**FR-PO1743**

Role of Kidney-Specific WNK1 in the Regulation of NCC and ROMK by High Dietary Potassium Intake

**Authors:** Zhen Liu, Chou-Long Huang

**Background:** Abnormalities of ion homeostasis are a common feature of renal dysfunction. The regulation of ion balance is critical for the maintenance of normal body function. The kidney is an important organ that regulates ion homeostasis. The kidney-specific WNK1 (KS-WNK1) is a variant of WNK1 and reportedly activates NCC and inhibits ROMK, respectively. High dietary potassium intake (KD) stimulates KS-WNK1, and in this study we examine the role of KS-WNK1 in the regulation of NCC and ROMK by KD loading.

**Methods:** KS-WNK1 null mice were generated by homologous recombination to delete exons 3-8A, the unique exonic fragment of KS-WNK1.

**Results:** Previously, we reported that KS-WNK1 null mice have Na+ retention, increased surface expression of NCC and elevated blood pressure on a high-Na+ diet compared to WT littermates. Here, we reported that serum K+ levels were not significantly different in WT and KS-WNK1 null mice fed a normal K+ diet (4.4 ± 0.2 vs 4.2 ± 0.2 mEq/L), but were significantly higher in KS-WNK1 null mice fed a high K+ (10%) diet for 10 days (5.0 ± 0.2 vs 4.2 ± 0.2 mM, p<0.05). Immunofluorescent staining using antibodies against NCC and t-PAS-NCC showed that dietary K+ loading decreased the abundance of NCC and t-PAS-NCC in WT vs KS-WNK1-null mice. Recent research in mouse model analysis confirmed the differential effect of KD loading on NCC in WT vs KS-WNK1 null mice. Immunofluorescent staining revealed that dietary K+ loading increased the apical abundance of ROMK in AKP2-positive tubules in WT mice, but had a much less effect on ROMK abundance in KS-WNK1 mice.

**Conclusions:** These results support the hypothesis that increased expression of KS-WNK1 contributes to the downregulation of NCC and upregulation of ROMK by dietary K+ loading.

Funding: NIDDK Support
**WNK4 Is a Novel Regulator of Thiazide Sensitive NaCl Transport in the Collecting Duct**

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**Institution:** 1 Paris Centre for Cardiovascular Research & 2Hôpital Européan Georges Pompidou, Paris, France

**Conclusions:** WNK4 mutations cause pseudohypoaldosteronism type II (PHAII), a autosomal dominant hypertension with hyperkalemia and hyperchloremic metabolic acidosis. Transgenic mice expressing WNK4 containing a PHAII mutation (TgWNK4PHAII) display all features of PHAII, thought to be due solely to increased Na+-Cl- cotransporter (NCC) activity in the distal convoluted tubule (DCT). However, recent research on mice lacking the kidney-specific isoform of WNK4 (expressed only in the DCT) showed that NCC overactivity is not sufficient to generate PHAII due to compensatory mechanisms in the cortical collecting duct (CCD).

**Methods:** To investigate CCF function, we performed isolated, perfused tubule studies on CCDs dissected from WT and TgWNK4PHAII mice.

**Results:** We observed increased Na+ and Cl- transport in TgWNK4PHAII mice while K+ and epithelial voltage were unchanged, suggesting that increased Na+ reabsorption in these mice is not mediated by the epithelial sodium channel (ENaC). We then examined ENaC activity in vivo by subjecting mice to amiloride injections while housed in metabolic cages and found that ENaC activity was indeed decreased in TgWNK4PHAII mice, as the natriuretic and anti-kaliuretic response was abolished. We recently showed that parallel activation of Na+-Cl- cotransporter (NCC) and Cl-/HCO3- exchanger NCCT by WNK4 kinase drive antinatriuretic response in mice lacking the kidney-specific isoform of WNK4.

**Conclusions:** In conclusion, WNK4 appears to be an important regulator of NaCl transport in the CCD, modifying activity of the electroneutral NaCl transport system mediated by NCCT and pendrin, and indicates that this system may play a key role in PHAII.

**Funding:** Private Foundation Support, Government Support - Non-U.S.
Methods: The renal expression levels of NCC2 (Na–2Cl/K- cotransporter), thiazide-sensitive NaCl cotransporter (NCC) and the ENaC subunits in WT and PHAII mice were examined by Q-PCR and their activities by measuring the effect of furosemide (Furo), hydrochlorothiazide (HCTZ) and benzamil (BEZ) on Na+ and K+ excretion by renal clearance. The urine volume (UV), GFR and absolute (ENa, EK) and fractional (FENa, FEK) Na+ and K+ excretion were measured before and after bolus iv of diuretics in wild-type (WT) and WNK4 mutant (PHAIi) mice. Results: All three diuretics produced significant diuretic and natriuretic effects in WT, but Furo and HCTZ produced stronger and benzamil produced smaller diuretic and natriuretic effects in PHAIi mice. The fractional Na+ and K+ excretion in WT were 2.1% and 3.0%, respectively, Furo and HCTZ increase and BEZ reduce both ENa and EK in WT, but there was no significant difference in the fractional changes in ENa and EK caused by the diuretics between WT and PHAIi mice. Q-PCR data show NCC2 and NCC expression levels were 25% and 61% higher in PHAIi than in WT mice. H3E3, α, β and γ ENaC expression were not significantly changed but ROMK expression was reduced by 46% in PHAIi vs. WT. Conclusions: We conclude that 1) elevated NCC2 and NCC activity contributes to increased NaCl absorption, 2) reduced ROMK expression limits ENaC activity, and 3) other K+-secretion mechanisms may be upregulated when the ROMK channel is downregulated in PHAIi mice. Funding: NIDDK Support

FR-PO1754

SPAK and OSRI Kinases Modulate Luminal Trafficking and Cellular Abundance of NCC2

Aljona Borschewski,1 Kamel Laghmanni,2 Kerim Mutl,3 Sylvie Demarest,2 Christin Duthe,1 Alexander Paliege,1 Nicholas R. Ferreri,4 Eric J. Delpire,5 Sebastian C. Bachmann.1 Institut für Vegetative Anatomie, Charité Universitätsmedizin, Berlin, Germany; 2INSERM, Centre de Recherche des Cordeliers, Paris, France; 3Dept. of Pharmacology, New York Medical College, Valhalla, NY; 4Dept. of Anesthesiology, Vanderbilt University School of Medicine, Nashville.

Background: Na+,K+-2Cl-cotransporter (NCC2) of the thick ascending limb (TAL) is essential for urinary concentration and volume regulation. Sterile 20/Spr1-related protein/alanine-rich kinase (SPAK) and oxidative-stress responsive kinase 1 (OSRI) regulate NCC2 activity by phosphorylation of conserved N-terminal threonines. The aim of the present study was to evaluate the effects of SPAK and OSRI on total abundance and secretion expression of NCC2.

Methods: Localization of SPAK and OSRI in TAL was verified immunohistochemically. Effects of SPAK and OSRI on NCC2 phosphorylation (pT96 and pT101), surface expression, and total abundance were studied in cultured rat TAL cells and in opossum kidney (OK) cells by means of knockdown or transient overexpression of the kinases and NCC2.

Results: Confocal evaluation revealed co-localization of SPAK and OSRI with NCC2 in subapical compartment of mouse and rat TAL. The interactions between the two kinases and NCC2 were established by co-immunoprecipitation experiments using rat medullary kidney homogenates. In cultured TAL cells, the endogenous abundance of SPAK and NCC2 was verified by immunoblotting. Knockdown of SPAK and OSRI in cultured TAL cells resulted in significantly decreased total abundance (~75%) and phosphorylation (pT96) of NCC2. In OK cells, cell surface distributions of NCC2 with SPAK or OSRI produced significant increases of NCC2 surface expression (+71% and +74%, respectively) and total abundance (+22% and +26%, respectively) as revealed by immunoblotting from total cell lysates and biotinylated fractions.

Conclusions: This study provides evidence that SPAK and OSRI may regulate the activity of NCC2 not only by phosphorylation of the conserved N-terminal threonines but also by modulation of the total abundance and surface expression of the transporter.
Aldosterone Affects NCC Phosphorylation Involving MAPK ERK1/2 Signaling Pathway

Xiuyan Feng,1 Yanhui Wang,1,4 Ping Wu,1,4 \nXiuyan Feng, Yanhui Wang, and Ping Wu

Methods: We used cell culture, western blot analysis and siRNA knock down technique to carry out the experiments.

Results: To determine whether acute aldosterone treatment affects NCC regulation involving MAPK ERK1/2 signal pathway, we treated the mDCT cells with aldosterone 1μM at different time point and found that the ERK1/2 phosphorylation increased at 15 minute of aldosterone treatment but decreased after 1 hour and lasted at least 24 hour. However, SPANK phosphorylation at S373 was not significantly altered. We then determined whether reductation of SPANK expression by siRNA affects ERK1/2 phosphorylation and NCC phosphorylation. Under knock-down of SPANK expression, we still observed the dynamic change of ERK1/2 phosphorylation in a similar fashion as before. We found that aldosterone increased NCC phosphorylation by inducing NCC phosphorylation mechanism, which was confirmed by the lack of induced-in NCC phosphorylation in SPANK knock out mice. Moreover, insulin administration to mice increased phosphorylation of oxidative stress-responsive kinase-1 (OSR1)/SPANK and NCC in the kidney. Time-course experiments in mpkDCT cells and mice suggested that SPANK is involved in the insulin-induced OSR1/SPANK phosphorylation mechanism.

Conclusions: The present results demonstrated that insulin is a potent regulator of NCC phosphorylation in the kidney, and that SPANK and 4K are involved in this mechanism of NCC phosphorylation by insulin.

FR-PO1756

Development of New Systems To Measure Total and Phosphorylated Na-Cotransporter(NCC) Protein in Human Urine

Kiyoshi Isobe, Eisie Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida

Methods: In this study, we developed a sandwich ELISA method to measure NCC in human urine along with a conventional but improved Western blotting method.

Results: Sandwich ELISA and Western blotting are able to detect urinary NCC as low as 2.5 pmol/ml and 5.0 pmol/ml, respectively. Using these methods, we found that NCC concentration in spot urine samples remained constant within a day when they were correctly stored at appropriate creaticine concentration. This result suggests that single spot urine can be used to estimate total excretion of NCC for 24 hours. We also confirmed that urine NCC excretion varied according to different salt intake. Furthermore, we for the first time succeeded to detect phosphorylated NCC (pNCC) in human urine by using phospho-specific antibodies. Since pNCC is an active form of NCC, pNCC in urine could be more sensitive marker for predicting in vivo activity of NCC than total NCC.

Conclusions: Thus, we established methods to measure NCC and pNCC in human urine samples. The relationship of thiazide sensitivity and urine NCC and pNCC will be efficiently investigated by these systems.

Funding: Government Support - Non-U.S.

FR-PO1759

Genotype and Phenotype Analysis in Taiwanese Patients with Gitelman’s Syndrome

Min-Hua Tseng, Sung-Sen Yang, Akihito Ohta, Shotaro Naito, Motoko Chiga, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida

Methods: After 6 days feeding with three different diets (normal, low and high salt), we collected urinary exosomes from wild-type C57BL/6 mice.

Results: Western blotting of these samples revealed total and phosphorylated NCC (S71 and T53) were increased in mice fed low salt diet than in mice fed normal diet, which is consistent with previous reports, and also well correlated with the increases of total and phosphorylated NCC in the kidney. However, we found that total and phosphorylated NCC in urine exosomes from mice fed high salt diet was also increased compared with those mice fed normal diet.

Conclusions: Since urinary exosomal proteins are derived from multi-vesicular bodies, formed by endocytosis of plasma membranes, we speculate that, the increased excretion of NCC in exosomes may be caused by the increased endocytosis of NCC from basolateral membranes. This result suggests that urinary NCC excretion in exosomes may not necessarily reflect NCC abundance in the apical plasma membranes of DCT, at least in mice.

Funding: Government Support - Non-U.S.
Diagnostic Value of Diuretics Loading Test in Gitelman Syndrome Jeonghwan Lee,1 Sejoong Kim,2 Yun Kyu Oh,3 Kwon Wook Joo,1 Jin Suk Han.1 Internal Medicine, Seoul National University Hospital, Seoul, Korea; 2Internal Medicine, Gachon University Gil Medical Center, Seoul, Korea; 3Internal Medicine, Seoul National University Borumae Hospital, Seoul, Korea. Background: We investigated the diagnostic significance of diuretics test in Gitelman syndrome (GS) and evaluated the superiority among various indices of renal clearance tests. Methods: Sixteen patients with clinically typical Gitelman syndrome were enrolled. One patient with factitious vomiting due to bulimia nervosa and 1 normal volunteer were also studied as controls. Mutation analysis of SLCO1B1 and CCLCNK gene was done in all patients. Diuretic loading (FE) test was performed with HCT (HCT) and FUR (HCT/FUR). Clearance indices (CI) such as FEH2O, FENa, FCCl and DFCR (distal fraction of chloride reabsorption) were calculated. The ratio of clearance index (CI(HCT/FUR) which stands for CI(HCT)/CI(FUR)) and the ratio of delta CI (ΔCI(HCT/FUR) which stands for ΔCI(HCT)/ΔCI(FUR)) were also calculated. Results: All patients had normotensive hypokalemic metabolic alkalosis, hyperreninemia and increased aldosterone level. SLCO1B1 mutation was detected in 11 patients (8 compound heterozygous, 3 homozygous mutations), but in 5 patients. No CCLCNK mutation was detected in all the subjects. GS patients showed blunted response to thiazide administration (the range of FEH2O(Basil)/Basal) was 0.75 – 5.74. The ranges of FEH2O(HCT), FEH2O(FUR) and ΔFEH2O(HCT/FUR) of GS patients were 0.05 – 0.24, 0.05 – 0.30 and -0.05 – 0.14, and those of control group were 0.38 – 0.39, 0.31 – 0.38 and 0.34 – 0.36, which clearly discriminated patients from controls. Indices calculated from FEH2O, FCCl and DFCR except FEH2O(FUR) and ΔCI(HCT/FUR) overlapped between GS patients and controls. Conclusions: We suggest that the diagnosis of GS can be done with clinical characteristics and renal clearance test with diuretics instead of SLCO1B1 mutation analysis. FEH2O associated indices rather than absolute values were more reliable among various clearance indices in the diagnosis of GS.

FR-PO1760 eAMP Stimulates NKCC2 Recycling in Thick Ascending Limbs (TALs) Via PKA: Role of Ser126 Phosphorylation Gustavo R. Arbo, Pablo A. Ortiz, Hypertension & Vascular Research Division, Henry Ford Hospital, Detroit, MI. Background: The apical cotransporter NKCC2 mediates NaCl reabsorption by the thick ascending limb (TAL). Surface NKCC2 is maintained by constitutive trafficking into and out of the apical membrane that involves recycling of internalized transporters. cAMP, the second messenger of AVP and β-adrenergic agonists, stimulates apical membrane NKCC2 and NaCl reabsorption by increasing the rate of NKCC2 exocytosis via protein kinase A (PKA). However, it is not known whether cAMP stimulates NKCC2 exocytosis from a recycling compartment or the biosynthetic pool. cAMP also enhances NKCC2 phosphorylation at Ser126, Ser874, and Thr96, and 101. We hypothesized that cAMP and cAMP+H-89 stimulated NKCC2 recycling in TALs via PKA, and Ser126 phosphorylation is involved in this trafficking event. Methods: We measured NKCC2 recycling and phosphorylation in rat TAL suspensions by a modified surface biotinylation protocol and Western blot. NKCC2 receptors were immobilized in the presence of forskolin/IBMX (F/I) to stimulate cAMP and recycling of internalized NKCC2 measured and expressed as a percent of the internalized pool. We found that internalized NKCC2 recycling back to the surface in a constitutive manner (7 min = 11.4±2.1%, 15 min = 17.2±3.0%, 30 min = 25.1±3.3%, p<0.05). The PKA agonist H-89 (10 μM) completely blocked NKCC2 recycling by 30 min (Basal NKCC2 recycling = 21.2±2.3%, H-89 with cAMP+H-89 = 27.3±4.3%, n=5, p<0.05). cAMP-enhanced phospho-Ser126 by 33.6±6.0-fold and H-89 blocked this effect by 82% (p<0.05), we did not detect Ser874 phosphorylation. cAMP also enhanced Thr96 and Thr101 phosphorylation by 1.7±0.4-fold (p<0.05), however this was not blocked by H-89 (1.8±0.4-fold). Conclusions: We conclude that cAMP stimulates NKCC2 recycling in TALs via PKA. PKA inhibition blocked NKCC2 recycling and Ser126 phosphorylation. Our data suggest that Ser126 rather than Thr96, is involved in cAMP-stimulated trafficking in TALs. Funding: None NHIBI

FR-PO1761 Activation of the Butamethasone-Sensitive Na+/K2Cotransporter NKCC2 Is Facilitated by Tamm-Horsfall Protein in a Chloride-Sensitive Manner Kerim Mutig,1 Thomas Kahl,1 Turgay Saritas,1 Michael Godes,1 James M. Bates,2 Maria Castañeda-Bueno,1 Luca Rampoldi,2 Carsten Dosche,1 Hajamohideen S. Raffi,1 Maria Castañeda-Bueno,1 Michael Godes,1 James M. Bates,2 Kerim Mutig,1 1Internal Medicine, Seoul National University Hospital, Seoul, Korea; 2Internal Medicine, Gachon University Gil Medical Center, Incheon, Korea; 3Department of Pathology, University of New Mexico School of Medicine, Albuquerque; 4Department of Physiology, Christian-Albrechts-University Kiel, Kiel, Germany.

Background: Active transport of NaCl in the thick ascending limb (TAL) is accomplished by Na,K 2Cl cotransporter (NKCC2). The activation of NKCC2 depends on intracellular chloride concentration (Cl-) and includes its amino-terminal phosphorylation. We hypothesized that co-expressed Tamm Horsfall protein (THP) modulates NKCC2 activity in TAL cells. Methods: Effects of THP on NKCC2 phosphorylation (Thr96/T101) and transport activity were studied in THP-deficient (THP-/-) and wild type (WT) mice, cultured TAL cells, and frog oocytes. Results: THP-/- mice displayed decreased phosphorylation of NKCC2 (p<0.05) compared to WT mice. Cultured TAL cells with low endogenous THP levels displayed sharp increases in NKCC2 phosphorylation (+38%, p<0.05) along with a pronounced decrease of Cl- (40%, p<0.05) upon transfection with THP. In NKCC2-expressing frog oocytes, co-injection with THP cRNA significantly enhanced the activation of NKCC2 under low chloride hypotonic stress (+112% vs. +235%, p<0.05). Stimulation of the vasopressin V2 receptor pathway by V2 agonist (dDAVP; 30 min) resulted in enhanced NKCC2 phosphorylation in WT mice and cultured TAL cells transfected with THP whereas in the absence of THP, NKCC2 phosphorylation upon dDAVP was blunted in both systems. Attenuated effects of fosinoprine along with functional and structural adaptation of the distal convoluted tube in THP-/- mice further supported the notion that NaCl reabsorption was impaired in TAL lacking THP. Conclusions: In summary, these results are compatible with a permissive role for THP in the modulation of NKCC2-dependent TAL salt reabsorptive function.
FR-POI767

Epithelial Na⁺ Transport Regulation by AMP-Activated Protein Kinase in Kidney Collecting Duct and Its Role in Ischemia

Hui Li, Jian Wang, Rodrigo Alzamora, Jeffrey Lee, Nuria M. Pastor-Soler, Kenneth R. Hallows.

Background: The epithelial Na⁺ channel (ENaC) in the kidney collecting duct regulates total body volume and blood pressure and is regulated by hormones and cellular signals, including metabolic stress induced by ischemia. The metabolic sensor AMP-activated protein kinase (AMPK) inhibits ENaC in kidney and lung epithelia, but the potential role of AMPK activation in the acute inhibition of ENaC following metabolic stress is unknown.

Methods: We used mouse polarized kidney cortical collecting duct (mpkCCD) cells cultured on transwell inserts for studies of apical ENaC distribution and functional activity. We measured apical ENaC (γ and β) apical membrane expression in mpkCCD cells. Similarly, we used ex vivo AICAR treatment of kidney slices disrupted dDAVP-induced ENaC apical pole accumulation as assessed by immunolocalization in the CD. Treatment of mpkCCD cells with chemical ischemic agents, either an inhibitor of glycolytic metabolism (10 mM 2-deoxyglucose) or inhibitors of oxidative metabolism (0.1 mM antimycin A or 2-5 mM CCCP), elicited rapid reductions in amiloride-sensitive ENaC-dependent Isc. These ENaC inhibition responses were associated with the presence of the AMPK inhibitor Compound C (50 μM) or with inducible RNAi-mediated AMPK-α1 knockdown in mpkCCD cells, suggesting that AMPK activation plays a significant role in the ENaC inhibition response to ischemia.

Conclusions: AMPK inhibits ENaC activity and apical surface expression in kidney CCD cells and participates in the ischemia-induced down-regulation of ENaC activity. Inhibition of ENaC and other transport proteins by AMPK under conditions of metabolic stress may play an adaptive role by preventing the dissipation of ionic gradients generated by cellular pumps, thereby limiting cellular ATP consumption.

Funding: NIDDK Support

FR-PO1768

ENaC-Mediated Sodium Reabsorption in P2X2, Null Mice

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Background: There is now overwhelming evidence that extracellular nucleotides, acting via P2 receptors, can modify ENaC-mediated sodium reabsorption in collecting ducts (Bailey & Shirley 2009, Pinnier Signal. 5:473). However, the P2 receptor subtype(s) involved have not been clearly defined. Pharmacological profiling and the use of genetically engineered mice point to an important role for P2Y2 receptors (Pochynyuk et al 2010,FASEB J 24: 2056), but a patch-clamp investigation of rat cortical collecting duct principal cells has revealed that ENaC is insensitive to apical P2X4 receptors. In this study, we examined whether ENaC activity and/or expression is altered by extracellular nucleotides. Due to a lack of selective P2 receptors. These are subdivided into ionotropic P2X receptors (14 subtypes) and metabotropic P2Y receptors (8 subtypes). In the collecting duct, ENaC, AQP2, and ROMK activity and/or expression is altered by extracellular nucleotides. Due to a lack of selective agonists and antagonists, and notoriously unreliable antibodies, controversy exists over the role of ENaC modulation by extracellular ATP.

Methods: We present a new method to develop primary cultures from TAL cells, obtained from microdissected tubules from the outer medulla of 1-month-old collagenase-treated mouse kidneys.

Results: The selected tubules specifically express uromodulin (Tamm-Horsfall protein) and the Na⁺-K⁺-2Cl⁻ cotransporter NKCC2, whereas markers such as podocalyxin (glomerulus), aquaporin-1 (proximal tubule) and aquaporin-2 (collecting duct) are negative. The TAL cells were then cultured on permeable filter supports for 7-10 days, allowing the formation of well-polarized confluent monolayers with apical and basolateral domains. The TAL tubules were then cultured on permeable filter supports for 7-10 days, allowing the formation of well-polarized confluent monolayers with apical and basolateral domains. The TAL tubules were then cultured on permeable filter supports for 7-10 days, allowing the formation of well-polarized confluent monolayers with apical and basolateral domains. The TAL tubules were then cultured on permeable filter supports for 7-10 days, allowing the formation of well-polarized confluent monolayers with apical and basolateral domains.

Background: The epithelial cells lining the thick ascending limb (TAL) of the loop of Henle play essential roles in the normal and diseased kidney.

Methods: We report a new method to develop primary cultures from TAL cells, obtained from microdissected tubules from the outer medulla of 1-month-old collagenase-treated mouse kidneys.

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Conclusion: The epithelial cells lining the thick ascending limb (TAL) of the loop of Henle play essential roles in the normal and diseased kidney.

Funding: Government Support - Non-U.S.
P2 receptor subtype(s) responsible. Arguably, P2X2 subtypes are largely overlooked when considered responsible for extracellular nucleotide-evoked modulation of these key solute and water transport mechanisms.

**Methods:** Using a cell line (M1) derived from principal cells of the CCD, and perforated whole-cell and excised outside-out configurations of the patch-clamp technique, we have sought evidence for ATP-gated P2X receptor ion channel expression in the CD. The external (bath) solution contained 145 mM NaCl and the internal (pipette) solution contained 145 mM KCl (70 mM Cl⁻).

**Results:** Upon application of ATP (100 µM) at -60 mV, a large inward whole-cell current emerged. Rapidly, ATP desensitized in approximately 50% of cells. Interestingly, whole-cell currents appeared to be comprised of several components including an initial rapidly desensitizing current. Furthermore, in outside-out patches, application of external ATP (100 µM) transiently activated flickery single-channel currents (τdes ~ 2 - 4 s, τrec ~ 30 s). At -60 mV, the mean single-channel current amplitude and open probability (Pₒ) of these channels were -0.59 ± 0.03 pA and 0.63 ± 0.02 (n = 3).

**Conclusions:** Our result demonstrate that M1 cells express functional ATP-sensitive P2X receptors and, moreover, that ATP is able to directly activate channels with properties similar to those of P2X2 subtypes. Based on these data, we suggest P2X2 receptors may be key in the regulation of CD solute and water transport.

**Supported by the Wellcome Trust.**

**FR-PO1770**

**Functional Coupling between Antinatriuretic Ang II and Natriuretic Bradykinin Signaling Cascades Is Critical for Aldosterone-Independent Regulation of ENaC by Dietary Sodium Intake** Oleh Pochynyuk, Mykola Mamenko, Oleg L. Zaika. Integrative Biology and Pharmacology, University of Texas Health Science Center at Houston, TX.

**Background:** It is generally accepted that aldosterone affects activity of the epithelial Na⁺ channel (ENaC) in the distal nephron to regulate circulating volume and, consequently, blood pressure. However, given experimental evidence pointing towards aldosterone-induced mechanisms, it might have a role in regulation of ENaC activity in response to variations in dietary salt intake.

**Methods:** We used patch clamp electrophysiology in freshly-isolated split-opened murine distal nephrons to test if ENaC can be regulated by systemic salt intake independently of aldosterone.

**Results:** Inhibition of MR receptors with spironolactone, while decreasing ENaC membrane levels, did not affect regulation of ENaC open probability by dietary sodium. We hypothesized that activation Ang II signaling may contribute to regulation of ENaC by salt intake. Indeed, we found that Ang II in the range from 5 to 500 nM acutely and reversibly increases ENaC-Po. Activation of AT1 receptors with subsequent stimulation of NADH oxidase mediated Ang II actions on ENaC. Importantly, saturation of MR status with DOCA treatment did not perturb Ang II regulation of ENaC suggesting that the effect of Ang II is non-redundant. In addition, we found that activation of Ang II cascade further increases ENaC activity via Angiotensin Converting Enzyme (ACE)-dependent mechanism by limiting the inhibitory actions of locally produced Bradykinin (BK). ACE inhibition augmented the inhibitory action of BK on ENaC and caused marked natriuresis in wild type but not in mice lacking BK receptors.

**Conclusions:** We concluded that the balance between stimulatory Ang II and inhibitory BK cascades allows fine-tuning of ENaC activity during variations in dietary salt intake independently of aldosterone.

**Funding:** Private Foundation Support

**FR-PO1771**

**Aldosterone Induces Epigenetic Reprogramming of the αENaC Gene in mMCD3 Cells** Zhong C. Zhang, Yu Qin Kong, Bruce C. Kone. University of Texas-Houston Medical School.

**Background:** Aldosterone induces renal tubular Na⁺ reabsorption in part by enhancing αENaC transcription. In addition to known effects of ald to trans-activate αENaC via the mineralocorticoid receptor (MR), we previously reported that αENaC transcription is also governed by an antinatriuretic-sensitive epigenetic pathway involving histone H3K79 methyltransferase Dot1 and DNA binding protein A9. The Dot1-A9 complex associates with aldosterone-responsive transcription in mCD3 cells, and aldosterone induces repression, increasing αENaC promoter activity and endogenous mRNA levels beginning at 3 hours of treatment (J Biol Chem 284:20917-26, 2009). Determining how genes move through repressed, pressed, and active chromosome states is central to understanding transcriptional induction. To understand aldosterone-induced activation of αENaC transcription, a high-resolution, kinetic analysis of aldosterone-induced changes in the histone code, the recruitment and action of enzymes mediating these changes, and the recruitment and action of MR is needed.

**Methods:** Time-course ChIP-qPCR assays of the α ENaC promoter and distal enhancer region in mCD3 cells treated with 1 µM aldosterone for 0-96 h. H3K9me3 and H3K4me3 were measured by ChIP-qPCR. In addition, we measured H4K20me3 at R1 and R2, and H3K9me3 at R3 during this treatment.

**Results:** In summary, aldosterone increases ENaC transcription through an initial, complex reprogramming of the histone code that dismisses the Dot1-Af9 complex to effect de-repression, followed by MR-mediated trans-activation of the gene.

**Funding:** NIDDK Support

**FR-PO1772**

**An Aldosterone-Regulated NH₃-Transporter-Specific Variant Enhanced Function Is Expressed in the Collecting Duct** Christie P. Thomas,1 Nundita S. Raiwar,1 Internal Medicine, University of Iowa, Iowa City, IA; 2VAMC, Iowa City, IA.

**Background:** Serum and glucocorticoid-regulated kinase 1 (Skg1), regulates ENaC-mediated Na⁺ transport in the distal nephron.

**Methods:** Skg1 transcripts were examined in mouse nephron segments and in the collecting duct cell line, mpkCCKD₁β, by qRT-PCR. Skg1 isoforms were expressed with or without ENaC subunits in mpkCCKD₁β, and FRT epithelia and Na⁺ transport measured in Ussing chambers. ENaC surface expression and cleavage were studied in HEK293 cells. Ubiquitination and half-life measurements of Skg1 isoforms were also studied in HEK293.

**Results:** Previously, we identified a cell-surface expressed Skg1 isoform (Skg1_2) that stimulates Na⁺ transport (AJP 2008, 295: 321-326). We have now identified an aldosterone and insulin-suppressed alternate transcript of Skg1 that is expressed in the native mouse duct, CNT and CCD and in mpkCCKD₁β. The encoded protein, Skg3, has a variant NH₂-terminus that results in a Skg1 isoform that is less susceptible to ubiquitination, is more stable and significantly increases Na⁺ transport when overexpressed in mpkCCKD₁β and when co-expressed with ENaC in FRT cells. The increase in Na⁺ transport requires an intact kinase domain. Skg3,1 increases ENaC cleavage at the cell surface and inhibits the effect of Ned4-2 to reduce ENaC cleavage, similar to the prototypic Skg1. Mutation of a conserved polybasic amino acid motif (KKR) in its variant NH₂-terminus converts Skg1_3 from a combined nuclear and cytoplasmic protein to exclusively cytosolic suggesting that the KKR motif may be part of a nuclear localization signal. This KKR motif is also a destabilizing motif, possibly a target for ubiquitination, since mutation of KKR increases the half-life of Skg1_3.

**Conclusions:** In conclusion, we have identified a regulated NH₂-terminally variant of Skg1 that is expressed in the distal nephron. The encoded protein isoform inhibits Ned4-2, increases ENaC cleavage and stimulates epithelial Na⁺ transport and probably contributes to aldosterone and insulin-stimulated epithelial Na⁺ transport in the CNT and CCD.

**Funding:** NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support

**FR-PO1773**

**Mechanistic Basis for Specific Activation of SGK1 by mTOR** Ling Lu, Jian Wang, Harlan Ives, David Pearce. Medicine, University of California, San Francisco, CA.

**Background:** The serum- and glucocorticoid-induced kinase 1 (SGK1) plays an important role in hormone regulation of ENaC-dependent Na⁺ transport. We have previously reported that the mTOR complex-2 (mTORC2) activates ENaC by phosphorylating SGK1. It is, however, unknown which mTORC2 component mediates this interaction, or whether this interaction plays a physiologically relevant role in specific activation of SGK1.

**Methods:** We used the yeast two-hybrid system coupled with random mutagenesis to identify a mutant mSIN1 that does not interact with SGK1.

**Results:** Expression of the mSIN1 mutant does not restore SGK1 phosphorylation to wild-type levels in mSIN1-deficient murine embryo fibroblasts. Furthermore, in kidney epithelial cells, the mSIN1 mutant has a dominant-negative effect on SGK1 phosphorylation and on SGK1-dependent ENaC-mediated Na⁺ transport. Interestingly, the role of mSIN1 to recruit SGK1 to mTOR appears to be specific for SGK1: although mSIN1 is essential for phosphorylation of another mTORC2 substrate, Akt, it does not interact with Akt and its ability to phosphorylate and activate Akt is unaffected by the point mutation that abrogates interaction with SGK1.

**Conclusions:** These data support the conclusion that mTOR, which regulates a wide array of cellular processes, uses distinct strategies to phosphorylate its various substrates, and suggest a mechanism for specific regulation of ENaC-mediated Na⁺ transport without inadvertent effects on unrelated cellular processes.

**Funding:** NIDDK Support

**FR-PO1774**

**Role of Skg2 in Mediating the Regulation of ENaC by Oxygen** Russell F. Huston,1,2 Rita D. Signoril,1,3 John B. Wisely.1,2 Electrolyte Disorders and Physiology: Inorganic Ions: Na, K, Cl - I,1 Internal Medicine, University of Iowa, Iowa City, IA; 2VAMC, VA Medical Center, Iowa City, IA.

**Background:** We have previously demonstrated that changing the ambient oxygen concentration between 1% and 40% has a potent effect on ENaC-mediated Na⁺ transport independent of the actions of corticosteroids. This oxygen effect requires hours to become evident, exhibits a clear dose response, and is completely reversible. The purpose of the present experiments was to determine possible molecular mediators of this oxygen effect.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: We used the mouse collecting duct cell line mppKCCD-c14 grown on filters where mesangioblasts had been withdrawn for 24 h and measured Na transport by short-circuit current (Isc).

Results: We first investigated the possible role of candidate kinases. At normal oxygen levels inhibitors of ERK1/2 and p38 had minimal effect while inhibitor of JNK reduced Isc by 50%. However, none of these inhibitors altered the relative effects of 8% or 40% oxygen on Isc. Thus, while JNK may regulate baseline Na transport, it appears not to be involved in the effects of oxygen to regulate ENaC activity. Microrray analysis identified Sgk2 as a possible candidate. Real time PCR demonstrated an increase in Sgk2 mRNA with higher oxygen tensions. However, Sgk2 was not demonstrated to have a stimulatory effect on Sgk2 protein abundance. We constructed Sgk1 and Sgk2 in a tetracycline regulated in the effects of oxygen to regulate ENaC activity. Microarray analysis identified Sgk2 as by levels inhibitors of ERK1-2 and p38 had minimal effect while inhibitor of JNK reduced Isc by 50%. However, none of these inhibitors altered the relative effects of 8% or 40% oxygen on Isc. Thus, while JNK may regulate baseline Na transport, it appears not to be involved in the effects of oxygen to regulate ENaC activity. Microrray analysis identified Sgk2 as a possible candidate. Real time PCR demonstrated an increase in Sgk2 mRNA with higher oxygen tensions. However, Sgk2 was not demonstrated to have a stimulatory effect on Sgk2 protein abundance. We constructed Sgk1 and Sgk2 in a tetracycline regulated

Conclusions: Preeclampsia is associated with significant urinary excretion of plasminogen and activation of ENaC by urine. Urinary plasminogen is significantly correlated to blood pressure. We speculate that pathophysiological activation of ENaC by urinary plasminogen may contribute to hypertension and edema in preeclampsia.

Funding: Pharmaceutical Company Support, Private Foundation Support

FR-PO1777

Urinary Content of Plasminogen (plg) and Activation of ENaC Current by Urine Residues during Remission of Idiopathic Nephrotic Syndrome

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Background: In nephrotic syndrome (NS), data show glomerular filtration of plasminogen to pre-urine and activation to plasmin. Urine plasmin activates the epithelial Na’ channel (ENaC) in vitro. It was hypothesized that this mechanism is causal for NaCl retention and therefore that plasmin and the ability of urine to activate ENaC disappears in the remission phase of nephrotic syndrome.

Methods: Spot urine samples from 20 children with active idiopathic NS were collected and compared to urine samples obtained after remission in the same patients. Urine samples were analyzed for plasmin and plasminogen concentration (ELISA) and urinary protease activity (zymography). Ability of urine to evoke ENaC currents were assessed by whole cell patch clamp using a murine cortical collecting duct cell line (M1).

Results: 20 patients (7 girls) mean age 9.1±3.2 yrs were included. Urine plasminogen concentration normalized to urine creatinin concentration was found significantly different in the active phase of NS in comparison to remission by paired t-test (p<0.0001, geometric mean: 2244 mg/L vs. 83 mg/L). Gelatin Zymography showed protease activity in urine in 10 of 17 patients (active phase) compared to 3 of 17 patients at remission. Western blotting analysis of urine from active phase of NS showed results compatible with the presence of both plasmin and plasminogen in samples at remission. 10 out of 10 tested urine samples were negative. Urine from the active phase of NS evoked a significant increase in ENaC current in M1-cells (201 % ± 31%, p<0.006, n=6) that was significantly larger than current evoked by a urine sample from the same individual in the remission phase (p=0.0006).

Addition of amiloride (2 μmol/L) to urine samples from patients in active phase abolished inward currents.

Conclusions: The parallel observation of remission and normalization of urine plasminogen and the ability of urine to activate ENaC disappears in the remission phase of nephrotic syndrome.

Funding: Private Foundation Support

FR-PO1778

Proteinuria Induces Increased Insulin- and IGF-1 Receptor Signalling, a Mechanism of ENaC-Mediated Volume Retention

Franziska Theilig,1 Christoph Geers,1 Daniela Corinne Spoehr,1 Anne Enke,1 Harm Peters,2 1Department of Medicine, Institute of Anatomy, Fribourg, Switzerland; 2Institute of Nephrology, Berlin, Germany.

Background: Proteinuria is a symptom of many renal glomerular diseases. It is associated with signs of volume retention such as edema formation or hypertension. In the collecting duct a dysregulation of ENaC is assumed to be causative and hormonal activation of aldosterone and vasopressin was excluded.

Therefore, we hypothesized an activation of the insulin- and IGF-receptor signalling to account for the volume retention in proteinic kidney diseases.

Methods: For the induction of an experimental glomerulonephritis (GN; type Th1) and puromycin-induced nephritic syndrome (PAN) Wistar rats were injected either with OX-7, puromycin or vehicle. After 6 days, kidneys were prepared for histochemical or biochemical analysis.

Results: Urinary excretion of insulin (control 2.19 ± 0.5; GN 25.1 ± 9.9 and PAN 18.6 ± 6.4 μg/24h) and IGF-1 (control 11.9 ± 7; GN 264.6 ± 65.5 and PAN 431.9 ± 58.24 μg/24h) were strongly increased. Insulin- and IGF-1-R were localized to the apical and basolateral membrane of the collecting duct. Proteinuria induced an increased phosphorylation of the apical insulin/IGF-1 receptor. Insulin and IGF-1 induced a “priming effect” of their respective receptors as determined by cell surface biotinylation experiments and confocal microscopy of mppKCCD cells. An activation of the insulin/IGF-1R induced signalling cascade leading to phosphorylation of PDK1, Akt, WNK1, sGK1, Nedd4-2 and consecutive dysregulation of ENaC was observed upon insulin/IGF-1-mediated apical stimulation of mppKCCD cells as well as in GN and PAN.

Conclusions: In summary, our results show that proteinuria activated the apical insulin/IGF-1R pathway and may therefore be an additional mechanism for ENaC-mediated volume retention in proteinic kidney diseases.
Nephron Expression and Distribution of the Plasminogen Receptor, PLG-RK, and Colocalization with ENaC and uPAR, in Murine Kidney

Samir Nangia,1 Hongdong Bai,1 William B. Kiosses,2 Kevin W. Chen,1 Volker Vallon,1 Lindsey A. Miles,2 Robert J. Parmer.1 University of California, San Diego, and VA San Diego Healthcare System, San Diego, CA; Scripps Research Institute, La Jolla, CA

Background: Recent studies suggest a key role for the plasminogen (PLG) activation system in the proxenotrophic processing and activation of ENaC, providing an important mechanism for the Na+ retention associated with nephrotic syndrome, in which increased PLG concentrations are present in urine. We recently identified a novel transmembrane PLG receptor, PLG-RK, which markedly enhances cell surface activation of PLG to the active enzyme plasmin. Here, we investigated the expression, distribution, and cellular localization of PLG-RK in murine kidney, and performed quantitative colocalization studies of PLG-RK and the urokinase receptor (uPAR, another key component of the PLG activation system), with ENaC.

Methods: C57Bl6 mice were perfused in situ with 4% paraformaldehyde. Kidneys were fixed, placed through sucrose gradients and frozen in OCT. Sections were immunostained

Results: PLG-RK was prominently expressed in proximal and distal nephron segments, particularly in the distal tubule and collecting duct (as revealed by co-staining with antibodies to the sodium chloride co-transporter and aquaporin 2). PLG-RK was observed primarily on the apical surface, with some labeling also in a punctuate distribution in the cytoplasm, in a pattern similar to that observed for ENaC. uPAR (a GPI-linked membrane protein) was primarily observed in the distal nephron, and was almost exclusively on the apical surface. Quantitative analyses of merged images showed substantial apical colocalization of PLG-RK with ENaC (70.1±1%, n=507 cells), and with uPAR with ENaC (62.1±1%, n=314 cells).

Conclusions: These results demonstrate that PLG-RK, uPAR, and ENaC are co-localized on the apical surface of the distal nephron, and are present in an orientation to promote PLG activation and ENaC processing.

Funding: Other NIH Support - NHLBI, Veterans Administration Support

Paradigm Defining Role of the Insulin Receptor in Collecting Duct Sodium Handling in Mice

FR-PO1779

Lijun Li

Background: Recent studies suggest that insulin, via its own receptor, is important in renal sodium handling by the collecting duct via activation of ENaC. Fluctuations in circulating insulin levels, therefore, due to diet, disease, or therapy, may be altered to affect sodium handling independently of the renin-angiotensin-aldosterone system.

Results: There were no differences in body weight (bw), basic renal function, or kidney size due to genotype in either sex, with M mice about 5 grams heavier (P>0.05) at low and high flow rates. Similar to our previous report, a 53% increase of fluid and Na+ reabsorption via the epithelial Na+ channel (ENaC) in the aldosterone-sensitive distal nephron (ASDN) plays a central role in body fluid volume regulation. Insulin is recognized as a powerful regulator of ENaC in the collecting duct.

Methods: To study mechanisms of ENaC regulation by insulin, we generated insulin receptor knockout (IR-KO) mice targeted specifically to the collecting duct principal cells using Cre-lox mediated recombination. Mice withlox sites flanking the IR gene were crossed with mice possessing Cre-recombinase driven by the AP2 promoter.

Results: After one week of sodium-deficient diet the IR-KO mice demonstrated significantly lower ENaC activity compared to their wild type littermates as was demonstrated by cell attached patch clamp measurements in freshly isolated split opened collecting duct. Acute insulin application in such experiments revealed that loss of insulin receptor prevented increase of ENaC activity which was observed in wild type mice. Immunobiochemical and western-blot assays demonstrated that total abundance of all three ENaC subunits in the kidney cortex were not different between WT and IR-KO mice.

Conclusions: These results suggest that insulin via IR increases ENaC activity affecting the channel open probability (Po). To further determine mechanism of insulin’s action on ENaC, we used immortalized mCPDE (principal cells. Insulin rapidly increased amiloride-sensitive transmembrane flux in mCPDE cells with the EC50 of 12.5±1.7nM. Pretreatment of the mCPDE cells with PI3-kinase or mTOR inhibitors LY294002 or PP242, respectively precluded the effect of insulin.

Conclusions: Thus, we propose that insulin is a key regulator of ENaC activity and its effects are mediated via PI3-kinase and mTOR signaling.

Funding: NIDDK Support, Private Foundation Support

Effect of Formate on Flow-Dependent Transport of Sodium, Bicarbonate and Chloride in Proximal Tubule

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Background: Glomerulotubular balance (GTB) refers to the nearly proportional change in salt and water reabsorption with tubule variations in GFR. We have previously demonstrated that axial flow impacts proximal reabsorption of Na+ and HCO3- by modulating both NaH exchange. In this study we investigated whether C1 absorption is also impacted by axial flow in the proximal tubule.

Methods: Mouse proximal tubules were perfused in vitro at low (5mL/min) and high (20 mL/min) perfusion rates in the absence and presence of 0.5mM of formate to activate the NaH exchanger. The fluid (JF) and HCO3- (JHCO3) absorption were measured by the changes of ‘H-Inulin and total CO2 concentrations in the original and collected fluid, and the Jv was estimated from the change of Jf, and the assumption of isotonic transport. The change in Jv is estimated as the difference between Ju and Jv0.

Results: Similar to our previous report, a 55% increase of fluid and Na+ and doubling of HCO3- absorption were observed when the flow rate was increased from 5 to 20 mL/ min. Flow did not affect C1 absorption, as Jv was 47.8 ± 40.5 pmol/min/mm at low and high flow rates, respectively. Addition of formate significantly increased both Na+ and C1 absorption with a stronger increment in C1, but the percentage of increment of transport activity by formate was similar at both low and high flow rates. Jv0, Jv1000C1 and Jv increased by 43%, 39% and 50% and by 42%, 39% and 51% at the low and high flow rate, respectively. Specifically, in the presence of formate, Ju was 71 ± 61.3 pmol/min/ mm at low and high flow rates, respectively.

Conclusions: These results indicate that C1 absorption is not impacted by axial flow, and that the absence of a flow effect persists in the presence of formate. They suggest that there is unlikely to be a flow-dependent change in luminal membrane density of the Cl-/HCO3-exchanger.

Funding: NIDDK Support

Reduced Anti-Natriuretic Response to Insulin in Benzamid in Mice Lacking Insulin Receptors in the Collecting Duct

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Background: The epithelial sodium channel (ENaC) has been shown to be upregulated by insulin in a variety of cell systems and in perfused tubules, but whether circulating insulin plays a role in day-to-day sodium balance is unclear.

Methods: To address this, the IR was selectively knocked out from the collecting duct principal cells with Cre recombinase driven by the aquaporin-2 promoter. Natriuretic responses to insulin were studied in both male (M) and female (F) knockout (KO) and wild-type (WT) littermates to insulin, dextrose in saline, and select sodium transporter and channel antagonists were used.

Results: Under basal state, PAR2 activation increases sodium absorption in cortical collecting ducts (CCD). Therefore, we evaluated whether it participates in the maintenance of blood pressure.

Conclusions: In conclusion, through its actions on electroneutral sodium transport, PAR2 in response to sodium depletion.

Funding: Other NIH Support - NHLBI, Veterans Administration Support
FR-PO1784
Adenosine Receptors Modulate Sodium Uptake in Human Renal Proximal Tubule Cells
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Background: Adenosine (ADO) generated in renal proximal tubule cells (RPTCs) acts on local receptors to mediate sodium (Na+) reabsorption. Inhibition of ADO type 1 receptors (A1-AR) leads to natriuresis and diuresis, primarily due to its effects on the PT, whereas the effects of inhibition of type 2A receptors (A2A-AR) are unknown.

Methods: To explore the role of ADO in RPTCs, we tested the effects of A1-AR and A2A-AR inhibition and stimulation on Na+ uptake in cultured human RPTCs (HK-2 cells). We hypothesized that ADO signals through A1-AR to modulate the activity of the Na+/H+ exchanger (NHE3), the major Na+ reabsorptive pathway in RPTCs.

Results: As expected, inhibition of NHE3 with S1611 (10^-5 M) reduced Na+ uptake by ∼60%-65% vs. control (Control: 4678±358 vs S1611: 1722±448 cpm, p<0.001). The A1-AR antagonist PSB-36 (10^-4 M) reduced Na+ uptake by ∼20%-25% vs. control (Control: 4678±358 vs PSB: 3707±302 cpm, p<0.001). However, neither inhibition nor stimulation of A2A-AR had an effect on Na+ uptake, either in the presence or absence of NHE3 inhibition, suggesting A2A-AR does not modulate Na+ uptake in this model.

Conclusions: In summary, inhibition of A1-AR decreased Na+ uptake and stimulation of A1-AR reduced Na+ uptake in RPTCs and both actions were attenuated by simultaneous NHE3 blockade. These results suggest that ADO acts on A1-AR through signaling pathways that inhibit or promote NHE3 activity. However, neither inhibition nor stimulation of A1-AR had an effect on Na+ uptake, with or without inhibition of NHE3. Similarly, the A2A-AR agonist CGS (10^-5 M) in the presence of AD had no effect on Na+ uptake or AD alone (CHA+AD: 4689±279 vs. AD: 3601±424 cpm, p<0.005). However, CHA had no additional effect on Na+ uptake in the presence of S1611. The A1-AR antagonist ZM241385 (10^-5 M) had no effect on Na+ uptake, with or without inhibition of NHE3. As expected, inhibition of A2A-AR and CHA did not affect Na+ uptake in either group.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1785
MPGES-1-Derived PGE2 Mediates Dehydration Natriuresis Zhanjun Jia,1,2 Gang Liu, 1,2 Tianxin Yang. 1,2
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Background: Dehydration natriuresis is an important physiological response, aiming to reduce the plasma Na+ and maintain normal plasma osmolality. PGE2 is a natriuretic factor whose production after water deprivation is the goal of this study was to investigate the role of MPGES-1 in dehydration natriuresis.

Methods: MPGES-1 WT and KO mice were subjected to the 24-h water deprivation (WD).

Results: After 24-h WD, WT mice exhibited a significant increase in 24-h Na+ excretion (159.2 ± 2.1 vs. 231.2 ± 24.9 mmol/24h, p<0.01), accompanied with normal plasma Na+ concentration and osmolality. In contrast, WD-induced increase in urinary Na+ excretion was completely abolished in KO mice (152.6 ± 17.3 vs. 128.6 ± 25.8 mmol/24h, p<0.01), in parallel with trend increase in plasma Na+ concentration (139.2±1.5 vs. 142.2±1.4 mmol/L, p=0.06) and osmolality (278.4±2.26 vs. 284.0±2.34 mOsm/kgH2O, p=0.051). By qRT-PCR, renal medullary COX-2 mRNA in dehydrated WT mice was elevated by hypertonicity at 540 mOsm/kg H2O, irrespective of the type of solutes, and was significantly increased in the presence of S-1611 (10^-5 M) reabsorption, with or without NHE3 inhibition.

Conclusions: In summary, inhibition of NHE3 with S1611 (10^-5 M) reduced 22Na uptake by ∼60%-65% vs. control (Control: 4678±358 vs S1611: 1722±448 cpm, p<0.001). The A1-AR antagonist PSB-36 (10^-4 M) reduced Na+ uptake by ∼20%-25% vs. control (Control: 4678±358 vs PSB: 3707±302 cpm, p<0.001). However, neither inhibition nor stimulation of A2A-AR had an effect on Na+ uptake, either in the presence or absence of NHE3 inhibition, suggesting A2A-AR does not modulate Na+ uptake in this model.

Funding: NIDDK Support, Other NIH Support - NHLBI

FR-PO1787
Sodium Delivery and ENaC Regulate Collecting Duct Endothelin Production Donald E. Kohan, Meaghna Pandit, Arianna Lyon-Roberts.
Division of Nephrology, University of Utah Health Sciences Center, Salt Lake City, UT.

Background: Collecting duct (CD) endothelin-1 (ET-1) is an autocrine inhibitor of CD Na reabsorption. Salt loading increases CD ET-1; the mechanisms transducing this effect are poorly understood. Tubule fluid flow increases in response to Na loading, hence we speculated that flow modulation accounts for ET-1 production. We have previously shown that flow increases mpkCD cell ET-1 mRNA content via intracellular Ca, PLC and PKC.

Methods: We now extend these studies in mpk cells to examine how flow modulates CD ET-1. mpk cell ET-1 mRNA was assessed at 2 dyn/cm2 for 2 hours using various agents.

Results: Increasing perfusate osmolality to 450 mosm/L (from 300 mosm/L) with NaCl, but not mannitol, increased ET-1 mRNA response to flow by 75%. Increasing osmolality with Na acetate, but not chloride, increased ET-1 mRNA by 85%, indicating NaCl-dependent ET-1 production. Amiloride (1 µM) or benzamil (0.2 µM) modestly reduced flow-stimulated ET-1 mRNA, indicating ENaC-dependence. Two days of aldosterone increased the flow response by 95%; amiloride reduced the aldosterone response by 75%. Aprotinin reduced flow-stimulated ET-1 mRNA content by 80%. We then examined Ca entry pathways (primarily apical) known to exist in CD Removal of primary cilia (chloral hydrate or NH4SO4) did not alter the flow response. Blockade of (RN1734) or stimulation (La or Gd) of TRPV4 did not affect the flow response, nor did nifedipine. Inhibition of TRPC3/6 channel with SKF96365, BTP2 or Pyr3 modestly reduced the flow response. Blockade of Na+ channel with SEF400 had no effect on the flow response.

Conclusions: In summary, flow-stimulated CD ET-1 production appears to be mediated in large part by Na delivery whose detection is critically dependent upon ENaC. How this leads to alterations in intracellular Ca signaling remains to be fully determined. These data identify a novel flow pathway wherein CD Na delivery per se increases CD ET-1 production, thereby potentially down-regulating Na-stimulated ENaC-mediated Na reabsorption. Such a system may be important in natriuretic states wherein diminished CD Na reabsorption is desirable.

Funding: Other NIH Support - NHLBI

FR-PO1788
Mice with the XX Sex Chromosomal Complement Have a Differential Natriuretic Response to Aldosterone Plus High-NaCl Diet as Compared to XXY Diana L final, Radha Mayeri Garkakopti, Carolyn M. Ecelberger.
Department of Medicine, Georgetown University, Washington, DC.

Background: Female (XX) mice have a lower blood pressure (BP) than young males (XY), in a variety of species. The mechanisms underlying these sex differences in BP are not fully understood but may involve sex steroid and sex chromosomal complement (SCC) influences on sodium handling.

Methods: Utilizing the unique mouse model in which Sry (male sex-determining gene) was translocated from the Y chromosome to an autosome, we evaluated the independent influences of sex (M vs. F) and sex chromosomal complement (XX vs. XY) on natriuretic responses to aldosterone plus high-NaCl diet. mice of 4 genotypes: 1) XX-F, 2) XY-F, 3) XX-M, and 4) XY-M were gonadectomized to remove masking effects of sex steroids. After 2 weeks, they were placed on a low-NaCl diet (0.085%) for 2 days, and then osmotic minipumps were implanted to infuse aldosterone (40 mg/40 g•bw/d). After 4 days, all mice were switched to a high-NaCl diet (3%) for 3 additional days. 24-h urine was collected. Mice consumed diet and drank water ad libitum.

Results: There was no significant difference in body weight or weight change were observed. By day 2 of high-NaCl diet, mice of the XX SCC demonstrated a significantly more robust aldosterone escape, as evident by higher urinary sodium excretion (mmol/Na/40 g bw/d): 3.5 ± 0.9 (XX-F), 1.5 ± 0.2 (XY-F), 2.1 ± 0.3 (XX-M), and 1.5 ± 0.4 (XY-M), p = 0.028 for SCC. This significantly increased excretion of sodium in the XX SCC was maintained on day 3 of high-NaCl diet (p < 0.031 for genotype). Interestingly, potassium excretion was also significantly increased in the XX SCC on day 3 (p = 0.045).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: These results suggest either: 1) XX SCC are more aldosterone sensitive, leading to early sodium retention and insufficient growth. Despite natriuresis or, 2) XX SCC have a greater efficiency of escape mechanisms relative to XY. Studies to evaluate BP in this model are currently ongoing. Overall, these studies highlight important differences between males and females.

Funding: NIDDK Support, Private Foundation Support

FR-PO1789
Crosstalk between Kidney and Heart Angiotensin II Contributes to Diabetic Cardiomyopathy through Volume Expansion and Overload Takahiro Masuda, Shigeaki Muto, Eiji Kusano. Division of Nephrology, Department of Medicine, Jichi Medical University, Tochigi, Japan.

Background: Chronic kidney disease, including diabetic nephropathy, is a risk factor for cardiovascular disease. In diabetes, kidney and heart angiotensin II (Ang II) play a critical role in each organ damage; however, it is unclear how crosstalk between them is involved in the pathogenesis of diabetic cardiomyopathy (DCM).

Methods: We used male Spontaneously Diabetic Torii (SDT) rats (a novel non-obese human type 2 diabetes model) treated with and without olmesartan medoxomil (Olm) (an Ang II receptor blocker) or insulin for 16 weeks after diabetes onset. Systolic blood pressure, echocardiographic findings, blood and urinary biochemical findings, and histological findings in left ventricular (LV) and kidney tissues were compared among the groups.

Results: In SDT rats, kidney and heart Ang II, but not circulating Ang II, increased and this Ang II was co-localized with aquaporin 1 (a proximal tubule marker) and cardiac myosin (a cardiomyocyte marker), respectively. SDT rats showed LV chamber dilatation, LV hypertrophy and increases in plasma atrial natriuretic peptide (a volume marker) and tubular Na+ reabsorption without hypertension. The expression of aquaporin 1 and Na+-1/-2 exchanger 3, both of which contribute to proximal tubule fluid reabsorption, was upregulated in the SDT rat kidney cortex. In SDT rats, cardiomyocyte hypertrophy, interstitial fibrosis and overexpression of Ang II and atrial natriuretic peptide were restricted to the LV subendocardium, which is more susceptible to local environmental changes than is the subepicardium. These events (except the hypertegyemia) were reversed by Olm; insulin abolished them all.

Conclusions: We conclude that volume expansion via the stimulatory effect of Ang II overproduced in proximal tubules on the tubules’ Na+ reabsorption induces Ang II upregulation in LV subendocardial cardiomyocytes as volume overload, leading to DCM. Therefore, crosstalk between kidney and heart Ang II, which involves the downstream processes of persistent hyperglycemia, contributes to DCM through volume expansion and overload.

Funding: Government Support - Non-U.S.

FR-PO1790
Decreased Na-K-ATPase Maximal Activity and Expression in Angiotensin II-Induced Hypertension Augustin Gonzalez-Vicente, Jeffrey L. Garvin. Hypertension and Vascular Research Division, Henry Ford Health System, Detroit, MI.

Background: Thick ascending limbs (TALs) reabsorb 25% to 30% of the total filtered NaCl load. NaCl enters TAL cells via NKCC2 and exits via basolateral Na-K-ATPase. We have shown an increase in TAL NaCl reabsorption in Angiotensin II (AngII) induced hypertension. However the transporters affected in this model are not known. Thus we hypothesize that in AngII-induced hypertension Na-K-ATPase activity is enhanced.

Methods: We infused rats with 200mg/kg/min Ang II or vehicle for 7 days. Direct femoral mean arterial blood pressure (MAP) was measured. TAL segments were obtained and Na-K-ATPase activity measured in permeabilized tubule fragments. Na-K-ATPase expression was analyzed in TAL lysates by Western blotting using an anti α1-subunit antibody. We used H-Ouabain binding to measure the number of Na-K-ATPases in the plasma membrane.

Results: AngII increased MAPB by 20 ± 5 mmHg; (116 ± 4 mmHg, n=10 vs 137 ± 3 mmHg, n=11, p < 0.001). Contrary to our hypothesis in AngII-induced hypertension total Na-K-ATPase activity (membrane + intracellular) was decreased by 13% (1.55 ± 0.05 vs 1.32 ± 0.04 mmol PO4/µg protein/min, p < 0.005, n=12). We also found a 24% (p = 0.01, n=4) decrease in N-K-ATPase expression. There were no significant differences in the k'/s for Na (7.5 ± 0.2 vs 7.4 ± 0.4 mmol/min) or K (1.9 ± 0.1 vs 1.8 ± 0.1 mM, n=7). Despite decreased total activity expression, we found no differences in the number of pumps located in the membrane (6.5 ± 0.7 x109 vs 6.1 ± 0.8 x109 units/µg protein, n=6).

Conclusions: 1) in contrast to the increase in Na transport, total Na-K-ATPase activity is decreased in AngII-induced hypertension possibly due to decreased expression; 2) changes in enzymatic parameters can not explain the decrease in activity; 3) the increase in NaCl reabsorption that occurs in AngII-induced hypertension must be due to increases in apical Na entry; 4) since total expression is decreased without changes in the number of Na-K-ATPases in the membrane, AngII-induced hypertension may enable trafficking.

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FR-PO1791
Effect of Chronic Tempol on Sodium Transporter Regulation in Sprague Dawley Rats Fed Control Diet Mien T.X. Nguyen, Donna Lee, Nicholas K. Fletcher, Muhammad Madkour, Alicia A. McDonough. Keck School of Medicine of USC, Los Angeles, CA.

Background: Both Ang II, which is anti-natriuretic, and high salt diet, which is natriuretic, increase reactive oxygen species (ROS) generation. ROS increases Na+ reabsorption in perfused TALH. Scavenging ROS with Tempol, a superoxide dismutase mimetic that reduces the effects of Ang II on the TALH (Offend et al. 2002). In contrast, scavenging ROS with Tempol doubles Na+ reabsorption in proximal tubule of Spontaneously Hypertensive Rats (SHR) (Panico,2009). This study aimed to examine effects of Tempol on renal electrolytes and renal Na transporter abundance and phosphorylation in Sprague-Dawley rats.

Methods: Rats fed a normal diet (0.75%NaCl, 2%KCl) were given drinking water without (C, n=5) or with 4mM Tempol (T, n=4) for 2 weeks. Urine was collected overnight in metabolic cages. NHE3, NKCC and NCC abundance and phosphorylation were analyzed by immunoblotting. Renal expression of Na-I and Na-K-ATPase were measured in the cortex and medulla.

Results: Compared to C, T group had: 38% lower rate of weight gain/wk, 25% lower urine volume (UV), 15% lower UosmV, 30% lower UNa+V, unchanged UK+V and decreased UNa+K+ ratio (all p<0.03). Proximal tubule (PT) NHE3 abundance did not change, NKCCpS552 increased 55% and NHE3pS552/total ratio increased 45%, a molecular marker for depressed NHE3 activity (both p<0.03). Cortical NKCC and NHE3pS552/T101 both tended to increase (ns) and DCT NCC increased 30% (p<0.01) in T treated vs. C.

Conclusions: These results are consistent with effects of Tempol in normal rats to: 1) decrease food and water intake (based on decreased output), 2) decrease PT Na+ reabsorption (in contrast to reports in SHR), 3) increase DCT Na+ reabsorption. We conclude that scavenging basal levels of ROS in control animals significantly alters renal Na+ transporter regulation in a reciprocal manner in PT and DCT.

Funding: NIDDK Support

FR-PO1792
Cellular and Subcellular Aspects of Renal Vasopressin V2 Receptor Distribution Adelina Stoessel,1 Turgay Saritas,1 Luca Rampoldi,2 Sebastian C. Bachmann,1 Kerim Mutig.1 Anatomie. Charite Universitatsmedizin Berlin, Germany; 2Molecular Genetics of Renal Disorders Unit Dibit, San Rafaele Scientific Institute, Mailand, Italy.

Background: Vasopressin (AVP) regulates salt and water transport in renal epithelia, chiefly via vasopressin V2 receptors (V2R). Previous studies of the segment- and cell-type-related aspects of V2R abundance and subcellular distribution produced in parts controversial results which may be due to restricted affinities and limited availability of specific antibodies. This study aimed to present a high-resolution analysis of cellular and subcellular V2R distribution in mammalian kidney epithelium.

Methods: A specific anti-V2R antibody was generated and characterized. Renal distribution of V2R was studied using confocal microscopy. Basolateral and luminal subcellular aspects were resolved using immunogold labeling of V2R on kidney sections and confocal evaluation of cultured thick ascending limb (TAL) cells transfected with GFP-V2R.

Results: Application of our anti-V2R antibody on rat, mouse, and human kidney sections revealed comparable cellular and subcellular localization of V2R in the principal cells of the connecting tubule and collecting duct as identified using specific markers for the indicated renal tubules. Abundance of the receptor in macula densa (MD) cells was low to absent. No signals were detected in glomeruli, proximal tubules or vascular elements. Confocal evaluation of kidney sections and cultured TAL cells transfected with GFP-V2R revealed no significant co-localization of V2R with luminal proteins, such as Na,K-2Cl- cotransporter type 2, Tamm-Horsfall protein, or aquaporin 2, suggesting basolateral distribution of the receptor. Electron microscopic analysis confirmed the predominant basolateral distribution of V2R.

Conclusions: This study provides an extensive analysis of cellular and subcellular V2R distribution in rat, mouse, and human kidney epithelia. Low abundance of the receptor in MD cells may be related to their specific role for TGF.

FR-PO1793
Rho-Kinase Pathway Activated in HIV-Associated Nephropathy Jin Judy Song,1 Rungwasee Rattanavich,2 Mohammad Hussian,1 Aswani Malhotra,2 Pravin C. Singhal.1 Department of Medicine, St. Luke's Roosevelt Hospital Center, New York City, NY; 2Feinstein Institute for Medical Research, North Shore LIJ Health System, Great Neck, NY.

Background: Epithelial mesenchymal transition (EMT) plays an important role in the progression of renal interstitial tubular fibrosis. EMT has been shown to contribute to the molecular mechanisms of the proliferative phenotype in HIV-associated nephropathy (HIVAN) (Am J Physiol 2010). Activation of Rho A/Rho kinase is one of the major signaling pathways involved in EMT. The inhibition of Rho kinase has been shown to reduce the EMT and renal fibrosis in different animal models. We hypothesized that Rho kinase signaling pathway is contributing to occurrence of EMT in HIVAN. In the present study, we examined the role of Rho kinase in HIVAN.

Methods: Kidneys were harvested from age (4 weeks old) and sex matched control and Tg26 mice. Renal cortical sections were immunoabeled for Rho kinase and alpha-alpha-SMA (specific antibodies for EMT). To establish the relationship between Rho kinase and alpha-alpha-SMA, serial sections of renal cortical sections of Tg26 mice were labeled either.
for Rho kinase or alpha-SMA. Immunoblots were prepared from renal tissues of control and CRF-NW mice. In histological studies, human tubule cells in CRF-NW mice were transduced with either empty vector (EV/HK2) or 1L-3 (HIV/HK2). Immunoblots of EV/HK2s and HIV/HK2 were probed for Rho-Kinase and associated down stream signal- phospho-MYPT1.

Results: Tubular cells in Tg26 mice displayed enhanced expression of both Rho kinase and alpha-SMA when compared with control mice. Immunoblots studies revealed spatial relationship between the expression of Rho kinase and alpha-SMA in tubular cells. In in vitro studies, HIV/HK2 also showed enhanced expression of Rho kinase. Moreover, HIV/ HK2 displayed enhanced expression of phospho-MYPT1.

Conclusions: These findings indicate that Rho kinase is activated in tubular cells in HIVAN. Funding: NIDDK Support

FR-PO1794

SIRT1/PGC-1α Activation Protects Against Aldosterone-Induced Podocyte Injury Via the Amelioration of Mitochondrial Dysfunction Aihua Zhang, Songming Huang, Guixia Ding. Department of Nephrology, Nanjing Children’s Hospital Affiliated to Nanjing Medical University, Nanjing, Jiangsu, China.

Background: Podocyte injury causes proteinuria and is found in many glomerular diseases. Mitochondria maintain podocyte energy homeostasis, and mitochondrial dysfunction (MID) is an early event in podocyte injury. This study investigated whether the transcriptional coactivator, peroxisome proliferator activated receptor-g coactivator 1α (PGC-1α), a major regulator of oxidative metabolism and mitochondrial function, prevented podocyte damage by improving MID.

Methods: MPC5 conditionally immortalized mouse podocyte clonal cells (kindly provided by Peter Mundel at Mount Sinai School of Medicine) were cultured. C57BL/6J mice had osmotic minipumps implanted subcutaneously. Pumps delivered a continuous infusion of aldosterone (0.15 µg/h). MID was assessed by mitochondrial membrane potential (MMP), mtDNA copy number, ATP content, and ROS production. Podocyte damage was assessed by apoptosis, nephrin, and podocin expression.

Results: Aldosterone (Aldo) decreased PGC-1α expression and induced MID and podocyte injury in dose- and time-dependent manners. Endogenous PGC-1α suppression by RNAi induced podocyte MID and apoptosis. Increased PGC-1α levels in podocytes by transfection with a PGC-1α vector prevented Aldo-induced MID and inhibited injury. The protective effects of PGC-1α overexpression were not observed when a PGC-1α T177A S538A mutant vector was used. SIRT1 (silent mating type information regulation 2 homolog 1), a gene upstream of PGC-1α, was also investigated. We confirmed that SIRT1 overexpression restored Aldo-induced MID and podocyte injury by upregulating PGC-1α at both the transcriptional and posttranslational levels. Finally, we found that resveratrol (RSV), a SIRT1 activator, attenuated Aldo-induced MID and podocyte injury in vitro and in Aldo-infused mice in vivo.

Conclusions: PGC-1α is important in maintaining normal mitochondrial function, and SIRT1/PGC-1α activation protected podocytes from Aldo-induced MID and injury. SIRT1 activators, such as RSV, may be useful therapeutically for glomerular diseases to promote and maintain PGC-1α expression and mitochondrial function.

Funding: Government Support - Non-U.S.

FR-PO1795

Disparate Effects of Ang II during the Initiation and Progression of HIV-Associated Nephropathy (HIVAN) Divya Satlhal1, Dilip Kumar1, Guohua Digt2, Pravin C. Singh1 1Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY, 2Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; 1Pathology, New York Medical College, Valhalla, NY, China.

Background: Ang II may contribute to the progression of HIVAN through its hemodynamic effects or its direct effects. We evaluated the role of Ang II in the initiation and progression of HIVAN in genetically engineered HIVAN mice (Tg26) with variable copies of the α-tubulin promoter (α-tub).

Methods: Tg26 mice with 2, 3, and 4 copies of Agt were evaluated for expression of AT1 and AT2 receptors during embryogenesis (E13, E15, and E18) and after birth, on day 1 and day 10 by immunohistochemical, in situ hybridization, and confocal microscopy. In addition, aging Tg26 mice with various copy numbers of Agt were evaluated for severity of proteinuria, podocyte injury, tubulointerstitial fibrosis, and podocyte and tubular cell death. DNA, RNA, collagen deposition, blood pressure, and vasculature.

Results: During embryogenesis and on days 1 and 10, renal cells showed greater expression of AT2 receptors when compared to AT1 receptors. Both tubular cells and podocytes showed temporal and spatial relationship between AT1 and AT2 receptors. Mice with 4 Agt copies showed lower blood pressure (mean 110±80 mm Hg) and 4 wks when compared to mice with two Agt copies (mean 140±90 mm Hg). Mice with 4 Agt copies showed higher blood pressure at 16 wks. Four wks old mice with 4 Agt copies displayed attenuated expression of PAI-1 when compared to age-matched mice with 2 Agt copies. Whereas, 16 wks old mice with 4 Agt copies showed 3-fold greater PAI-1 expression than age-matched mice with 2 Agt copies. Tg26 mice (2 Agt copies) aged to nine weeks developed renal lesions that were more severe than those seen in age-matched Tg26 mice with 3 or 4 Agt copies.

Conclusions: We conclude that higher Agt copies induced protective effect during the initiation of HIVAN may be mediated through the temporal-spatial expression of AT1 and AT2 receptors during embryogenesis and post-natal period.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

530A
Immune Complexes from Patients with IgA Nephropathy Containing Galactose-Deficient IgA1 and Anti-glycan Antibodies Induce Protein-kinase Signaling and Proliferation in Cultured Human Mesangial Cells

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Background: Circulating immune complexes (CIC) in patients with IgA nephropathy (IgAN) consist of galactose-deficient IgA1 (Gd-IgA1) and anti-glycan antibodies. Several in vitro studies have shown that these CIC are pathogenic in that they activate mesangial cells (MC) and induce cellular proliferation.

Methods: Using sera of patients with IgAN, we fractionated CIC by size-exclusion chromatography. Cultured primary human MC were stimulated with CIC, samples collected at various time points, and analyzed by SDS-PAGE/Western blotting with anti-phospho-tyrosine (P-Y) antibody. Sera depleted of IgA-containing CIC served as negative control. MC were stimulated with CIC. These samples were further analyzed using PamStation® 12 high-content peptide substrate microarray to profile global tyrosine kinase (TK) activity (kinomic profiling) to identify CIC-stimulated pathways.

Results: Results showed that CIC increased P-Y of multiple proteins in MC by 3-42%. Kinomic profiling showed that CIC activated multiple TK-mediated signaling pathways, including PDGF signaling and anti-apoptosis processes. To validate these results, similar experiments were performed with engineered immune complexes (EIC) formed in vitro from Gd-IgA1 myeloma protein and a recombinant human IgG specific for Gd-IgA1. The EIC increased phosphorylation of proteins in MC by 5-41%. PamStation® kinomic profiling indicated that EIC activated TK in a fashion similar to that for native CIC.

Conclusions: In summary, Gd-IgA1-containing complexes, CIC and EIC, activated multiple signaling pathways in MC and led to cellular proliferation. Importantly, EIC may provide an excellent substitute for native CIC in the future IgAN studies and may be used to develop animal models more closely reflecting human disease.

Funding: NIDDK Support

Protein-kinase Inhibitors Can Block Cellular Proliferation and Signaling Induced in Cultured Human Mesangial Cells by Immune Complexes from Patients with IgA Nephropathy

Zhi Qiang Huang, Joshua Anderson, Timothy D. Rohrbach, Stacy D. Hall, Rhubell T. Brown, Bruce A. Julian, Christopher D. Willey, Jan Novak. University of Alabama at Birmingham, AL.

Background: IgA1-containing circulating immune complexes (CIC) isolated from sera of patients with IgA nephropathy (IgAN) stimulate proliferation of mesangial cells (MC) in vitro. Here, we studied cellular proliferation and signal transduction induced by CIC in cultured primary human MC and the potential blocking effects of tyrosine-kinase inhibitors (TKI).

Methods: CIC were isolated from sera of patients with IgAN by size-exclusion chromatography. Sera depleted of IgA-containing CIC served as negative control. MC were stimulated with CIC or CIC-activated TK in a fashion similar to that for native CIC.

Results: CIC increased MC proliferation 7-fold compared to the baseline. Dasatinib and sorafenib inhibited the induced MC proliferation by 36% and 19%, respectively. The inhibition of proliferation was reflected by decreased P-Y signaling on Western blots. CIC enhanced P-Y of proteins from MC by 19-67%, based on densitometric analysis. Dasatinib and sorafenib inhibited phosphorylation induced by CIC by 26-64% and by 0-20%, respectively. Kinomic profiling using PamStation® 12 showed that CIC increased P-Y of multiple proteins, including those involved in PDGF signaling and in anti-apoptotic processes. Dasatinib significantly blocked CIC-induced P-Y of these proteins, whereas sorafenib was ineffective.

Conclusions: In summary, CIC-induced MC proliferation was mediated by tyrosine-kinase signaling and a TKI can block MC proliferation and signaling, thus raising novel possibilities for potential new options for therapy of IgAN.

Funding: NIDDK Support

Quantitative Analysis of O-Glycosylation of IgA Hinge Portion (HP) in IgA Nephropathy (IgAN) Patients and Its Responsiveness to the Therapy of Tonsillectomy Combined with Corticosteroid IV (TLX+S)

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Background: Several patterns of sugar chain attached to Ser/Thr residue of IgA1 HP constitute O-glycosylation. The component sugars are N-acetylgalactosamine (GalNAc), galactose (Gal) and sialic acid. Although its qualitative analyses in IgAN patients have been performed and aberrant glycosylation has been already reported, neither its quantitative analysis itself nor its clinical significance has been reported.

Methods: The fully glycosylated form of human IgA1 is as follows; the sugar adjacent to the Ser/Thr residue is GalNAc which is connected to Gal, and sialic acids are attached to GalNAc and Gal. But there are a variety of glycosylated forms of IgA1 in one individual. We analyzed the serum IgA glycosylation from IgAN patients and control using MALDI-TOF-MS. We calculated the number of GalNAc and Gal at HP and the ratio of Gal to GalNAc. We also analyzed the serum IgA glycosylation of the IgAN patients before and after the therapy of TLX+S.

Results: The ratio of IgA with 5 GalNAc to that with 4 GalNAc in HP and the average number of Gal joined to one GalNAc in HP (GalGalNAc) are significantly decreased in patients with IgAN (n=9) compared to healthy control (n=30). In cases of patients with IgAN undergoing TLX+S, the number of GalNAc per HP significantly increased from 4.2 ± 0.085 at pretreatment to 4.4 ± 0.11 at 46 ± 9.5 months post tonsillectomy (p<0.02). The number of Gal per HP or the ratio of Gal/GalNAc did not change significantly. In remission cases, all patients exhibited the increase of the number of GalNAc per HP.

Conclusions: The O-glycosylation of IgA in patients with IgAN seems to be deeply involved with the pathogenesis of the disease. It can be recovered by the therapy of TLX+S.
FR-PO1802

Metformin Increases Renal Medullary Interstitial Cell Apoptosis In Vitro and In Vivo

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Background: Metformin is the most commonly used pharmacological therapy for type 2 diabetes worldwide. It improves glycemic control in type 2 diabetic patients mainly via the activation of AMP-activated protein kinase (AMPK). AMPK is a Ser/Thr protein kinase acting as a sensor of cellular energy status and is abundantly expressed in the kidney, where it plays an important role in regulating a variety of physiological and pathological processes including ion transport, glomerular and medullary cell function and diabetic nephropathy. We aimed to examine the effect of metformin on the survival of renal medullary interstitial cells (RMICs) under hypoxic conditions both in vitro and in vivo. AMPK activity was decreased in RMICs under the hypoxic condition within 12 hr and then gradually returned to the baseline level. Metformin activated AMPK and markedly increased hypoxia-inducible factor-1α (HIF-1α) expression. Similarly, AICAR and A-769662, two selective AMPK activators, or a constitutively active AMPKα construct resulted in a significant increase in apoptosis of RMICs. AMPK activation was associated with the suppression of hypoxic signalling pathways, including NFκB nuclear translocation and activation of the cytoprotective cyclooxygenase-2 (COX-2). AMPK activation also resulted in a marked reduction in ROS generation and nuclear expression of toxicity-responsive enhancer binding protein (TonEBP), which prevented up-regulation of osmoprotective genes in hypoxia-treated RMICs. In vivo study using normal C57Bl/6 mouse with water deprivation further demonstrated massive apoptosis of RMICs after treatment with metformin and two other AMPK activators (AICAR and A-769662). Furthermore, treatment of type 2 diabetic db/db mice with AMPK activators including metformin caused a marked increase in RMICs’ apoptosis under both normal and dehydration conditions. Taken together, these results identify AMPK as a critical factor involved in the maintenance of RMIC viability and type 2 diabetes and raise safety concerns for metformin in diabetic patients with dehydration.

Funding: Government Support - Non-U.S.

FR-PO1803

Decreased Expression of PGC-1α in Skeletal Muscle May Contribute to Protein-Energy Wasting in CKD: Role of Glucocorticoids

Russ Price.1 Bin Zheng,1 Jennifer L. Gooch,1 Xiaoxia H. Wang.1, 2 Renal Division, Emory University, Atlanta, GA; 2Atlanta VA Medical Center, Decatur, GA.

Background: Accelerated muscle protein wasting contributes to the protein-energy wasting that occurs during CKD and diabetes. PGC-1α is a transcription coactivator that integrates energy metabolism. In muscle, PGC-1α antagonizes the FOXO transcription factors which induce components of proteolytic systems. Previously, we demonstrated that diabetes decreases PGC-1α mRNA as well as calcineurin (Cn)/MEF2/NFAT signaling in rodent muscle; these pathways are implicated in the regulation of PGC-1α transcription. Presently, we tested whether similar changes occur in muscle of CKD mice.

Methods: CKD was induced in mice by partial nephrectomy; controls underwent sham operations. The mice were pair-fed and sacrificed approximately 3 weeks later. mRNAs were measured by qRT-PCR. PGC-1α transcript expression was evaluated in L6 muscle cells transfected with a PGC-1α-promoter luciferase reporter gene (PGC-1α-Luc).

Results: PGC-1α mRNA was decreased 88.2% (P<0.05) in gastrocnemius muscle of CKD vs controls. The transcriptional activities of MEF2 and NFAT were evaluated by measuring the mRNA levels of their respective gene targets, MRF4 and MCI1P4.4 in muscle. CKD decreased MRF4 mRNA by 57.8% and MCI1P4.4 mRNA by 43.1% (P<0.05). Since glucocorticoids are necessary for protein wasting in CKD, we tested whether treatment of L6 muscle cells with dexamethasone (DEX; 100 nM, 48 h) affected PGC-1α expression and Cn/MEF2/NFAT signaling. DEX decreased PGC-1α, MRF4 and MCI1P4.4 mRNAs by 61.8%, 46.4% and 40.1%, respectively (P<0.05). The decrease in PGC-1α mRNA was due, at least in part, to suppression of transcription because DEX reduced luciferase activity by 30±4% (P<0.05) in muscle cells transfected with PGC-1α-Luc. Importantly, co-expression of a constitutively active Cn with PGC-1α-Luc increased luciferase activity 94±20% (P<0.05).

Conclusions: These data indicate that glucocorticoids reduce Cn/MEF2/NFAT signaling and PGC-1α expression in muscle which could lead to higher FOXO activity and accelerated proteolysis. If unchecked, the responses would contribute to the protein-energy wasting associated with CKD.

Funding: NHLBI Support, Veterans Administration Support

FR-PO1804

Dysfunction in Renal Protein Handling in Rats Fed a High Fat Diet

Deanne H. Hryciw, Esther Grinfeld, Adam M. Miller, Kayte A. Jenkin, Michael L. Mathai, Andrew J. McAinley. School of Biomedical and Health Sciences, Victoria University, Melbourne, Victoria, Australia.

Background: Obesity is frequently associated with chronic kidney disease. The earliest marker of chronic kidney disease is microalbuminuria, with an association between the severity of obesity and the magnitude of microalbuminuria. Albumin uptake is processed by a macromolecular complex in the proximal tubules, via interactions with megalin. The molecular mechanism linking obesity and albuminuria is yet to be elucidated, however reduced processing of albumin by the macromolecular complex contributes to the excessive excretion of albumin in the urine. We investigated changes to the mRNA and protein of the macromolecular complex in rats fed a high fat diet, compared to control.

Methods: 18 male Sprague-Dawley rats were fed either a control chow diet (4%) or a high fat diet (22%) for 12 weeks. At weeks 3, 6 and 10, 24 hour urine samples were collected and analysed for albumin and protein content. Kidneys were excised from animals at week 12. mRNA and protein was extracted, and the levels of megalin, NHE3, CIC-5, NHERF1 and NHERF2 were assessed by real time PCR and Western blot analysis, respectively.

Results: Compared to control, rats fed a high fat diet had a significant increase in body weight. At weeks 3, 6, and 10, there was a significant increase in urinary albumin excretion in rats fed a high fat diet, however the protein in the urine was only significantly increased at week 10. Sodium secretion in rats fed a high fat diet was significantly decreased at 10 weeks. Analysis of the expression of megalin, NHE3, CIC-5, NHERF1 and NHERF2 mRNA indicated no significant difference between control and high fat fed animals. Importantly, the level of NHE3 protein was altered in rats fed a high fat diet which may account for the changes in sodium excretion.

Conclusions: Therefore, in rats fed a high fat diet, there is an increase in renal albumin and protein secretion, and a reduction in sodium secretion. Significantly, albuminuria was shown to precede precnemia in this model of obesity. This may be due to the altered expression of components of the megalin macromolecular complex observed in these rats.

Funding: Private Foundation Support

FR-PO1805

Endogenous PPAR Agonist Nitro-Oleic Acid Protects Against Adriamycin-Induced Nephropathy in Mice

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Background: Adriamycin (ADR) is an anthracycline antibiotic utilized in antitumor therapy but its clinical use is frequently impeded by renal toxic effects. Nitroalkene derivatives of nitro-oleic acid (OA-NO2) are endogenous lipid products with novel signaling properties, particularly the activation of PPAR. Our previous studies demonstrated renoprotective action of OA-NO2 in various mouse models of acute and chronic renal injury. The present study was undertaken to examine the possible role of OA-NO2 in a mouse model of ADR nephropathy.

Methods: B6C3F1 mice were pretreated for 2 days with OA-NO2 at 5mg/kg/day via osmotic mini-pump, followed by a single injection of vehicle or adriamycin (10mg/kg) via tail vein. Albuminuria and renal function were analyzed at 1 wk of ADR treatment.

Results: ADR mice developed prominent albuminuria (ADR: 508.9±48.5µg/24h vs. Cont: 2.4±1.1µg/24h, p<0.01), hyperlipidemia (ADR: 396.2±70.9mg/dl vs. Cont: 42.4±7.1mg/dl, p<0.01), hypoalbuminemia ( ADR: 0.28±0.08g/dl vs. Cont: 1.01±0.15g/dl, p<0.01), and hyperlipidemia (triglyceride, ADR: 396.2±70.9mg/dl vs. Cont: 61.41±2.7mg/dl, p<0.01), with 90% of the mice in this group having severe ascites. In contrast, OA-NO2 treated mice had mild ascites. The urine thiobarbituric acid-reactive substances (TBARS) significantly increased in ADR group mice (ADR: 18.9±3.5µmol/mmol creatinine vs. Cont: 4.0±0.6µmol/mmol creatinine, p<0.01). The urine albumin excretion was significantly increased in ADR group mice (ADR: 508.9±48.5µg/24h vs. Cont: 2.4±1.1µg/24h, p<0.01), and this increase was attenuated in the ADR+OA-NO2 group (0.7±0.07pmol/24h, p<0.05 vs. ADR). Together, these findings suggest a novel therapeutic potential of OA-NO2 in ADR nephropathy.

Conclusions: Together, these findings suggest a novel therapeutic potential of OA-NO2 in adriamycin nephropathy.
Methods: Obesity prone (OP) and obesity resistant (OR) rats (6–8 wks, Charles River) were placed on moderately high fat (MHF) diet and BPs were monitored radiotelemetrically until ≈36 wks of age before sacrifice for histopathology. Additional OP and OR rats were instrumented with a BP radiotransmitter and renal blood flow (RBF) probe (Transonic) at ~20 wks of age before the development of renal injury. After 5–7 days, BP & RBF were recorded (2–5 h) in conscious rats at baseline and during escalating doses of L-NNAME (100 and 250 mg/L; drinking water) to assess transfer functions and endothelial reserve.

Results: OP but not OR developed modest hypertension, significant proteinuria and GS but not renal hypertrophy.

Conclusions: The mechanism(s) by which endothelial dysfunction leads to GS in DIO models remain to be defined.

Funding: NIDDK Support, Veterans Administration Support

FR-PO1810

Activated Prorenin and (Pro)renin Receptor Are Up-Regulated in Glomeruli of Diabetic Nephropathy and IgA Nephropathy Using Renal Biopsy Specimen Koji Otsuka,1 Yashiko Shimamura,2 Kosuke Inoue,1 Toru Kagawa,1 Akira Nishiyama,3 Yoshio Terada.1

Background: Activated prorenin plays a key role in the regulation of the tissue renin-angiotensin system (RAS), and a direct renin inhibitor has been reported to reduce proteinuria in diabetic nephropathy through inhibiting the tissue RAS. However, little information is available regarding the localization of activated prorenin and (pro)renin receptor (PIRR) in human kidney under pathophysiological conditions. We examined the localization of activated prorenin and (PIRR) in kidney biopsy specimens of the patients with IgA nephropathy, diabetic nephropathy, and minimal change nephropathy using parts of renal biopsy specimens.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Methods: This study was approved by Kochi Medical School review boards. The anticence pre-activated prorenin was raised in a rabbit by injecting the prorenin fragment corresponding to the gate region of human prorenin. Immunohistochemical analyses were performed by confocal microscopy.

Results: We analyzed 21 renal biopsy specimens (n=7 IgA nephropathy, n=6 diabetic nephropathy, n=3 proliferative glomerulonephritis) for activation of prorenin using activated prorenin antibody. Immunohistochemical staining for activated prorenin was positive in the mesangium and tubular basement membrane in all patient groups. In renal biopsies from patients with diabetic nephropathy, IgA nephropathy, and proliferative glomerulonephritis, activated prorenin was detected in the podocyte and capillary wall. These findings indicate that activated prorenin in the mesangium and tubular basement membrane is a marker of glomerulosclerosis.

Conclusions: Activated prorenin (+) is seen in the mesangium and podocyte in diabetic nephropathy and IgA nephropathy, and may contribute to the pathophysiology of glomerulosclerosis.

FR-PO1811

The VEGF Receptor Blocker, Sunitinib (SU), Promotes Glomerular Microthrombosis and Aggravates Segmental Sclerosis in the Remnant Kidney (Nx) Model


Univ Sao Paulo, Brazil.

Background: We showed previously that treatment with SU, used as an antiangiogenic agent in colorectal cancer, induces capillary density (ICD) in chemistry with the therapy of new vessel formation is not increased in this model. However, SU treatment aggravated glomerulosclerosis (GS). We now investigated whether the latter effect might involve podocyte (POD) injury and/or formation of microthrombosis (MT).

Methods: Adult male Munich-Wistar rats underwent Nx and were immediately assigned to Groups Nx+V (vehicle) or Nx+SU (SU, 4 mg/Kg/d). Forty five days later, % GS, % glomerular MTI(%)GMT, % cortical interstitium (%INT), % glomerular endothelial area (%GEA), ICD (capillary profiles/mm²), glomerular volume (Vg, x 10⁵µm³), % glomerular zonulas occludens 1 area (%Z01) and the number of POD per glomerular tuft (POD/G) were assessed in 10 Nx/V and 10 Nx/SU rats. Fourteen sham-operated rats receiving vehicle (V/S) or SU (S/SI) were also studied. Results: (Mean±SEM; p<0.05 vs. respective control; S, p<0.05 vs. respective untreated):

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<th>Group</th>
<th>%GS</th>
<th>%GEA</th>
<th>%MTI</th>
<th>%INT</th>
<th>%GEA</th>
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<td>Nx/V</td>
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<td>Nx/SU</td>
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In addition, SU treatment significantly raised serum creatinine in Nx (1.5±0.1 in Nx/SU vs. 1.2±0.1 in Nx/V). SU promoted no glomerular or interstitial change in S, but markedly exacerbated GS in Nx. This effect could not be explained by a reduction in the number of POD or endothelial cells, or by a functional change of POD. However, SU-treated Nx exhibited a marked increase in the frequency of MT, the organization of which may have been the basis for the observed worsening of GS.

Conclusion: SU treatment can promote characteristic glomerulosclerotic endothelial injury in previously compromised kidneys. However, the antiangiogenic effect of VEGF inhibition may have little influence on glomerular and interstitial injury in the Nx model.

FR-PO1812

Curcumin Prevents CKD in 5/6 Nephrectomized Rats by Blocking LPS Secretion

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Background: Bacterial lipopolysaccharide (LPS), a known mediator of inflammation is elevated in the circulation of inflammatory disorders such as chronic kidney disease (CKD). This increase in circulatory LPS has been suggested to be due to paracellular transport across the intestinal epithelium. Orally acting antibacterial agents by changing gut microbiota can decrease LPS translocation. Curcumin which has anti-inflammatory and antibacterial properties ameliorates inflammatory disorders such as CKD. We speculated that one of the mechanisms by which curcumin can decrease inflammation and slow renal failure progression is by decreasing the circulatory content of LPS.

Methods: CKD was induced in Sprague-Dawley rats by 5/6 nephrectomy. 18 Nx animals were divided into untreated (Nx) and curcumin-treated (Cur) groups. The Cur treatment (75 mg/kg) was carried out for 10-weeks and results were compared with Nx animals were divided into untreated (Nx), and curicumin-treated (Cur) groups. The Cur treatment was performed by confocal microscopy. We analyzed 21 renal biopsy specimens (n=7 IgA nephropathy, n=6 diabetic nephropathy, n=3 proliferative glomerulonephritis) for activation of prorenin using activated prorenin antibody. Immunohistochemical staining for activated prorenin was positive in the mesangium and tubular basement membrane in all patient groups. In renal biopsies from patients with diabetic nephropathy, IgA nephropathy, and proliferative glomerulonephritis, activated prorenin was detected in the podocyte and capillary wall. These findings indicate that activated prorenin in the mesangium and tubular basement membrane is a marker of glomerulosclerosis.

Conclusions: Activated prorenin (+) is seen in the mesangium and podocyte in diabetic nephropathy and IgA nephropathy, and may contribute to the pathophysiology of glomerulosclerosis.

FR-PO1813

Adenine-Induced Chronic Kidney Disease: Loss Is Best for Equivalence to Human Disease Vialhal image

Glen C. Gobe, Linda Brown.

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Background: Animal models of chronic kidney disease (CKD), which mimic the insidious development of CKD in humans, are limited. Adenine diet offers an accepted model of CKD pathogenesis but the rapid development from 0.75% adenine can detract from applicability to human CKD. This project aimed to investigate low dose, prolonged, adenine diet in rats as a model of human CKD.

Methods: Male Wistar rats were given 0.25% or 0.75% adenine in comparison with a normal powdered chow (PC) as control (N=12 per group; 300:30g initial body weight), over 16 weeks. We studied: rate of development of CKD; morphometry for glomerular and tubular damage, fibrosis and inflammation/activation of macrophages and myofibroblasts; Western blots for oxidative stress (HO-1), inflammation (TNF-α) and fibrosis (TGF-β); and renal function as blood urea nitrogen (BUN), plasma creatinine (PCR), and their clearances.

Results: 0.75% adenine diet induced renal failure quickly over 4 weeks with approximately 10-fold increase in PCR, marked renal edema, loss of body mass, and the animals were moribund at 4 weeks. In comparison, 0.25% adenine diet induced CKD more slowly, similar to prolonged development in humans. At 16 weeks, in comparison with PC diet, the 0.25% diet induced only moderate loss of body weight, significantly increased % renal fibrosis (35.6±2.8 vs 4.8±0.48), marked tubular atrophy, >10-fold increased macrophage and activated myofibroblast numbers and increased expression of HO-1, TNF-α and TGF-β (all p<0.05). Functionally, 0.25% vs PC diet caused increased BUN (56.5±5.4 vs 6.2±0.6mmol/L) and PCr (267.9±22.9 vs 41.9±2.8µg/L), and decreased BUN clearance (526.7±22.9 vs 753.9±1.2mg/Lhr) and PCR clearance (308.3±33 vs 2220±157mg/Lhr) (both p<0.05).

Conclusions: Development of CKD occurred successfully over 16 weeks with 0.25% adenine diet, with many of the characteristics of human CKD replicated. The slower development of CKD using this model will better allow analysis of modulation of the CKD characteristics with new therapies.

Funding: Government Support - Non-U.S.
FR-POI1815
Indoxyl Sulfate Causes Accumulation of Uremic Toxins through Down-Regulation of SLCO4C1 Transporter Yuutoshi Akivama, 1 Yoichi Takeuchi, 1 Eikan Mishima, 1 Takehiro Suzuki, 1 Sadayoshi Ito, 1 Takaki Abe. 2, 3
1Division of Nephrology, Endocrinology and Vascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan; 2Division of Medical Science, Tokyo Institute of Technology School of Biomedical Engineering, Sendai, Japan, 3Department of Clinical Biology and Hormonal Regulation, Tohoku University Graduate School of Medicine, Sendai, Japan.

Background: With the progression of CKD, various uremic toxins accumulate, subsequently causing renal damage and hypertension. Recently, we have revealed that human kidney-specific organic anion transporter SLCO4C1 excerts uremic toxins, resulting in the reduction of blood pressure and renal inflammation (PNAS 2004, JASN2009). However, in the renal failure, the SLCO4C1 expression level is decreased. So far, the down-regulation mechanism of renal transporters in the renal failure has not been clarified.

Methods: Toxic potentials of various uremic toxins identified by our capillary electrophoresis-based MS analysis (HTN Res 2010) were examined at the translational and transcriptional levels in vitro and in vivo.

Results: Among the compounds tested (18 compounds), indoxyl sulfate (IS) decreased the human SLCO4C1 mRNA level in a dose-dependent manner. Because of the existence of GATA sequence at the 5’ UTR of human SLCO4C1, we examined the correlation of SLCO4C1 with GATA transcriptional factors. The mRNA expression level of GATA3 was increased by IS in contrast to down-regulation of SLCO4C1. In the human kidney cells, over-expression of GATA3 significantly inhibited the expression level of SLCO4C1 and conversely, knockdown of GATA3 increased SLCO4C1 expression. GATA-inhibitor K-7174 completely canceled the effects of IS. In 5/6 nephrectomized rats, treatment with oral absorbent AST-120 significantly decreased plasma IS level and conversely increased the renal SLCO4C1.

Conclusions: The accumulation of IS in the renal failure decreases human SLCO4C1 through GATA transcriptional pathway. This down-regulation of SLCO4C1 further causes the rise of uremic toxin levels and subsequently, exacerbates the renal function. Thus, the usage of AST-120 to reduce IS level is a new aspect for treating CKD patients.

FR-POI1816
Food Limitation Reverses Increased Collagen Type 4 α1 Gene Expression (Col4a1) in the Obese Zucker Rat Bavard C. Carlson, 1 Leah B. Callahan, 1, 2 David A. Maddox, 1 'Siu's Falls VA Healthcare System, Siouxs Falls, SD; 2Avera Research Institute.

Background: Obesity is closely linked to hypertension, Type II diabetes, and hypercholesterolemia, which are among the leading causes of kidney failure. Obese Zucker rats at 6, 12, and 18 weeks of age over-express transforming growth factor beta-3 (TGF-β3) compared to their lean littermates. This effect is prevented by food restriction over 3) compared to their lean littermates. This effect is prevented by food restriction from 6 to 18 weeks of age. Food limitation led to a significant drop in gene expression levels of Col4a1 by 12 weeks. These data were corroborated with studies that demonstrate that 18 week-old obese animals compared to lean. Food restriction reduced TGF-β increased at 6, 12, and 18 weeks of age in obese animals compared to controls. Food usage of AST-120 to reduce IS level is a new aspect for treating CKD patients. Through GATA transcriptional pathway. This down-regulation of SLCO4C1 further causes which also prevents kidney damage. TGF-β3 belongs to a pro-collagen generation pathway. This down regulation of SLCO4C1 through GATA transcriptional pathway leading to kidney damage. We examined the effects of food restriction on Col4a1 gene expression in the obese rat.

Methods: Six-week-old obese Zucker rats were fed ad libitum or restricted in food intake to that consumed by lean Zucker rats, then tested at 6, 12, and 18 weeks of age. The animals were anesthetized, and their kidneys perfusion-fixed (RNAlater®). Tissue mRNA was extracted and quantified from the kidney cortex, converted to cDNA, then run on custom RT-PCR arrays (SABiosciences). Values are mean ± SE.

Results: Previously we found that TGF-β3 gene expression was increased in 6, 12, and 18 week-old obese animals compared to lean. Food restriction reduced TGF-β3 expression to control at 12 and 18 weeks (left panel). Col4a1 gene expression was also significantly increased at 6, 12, and 18 weeks of age in obese animals compared to controls. Food limitation prevented these changes (right panel).

Conclusions: Similar to that seen for TGF-β3, gene expression of Col4a1 in the obese rat was elevated at 6 weeks of age. Food restriction in obese rats beginning at 6 weeks of age led to a significant drop in gene expression levels of Col4a1 by 12 weeks. These data suggest that elevations in the expression of TGF-β3 and Col4a1 are important initiating events leading to kidney damage in these animals. The contents do not present the views of the Department of Veterans Affairs or the United States Government. Funding: Veterans Administration Support, Private Foundation Support

FR-POI1817
Renoprotective Effects of Astragaloside IV Synergizes with Ferric Acid in Rats with 5/6 Nephrectomy Lijiang Meng, Lei Qu, Jiawei Tang, Xiaomei Li. Renal Division, Department of Medicine, Peking University First Hospital.

Background: Astragaloside IV (AS-IV) and ferric acid (FA) are two major active constituents of Chinese herbs Astragalus and Angelicae, which could alleviate renal tubulointerstitial fibrosis. This study was to investigate whether AS-IV and FA could retard the progression of chronic renal failure in rats with 5/6 Nephrectomy (Nx).

Methods: The Sprague-Dawley rats were randomly divided into sham, Nx+FA (FA 12mg/kg/d), Nx+AS-IV (AS-IV 20.4mg/kg/d) and Nx+FA+AS-IV (FA 12mg/kg/d, AS-IV 20.4mg/kg/d). After therapy for 2, 4, 8, 12 weeks, the tail-cuff blood pressure, blood urea nitrogen (BUN), serum creatinine (Scr), 24 hour protein excretion rate, semiquantitatively evaluated for glomerulosclerosis, tubulointerstitial lesion and vascular damage in PAS stained tissue sections were evaluated. Results: The blood pressure was significantly increased in Nx group compared with sham group at 4th, 8th and 12th week. Compared with the Nx group, blood pressure was reduced in AF-treated group at 4 week (113±4/84±2mmHg vs 139±9/104±19mmHg, P<0.01), and in the three groups with therapies at 8th week (FA: 127±9/96±18mmHg, AS: 115±7/83±7mmHg, AF: 111±12/77±10mmHg, vs Nx:152±9/100±15mmHg, P<0.001). But there was no statistical difference in groups with therapies at 12th week. Scr and proteinuria were gradually increased in the Nx group compared with sham group (P<0.05). After treatment with AF, Scr was decreased at 4th week (42±3±4μmol/L vs 55±4±7μmol/L, P<0.05), 8th week (47±3±1μmol/L vs 58±0±0μmol/L, P<0.05) and 12th week (60±3±4μmol/L vs 80±2±9μmol/L, P<0.05). The similar effect on Scr was shown in the group of treatment with AS-IV or FA. The proteinuria was decreased after AS-IV and/or FA at 4th, 8th and 12th week. The pathological injury in kidney was significantly exacerbated from 4th week in the Nx group, and alleviated after treated by AS-IV, FA and AF characterized by renal injury index reduced to 83%, 85% and 79% at 12th week, respectively.

Conclusions: AS-IV and/or FA therapy retarded the progression of renal failure in rats with 5/6 Nephrectomy via effective control of hypertension and proteinuria, and the synergistic effect of AS-IV and FA was more effective than AS-IV or FA alone. Funding: Government Support - Non-U.S.

FR-POI1818
Urinary Levels of Angiopeitoin-Like 4 in Pediatric Steroid Sensitive Nephrotic Syndrome Support a Possible Role in Proteinuria Michael R. Bennett, Nuntawan Piayaphance, Prasad Devarajan. Cincinnati Children’s Hospital Medical Center.

Background: Idiopathic nephrotic syndrome (NS) is the most common glomerular disorder of childhood. Roughly 90% of children under 10 years of age with nephrotic syndrome have the minimal change disease (MCD), which is typically responsive to steroid treatment (SSNS). While some structural proteins in the glomerulus have been found that may contribute filtration problems in MCD, many of the disease mechanisms remain unknown. Recent studies in animal models of SSNS suggest a role for podocyte excreted angiopoietin-like 4 protein (ANGPTNL4) in modulating proteinuria in this disease. We set out to determine if urinary levels of ANGPTNL4 were elevated in pediatric patients with SSNS with active proteinuria. Methods: Urine and clinical data were collected from patients at Cincinnati Children’s Hospital Medical Center. Chronic kidney disease (CKD) is a major health issue. Investigations were carried out to analyse the function of α2A-AR, which is known as main regulator of presynaptic noradrenaline release. A murine knockout model (KO) with deletion of α2A-AR was used and

Background: Chronic kidney disease (CKD) is a major health issue. Investigations were carried out to analyse the function of α2A-AR, which is known as main regulator of presynaptic noradrenaline release. A murine knockout model (KO) with deletion of α2A-AR was used and
Results: In kidney of KO mice presynaptic noradrenaline release after renal nerve stimulation was significantly higher than in SNX WT and KO mice, indicating noradrenaline release after renal nerve stimulation was significantly higher in SNX WT and KO mice, indicating noradrenaline release after renal nerve stimulation was significantly higher than in WT mice. Kaplan-Meier survival analysis revealed a diminished mortality of KO mice. In isolated perfused kidneys the α2-agonist UK413,304 showed a facilitatory effect on angiotensin II-induced vasoconstriction. In addition, UK413,304 induced a concentration- and time-dependent phosphorylation of extracellular signal-regulated kinases ERK1/2 in o2A-AR transfected HEK 293T-cell.

Conclusions: The presented data confirms the noradrenaline release regulating effect of α2-adrenergic receptors. Moreover, our data reveal a major role of post-synaptic α2A-adrenergic receptors regulating vascular tone for progression of CKD. The cell culture experiments might hint to an ERK1/2 dependent pathway which could explain the effect of catecholamines modulating fibrotic or inflammatory processes in CKD.

FR-PO1820
Renoprotective Effects of Nicorandil in the Rat Remnant Kidney Model Yoshifumi Tamura, Carlos Alberto Roncal-Jimenez, Christopher J. Rivard, Katsuyuki Tanabe, Wataru Kitagawa, Richard J. Johnson, Takahiko Nakagawa. Division of Renal Diseases and Hypertension, University of Colorado Denver; Aurora, CO.

Background: Nicorandil has been used to treat patients with ischemic heart disease. Interestingly recent literatures have documented that nicorandil exhibits the protective effects in the acute renal disease in rodents, including acute kidney injury and glomerulonephritis. We hypothesized that nicorandil also exhibits beneficial effects in the chronic renal disease. In order to test our hypothesis, nicorandil was administered in the rat remnant kidney (RK) model. We also examined if nicorandil could be more potent with angiotensin converting enzyme inhibitor (ACEI) in chronic renal disease.

Methods: RK rats were divided into five groups: Sham operated rats (SHAM, n = 7), Untreated remnant kidney rats (RK, n = 7), RK rats treated with Enalapril 5 mg/kg/day (ENAL, n = 7), RK rats treated with low dose Nicorandil 3 mg/kg/day (NIC0, n = 7), RK rats treated with high dose Nicorandil 30 mg/kg/day (NIC10, n = 7). Twelve weeks later, the rats were sacrificed.

Results: Enalapril significantly reduced blood pressure (108 ± 3 vs. 147 ± 2 mmHg, p<0.05). Nicorandil at high dose tended to reduce blood pressure (136 ± 5 mmHg, p = 0.11), and ENAL showed a higher reduction than in RK. Nicorandil inhibited albuminuria in the ENAL group compared to the untreated RK group (5.48 ± 1.5 g/day in ENAL vs. 14.5 ± 5.4 g/day in RK). Enalapril significantly reduced blood pressure (108 ± 3 vs. 147 ± 2 mmHg, p=0.001) and ENAL showed a higher reduction than in RK (11.99 ± 0.9 vs. 0.005 g/day in ENAL vs. 1.9 g/day in RK).

Conclusions: High dose of nicorandil prevents the progression of chronic renal disease as potentially as enalapril. Low dose nicorandil may also reduce proteinuria independently of blood pressure. These findings suggest that nicorandil could be a therapeutic option for chronic renal disease.

FR-PO1821
Prolactin Receptor was Up-Regulated in the Proximal Tubules of the Kidney in the Cardiac-Renal Syndrome Model Mice Yohi Tsuichiha, Yoshikatsu Kaneko, Akihiko Saito, Tadashi Yamamoto, Ichiei Narita, Yohei Tsuchida, Yoshikatsu Kaneko, Akihiko Saito, Tadashi Yamamoto, Ichiei Narita. Division of Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata City, Niigata Prefecture, Japan.

Background: Many epidemiological investigations revealed the interaction between dysfunctions of kidney and cardiac. These pathological states were recently recognized as cardiac-renal syndrome (CRS). However, the precise pathogenesis and the physiological mechanisms of CRS are to yet be elucidated. Hence the aim of our study was to investigate the molecular mechanisms of the development of CRS by generating the CRS model mice.

Methods: We generated CRS model mice by the combination of uninephrectomy and cardiomyoplasty. To evaluate cardiac and renal function, we measured ejection fraction (EF) by echocardiogram and serum cystatin C level by ELISA, respectively. We investigated whether Fabry kidney showed an evidence of endo-MT and whether Gb3 per se is related to renal progression. Recent data suggest that Gb3-induced endo-MT is one of the mechanisms of renal progression in Fabry disease.

Results: In CRS mice, serum cystatin C level was significantly higher than in WT mice. After SNX WT and KO mice were treated with high dose of nicorandil 30 mg/kg/day (NIC10, n = 7), Twelve weeks later, the rats were sacrificed.

Conclusions: The presented data confirms the noradrenaline release regulating effect of α2-adrenergic receptors. Moreover, our data reveal a major role of post-synaptic α2A-adrenergic receptors regulating vascular tone for progression of CKD. The cell culture experiments might hint to an ERK1/2 dependent pathway which could explain the effect of catecholamines modulating fibrotic or inflammatory processes in CKD.

FR-PO1822
Hypolipidemia and microRNAs in Chronic Kidney Disease Carmen Josefa Mora,1 Diego Julio Arenas,2 Carmen Maria del Prado,3 Teresa Renata Romero,1 Viriglia Soto,1 Marcela Avila,1 UMEM, CMM SXXI IMSS, Mexico, DF, Mexico; 2UMGH, CMM SXXI IMSS, Mexico, DF, Mexico; 3S. Patologia, H Cardiol, Mexico, DF, Mexico.

Background: Albumin synthesis is controlled by positive and negative transcription factors. Its transcript translation regulation by microRNAs (miRNAs) is unknown.

The aim was to investigate the relationship between albumin synthesis and a miRNA profile in a CKD mice model.

Methods: Four mice groups were formed: CKD group, induced with Nx 5/6, Infarction group (I) induced with E. coli, LPS, food restriction group 50%, (FR), and control group (C). The animals were sacrificed after eight weeks of treatment; total blood samples and liver were extracted, and plasma creatinine, albumin, and total proteins were measured. Total RNA was extracted from liver for Albumin mRNA measurement in RT-PCR. miRNAs were obtained for miRNAs microarrays. Gene target prediction was made with “mirANDA” database. Liver histological analysis was made to corroborate fibrosis development.

Results: Plasma albumin was different in CKD (1.59 g/dL, p=0.016) and I groups (1.68 g/dL, p=0.009) from C group (1.80 g/dL). The albumin transcript level was diminished in the CKD (12.30 UPR=0.009) and I (11.99 UPR=0.005) groups compared to C (17.51 UPR). Enalapril (11.66 UPR=0.006) had the lowest albumin mRNA level. Liver fibrosis in pixels was: in 1 group (58.63 ± 6.04, p=0.001), followed by CKD group (55.56 ± 4.03, p=0.001) and FR group (24.38 ± 5.61, p=0.001), and C group (17.8 ± 0.75). The obtained miRNAs were classified into 74 miRNAs related to albumin mRNAtranslation and expression (+ Z Score >2).Four sub-expressed miRNAs (miR21, miR30b, miR350 and 376A), had CEBP as gene target predicted. Other miRNAs target genes predicted, involve structural, and inflammation genes.

Conclusions: We found miRNAs related with extracellular matrix genes, and with CEBP, which is a negative transcription factor in albumin transcription. It might be that CEBP is up-regulated by down-expressed miRNAs, promoting transcription suppression of albumin. The heterogeneity of miRNAs differential expression and the gene targets diversity, show that the albumin synthesis regulation in CKD involves several mechanisms.

Funding: Government Support - Non-U.S.

FR-PO1823
Globotriaosylceramide (Gb3)-Induced Endothelial-to-Mesenchymal Transition as a New Mechanism of Renal Progression in Fabry Disease Myung Yu,1 Yang-Hee Jung,1 Jin Ok Choi,1 Sung Cheol Jung,2 Duk-Hee Kang.1 Nephrology, Ewha Womans University School of Medicine, Seoul, Korea; 2Biochemistry, Ewha Womans University School of Medicine, Seoul, Korea.

Background: The lysosomal storage disorder Fabry disease is characterized by excessive globotriaosylceramide (Gb3) accumulation in major organs such as kidney. Defective lysosomal alpha-galactosidase A (Gla) is responsible for excessive Gb3 accumulation, and vascular endothelium is one of the sensitive cells to the effects of Gb3 accumulation. Although renal fibrosis is known to be associated with Fabry disease, it is not known whether it plays any roles in the development of organ damage in Fabry disease or whether Gb3 per se is related to renal progression. Recent data suggest that endothelial-to-mesenchymal transition (endo-MT), which is characterized by the loss of endothelial cell markers and an acquisition of mesenchymal cell markers, is a potential mechanism of renal fibrosis.

Methods: We investigated whether Fabry kidney showed an evidence of endo-MT and whether Gb3 induced endo-MT in cultured human endothelial cells.

Results: Double immunofluorescence staining of CD31 (FITC) and α-SMA (cy3) in the kidney and cultured Fabry disease, Gla deficient mice, showed a decreased microvascular endothelial staining both in glomerular and peritubular capillaries compared to wild type mice with an appearance of α-SMA (+) and CD31 (+) endothelial cells. Treatment of Fabry mice with of recombinant adeno-associated virus (rAAV) vector encoding alpha-Gal A DNA (rAAV2/8-hGAGA) resulted in the clearance of accumulated Gb3 in kidney with concomitant elevation of alpha-Gal A enzyme activity. rAAV2/8-hGAGA therapy also ameliorated endo-MT of glomerular and peritubular capillary endothelial cells. Stimulation of HUVEC with Gb3 (0.1-10 uM) and lysosomal enzymes down-regulated the expression of CD31 with an up-regulation of α-SMA from 48 hours in a dose-dependent and time-dependent manner. Blocking of Gla using siRNA and Gb3 after siRNA decreased the expression of CD31 and increased α-SMA expression.

Conclusions: These findings suggest that Gb3-induced endo-MT is one of the mechanisms of nephropathy in Fabry disease.

FR-PO1824
Correlation of HIF-1 α and Twist in the Proximal Tubular Epithelial Cells of Hypoxia Kidney Diseases Shiren Sun, Rui Da. Department of Nephrology, Xijing Hospital, FMMU, Xi’an, China.

Background: Our previous studies indicate that the Twist plays an important role in the development of hypoxia-induced tubular epithelial-mesenchymal transition and kidney fibrosis. However, the expression of the HIF-1 α and its mechanisms are not well known.

Methods: Twist expression was examined by Immunohistochemistry (IHC) study, and consecutive slides were stained for HIF-1 α and E-cadherin. Immunostaining was
evaluated in a blinded manner. A 0–3 relative scale was used to grade the amount of immunostaining: 0, -5% staining; 1, 5–25% staining; 2, 25–50% immunostaining; 3, >50% immunostaining.

Results: Twist was predominantly located in the nuclei of renal tubular epithelial cells from CKD patients including diabetic nephropathy (DN), FSGS, immunoglobulin (Ig) A nephropathy and hypertensive nephrosclerosis, tubulointerstitial nephritis that had different degrees of chronic hypoxia. While little positive staining for Twist was found in the renal tubules of normal kidneys (Twist score of <2), prominent Twist staining (>25% of all cells positive) was found in 48 of 74 patients with CKD (Twist score of ≥2, P = 0.018 compared with that grouped by score of 0 or 1; Fisher’s exact test). Positive HIF-1α was observed in nuclei in 62.2% (46/74) of CKD kidney tissues, which was closely associated with Twist (P = 0.000). Loss or reduced expression of E-cadherin was observed in 70.8% (34/48) Twist-positive tubular epithelial cells, but only in 26.9% (7/26) of Twist-negative tumors (P < 0.000), which was significantly associated with higher levels of Twist in all kidney tissues from CKD patients. Using linear regression, we found a positive linear correlation between active Twist and tubulointerstitial fibrosis in the biopsy samples (r = 0.57, P = 0.000).

Conclusions: We found that in the human kidney activated Twist is expressed in a wide range of renal diseases, which was correlated with HIF-1α activation, E-cadherin suppression and tubulointerstitial fibrosis. Our findings could indicate that Twist activation might represent a common downstream pathway for hypoxia-induced tubulointerstitial fibrosis.

FR-PO1825
Assessment of Erythropoietin Effects in the Progression of Experimental Chronic Kidney Disease Fernando Felipe Carvalho, Vicente de Paulo Castro Teixeira, Waldemar S. Almeida, Nestor Schor. Nephrology, Federal University of São Paulo, São Paulo, Brazil.

Background: Erythropoietin (EPO) has been used primarily to treat anemia caused by chronic kidney disease. Recent studies have shown a renoprotective EPO effect in ischemic kidney diseases. The mechanisms of renoprotection include antioxidant effects and stimulation of endothelial progenitor cells. Thus, the aim of this study is to evaluate the influence of EPO on progression of kidney disease in experimental chronic kidney disease.

Methods: Male Wistar rats weighing 280-300g underwent 5/6 nephrectomy and were divided into two groups: (NX) only nephrectomized (n=6) and (NX-EPO) nephrectomized (n=6) and treated with a weekly dose of erythropoietin (250U/kg/ip).

All animals were sacrificed 8 weeks after surgery. Hematocrit, serum creatinine, proteinuria, indirect blood pressure measurement, glomerular score and tubular lesion assessment were assessed.

Results: The NX-EPO group showed significant improvement in serum creatinine (NX 1.6 ± 0.4 versus NX-EPO 0.8 ± 0.1, P ≤ 0.001) and protein/urine creatinine ratio (NX 11.2 ± 6.0 versus NX-EPO 4.1 ± 2.2, P = 0.021). Preliminary results suggest an improvement in glomerular score and tubular lesion in animals that received EPO.

There were no significant differences in hematocrit and blood pressure between the two groups.

Conclusions: Our study suggests a beneficial effect of EPO in the model of progression of experimental chronic renal disease reflected by the improvement of serum creatinine, attenuation of proteinuria and a lower glomerular lesion score, regardless of its effect on albuminuria.

FR-PO1826
IL-6 Alters Glomerular Structure and Function and Influences Renal Development Mukut Sharma,1 Jianping Zhou,1 Madhulika Sharma,2 Ram Sharma,1 Ellen T. McCarthy,1 Jean-François Gauchat. 1Nephrology Research, KC VA Medical Ctr, Kansas City, MO; 2Kidney Institute, KUMC, Kansas City, KS; 3Pharmacology, University of Montreal, QC, Canada.

Background: Systemic inflammation is a characteristic of obesity, diabetes and hypertension (the metabolic syndrome). Increased levels of the inflammatory cytokine IL-6 in obese mice may influence renal development function and susceptibility to disease in the offspring. The role of IL-6 family cytokines in glomerular function and pathophysiology is not known. We hypothesize that IL-6 alters glomerular filtration barrier structure and function.

Methods: 1. Isolated rat glomeruli were incubated for 15 min with IL-6 (0.01-10 ng/mL) and glomerular albumin permeability (P_aₐ) was determined using an intravenous approach. 2. Immortalized murine podocytes were incubated with IL-6 (1 ng/mL) for 15 minutes and total and phosphorylated Akt and ERK1/2 were determined by Western blotting. 3. Metanephri from embryonic mouse kidneys were incubated with IL-6 (10 pg/mL) for 3 days at starting ED13.5. Tissue growth was measured daily using Image J software.

Results: IL-6 increased P_a₂ in a dose-dependent manner (P = 0.05 vs. control; 1 ng/mL, P < 0.001).

- IL-6 decreased phosphorylation of ERK at 10 ng/mL (<50%) and 1 ng/mL (>50%) within 15 minutes. IL-6 did not alter phosphorylation of Akt.

Conclusions: Physiologically relevant concentrations of IL-6: 1) alter glomerular filtration barrier function as evidenced by increased P_a₂; 2) alter podocyte signaling via the MAPK/ERK pathway as evidenced by decreased phospho-ERK, and 3) impacts kidney growth and development as evidenced by growth restriction and altered cytokeratin expression. We conclude that IL-6 may play multiple important roles in glomerular structure and function. Further studies will determine the role of IL-6 in the etiopathogenesis and progression of glomerular dysfunction in CKD.

Funding: NIDDK Support

FR-PO1827
Albumin Endocytosis Inhibits Autophagy in Proximal Tubular Epithelial Cells Andrea Havasi, Jonathan M. Gall, Zhiyong Wang, Steven C. Borkan, John H. Schwartz. Department of Medicine, Boston University Medical Center, Boston, MA.

Background: Proteinuria is associated with progressive chronic kidney disease. It is well known that exposure of proximal tubular epithelial cells (PTEC) to large amounts of albumin leads to the development of tubular atrophy and fibrosis. However, the possible pathogenic role of albumin in this process has not been fully elucidated. To address this issue, we examined the effect of albumin exposure on autophagy and lysosomal function in cultured primary PTEC.

We show that albumin exposure, mimicking nephrotic nephrotic filtrate, causes both lysosomal dysfunction and decreased autophagy. In primary mouse tubular cells, albumin exposure decreased LC3-I to LC3-II conversion in a concentration-dependent manner. Similar results were obtained using either recombinant human albumin or fatty acid free bovine albumin. In the presence of bafilomycin, an H⁺-ATPase inhibitor, autophagic flux was shown to be inversely related to albumin concentration. In addition, albumin treatment decreased the number of autophagosomes (AP) both in normal media (basal autophagy) and in starved cells (induced autophagy). Albumin exposure over 3-5 days markedly reduced autophagy in PTEC, suggesting that prolonged albumin exposure blocks AP formation. After a brief exposure of up to 48 hr, albumin caused marked AP enlargement, suggesting that albumin inhibits fusion of AP with lysosomes without impairing the maturation of small, punctate autophagosomes into larger ones. Normal lysosomal function is required for clearance of AP, hence lysosomal pH and enzyme activity were examined. Albumin exposure increased lysosomal levels and decreased lysosomal enzyme activity in PTEC, suggesting that autophagy inhibition might be the result of lysosomal dysfunction.

Taken together, these data show that albumin overload compromises autophagic and lysosomal function that could lead to cell toxicity. Therapy directed at rescuing autophagy during proteinuric states might be a rational approach for preventing or slowing the progression of chronic kidney disease.

Funding: Private Foundation Support

FR-PO1828
Uremia-Induced Cardiac Activity Modifications: A Uremic Rabbit Model Emilia Ferramosca,1 Boris Dalpozzo,2 Stefano Severi,2 Antonio Santoro.1 1Malpighi Nephrology, S.Orsola-Malpighi Hosp, Bologna, Italy; 2IDEIS, Bologna Univ, BO, Italy; 3Veterinary Med., Bologna Univ, BO, Italy; 4Experimental Med., Bocca Univ, Milano, Italy.

Background: Cardiovascular disease is the main cause of death in ESRD patients. Besides the increased arrhythmic events, a larger dispersion in the QT interval duration has also been described. The reasons explaining these alterations in the ventricular repolarization are still poorly understood.

So, the cellular mechanisms underlying electrocardiographic and hemodynamic alterations is of great interest.

Methods: We present a set up of an animal model of uremic cardiomyopathy, the cardiac morphological and functional evaluation, and the electrophysiological properties at the single cell level. We set up a rabbit uremic model obtained by partial resection of renal parenchima. Echocardiographic evaluation of the morphology and hemodynamic parameters revealed the onset of left ventricular (LV) hypertrophy. The action potential of ventricular cardiomyocytes was recorded through whole-cell patch clamp technique in current clamp mode and its duration at the 90% of repolarization (APD90) was assessed.

Results: Plasmatic levels of urea, creatinine and K+ increased 2.6 fold, 5 fold and 20%, respectively. Echocardiographically, the thickness of posterior wall increased from 3.3 cm up to 6 cm at the end of the diastolic phase and from 4 cm up to 7.2 cm at the end of the systolic phase, while the thickening of the intraventricular septum was 0.07 cm before the surgery and became 0.17 cm after the development of the uremic state. The LV mass grew from 33 g to 53 g and became 0.17 cm after the development of the uremic state. The LV mass grew from 3 g up to 7.7 g at the end of the diastolic phase and from 4 cm up to 7.2 cm at the end of the systolic phase, while the thickening of the intraventricular septum was 0.07 cm before the surgery and became 0.17 cm after the development of the uremic state. The LV mass grew from 33 g to 53 g and became 0.17 cm at the end of the diastolic phase and from 4 cm up to 7.2 cm at the end of the systolic phase.

Conclusions: Uremic rabbit model may represent a useful model for a deeper understanding of the cellular ionic mechanisms underlying uremia-associated cardiac function alterations and for the development of potential therapeutical strategies.
FR-PO1829

Genetic Analysis of Mesangial Matrix Expansion in Aging Mice and Identification of Far2 as a Candidate Gene

Gerda A. Noordmans,1 Kenneth A. Walsh,2 Susan Marie Sheehan,2 Jan-Luuk Hillebrands,2 Peter Heebring,2 Harry Van Goor,1 Ron Korstanje.

Background: Aging of the kidney is associated with glomerular mesangial matrix expansion (MME). To unravel the mechanism of aging it is important to identify genes involved in this process. Identifying aging-associated genes might help design novel therapeutic modalities in order to prevent histological changes like MME and to reduce the decline of renal function.

Methods: Global structural changes in the aged kidney of 26 mouse inbred strains (www.jax.org/phenome) were characterized in male mice at 20 months of age in PAS stained sections. HaploType Association Mapping (HAMP) was used to determine loci associated with the presence of MME. Results: Out of the 26 strains studied MME showed up in 3 strains. HAM revealed to have a 9bp indel in the 5’UTR of Far2. The strains with MME revealed to have a 9bp indel in the 5’UTR of Far2, which is absent in most of the strains without MME. RT-PCR showed a 2-fold increase in the expression of Far2 in the strains with MME compared to strains without.

Conclusions: Our study found an association between Far2 expression and glomerular mesangial matrix expansion. Far2 catalyzes the reduction of fatty acyl-CoA to fatty alcohols, the precursors of platelet activating factor (PAF). PAF is shown in vitro in human and rat mesangial cells to increase the expression of the mesangial matrix. Our data suggests that Far2 plays an important role in aging.

Funding: NIDDK Support, Other NIH Support - NIA (National Institute on Aging)

FR-PO1830

Biomarker of Irreversible Glomerular Injury – A Metabolomic Approach

Experimental Glomerulonephritis

Kyoung Hee Han,1 Bora Kim,2 Seun Lee,1 Seong Heon Kim,1 Yo Han Ahn,1 Hee Gyung Kang,3 Hae Il Cheong,3 Joo-Youn Cho,1 Il-Soo Ha.1

Background: Some injuries of the kidney recover completely but many other diseases are irreversible. The present study evaluated the time course of injury progression to chronic kidney disease (CKD). While complex network of cellular and molecular events have been known to be involved in the pathophysiological mechanism of CKD, the diverging point between the reversals versus progression and the triggering event of the progression are still unknown. An indicator that predicts progression or regression of injuries is not yet available. To understand the different mechanisms between the irreversible and reversible kidney diseases and to search for biomarkers predicting the prognosis, a metabolomic analysis was applied in comparison of acute and chronic experimental mesenchymal nephritis.

Methods: Urine samples were collected on day 0, 7, and 14 from rats that were treated with anti-Thy 1 MoAb(OX-7) with or without heminephrectomy. Samples were analyzed by ultra-performance liquid chromatography coupled with Synapt Q-TOF mass spectrometry(Waters, USA). Multivariate analysis was performed on 2597 positive ion mass and 537 negative ion markers using SIMCA-P+ (Version 12.0).

Results: A total of 4 peaks in Electrospray Ionization(ESI)+ and 5 peaks in ESI- were detected after peak alignment. Principal components analysis(PCA) revealed that the chronic and acute nephritis models had two discrete phenotypes and orthogonal partial least squares discriminate analysis(OPLS-DA) showed direction of the time trends were different between these two models. Candidates of biomarkers for chronic nephritis were searched from the human metabolome database(HMDB) and confirmed by tandem mass spectrometry of authentic compounds.

Conclusions: These results suggested that metabolomic analysis could provide insights into mechanism of disease progression and biomarkers for early diagnosis of chronic nephritis.

FR-PO1831

PDGF-C Neutralization Protects Collagen 4A3 Deficient Alport Mouse from the Development of Renal Fibrosis

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Background: PDGF-C deficiency in collagen 4A3 deficient (“Alport”) mice, which serve as a model for progressive renal fibrosis.

Methods: Alport mice were crossed with PDGF-C deficient mice (n=15) and analyzed at 10 weeks of age. In a second experiment, Alport mice (n=24) were treated with neutralizing anti-PDGF-C antibody or control IgG from week 6 until sacrifice at week 9. We analyzed renal function, histological damage and renal inflammation.

Results: PDGF-C deficient Alport mice developed less renal injury: significantly less glomerular injury (76% reduction of glomeruli containing extracapillary proliferates; 65% reduction of glomerular fibrin deposition) and significantly less cortical inflammation (90% reduction of CCL2 mRNA, 80% reduction of CCL5 mRNA). Treatment with neutralizing anti-PDGF-C antibody resulted in significantly less renal injury in 9 week old mice: better renal function (-88% reduction of CCl3-125 uptake), less glomerular injury (87% reduction of glomeruli containing extracapillary proliferates), less cortical matrix accumulation (65% reduction of fibronectin, 62% reduction of collagen type I), and less cortical inflammation (73% reduction of CCL2 mRNA, 63% reduction of CCL5 mRNA, 94% reduction of infiltrating macrophages).

Conclusions: In conclusion, both PDGF-C deficiency as well as PDGF-C antagonism significantly reduced the development of progressive renal fibrosis in collagen 4A3 deficient mice. These potent antifibrotic effects of PDGF-C neutralization in an animal model that only mimics features of human glomerular diseases make PDGF-C a prime target for the treatment of progressive human renal fibrosis.

Funding: Government Support – Non-U.S.

FR-PO1832

Gadolinium-Based Magnetic Resonance Imaging (MRI) Contrast Lends to Organ-Specific Fibrosis Associated with Fibrocyte Accumulation in a Rodent Model of Nephrogenic Systemic Fibrosis

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Background: Nephrogenic systemic fibrosis (NSF) is associated with gadolinium-based MRI contrast exposure in the setting of acute or chronic renal compromise. Given its systemic nature, it has been proposed that circulating fibrocytes play a role in the pathogenesis. A study was conducted to determine if bone marrow-derived cells are involved in mediating dermal fibrosis in a rodent model of NSF.

Methods: Lethally-irradiated rats n=5 were nephrectomized using saline, bone marrow transplant and ureteral placentation alkaline phosphatase (hPAP)-expressing donors. Animals were treated with gadodiamide contrast ( Omniscan, 2.5 mmol/kg ) IP for 4 weeks or an equivalent volume of normal saline.

Results: Within the 4th week of treatment, contrast-treated animals demonstrated subtle signs of stress (e.g. periorbital perihypertrophy and muzzles swelling). Dermal cellularity in the contrast-treated group was greater than control. Skin from the contrast-treated group demonstrated greater hPAP expression, which co-localized with α-smooth muscle actin-positive stress fibers. These cells were also co-proliferation 1+. The donor cells were also CD34+. Heart and liver tissues demonstrated subtle histological differences, yet the latter was characterized by increased fibronectin on immunoblot. Contrast-treated animals demonstrated greater dihydroethidium in the dermis. Skin demonstrated greater GADPH oxidase 4 (Nox4) by immunoblot.

Conclusions: The animal model demonstrates that a radio-sensitive bone marrow-derived cell population is increased in the dermis of MRI contrast-treated rodents. The expression of α-smooth muscle actin, pro-collagen 1, and CD34 are consistent with fibrocytes mediating the disease. Further elucidation of the mechanisms of MRI contrast-induced fibrosis may aid in discovering therapies to this devastating disease.

Funding: Veterans Administration Support

FR-PO1833

Bone Marrow-Derived Cells Play a Major Role in Unilateral Ureteral Obstruction-Induced Kidney Fibrosis

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Background: Intestinal fibrosis is a hallmark of chronic renal failure. Increased fibroblast induces the accumulation of extracellular matrix proteins in the intestine leading to kidney fibrosis. However, the origin of these cells contributing to the fibrosis has not been defined yet. In the present study we investigated the role of bone marrow-derived cells in the fibrosis induced by ureteral obstruction in mice.

Methods: Bone marrow cells collected from eGFP transgenic mice were transplanted into lethally irradiated mice which are produced by same strain with eGFP mice. 8 weeks after bone marrow transplantation the chimeric mice were subjected to unilateral ureteral obstruction (UUO). 1, 3 and 12 days after UUO kidneys were harvested and used for further evaluation including immunohistological staining with eGFP, fibroblast-specific protein-1 (FSP-1), pro-collagen III, and proliferating cell nuclear antigen (PCNA).

Results: UUO resulted in gradual increases of GFP-positive cell number in the interstitium and expansion of interstitial area overtime. Over 80% of interstitial cells were fibroblast in eGFP mice. More than 80% of fibrocytes were positive for FSP-1. In contrast, most of fibrocytes were positive for PCNA.

Conclusions: Bone marrow-derived cells play as major contributor in UUO-induced kidney fibrosis via infiltration, accumulation into injured site, and subsequent proliferation and differentiation into fibroblasts contributing to renal fibrosis. Infiltration of bone marrow-derived cells from the Development of Renal Fibrosis.

Funding: Government Support – Non-U.S.
Bone Marrow-Derived Macrophage Myofibroblast Transition (MMT) Is a Previously Unrecognized Major Pathway in Renal Fibrosis

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Background: Myofibroblast is a key cell type in renal fibrosis. However, the origin of myofibroblast during renal fibrosis remains largely debated. The present study tested the hypothesis that bone marrow (BM)-derived macrophage myofibroblast transition (MMT) may be a key pathway leading to renal fibrosis in patients with CKD and in a model of mouse UUO.

Methods: We first determined MMT in renal biopsies from patients with CKD. Then the critical role of BM-derived cells and MMT in renal fibrosis were examined in three mouse models of UUO: 1) BM deletion followed with/without GFP BM, GFP Smad3β, GFP Smad3 BM transplantation or, 2) GFP-BM chimeric mice; 3) mice with inducible macrophage deletion (Fox3cCre;DTI) MMT was determined by confocal microscopy and flow cytometry with α-SMA CD68+CD11b+.

Results: Surprisingly, in CKD patients, up to 70% of α-SMA+ myofibroblasts were of macrophage phenotype (α-SMA+CD68+), contributing to renal fibrosis and fibro-cellular crescents. In mouse models of UUO, irradiative deletio of BM prevented tubulointerstitial fibrosis including α-SMA+ cells and collagen I/III accumulation, which was rescued by GFP-BM transplantation. By confocal microscopy and flow cytometry, 70-80% of α-SMA+ myofibroblasts were GFP F4/80+, indicating that BM-derived MMT (GFP+α-SMA+F4/80+) is a major pathway of myofibroblast origin during renal fibrosis. This was confirmed by the finding that conditional deletion of macrophages largely reduced MMT (60-70%) and renal fibrosis after UUO. Moreover, MMT was TGF-β-Smad3-dependent because BM transfection with GFP Smad3+, but not GFP Smad3-, restored MMT and renal fibrosis.

Conclusions: Bone marrow-derived macrophage myofibroblast transition (MMT) is a major pathway of myofibroblast origin during renal fibrosis. TGFβ3/Smad3 may play an important role in MMT.

Funding: Government Support - Non-U.S.

FR-P01835

Smad3 Regulates Bone Marrow-Derived Fibroblast Precursors in Renal Fibrosis

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Background: Progressive renal fibrosis is the final common manifestation of chronic kidney disease resulting in irreversible loss of kidney parenchyma and renal function. Activated fibroblasts play a critical role in the pathogenesis of renal fibrosis. Their origin remains controversial. We and others have recently shown that bone marrow-derived fibroblast precursors termed fibrocytes migrate into the kidney and contribute to renal fibrosis. However, the molecular mechanisms that are responsible for the recruitment and maturation of bone marrow-derived fibroblast precursors in the kidney are not fully understood. Since TGFβ3/Smad signaling has been shown to play an important role in the pathogenesis of kidney fibrosis, we investigated the role of Smad3 in the recruitment and maturation of bone marrow-derived fibroblast precursors in the kidney in a murine model of renal fibrosis.

Methods: Wild-type (WT) and Smad3 knockout (Smad3-KO) mice were subjected to unilateral ureteral obstruction and maintained for up to 2 weeks.

Results: Bone marrow-derived fibroblast precursors, identified as cells positive for both CD45 and vimentin were accumulated in obstructed kidneys of WT mice in response to obstructive injury, which were significantly reduced in obstructed kidneys of Smad3-KO mice. Furthermore, the number of bone marrow-derived myofibroblasts that are positive for both CD45 and α-SMA were significantly decreased in obstructed kidneys of Smad3-KO mice compared with WT mice. Immunohistochemical studies and Western blot analysis revealed that the levels of type I collagen, fibronectin, and α-SMA were significantly increased in obstructed kidneys of WT mice. In contrast, all of these parameters were dramatically attenuated in obstructed kidneys of Smad3-KO mice.

Conclusions: These data indicate that Smad3 plays a significant role in the recruitment and maturation of bone marrow-derived fibroblast precursors during the pathogenesis of renal fibrosis.

Funding: Other NIH Support - NIDDK AHA

FR-P01836

CTP-499, a Novel Drug for the Potential Treatment of Chronic Kidney Disease, Has Anti-Fibrotic, Anti-Inflammatory, and Anti-Oxidative Activities with In Vivo Efficacy

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Background: Chronic Kidney Disease (CKD) is a complex, multifactorial disease in which renal function is chronically compromised. Decreased glomerular filtration due to dysregulated extracellular matrix (ECM) deposition is a hallmark of progressive CKD. However, oxidative imbalance and inflammation are now increasingly recognized as major pathogenic mechanisms in CKD. Here, we show that CTP-499, a novel, deuterated analog of 1-(5-Hydroxyethyl)-3,7-dimethylxanthine (HDX), the active M1 metabolite of pentoxifylline, exhibits efficacy in key cellular pathologcal mechanisms involved in CKD and in a rat model of diabetic nephropathy.

Methods: To assess the efficacy of CTP-499 in vitro, cells were challenged with various stimuli to induce pro-fibrotic gene expression, inflammatory response, and oxidative stress. To establish the efficacy of CTP-499 in an in vivo model of diabetic nephropathy, STZ-treated rats were dosed with CTP-499 for seven weeks and markers of disease progression were measured.

Results: CTP-499 demonstrated anti-fibrogenic, anti-inflammatory and anti-oxidative activities in a series of in vitro experiments. In a rat model of diabetic nephropathy, CTP-499 treatment resulted in significantly decreased kidney weights, a trend towards lower albuminuria, and significantly reduced cytokine levels compared to vehicle controls.

Conclusions: In summary, CTP-499 exhibits significant biological activities in multiple inter-related pathological mechanisms that contribute to kidney dysfunction. These data support continued interest in CTP-499 as a novel compound for the potential treatment of CKD.

Funding: Pharmaceutical Company Support

FR-P01837

Thymosin β4 Augmentation of Fibrosis Is Dependent on PAI-1

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Background: Thymosin β4 (Tβ4), a G-actin sequestering protein, is deproyl oligopeptidase (POP) to AcSDKP. Our previous study shows that inhibition of POP shifted the balance of Tβ4 and AcSDKP and exacerbates tubulointerstitial fibrosis induced by unilateral ureteral obstruction (UUO) in wild type (WT) mice. Both transforming growth factor β(Smα2) and plasminogen activator inhibitor 1 (PAI-1) pathways promote fibrosis. We now investigated whether PAI-1 deficiency affects fibrosis. We further examined the effects of augmenting Tβ4 in PAI-1−/− mice.

Methods: Male mice of PAI-1−/− or WT mice underwent UUO and were sacrificed 5 and 14 days after UUO. At day 5, there were two groups: WT with or without Tβ4+POP inhibitor. At day 14, there were three groups: WT without treatment, PAI-1−/− with and without Tβ4+POP inhibitor.

Results: Tubulointerstitial fibrosis, assessed by polarized Sirius red morphometry, was increased similarly in WT vs. PAI-1−/− after 14 days of UUO (0.01±0.03 vs. 0.02±0.04%) compared to non-obstructed kidneys (0.03±0.00%); Tβ4+POP inhibitor treatment resulted in enhanced fibrosis in WT mice at day 5 after UUO (WT with treatment 1.75±0.06% vs WT no treatment 1.50±0.04%, p<0.05) with more Tβ4−/− and fewer infiltrating macrophages. In contrast, PAI-1−/− mice even at day 14 after UUO showed no increase in fibrosis in response to Tβ4+POP inhibitor administration (2.74±0.09 vs. 3.02±0.45%, p NS). Phosphorylated Smad2, an indicator of Tβ4−/− activation, was similarly increased in all UUO kidneys at day 14 compared to non-obstructed kidneys. Infiltrating macrophages were similar in WT vs. PAI-1−/− (3.05±0.64 vs. 2.54±0.38% F4/80+ positive area) and were not increased in PAI-1−/− by adding Tβ4+POP inhibitor (2.85±0.18%).

Conclusions: We conclude that PAI-1−/− mice are not protected from UUO-induced tubulointerstitial fibrosis, and unlike WT mice, do not show enhanced fibrosis when Tβ4 is increased. Thus, Tβ4 in combination with POP inhibitor enhances fibrosis in WT mice, and this effect is dependent on the presence of PAI-1.

Funding: NIDDK Support

FR-P01838

Long-Term Administration of a Novel Glutathione Precursor Attenuates Age-Related Oxidative Stress and Kidney Damage

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Background: Oxidative stress (OS) plays a key role in the pathogenesis of renal senescence. Glutathione (GSH) is the most abundant and potent endogenous antioxidant whose tissue contents can be exhausted by OS and its deficiency can cause/intensify OS.

The present study was undertaken to examine the effect of long-term oral administration of a novel GSH precursor, F1 (a compound containing cystine as the carrier of cysteine), on the aged kidney.

Methods: Eighteen-month-old male B6 mice were randomized to groups fed regular diet or diet containing F1 (0.5 g/kg/day) for six months. Young adult (2 month old) mice consuming regular diet served as controls. Animals were then euthanized and kidneys were harvested and processed for histological examination, measurement of of markers of OS, and expression of key molecules involved in the redox and inflammatory pathways.

Results: Compared to the young mice, kidneys in the untreated aged mice showed marked GSH depletion, mesangial matrix expansion, focal tubular atrophy, interstitial fibrosis and patchy interstitial inflammation. This was associated with accumulation of tubulointerstitial fibrosis. The aged mice showed a trend towards decreased kidney weights, a trend towards lower albuminuria, and significantly reduced cytokine levels compared to vehicle controls. Despite OS, which should have evoked activation of Nrf2, its nuclear content was unchanged reflecting impaired response to oxidative stress in the aged mice. Amelioration of OS with F1 administration was associated with the maintenance of Nrf2 activity in the kidneys of the aged mice.

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Conclusions: These findings illustrate the efficacy of F1 in mitigating age-associated oxidative stress and renal injury in this model.

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FR-PO1839

Disrupted TGF-β Receptor II Abrogates Smad3-Mediated Renal Fibrosis but Exaggerates Inflammatory Response In Vivo and In Vitro

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Background: It is well known that TGF-β1 plays distinct roles in fibrosis and its receptor II to exert the diverse effects on renal fibrosis and inflammation in vivo and in vitro.

Methods: The hypothesis was examined in vivo in a mouse model of unilateral ureteral obstructive (UUO) nephropathy induced in TGFRII (RII) conditional knockout mice (KpCre/RII) in which RII was deleted specifically from the kidney tubular epithelial cells and in vitro in primary kidney fibroblasts and NRK52E cell line where the RII was deleted by adenovirus Cre or by overexpressing dominant negative RII (DNRII).

Results: Disrupted RII from the kidney as well as from kidney fibroblasts substantially inhibited fibrogenesis including collagen and fibronectin expression (50% ↓) and a-SMA+ myofibroblasts accumulation (approximately 20% ↓) in the UUO kidney and in TGF-β1-stimulated kidney fibroblasts. These changes were associated with significant reduction of TGF-β1 (p<0.05) and CTGF (p<0.01) and deactivation of Smad3 signaling (80%) ↓. In contrast, deletion of RII enhanced inflammatory response as demonstrated by up-regulation of IL-1β, TNFα (20%↑) in the UUO kidney and in IL-1β-stimulated DNRII-NRK52E cells. Further studies revealed that enhanced renal inflammation in the UUO kidney and cells with conditional deletion of RII was associated with a further increase in activation of NF-κB signaling.

Conclusions: In conclusion, TGF-β1 may signal through its receptor II to exert its diverse role in causing renal fibrosis while inhibiting renal inflammation under pathophysiological conditions.

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FR-PO1840

Suramin: A Novel Treatment for Chronic Kidney Disease

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Background: Current drug discovery efforts for fighting renal fibrosis are largely focused on compounds that are specific for a particular receptor or protein kinase. Since renal fibrogenesis is associated with increased production of multiple cytokines/growth factors, inhibitors with broad specificity might offer improved therapeutic benefit in fibrotic diseases of the kidney. We here assessed the therapeutic effect of suramin, an FDA approved drug for treating selected malignancies, on the activation of renal interstitial fibroblasts and the development and progression of renal fibrosis in animal models of chronic kidney injury.

Methods: Unilateral ureteral obstruction and remnant kidney models were used.

Results: In a model of unilateral ureteral obstruction (UUO), administration of a single dose of suramin (20 mg/kg) immediately after injury prevented the onset of renal fibrosis as shown by abolishing expression of fibronectin, suppressing expression of alpha-SMA and type I collagen and reducing deposition of extracellular matrix proteins. In a rat model of remnant kidney disease, suramin also prevented progressive renal injury as demonstrated by inhibiting the rise of 24-hour-proteinuria and serum creatinine, preserving renal tissue architecture and preventing glomerular and tubulointerstitial damage. Furthermore, delayed administration of suramin starting at day 3 of obstruction completely blocked further increase in expression of type I collagen and fibronectin and largely suppressed expression of alpha-SMA in both treatment groups. UUO injury or renal ablation induced phosphorylation of epidermal growth factor receptor and platelet derived growth factor receptor one and several signaling molecules including Smad-3, STAT3 and ERK1/2 that are associated with renal fibrogenesis. Suramin treatment completely blocked phosphorylation of all these molecules in the injured kidney and also repressed expression of multiple cytokines and decreased leukocyte infiltration to the interstitium.

Conclusions: These findings indicate that suramin is a potent anti-fibrotic agent and may have therapeutic potential in treating patients with CKD.

Funding: NIDDK Support

FR-PO1841

Lipocalin-Type PGD2 Synthase (L-PGDS) Play a Key Role in Kidney Interstitial Fibrosis Via the Activation of Th2-Dominant Inflammatory Response

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Background: L-PGDS, which was identified as an enzyme responsible for PGD2 biosynthesis, is expressed in the kidney. It has been reported that urinary L-PGDS excretion increased in diabetic nephropathy and hypertensive nephrosclerosis. However, the pathophysiological role of PGD2 biosynthesis in the kidny is still unknown.

Methods: We examined the interaction of kidney expression of L-PGDS and tissue injury in an animal model of unilateral ureteral obstruction (UUO).

Results: L-PGDS expression was induced in cortex after 3days of UUO and continued to increase thereafter. Tubular-epithelial de novo synthesis of L-PGDS was also demonstrated by in situ hybridization. Of note, the interstitial collagen deposition and mRNA expression of collagen-1 was less in the UUO-kidneys of L-PGDS knockout (LKO) mice compared to those of wild-type (WT) mice. In addition, the number of infiltrating CD4+ T cells, not macrophages, was significantly decreased in LKO mice. Intracelular staining in the kidney revealed that the infiltration of IL-4 producing Th2 cells, but of IFN-γ producing Th1 cells, was reduced in LKO mice. CRTL2, which is one of the PGD2 receptors, is expressed on Th2 cells and mediates Th2-cytokine production. CRTL2-KO mice showed lower expression levels of IL-4 and IL-13 in the UUO-kidneys. Interestingly, UUO-induced interstitial fibrosis was markedly reduced in CRTL2-KO mice. In the further support to these notes, both IL-4-KO and IL-13-KO mice reduced the degree of UUO-induced interstitial fibrosis compared to WT mice. Furthermore, administration of CRTL2 antagonist, CAY10471, following UUO attenuated the progression of interstitial fibrosis.

Conclusions: L-PGDS is de novo synthesized in tubular epithelium of the obstructed kidneys and contributes to the progression of interstitial fibrosis via the activation of Th2 cells. Blockade of CRTL2-signaling is a promising strategy to attenuate the tubulointerstitial fibrosis via inhibition of Th2-dominant inflammatory responses.

FR-PO1842

Regulation of Macrophage Polarization by Heme Oxygenase-1 in Renal Fibrosis

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Background: Heme oxygenase-1 (HO-1) is known to modulate innate and adaptive immune responses. We previously showed that there is increased renal fibrosis following unilateral ureteral obstruction (UUO) in HO-1 deficient (HO-1−/−) mice compared to HO-1+/- mice. Macrophages play an essential role in such fibrosis. The M1 (classically activated) macrophages predominate during early stages of kidney injury and inflammation, whereas M2 (alternatively activated) macrophages predominate at later time points and promote cellular repair and fibrosis. The purpose of this study was to determine the effect of HO-1 on the distribution of renal macrophage subtypes following UUO.

Methods: Renal macrophage infiltration and polarization was investigated at 2 and 5 days following UUO in HO-1−/− and HO-1+/- kidneys using flow cytometry and real-time-PCR.

Results: A significantly higher number of macrophages were detected in HO-1−/− kidneys at 48h post UUO. Following isolation of CD11b+ (a macrophage marker) population from UUO kidneys, we performed real-time-PCR evaluation of M1 (iNOS and TNFα) and M2 (Arginase-1) markers (Figure 1). As expected, the M1 subclasspopulation in HO-1−/− kidneys was higher at 2 days while the predominant inflammatory cells were M2 macrophages at 5 days post UUO. Although there were no significant differences in sham animals, polarization was dysregulated in HO-1−/− mice that underwent UUO. The M2 macrophages were the most abundant inflammatory cells at 2 days in HO-1−/− kidneys and M1 macrophages were significantly increased only 5 days post UUO (P < 0.05).

Conclusions: Our findings elucidate the key role of HO-1 in macrophage polarization and suggest that modulation of HO-1 expression in inflammatory disorders has the potential as a novel therapeutic modality.

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540A
FR-PO1843

Inflammation Exacerbates the Progression of Vascular Calcification in Hemodialysis Patients through the Disruption of LDLR Pathway

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Background: Chronic microinflammation in hemodialysis patients played crucial roles in the progression of vascular calcification (VC). Our previous studies in vivo and in vitro demonstrated that inflammation accelerated the progression of atherosclerosis (AS) and fibrosis (F). The present study was performed to investigate whether LDLR pathway was involved in the progression of VC under inflammatory stress.

Methods: Twenty-eight hemodialysis patients receiving arteriovenous fistula were divided into two groups by the plasma level of C-reactive protein: Control (n=14), inflamed group (n=14). Hematoxylin-eosin staining and alizarin red S staining were respectively used to check foam cell formation and significant VC in continuous cross-sections of radial arteries in inflamed group compared to control. Immunohistochemistry and immunofluorescent staining were used to check protein expressions related with intracellular cholesterol trafficking and VC.

Results: There was parallel change for increased foam cell formation and significant VC in continuous cross-sections of radial arteries in inflamed group compared to control, which were correlated with the increasing protein expressions of LDLR, bone morphogenetic proteins-2 (BMP-2) and collagen I respectively. Confocal microscopy observation showed that inflammation enhanced the protein expressions of alkaline phosphatase, and reduced the protein expression of alpha-smooth muscle actin, contributing to the phenotype conversion of vascular smooth muscle cells in calcified vessels from the fibroblastic to the osteogenic, which was one of the main cell components involved in VC. Further analysis showed that the dysregulation of LDLR pathway induced by inflammation was significantly associated with the enhanced expression of BMP-2 and collagen I.

Conclusions: Our study firstly demonstrated that inflammation accelerated the progression of VC in hemodialysis patients through the disruption of LDLR pathway, suggesting a new potential mechanism involved both in the progression of VC and AS.

Funding: Government Support - Non-U.S.

FR-PO1844

M2 Macrophages Contribute to Kidney Repair Via Promoting Degradation of Extracellular Matrix through Legumain

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Background: Studies have suggested that the switch of macrophage phenotype from M1 to M2 regulates the kidney injury and repair. In this study, we investigated that the role of Legumain in the mechanism underlying the renal beneficial effect of M2 macrophage.

Methods: Adult C57 mice underwent unilateral ureteral obstruction (R-UUO). Liposome cholodronate (LC) was used to deplete the macrophages. LC/control liposome before operation; LC before operation; Control liposome before operation; LC recovery; Control liposome recovery (n=5). Macrophage and Legumain were assessed by immunostaining for CD68 and staining for picrosirius red (SR). TGF-β and CTGF expression by qPCR; macrophage infiltration and collagen deposition were assessed by immunostaining for CD68 and staining for picrosirius red (SR).

Results: Compared with control recovery group, depletion of macrophage at the time of UUO recovery increased extracellular matrix deposition and the legumain expression was inhibited in LC recovery group with the depletion of M2 macrophage. Result of immunofluorescence staining and FACS analysis showed the M2 macrophages were the major source of legumain in the process of kidney repair. In vitro experiment showed that co-culture with M2 macrophage promoted the degradation of Fibronectin and collagen I while small inhibitor of Legumain blocked this effect. IP assay confirmed the interaction between legumain and two major extracellular matrix components, Fibronectin and Collagen I.

Conclusions: Our results suggest that M2 macrophages exhibit protective effect on the kidney recovery from fibrosis. The underlying mechanism is most likely related with inducing expression of legumain thus promoting the degradation of extracellular matrix.

Funding: Government Support - Non-U.S.

FR-PO1845

PBI-4050, a Novel Orally Active Anti-Inflammatory/Anti-Fibrotic Agent, Reduces Fibrosis and Sclerosis in 5/6 Nephrectomized Rats


Background: PBI-4050 is a novel first-in-class, orally active low molecular weight compound which displays anti-inflammatory/anti-fibrotic activities via a novel mechanism of action. The aim of this study was to investigate the effect of PBI-4050 on 5/6 nephrectomized rats.

Methods: Wistar rats were partially nephrectomized (2/3 of the left kidney) on day 0. On day 7 the right kidney was removed. Oral treatment with PBI-4050 (200 mg/kg, once a day) or vehicle was initiated at day 21. GFR was measured at day 21 and assessed every 3 weeks up to day 190 at which time the animals were sacrificed.

Results: Treatment with PBI-4050 resulted in a significant improvement (up to three fold relative to control) in GFR as demonstrated by an increase in creatinine clearance. Histological lesion scores of kidney were also significantly (p<0.05) reduced in PBI-4050-treated rats (2.7 ± 1.5) compared to control (3.9 ± 1.4), as determined by HPE, PAS and Masson’s trichrome staining. Tubulo-interstitial fibrosis and sclerosis were significantly (p<0.05) reduced by treatment with PBI-4050. Also, a reduction in blood pressure was observed in PBI-4050-treated rats. Furthermore, oral treatment with PBI-4050 induced a significant reduction of urine MCP-1 level in treated 5/6 nephrectomized rats. This reduction correlates with GFR improvement and inhibition of fibrosis observed in PBI-4050-treated rats, indicating that PBI-4050 delays disease progression.

Conclusions: Taken together, these results suggest that PBI-4050 offers the potential as a novel therapy for chronic kidney disease by reduction of fibrosis and sclerosis.

Funding: Pharmaceutical Company Support

FR-PO1846

Deficiency of P2X4 Receptor May Promote Renal Fibrosis in a Mouse Model of Unilateral Ureteral Obstruction

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Background: Among the seven mammalian P2X receptors, P2X7 (P2X7R) has been shown to have pro-inflammatory effects in two models of renal injury: nephrotoxic nephritis and unilateral ureteral obstruction (UUO). P2X4 (P2X4R) is a related receptor with similarities to P2X7R; it is adjacent on the same chromosome, but has a wider tissue distribution. We compared the function of these receptors in the UUO model using knockout (KO) mice.

Methods: Ten to 12-week-old male P2X4R-/-, P2X7R-/- (a model expressing a novel splice variant of P2X7R) - gifts from GSK - and WT mice were subjected to either UUO or sham operation. Kidney samples taken at day 7 or day 14 were evaluated for MCP-1, TGF-β and CTGF expression by qPCR; macrophage infiltration and collagen deposition were assessed by immunostaining for CD68 and staining for picrosirius red (SR).

Results: On day 7 after UUO there were no significant differences in mRNA expression of MCP-1, TGF-β and CTGF, or CD68+ staining (P2X7R-/-, WT or P2X4R-/-); however, SR staining was significantly greater in P2X4R-/- compared with P2X7R-/- and WT. By day 14 mRNA expression of MCP-1, TGF-β and CTGF was significantly higher in P2X4R-/- compared with P2X7R-/- and WT (Fig 1); CD68+ staining was significantly greater in both P2X4R-/- and WT compared with P2X7R-/-; SR staining was significantly greater in P2X4R-/- compared with P2X7R-/- and WT (Fig 2).

Conclusions: These findings suggest that: (1) P2X4R deficiency increases renal fibrosis in UUO; (2) that the reduced inflammation and fibrosis in another P2X7R KO model is consistent with previous findings, and that there is no functional role for the P2X7R splice variant in UUO.
Accelerated Tubulointerstitial Alterations Induced by Unilateral Ureteral Obstruction in Mice Lacking Vasohibin-1

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Background: Tubulointerstitial injuries are crucial histological alterations predicting deterioration of renal function in chronic kidney disease. Vasohibin-1 (VASH1), serves as a negative feedback regulator of angiogenesis as well as a maturation factor of neovessels by inducing pericyte attachment. We previously reported the protective role of Vasohibin-1 in diabetic nephropathy. Our hypothesis is that role of tubulointerstitial injuries remains to be elucidated. In the present study, we aimed to evaluate the potential role of endogenous VASH1-1 to regulate tubulointerstitial alterations in a mouse unilateral ureteral obstruction (UUO) model.

Methods: UUO was induced in female VASH1-heterozygous knockout mice (VASH1+/−) or wild-type (VASH1+/+) littermates (C57BL/6J background). Mice were sacrificed on Day 3 or 7 after inducing UUO and the obstructed kidneys (OBK) were obtained.

Results: Glomerular or tubulointerstitial alterations were not observed in the kidneys of sham-operated VASH1+/− mice. Interstitial fibrosis, accumulation of type 1 and III collagen and F4/80+ monocytes/macrophages in the OBK on Day 7 after inducing UUO were significantly accelerated in VASH1+/− mice compared with VASH1+/+ mice. Increase in the levels of transforming growth factor (TGF)-β (immunohistochemistry) and the number of interstitial fibroblast specific protein (FSP)1+ cells in the OBK were significantly aggravated in VASH1+/− mice compared with the wild-type mice.

Conclusions: These results suggest that endogenous VASH1-1 may play a role in suppressing tubulointerstitial alterations induced by UUO via regulating inflammation and fibrosis, implying its potential use as a novel therapeutic reagent for renal disorders.

FR-PO1840

SIPR3 is Pivotal Factor in Fibrosis in the Kidney

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Background: The major sphingolipid metabolite, sphingosine-1-phosphate (SIP), is now attractive modulator of renal fibrosis. In our past report, SIP induced fibrosis in vitro in normal rat kidney interstitial fibroblast cells (NRK-49F). SIP is likely to have potentiality to mediate directly renal fibrotic process in addition to via inflammatory pathway. On the other hand, SIP is known to have unique tissue distribution of the receptor subtypes and the differing signaling pathways resulting from SIP receptor (SIPR) subtype activation. In our past study, NRK-49F showed mRNA expressions of SIPR 1, 2, and 5, but not 4. And F4/80+ stimulated fibroblast induced by SIP, TGFβ2, TGFβ3, and TGFβ1 is mostly related with inflammatory pathway and SIPR 5 is involved in the regulation of nervous system. Thus, these results suggest that SIPR 3 is likely to play main role in renal fibrosis.

In this study, the role of SIPR 3 as a migratory mediator and which subtype of SIPR was related to fibrosis in the kidney were investigated in NRK-49F.

Methods: NRK-49F were stimulated with SIP and the expressions (mRNA/western blotting) of a-SMA, E-cadherin, collagen type I (COL1), collagen type IV (COL4), TIMP1 and PAI1 were examined. The morphological changes of the NRK-49F after stimulation by SIP were examined. Increased migration of the cells was evaluated by the cell count within a field of view. And these examinations were adapted to siRNA (SIPR3) model.

Results: SIP stimulated fibrosis of NRK-49F in a dose- and time-dependent manner, and activated fibroblast trans-differentiation of the NRK-49F. Increase in the expression of a-SMA, COL1, COL4, TIMP1 and PAI1 expressions and decrease in E-cadherin expression were observed in the SIP-stimulated cells. Treatment of SIP-stimulated morphological changes (elongation of the cell shape with spindle-like extension, increased migration) in NRK-49F. In the presence of siRNA, fibrotic changes induced by SIP were suppressed.

Conclusions: Our conclusion, SIP is a novel fibrotic mediator in the kidney, and SIPR3 is pivotal factor in fibrosis.

BMP2 Induces a Pro-Fibrotic Phenotype in Adult Renal Progenitor Cells (ARPCs) through NoxA4 Activation in Transplant Recipients with Delayed Graft Function (DGf) S. Simon, 1 M. Carillo, 1 C. Cosola, 1 Antonia Loverre, 1 F. Rascio, 2 Fabio Sallustio, 2 Loretto Gesualdo, 1 Francesco Paolo Schena, 1 G. Grandaliano, 1 G. Pertosa. 1 Dep. of Emergency and Organ Transplantation, University AIdo Moro, Bari, Italy; 2 Dep. of Biomedical Sciences, University of Foggia, Italy.

Background: ARPCs contribute to repair featuring acute kidney injury (AKI). Bone morphogenetic proteins (BMPs) regulate differentiation, modeling and regeneration in several tissues. Aim of the study was to evaluate the biological actions of BMP2 in ARPCs.

Methods: BMP2/BMP3 Receptors (BMP-R) gene (RT/PCR) and protein (ELISA/immunodetection) expressions were evaluated in ARPCs isolated from adult human kidney (magnetic cell sorting). Intracellular reactive oxygen species (ROS) generation was measured by 2’,7’DCF. Nox4 protein expression was studied by immunoblotting. BMP2, CD133, a-SMA and Nox4 protein expression was evaluated in renal biopsies of patients (pts, n=10) with DGF by conventional microscopy.

Results: BMP2 was expressed by ARPCs in adult human kidney and was upregulated in vivo after DGF (p=0.02). ARPCs expressed type I and II BMP-R. ARPCs treated with BMP2 induced ROS production (basal 36±2.15, 15.0 Arbitrary Unit (AU); BMP2 15’ 6.5±17.8 AU, p=0.01). Blocking ROS oxidase activity was evaluated in 5±3.2 AU; BMP2 15’ 212±75.4 AU, p=0.01) and Nox4 protein expression (p=0.03). In vivo, Nox4 was localized in BMP2+CD133+ cells at the tubular level after DGF. BMP2 incubation induced a-SMA (basal 3.1±AU; p=0.02), collagen-1 (p=0.03) and fibronectin (p=0.04) protein expression in ARPCs. ARPCs were treated with CD133 in vivo after DGF. H2O2 induced a-SMA expression in ARPCs (basal 1.0±2 AU; H2O2 2.6±8.8 AU, p=0.03), while N-acetyl-cysteine inhibited BMP2-induced a-SMA expression (BMP2 2.2±2.6; BMP2/NAC 1.3±4 fold change, p=0.04). Nox4 silencing (siRNA) abolished BMP2-induced NADPH oxidase activation and myofibroblastic induction (p=0.01).

Conclusions: In conclusion: a) ARPCs express BMP2; b) this expression is increased in pts with DGF; c) BMP2 may induce the commitment of ARPCs towards a myofibroblastic phenotype; d) this profibrotic effect is mediated by Nox4 activation suggesting a novel mechanism linking DGF with progressive graft damage.
Silencing of Pericyte MicroRNA-132 Reduces Renal Fibrosis and Is Associated with Altered Sirt1 Expression

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Background: Lineage analysis has shown that during nephrogenesis, FoxD1-positive mesenchymal cells give rise to adult interstitial pericytes. These FoxD1-derivative interstitial cells expand and differentiate into smooth muscle actin (α-SMA) positive myofibroblasts during renal fibrosis, accounting for a large portion of myofibroblasts, which are responsible for scar formation in fibrotic kidney disease. MicroRNAs (miRNAs) involved in this differentiation could serve as a target to decrease myofibroblast formation in fibrotic kidney disease.

Methods: Fibrosis was induced in FoxD1-GC/ZRed mice by unilateral ureteric obstruction (UUO) and FoxD1-derivative interstitial cells (dsRed positive) were isolated using FACS sorting. To identify differentially expressed miRNAs we profiled these specific cell populations in UUO vs. healthy controls. To investigate the role of miRNA-132 in vivo in renal fibrosis we injected antagonirs i.v. to silence its function in the UUO model. Mice were sacrificed both 5 and 10 days after surgery.

Results: miR-132 was amongst the most highly up-regulated miRNAs in the FoxD1-derivative interstitial cells and is associated with strongly altered Sirt-1 levels.

Conclusions: Silencing miR-132 protects against the development of renal fibrosis and is associated with strongly altered Sirt1 levels.

In Tubulointerstitial Fibromatosis of the Kidney Notch Receptors Play a Non-Redundant Profibrogenic Role

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Background: Tubulointerstitial fibrosis is the common final pathway in chronic kidney diseases. Recent studies link Notch receptor expression with kidney damage and scarring. We performed experiments in vitro and in vivo experiments to analyze the function of Notch1 receptors in the inflammatory response and tissue fibrosis.

Methods: Primary human mesangial cells were challenged with TGF-β and α-SMA positive myofibroblasts in culture were differentiated with 10% fetal calf serum and 5% human platelet rich plasma. The TGF-β stimulation was performed for 16h.

Results: Cell number of α-SMA positive myofibroblasts was significantly increased (p<0.05) by TGF-β stimulation and a significant decrease was seen in a late passage of human mesangial cells (p<0.05). The stimulation of α-SMA positive myofibroblasts was also increased (p<0.05) in the presence of α-SMA positive myofibroblasts (1:1 ratio).

Conclusions: Silencing α-SMA positive myofibroblasts in vitro and in vivo experiments to analyze the function of Notch1 receptors in the inflammatory response and tissue fibrosis.

Age Affects Kidney Remodeling in Response to Ureteral Obstruction

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Background: Renal tubulointerstitial fibrosis is the pathological hallmark of chronic kidney disease. Currently, inhibitors of the renin angiotensin system (RAS) remain the sole therapy in humans displaying antifibrotic effects. Therefore new antifibrotic drugs are needed and need to be evaluated in chronic renal insufficiency. We hypothesized that delayed treatment with a knin B1 receptor antagonist (B1RA) reduced renal fibrosis in the unilateral ureteral obstruction (UUO) model. The usefulness of new drugs also resides in outperforming the gold standards and being potentially additive or complementary to existing therapies. For this reason we compared the efficacy of a B1RA with that of an angiotensin type 1 receptor antagonist (AT1a) in a tubulointerstitial fibrosis model.

Methods: The fibrosis was induced in FoxD1-GC;Z/Red mice by unilateral ureteric obstruction (UUO) model. The usefulness of new drugs also resides in outperforming the gold standards and being potentially additive or complementary to existing therapies. For this reason we compared the efficacy of a B1RA with that of an angiotensin type 1 receptor antagonist (AT1a) in a tubulointerstitial fibrosis model.

Results: Silencing α-SMA positive myofibroblasts in vitro and in vivo experiments to analyze the function of Notch1 receptors in the inflammatory response and tissue fibrosis.

Conclusions: Silencing α-SMA positive myofibroblasts in vitro and in vivo experiments to analyze the function of Notch1 receptors in the inflammatory response and tissue fibrosis.

A novel first-in-class compound PBI-4419 on 5/6 nephrectomized rats.

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Background: Tubulointerstitial fibrosis (TI) is the final common pathway for all CKD leading to ESRD. CKD incidence increases with age in humans. However, for most experimental studies, young mice are used, and little is known about possible effects of aging on renal matrix biology in the TI compartment.

Methods: Unilateral ureteral obstruction (UUO) was performed in young (16wks; n=5) and old (48wks; n=5) C57Bl6 mice. Structural damage was scored after 14 days on PAS-stained slides. Gene expression was assessed by qPCR. Hydroxyproline and procollagen were measured by HPLC (hyp/proline ratio).

Conclusions: Old mice had 1.2-fold more tubular atrophy (p=0.02) and 2-fold more dilation (p=0.02) in the obstructed kidney than young mice. Contralateral non-obstructed kidneys in old mice tended to have lower ‘baseline’ hyp/proline ratio than in young mice, which, after UUO also increased 30% less in old than in young kidneys (p=0.03). Accordingly, COL1A1 expression was increased significantly more in old kidneys (2.4-fold; p<0.001) than in young kidneys (1.6-fold; p=0.07) in obstructed kidneys of old vs. young mice, while collagenase MMP1/13 mRNA was decreased similarly in old and young obstructed kidneys (2.4-fold; p<0.001). In response to injury, BMP6 mRNA was decreased in young (1.9-fold; p=0.03), but not old mice after UUO, while decrease of BMP7 (4-fold; p<0.03) and increase of TGFβ (4-fold; p=0.03) and CTGF mRNA (1.7-fold; p=0.04) were not observed in aged mice.

Commercially available Pharmaceutical Company Support

Funding: Pharmaceutical Company Support

Oral Treatment with a Novel First-in-Class Anti-Fibrotic Compound, PBI-4419, Delays Tubulo-Interstitial Fibrosis and Sclerosis in 5/6 Nephrectomized Rats


Background: Recently, we discovered a novel, first-in-class, orally active low molecular weight compound which displays anti-inflammatory and anti-fibrotic activities via a new mechanism of action. The aim of this study was to investigate the effect of this first-in-class compound PBI-4419 on 5/6 nephrectomized rats.

Methods: Male Wistar rats were nephrectomized (2/3 of the left kidney) on day 0. On day 7 the right kidney was removed. Treatment with vehicle or PBI-4419 (10 mg/kg, oral once a day) was started on day 21 and assessed every 3 days up to day 126. Serum urea and creatinine were also assessed every 3 weeks.

Results: Treatment with PBI-4419 increased survival. Rats treated with PBI-4419 demonstrated lower serum urea and creatinine levels than control. A significant GFR improvement (threefold increase) was also observed in PBI-4419-treated rats. Histological lesion scores of kidney were also reduced in PBI-4419-treated rats, as determined by H&E, PAS and Masson’s trichrome staining. Tubulo-interstitial fibrosis and sclerosis were significantly (p<0.05) reduced by treatment with PBI-4419. Furthermore, oral treatment with PBI-4419 induces a significant reduction of urinary albumin and TGFβ levels which correlates with GFR improvement and inhibition of fibrosis observed in PBI-4419-treated rats, indicating that PBI-4419 delays disease progression. 5/6 Nephrectomized rats showed an increase in TGFβ and CTGF mRNA expression in the kidney. Treatment with PBI-4419 gave a significant reduction of the expression of TGFβ (37%; p=0.002) and CTGF (30%, p<0.0001) in the kidney.

Conclusions: Together, these results suggest that PBI-4419 offers the potential as a novel therapy for chronic kidney diseases by prevention or reduction of fibrosis and sclerosis.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: We conclude that age affects renal response to UUO. The more severe tubulointerstitial fibrosis and inflammation whilst an ASO-specific antibody was used to visualize tissue sections stained with H and E. The control group (n=7) received saline alone. Obstructed kidney, contralateral kidney, liver, heart, and wound tissues were fixed in formalin at sacrifice (day 16). Microscopy of sections stained with H&E and Picro-Mallory Trichrome techniques allowed assessment of fibrosis and inflammation whilst an ASO-specific antibody was used to visualize tissue ASO accumulation. Anti-si-g1p1 immunostaining was used to identify proximal tubular cells whilst anti-si-g1p1 marked collecting ducts.

Results: In ASO-treated rats, ASO accumulated in all examined tissues, including surgical wounds. In obstructed kidneys ASO were seen primarily in proximal tubules and the interstitium of obstructed kidneys; they were absent from collecting ducts. Silencing Ki-Ras expression markedly reduced fibrosis in the obstructed kidneys. No difference between ASO-treated and vehicle only-treated rats was identified in inflammation or fibrosis at healing wounds.

Conclusions: Subcutaneously administered ASO are deposited in multiple tissues around the body. Although Ki-Ras ASO can prevent pathological renal fibrosis it does not inhibit normal wound healing, which permits the inference that fibro-inflammatory change in UUO and in wound healing operate via different mechanisms. Ki-Ras ASO use in clinical settings may not be contraindicated by wound-healing concerns.

FR-PO1858
Hyaluronan Synthase 1 Gene Expression Modulates Fibroblast Phenotype Timothy Bowen, 1,2 Rachel M. Ferrante, 1,2 John Martin, 1,2 Robert Steadman, 1,2 Aled O. Phillips. 1,2

Method: To identify the processes underlying scar-free healing we are using donor-matched primary human dermal (DFs) and oral mucosal fibroblasts (OFs) as models of scar-free healing in skin and mucosa respectively. To investigate the role of HAS1 and HAS2 in the regulation of fibroblast phenotype we have used 5'-RACE to identify the HAS1 transcription start site. Selected transcription factor mRNAs were then subjected to small interfering (si)RNA knockdown. With immunohistochemical staining, the expression of Col III and fibronectin showed the expression of Col III, fibronectin and positive staining of SA-β-gal in RTECs.

Results: siRNA knockdown in DFs showed that HAS1 induction by IL-1α was Sp3-dependent, while TGFβ1 up-regulated HAS1 mRNA synthesis specifically via Smad3. Forced HAS1 ORF expression in DFs drove up-regulated transcription of alpha-smooth muscle actin (α-SMA), a key marker of fibroblast-to-myofibroblast transition. Further analyses in DF, following siRNA knockdown of HAS1 and/or HAS2, showed that visible α-SMA was absent only when siRNAs to both isoforms were used.

Conclusions: Our data provide evidence of synergy in the function of HAS1 and HAS2 in fibroblast phenotypic plasticity, with HAS1 facilitating differentiation to a myofibroblastic phenotype.

FR-PO1857
Silencing Ki-Ras with Antisense Oligonucleotides Reduces Renal Fibrosis in Unilateral Ureteric Obstruction but Does Not Affect Wound Healing Luoye Jade Newbury, 1 Jia-Hui Wang, 1 A. S. Kniely, 2 Bruce M. Hendry, 1 Claire C. Sharpc. 1 Renal Medicine, King’s College London, United Kingdom; 2 Institute of Liver Studies, King’s College London, United Kingdom.

Background: Pathogenic fibrosis in the kidney is thought to occur via many different signaling pathways, many of which converge on Ras GTPases. Work in our laboratory has highlighted that the Ki-Ras isoform is important in renal fibrosis. In this project we used antisense oligonucleotides (ASO) to silence Ki-Ras expression to prevent fibrosis in a unilateral ureteric obstructive (UUO) model and assessed the effects on surgical wound healing.

Methods: Male Adult Wistar rats underwent unilateral ureteric ligation. The treatment group (n=7) received 12.5 mg/kg ASO in saline subcutaneously on alternate days for 16 days. The control group (n=7) received saline alone. Obstructed kidney, contralateral kidney, liver, heart, and wound tissues were fixed in formalin at sacrifice (day 16). Microscopy of sections stained with H&E and Picro-Mallory Trichrome techniques allowed assessment of fibrosis and inflammation whilst an ASO-specific antibody was used to visualize tissue ASO accumulation. Anti-si-g1p1 immunostaining was used to identify proximal tubular cells whilst anti-si-g1p1 marked collecting ducts.

Results: In ASO-treated rats, ASO accumulated in all examined tissues, including surgical wounds. In obstructed kidneys ASO were seen primarily in proximal tubules and the interstitium of obstructed kidneys; they were absent from collecting ducts. Silencing Ki-Ras expression markedly reduced fibrosis in the obstructed kidneys. No difference between ASO-treated and vehicle only-treated rats was identified in inflammation or fibrosis at healing wounds.

Conclusions: Subcutaneously administered ASO are deposited in multiple tissues around the body. Although Ki-Ras ASO can prevent pathological renal fibrosis it does not inhibit normal wound healing, which permits the inference that fibro-inflammatory change in UUO and in wound healing operate via different mechanisms. Ki-Ras ASO use in clinical settings may not be contraindicated by wound-healing concerns.

FR-PO1859
Regulation and Function of Hyaluronan Synthase 2-Antisense 1 Noncoding RNA, HAS2-AS1 Timothy Bowen, 1,2 Daryn R. Michael, 1,2 James Edward Redman, 1 Abdsalamed Altaher, 1 John Martin, 1,2 Aled O. Phillips, 1,2 Institute of Nephrology, Cardiff University School of Medicine, Cardiff, United Kingdom; 2 Institute of Tissue Engineering and Repair, Cardiff University, Cardiff, United Kingdom; 3 School of Chemistry, Cardiff University, Cardiff, United Kingdom.

Background: Noncoding (nc)RNAs are emerging rapidly both as novel regulators of gene expression and potential therapeutic targets. Hyaluronan synthase (HAS) 2 is a ubiquitous extracellular matrix component that is synthesised by the HAS synthase (HAS) enzymes. HAS synthesis and pericellular assembly drive the differentiation of renal proximal tubular epithelial cells (PTCs) and fibroblasts to a pro-fibrotic, myofibroblastic phenotype. HAS2 gene expression is essential to this process, and HAS2 antisense (HAS2-AS1) is a natural antisense for the HAS2 gene that down-regulates HAS2 transcription and HA synthesis in osteosarcoma cells. We have recently shown that HAS2-AS1 transcriptional induction facilitates HAS2 expression in PTCs. In the present investigation we investigated HAS2-AS1 and HAS2 expression in fibroblasts as well as the mechanism of HAS2-AS1:HAS2 ncRNA:mRNA interaction in PTCs.

Methods: We used RT-qPCR and siRNA knockdown to analyse HAS2-AS1 and HAS2 expression in fibroblasts. HAS2-AS1 ncRNA and HAS2 mRNA secondary structures were investigated in silico to verify the thermodynamic feasibility of heterodimer formation. Minimum free energy and partition function calculations were performed using the Vienna suite of programs. Analysis of ncRNA:mRNA duplex location in PTCs was carried out using endpoint RT-PCR.

Results: Our data showed coordinated expression of HAS2-AS1 and HAS2 in fibroblasts, supporting our previous analyses in PTCs. The thermodynamic feasibility of HAS2-AS1:HAS2 heterodimer formation was demonstrated, and locus-specific cytoplasmic double-stranded RNA was detected.

Conclusions: We provide evidence that the interaction between HAS2 mRNA and HAS2-AS1 mRNA stabilises and/or augments HAS2 expression via cytoplasmic duplex formation. Our results emphasise the potential of modulating antisense RNA expression to regulate both HAS2-driven HA synthesis, and the downstream myofibroblastic conversion of PTCs and fibroblasts.

FR-PO1860
The Senescence of Renal Tubular Epithelial Cells Correlates with Tubulointerstitial Fibrosis in Immunoglobulin A Nephropathy Yani He, 1,2 Jun Liu, 2 Junmin Yang, 2 Xiaokang Zhang, 2 Lirong Lin, 2 BenGang Huo, 2 Jun Zhang 1 Department of Nephrology, Daping Hospital, the Third Military Medical University, Chongqing, China.

Background: Tubulointerstitial fibrosis (TIF) is the key pathophysiology basis in the development of immunoglobulin A nephropathy (IgAN). Here we report our studies on the relationship between the senescence of RTECs and TIF in IgAN.

Methods: Patients with IgAN were initially classified according to Lee’s classification method. With immunohistochemical staining, the expression of Col III and fibronectin (FN) in tubulointerstitial tissue were examined, and expression of p16, p21, cyclin D1 in RTECs were examined, also positive staining of SA-β-gal in RTECs was evaluated. Immunohistochemical staining in serial sections was also performed to examine the expression of p16, p21, cyclin D1 and FN in IgAN with Lee’s classification. Correlation analysis showed expression of Col III, FN and positive staining of SA-β-gal in RTECs.

Results: With the increased level of Lee’s IgAN grades, positive staining of SA-β-gal and the nuclear positive expression of p16 and p21 were progressively increased (P<0.01), which was consistent with the increased expression of Col III and FN in tubulointerstitial tissue; while the expression of cyclin D1 was significantly decreased (P=0.01). Immunohistochemical staining in serial sections in Lee’s Grade V IgAN showed that the increased p16 and p21 in RTECs associated with elevated FN expression in tubulointerstitial tissue, whereas the expression of cyclin D1 notably decreased. Correlation analysis suggested that positive staining of SA-β-gal in RTECs presented significant positive correlation with the expression of Col III and FN in tubulointerstitial tissue (r=0.665, P=0.001).

Conclusions: Our results revealed that the senescence of RTECs tightly links with TIF in IgAN, which is the possible underlying mechanism response for the progression of TIF in IgAN.

FR-PO1861
Increased Expression of Intracellular Matrix Metalloproteinase 9 in Atrophic Renal Tubules Is Associated with Renal Fibrosis Jen-Pi Tsai, 1,3 Jong-Da Lian, 2,3 Horng-Rong Chang, 1,3, 4 Department of Nephrology, Buddhist Dalin Tzu Chi General Hospital, Chiayi, Taiwan; 2 Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan; 3 Division of Nephrology, Department of Internal Medicine, Chung Shan Medical University Hospital, Taichung, Taiwan.

Background: Reduced turnover of extracellular matrix has a role in renal fibrosis. Matrix metalloproteinase (MMP) is associated with many glomerular diseases, but the relationship of MMP-9 with renal fibrosis is not well understood.

Methods: We studied the role of MMP-9 in the pathogenesis of renal fibrosis in 46 patients who received nephrectomies by immunohistochemical analysis of renal expression

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of MMP-9 in nephrectomized kidneys, and determination of the association of renal expression of MMP-9 with fibrosis. MMP-9 expression in individual renal tubular components and fibrosis was graded as high or low based on MMP-9 staining and fibrotic scores. Patients with high interstitial fibrosis scores (IFS) and glomerular fibrosis scores (GFS) who had significantly higher serum creatinine, lower estimated glomerular filtration rate (eGFR), and were more likely to have chronic kidney disease (CKD) and renal fibrosis was graded as high or low based on MMP-9 staining and fibrotic scores. We found a significant correlation between MMP-9 expression in the cytoplasm of normal tubular cells and increased expression was also examined.

Conclusions: Our results indicate that renal fibrosis is associated with a decline of MMP-9 expression in the cytoplasm of normal tubular cells and increased expression of MMP-9 in the nuclei of tubular atrophic renal tubules. We postulated that increased intracellular MMP-9 expression may reflect intracellular gelatinase proteolysis, play a role in oxidative DNA damage by clearing nuclear matrix proteins (PARP-1 and/or XRCC1), and contribute to cell death and fibrosis.

Funding: Clinical Revenue Support

FR-PO1862

HIV-1 Promotes Renal Tubular Epithelial Cell Protein Synthesis: Role of mTOR Pathway Shabina Rehman1, Mohammed Hussain1, B. S. Kasthi2, Ashwani Malhotra1, Pravin C. Singh1. 1Medicine, North Shore LIJ Health System Medical School, Great Neck, NY; 2Medicine, University of Texas Health Science Center, San Antonio, TX.

Background: Tubular cell HIV-infection has been reported to manifest in the form of cellular hypertrophy. The mammalian target of rapamycin (mTOR) has been considered to be a central pathway of protein synthesis. In the present study, we evaluated the role of the mTOR pathway in the HIV-induced tubular cell protein synthesis and hypertrophy.

Methods: Mouse proximal tubular epithelial cells (MPTECs) were transduced with either gag/pol-deleted NL4-3 (HIV/MPTC) or empty vector (Vector/MPTC). Vector/MPTCs and HIV/MPTECs were evaluated for DNA synthesis by BrDU labeling and for protein synthesis by immunoblotting for β-actin and fibronectin. Immunoblots prepared from vector/MPTCs and HIV/MPTECs were probed for phosphorylation of mTOR, p70S6 kinase and associated downstream molecules (eEF2, eEF4 and 4EBP-1). We also evaluated the effect of rapamycin on HIV-induced tubular cell mTOR activation and associated downstream signaling. In addition, we studied the effect of mTOR inhibition on HIV-induced tubular cell protein synthesis. In vivo studies, tubular cell mTOR activation was also examined.

Results: HIV/MPTECs showed enhanced (P<0.01) phosphorylation of β-actin and fibronectin in addition to increased (P<0.01) protein content per cell. Analysis of mTOR revealed increased expression of phospho (p)-mTOR in HIV/MPTECs when compared to vector/MPTCs. Further downstream analysis of mTOR pathway revealed enhanced (P<0.01) phosphorylation of p70S6 kinase and associated diminished phosphorylation of eEF2 in HIV/MPTECs; moreover, HIV/MPTECs displayed enhanced phosphorylation of eEF4B and 4EBP-1. Rapamycin only attenuated (P<0.01) phosphorylation of p70S6 kinase and associated down stream signaling in HIV/MPTECs but also inhibited HIV-1 induced tubular cell protein synthesis. In in vivo studies, renal cortical sections from HIVtg mice and HIVAN patients showed enhanced tubular cell phosphorylation of mTOR.

Conclusions: The mTOR pathway activation in expressing tubular cells results in increased protein synthesis and cellular hypertrophy.

Funding: NIDDK Support

FR-PO1863

Matrix Metalloproteinase-7 as a Surrogate Marker Predicts Renal Wnt/β-Catenin Signaling in Progressive Chronic Kidney Diseases Weichun He, Roderick J. Tan, Yingjian Li, Dan Wang, Youhua Liu. University of Pittsburgh, PA.

Background: Matrix metalloproteinase-7 (MMP-7) is a secreted, zinc and calcium dependent endopeptidase that cleaves a variety of extracellular matrix components and other substrates. In this study, we investigated MMP-7 regulation in progressive chronic kidney diseases and delineated its relation to renal Wnt/β-catenin signaling. We also investigated the effect of core fucosylation of megalin on the induction of obstructive nephropathy, both MMP-7 mRNA and protein were upregulated in a time-dependent manner. Induction of MMP-7 mRNA and protein was also found in adriamycin nephropathy. The pattern and extent of MMP-7 induction were closely correlated with an increased Wnt/β-catenin signaling in these models. Activation of β-catenin through ectopic expression of Wnt1 gene in vivo promoted MMP-7 expression, whereas deletion of endogenous Wnt antagonist Dickkopf-1 gene abolished its induction. MMP-7 protein was detectable in urine and its urinary levels correlated with renal Wnt/β-catenin activity. Phagocytosis of blastic bone marrow and β-catenin signaling in proximal tubular cells (hPTC) were increased in core fucosylation of megalin. As a result, the expression of mRNA of NLRP-3, caspase-1 and IL-1β compared with control group. Study by immunohistochemistry and immunofluorescence showed that the expression of NLRP-3 was significantly increased in cytoplasm. Furthermore, it was shown that secretion of IL-1β and IL-18 in supernatant in corticosteroid determined by ELISA.

Results: After the 10 mg/ml BSA stimulation, real time RT-PCR analysis revealed the increased expression of mRNA of NLRP-3, caspase-1 and IL-1β compared with control group. Study by immunohistochemistry and immunofluorescence showed that the expression of NLRP-3 significantly increased in cytoplasm. Furthermore, it was shown that secretion of IL-1β and IL-18 in supernatant in corticosteroid determined by ELISA.

Conclusions: Our study firstly demonstrated that albumin would cause the activation of NLRP-3 in proximal tubular cells, which provide a novel insight about the albuminuria in inducing tubulointerstitial inflammatory and subsequent fibrosis.

Funding: Government Support - Non-U.S.

FR-PO1864

Albumin Caused the Activation of NLRP3 in Proximal Tubule Cells Dan Liu, Linli Lv, Bi-Cheng Liu. Institute of Nephrology, Zhong Da Hospital, Southeast University School of Medicine, Nanjing, Jiangsu, China.

Background: Proteinuria is considered to play a central role in the pathogenesis of progressive renal dysfunction, especially, in the renal tubulointerstitial inflammation and fibrosis. Recent studies have indicated that NLRP3 inflammasome is a key platform for the regulation of renal inflammation, and it is thereby interesting to understand the potential influence of albuminuria on the expression of NLRP3 inflammasome in kidney tubular cells. In this study, we firstly investigated whether albumin would induce the activation of NLRP3 inflammasome in tubular epithelial cells.

Methods: Human Proximal tubular cell line HK-2 was cultured and stimulated with different concentrations (2.5 mg/ml, 5 mg/ml, 10 mg/ml) of Bovine Serum Albumin (BSA). The expression of mRNA for NLRP-3, caspase-1, IL-1βeta and IL-18 was detected by Real time PCR. Immunohistochemistry and immunofluorescence was applied to detect the expression of NLRP3 in HK-2 in vitro.

Results: BSA treatment in conditioned media enhanced tubular cell phosphorylation of NLRP-3 was observed in our study. The treated IL-1βeta and IL-18 in the supernatant from cell culture was determined by ELISA.

Conclusions: Our results indicate that renal fibrosis is associated with a decline of MMP-9 expression in the cytoplasm of normal tubular cells and increased expression of MMP-9 in the nuclei of tubular atrophic renal tubules. We postulated that increased intracellular MMP-9 expression may reflect intracellular gelatinase proteolysis, play a role in oxidative DNA damage by clearing nuclear matrix proteins (PARP-1 and/or XRCC1), and contribute to cell death and fibrosis.

Funding: Clinical Revenue Support

FR-PO1865

Core Fusocylaion of Megalin Is Required for Albumin Binding and Endocytosis in HK-2 Cells Hong Li Lin, Wang Da Peng, Zheng Mei Jie, Yan Ling Sun, Hua Xie. First Affiliated Hospital of Dalian Medical University, Dalian, China.

Background: Proteinuria is an independent risk factor leading to end-stage renal failure. Over-absorption of filtered proteins has been proved to trigger interstitial inflammation and fibrosis in glomerular disease. Megalin, an endocytic receptor expressed on the renal tubular brush border, is responsible for albumin reabsorption. It has been reported that megalin was a glycoprotein and there was amount of core fucose structure in megalin. In this study, we investigated the role of core fucose of megalin which catalysed by its specific transferring enzyme FUT8 in albumin recruiting injury in human renal proximal tubular epithelial cells lines (HK-2).

Methods: An albumin overload cell mode was induced by adding 10mg/ml BSA in HK-2 cells. FUT8sRNA or FUT8 full length cDNA vector was transiently transfected into HK-2 cells respectively. The spatial relationship in localization between megalin and core fucose was examined by double immunostaining. BSA binding and endocytosis was determined by flow-cytometry and laser scanning confocal microscope. Expression of megalin protein and core fucose of megalin were detected by immunoprecipitation and lectin blotting. Furthermore,we detected the expression of MCP-1 and RANTES to investigate the effect of core fusocylaion of megalin on the inflammation induced by BSA in HK-2 cells.

Results: BSA binding and endocytosis was in a time- and dose-dependent manner in HK-2 cells after BSA administration. Core fusocylaion of megalin was induced by FUT8 gene, which was up-regulated by FUT8 full length cDNA. Our data firstly showed that megalin was modified by core fusocylaion, and furthermore the core fusocylaion of megalin was essential for its binding and endocytosis of albumin in HK-2 cells. Incubation with BSA led to a significant increase of MCP-1 and RANTES in HK-2 cells. Reducing expression of core glycosylation of megalin markedly decreased expression of MCP-1 and RANTES.

Conclusions: Core fusocylaion of megalin was required for albumin binding and endocytosis. Reducing expression of core glycosylation of megalin markedly decreased expression of inflammation factors in HK-2 cells induced by albumin overload.

Funding: Government Support - Non-U.S.

FR-PO1866

Oncostatin M Is a Novel Inhibitor of TGF-β1-Induced CTGF Expression in Human Proximal Tubular Cells Marko Pekala, Hua SARKOZI, Christine M. HAUSER, Susie-Jane NOPPERT, Andreas KRONBICHLER, Viktoria Maria HALLER, Gert J. MAYER, Herbert SCHRAMEK. Department of Internal Medicine IV, Nephrology and Hypertension, Innsbruck Medical University, Innsbruck, Austria.

Background: Matricellular proteins (MP) such as connective tissue growth factor (CTGF), thrombospondin-1 (TSP-1), tenasin-C (TNC), and SPARC (secreted protein, acidic and rich in cystein) have been implicated in the development of tubulointerstitial lesions in human and experimental diabetic nephropathy. This study investigated potential anti-fibrotic effects of the cytokine oncostatin M (OSM) in human proximal tubular cells (hPTC), particularly with regard to inhibition of pro-fibrotic events mediated by transforming growth factor-β1 (TGF-β1).

Methods: Cell culture, Western blot, real-time PCR. hPTC were infected in quiescent hPTH(+)/50% OSM diminished TGF-β1-induced expression of the transcriptional EMT mediator FoxC2. Real-time PCR analysis revealed time-dependent induction of CTGF, TSP-1, TNC, and SPARC in hPTC stimulated with TGF-β1 (10 ng/
Maternal Fetal Immunology; Department of Therapeutics, School of Medical Sciences, Jiaodong University, Yantai, China; 2Nephrology, First Hospital of Linyi University, Linyi, China.

Background: Recent studies have shown that inflammatory cytokines can initiate an fetal inflammatory response, which can lead to adverse pregnancy outcomes. Inflammation has been implicated in the development and progression of preeclampsia. The aim of the study was to examine the potential association between maternal serum inflammatory cytokines and preeclampsia.

Methods: We collected maternal serum samples from 143 healthy pregnant women and 71 pregnant women with preeclampsia at 20-24 weeks of gestation. The concentrations of serum TNF-α, IL-6, and IL-10 were measured using ELISA.

Results: The concentration of TNF-α was higher in pregnant women with preeclampsia compared to healthy pregnant women. The concentration of IL-6 was also higher in pregnant women with preeclampsia. However, the concentration of IL-10 was not significantly different between the two groups.

Conclusions: Our study suggests that maternal serum TNF-α and IL-6 levels may be associated with the development of preeclampsia. Further studies are needed to investigate the role of inflammatory cytokines in the pathogenesis of preeclampsia.
The Spectrin-Based Cytoskeleton Organizes Differential Functional Domains along the Mouse Nephrin

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Background: The spectrin-based cytoskeleton lines the intracellular side of membranes of many cell types, forming a molecular scaffold that links the actin cytoskeleton to many membrane-spanning proteins either directly or through its partner, ankyrin. Seven spectrin genes and three ankyrin genes (spectrin α1/α2, β1/β4 and ankyrin I (R), II (B) and III (G) are known to be vertebrates.

Methods: Their expression profiles were investigated using RT-PCR/q-PCR/Western analysis/immuno-fluorescence and electron microscopy in the mouse kidney.

Results: mRNA for seven of the ten-spectrin/ankyrin genes is found (all except for α1, β1 and IV spectrin). α1/βII are the most abundant spectrin transcripts and Ank3 (G) is the most abundant ankyrin. βII spectrin is predominantly expressed together with αII spectrin in the proximal tubules at the basolateral membrane and in coated vesicles near the lateral membrane, while IIII spectrin is expressed in distal segments of the kidney. AnkyrinR is predominantly found in the thick loop of Henle and the distal convoluted tubule, while AnkyrinR reactivity is observed in most renal tubules. In the glomeruli, α1/βII/III spectrins and ankyrinR are found in podocytes and capillary endothelial cells.

Conclusions: Together, the full repertoire of spectrin and ankyrin genes provide a molecular scaffold for apical and basolateral surfaces and for vesicular structures in both the proximal and distal renal tubules. We propose that the distinct distribution of spectrin and ankyrin proteins to intracellular, lateral or apical membrane domains suggest their involvement in the cellular location and properties of receptors, ion channels and transporters in kidney cells.

Funding: NIDDK Support

FR-PO1875

Role of Heme Oxygenase-1 in Mediating Epithelial-to-Mesenchymal Transition of Peritubular Mesothelium

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Background: Epithelial-to-mesenchymal transition (EMT) or mesothelial-to-mesenchymal transition of peritubular mesothelial cells has been regarded as an early mechanism of peritoneal fibrosis. The effects of HO-1 expression on Epithelial-to-mesenchymal transition, which plays a critical role in the development of peritoneal membrane fibrosis, are unknown and its roles in peritoneal fibrosis has not been studied yet.

Methods: We treated the human peritoneal mesothelial cells (HPMCs) with high glucose solution, HO-1 inducer (hemin, 10 μmol/l) and HO-1 antagonist (StPPX, 10 μmol/l). To further investigate the pure effect of HO-1 on EMT of mesothelium, Gene transfer of recombinant Adenovirus-harboring human HO-1 (Adv-HO-1 Gene) to HPMCs was done.

Results: Exposure of HPMCs to HG solution (30, 60, and 120 mM D-glucose) for 2 to 7 days increased the expression of mesenchymal markers such as α-smooth muscle actin(α-SMA) and vimentin, associated with an increase in the expression of epithelial markers, E-cadherin. HO-1 protein expression was decreased in the same situation. Treatment of HPMCs with HO-1 inducer, hemin (2.5, 5, 10, and 20 mM) showed a dosage-dependent amelioration of HG induced changes in markers of EMT with increase of expression of HO-1. Treatment of HPMCs with HO-1 antagonist(StPPX, 10 μmol/l) simultaneously with HG or after the treatment of HO-1 inducer reversed the effect of HO-1 inducer. Adenovirus-harboring human HO-1 (Adv-HO-1 Gene) transfer resulted in a significant increase in HO-1 protein expression and ameliorated HG-induced changes in expression of E-cadherin, α-SMA, vimentin. Consistent with the protein data, HO-1 treatment resulted in an amelioration of HG-induced changes in mRNA of those markers.

Conclusions: Taken together, these results suggest that HO-1 has a critical role in the modulation of peritoneal fibrosis, and, more important, the suppression of EMT. Modulation of EMT, a major contributor to peritoneal fibrosis, through the HO-1 pathway may provide a means for novel therapeutic interventions aimed at progressive peritoneal diseases.

Funding: Private Foundation Support

FR-PO1876

Mouse Renal Fibrosis after Unilateral Ureteral Obstruction Is Reduced by Klotho Protein Overexpression through Wnt Signaling Inhibition

Minoru Itoh,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andrea Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Klanke, 2 Andreas Hartner,1 Nada Cordasic, 2 Carlos Menendez-Castro, 1 Bernd Kl...
integins, we quantified integrin mRNA and protein abundance. Significant mRNA increases were observed for integrin α1 and α3 genes in CsA-treated cells. In contrast, glomerular immunochemical signals for both integrin α1 and integrin α3 were significantly upregulated in Alport podocytes whereas integrin α2 and integrin β1 were unchanged.

Conclusions: We conclude that the Alport GBM alters the normal expression of α3 and β1 integrins, which in turn may lead to cytoskeleton reorganization and other signaling events important for pathogenesis of the disease.

Funding: NIDDK Support

FR-PO1876

Prevention of Cyclosporin A-Induced Fibronectin Synthesis by Simvastatin in Rat Mesangial Cells

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Background: Cyclosporin A (CsA) is an effective immunosuppressant for prevention of rejection after kidney transplantation. CsA has nephrotoxicity leading to chronic allograft nephropathy and renal fibrosis. Simvastatin improves hypercholesterolemia after renal transplantation and preserves structures and functions of glomeruli. But, it is not well known how simvastatin acts on CsA induced fibronectin synthesis. We studied to investigate the effect of simvastatin(sina) on Smad pathway, which is the key pathway of CsA induced fibronectin synthesis in rat mesangial cells (RMCs).

Methods: Transforming growth factor-β1 (TGF-β1) was induced by CsA cultured RMCs. Optimal concentration and time of CsA were measured according to its concentration (0.1~10 μM) and TGF-β1 expression time (1~72 h) measured by ELISA. All RMCs were divided into six experimental groups: control, CsA (1 μM) alone, simva (1 μM) alone, simva (0.01 μM) pretreatment plus CsA 1 μM, and simva (10 μM) pretreatment plus CsA 1 μM. The level of cytokines such as phosphorylated Smad3 (phospho-Smad3), Smad7 and fibronectin proteins were measured by semiquantitative immunoblotting.

Results: TGF-β1 expression was maximally increased at 1 μM dose of CsA, 24 h after CsA treatment. After treatment with CsA alone, the level of TGF-β1 expression significantly increased (3.995 ± 1.272-fold, p=0.0035). Phospho-Smad3 expression and fibronectin production significantly increased after CsA treatment (2.900 ± 0.535-fold, p=0.006, 1.812 ± 0.223-fold, p=0.0085). When all the groups treated with CsA and simva were compared with those treated with CsA alone, phospho-Smad3 expression (p = 0.0010, 0.0001, 0.0003) and fibronectin production (p = 0.0064, 0.0005, 0.0002) significantly decreased. But, in simva 10 μM pretreatment CsA 1 μM group, the level of Smad7 expression was significantly increased compared with control (p=0.0316).

Conclusions: This study demonstrates that simvastatin may ameliorate CsA-induced fibronectin synthesis by modulating the TGF-β1/Smad pathway in RMCs. These results suggest that simvastatin may inhibit the development of nephrosclerosis and subsequent chronic CsA nephropathy.

FR-PO1877

Parathyroid Hormone Induces Endothelial-to-Mesenchymal Transition in Human Aortic Endothelial Cells

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Background: Cardiovascular disease (CVD) is the leading cause of death among patients with end-stage renal disease (ESRD). Recent studies have suggested that highly secreted parathyroid hormone (PTH) might be involved in the development of uremic vascular impairment. However, the underlie mechanism has not been well understood. Our previous study have showed that endothelial-mesenchymal transition (EndMT) involves in the cardiac fibrosis. In this study, we addressed the question of whether PTH as an inducer of EndMT, and subsequently contributes to the vascular sclerosis in ESRD.

Methods: Primary human aortic endothelial cells (HAECs) between passage 3 to 5 were stimulated with 10^-7 to 10^-3 mol/L PTH. Pathological changes were examined by confocal microscopy for co-expression of endothelium marker (CD31) and fibroblast markers (α-SMA and -SMA). The mRNA and protein expressions of CD31, FSP1 and α-SMA were detected by real-time PCR and western blot. Cellular ultrastructure was observed with electron microscopy.

Results: CD31 has been found to be expressed in HAECs and its mRNA and protein levels were significantly increased in PTH-dependent manners compared with control (P<0.05). Double staining indicated that a co-expression of CD31 and FSP1 when HAECs were exposed to PTH, and some cells acquired spindle-shaped morphologies accompanying with a loss of CD31 staining. Ultrastructural investigation showed HAECs incubated with PTH (10^-3 mol/L) have an increasing roughed endoplasmic reticulum.

Conclusions: We firstly demonstrated that PTH could stimulate HAECs developing phenotypic changes as EndMT, which may provide a novel insight that secondary hyperparathyroidism being involved in the vascular sclerosis in CKD.

Funding: Government Support - Non-U.S.
FR-PO1880

Soluble fms-Like Tyrosine Kinase 1 (sFLT) Correlates with Proteinuria in ANCA-Associated Vasculitis and Declines during Immunosuppressive Treatment

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Background: Fms-like tyrosine kinase-1 (FLT1) is one of major receptors for the vascular endothelial growth factor (VEGF). Its soluble form (sFLT1) may act as decoy receptor for VEGF in the circulation thereby reducing VEGF bioavailability. Hence, increased levels of sFLT1 may cause inactivation of VEGF and therefore leads to endothelial dysfunction.

Elevated serum levels of sFLT1 have been found in sepsis and preeclampsia and correlated with disease severity. In ANCA-associated vasculitis higher VEGF levels have been reported in patients with major disease activity compared to those with minor activity. The present study was therefore intended to determine the role of sFLT1 serum level in ANCA-associated vasculitis.

Methods: Plasma sFLT1 levels were measured with a commercially available ELISA in patients with ANCA-associated vasculitis at initial diagnosis and after 1, 3, 6 and 12 months (n=38). Disease activity was assessed in accordance with the Birmingham Vasculitis Activity Score (BVAS). Proteinuria, BVAS, C-reactive protein, creatinine and ANCA titres were recorded at baseline and during follow-up.

Results: In patients with active ANCA-associated vasculitis median levels of sFLT1 are elevated (108 (96.5-165.5) pg/mL, p < 0.05) compared to healthy controls (86 (71.88-115.5) pg/mL, p < 0.05) and compared to those without activity median level of sFLT1 were increased (p<0.05) and declined during follow-up.

Conclusions: sFLT1 serum level correlate with proteinuria in severe ANCA-associated vasculitis. sFLT1 is elevated before treatment and declines during immunosuppressive therapy. We therefore conclude that sFLT1 may act as a robust marker for disease activity.

FR-PO1881

Long-Term Observation of Clinicopathological Characteristics and Outcome of Japanese Patients with Pauci-Immune Crescentic Glomerulonephritis

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Background: Clinicopathological characteristics and outcome of Japanese patients with pauci-immune crescentic glomerulonephritis (CrecGN) are presumed to vary among periods. We examined characteristics and outcome of patients with CrecGN according to the periods.

Methods: From 1968 to 2010, a total of 100 patients who were diagnosed as CrecGN by renal biopsy in Kanazawa University Hospital and Kanazawa collaborative group was examined in this study. All cases were divided into group I (1968-1988, 18 cases, mean age 52.9±3.7, female 6, male 12), group II (1989-2001, 37 cases, mean age 66.6±4.2, female 21, male 16) and group III (2002-2010, 45 cases, mean age 67.5±1.5, female 24, male 21).

Results: Mean follow-up period was 1367±130 days. Neither blood pressure, the degree of hematuria, renal function nor titer of ANCA had statistical difference among three groups. While signs of active vasculitis and one had global and segmental glomerulosclerosis.

Conclusions: In conclusion, the patients with CrecGN were diagnosed at early phase of crescentic formation and outcome was improved in recent years.

FR-PO1882

ANCA Negative Pauci-Immune Glomerulonephritis in a U.S. Cohort

John Havill, Michael Kuperman, Abigail Thompson, Duvuru Geetha, Stephen M. Sozzo, Johns Hopkins Medical Institutions, Baltimore, MD.

Background: ANCA antibodies are thought to play an important role in the pathogenesis of ANCA-associated vasculitis. The lack of ANCA antibodies may indicate a variation in clinical presentation and outcomes of this disease.

Methods: We identified 78 patients from the Johns Hopkins Renal Pathology database between 1995 and 2008 with the diagnosis of pauci-immune glomerulonephritis (GN). Compared to ANCA negative and positive patients, we examined demographics, histological features at presentation, and subsequent treatment and correlated this with renal function at presentation and follow-up. Renal histology was reviewed by a pathologist blinded to clinical data.

Conclusions: Ours is the first USA based study to compare patients with ANCA negative and positive serology, and we showed these two groups had similar demographics, presentation, and outcomes in our 13 year experience. Percentage of tubulo-interstitial scarring was a strong negative, graded predictor of renal outcomes at 1 year for both groups.

FR-PO1883

Persistent or New Onset Microscopic Hematuria in Vasculitis Patients in Remission: Findings on Renal Biopsy

Duvuru Geetha, John Havill, Michael Kuperman. Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Hematia is considered a sign of active disease in patients with small vessel vasculitis requiring aggressive immunosuppressive therapy. In patients who are in apparent clinical remission, the presence of persistent or new onset microscopic hematuria after completion of immunosuppressive therapy and may after discontinuation of induction and maintenance therapy may be due to active renal vasculitis, chronic and healed vasculitis or other glomerular pathology.

Methods: We identified 78 patients from the Johns Hopkins Renal Pathology database between 1995 and 2008 with the diagnosis of pauci-immune glomerulonephritis (GN). Among these 78 patients, we identified patients who were in apparent clinical remission and underwent a renal biopsy for evaluation of persistent or new onset microscopic hematuria. Renal histology was reviewed by a pathologist blinded to clinical data.

Results: 8 patients with small vessel vasculitis, 7 ANCA positive and 1 ANCA negative underwent a renal biopsy at variable time periods after remission of vasculitis (6 months to 14 years) for persistent microscopic hematuria (n=5) or new onset microscopic hematuria (n=3). All patients were in apparent clinical remission at the time of renal biopsy. Of the three patients presenting with new onset vasculitis, two patients had crescentic IgA nephropathy and one patient had healed crescentic pauci-immune glomerulonephritis with fibrous crescents. Of the five patients with persistent hematuria, two patients had arteriolosclerosis with no evidence of active vasculitis, two had focal segmental glomerulosclerosis and no signs of active vasculitis and one had global and segmental glomerulosclerosis.

Conclusions: Microscopic hematuria in patients with renal vasculitis otherwise in remission could represent chronic glomerular injury from prior episode of vasculitis or may represent new glomerular pathology. We conclude that patients with small vessel vasculitis who are in apparent clinical remission presenting with persistent or new onset microscopic hematuria should undergo renal biopsy prior to escalation of immunosuppressive therapy.

FR-PO1884

Long-Term Outcome of Severe Alveolar Haemorrhage in Small Vessel Vasculitis: A Retrospective Cohort Study

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Background: Alveolar haemorrhage (AH) is an important manifestation of small vessel vasculitis (SVV) and the most common vasculitic cause of early death. We describe the characteristics and long-term outcome of severe AH due to SVV from two European centres.

Methods: A retrospective analysis of hospital notes on 867 patients with SVV presenting between 1990 and 2011. AH was defined as the presence of bilateral alveolar infiltrates on radiological imaging without an alternative explanation plus at least one of
the following: haemoptysis, increased CO diffusion capacity, bronchoscopic evidence of haemorrhage, or an unexplained drop in haemoglobin >2g/dl. AH was considered severe if either hypoxia or the need for blood transfusions were recorded.

Results: 59 patients (MF 36/23; median age 58 [18-81] years) were identified. Most (89.8%) were diagnosed with ANCA-associated vasculitis, six with anti-GBM disease. PR-3-ANCA was positive in 38 (64.4%), MPO-ANCA in 15 (25.4%, p<0.05 for MPO vs PR-3-ANCA), and anti-GBM in 9 patients (3 were also ANCA-positive). AH was the first disease manifestation in 54/59 (91.5%). Invasive assisted ventilation was required in 24 patients (40.7%). Renal involvement was present in 57 (96.6%), 33 (55.9%) required dialysis. Five-six (78.0%) were treated with plasma exchange (PE). At 6 months, 50/59 (84.7%) were alive, six died of active refractory vasculitis and 3 of infection. The mean follow-up was 50 months when 37 (62.7%) were alive. Mortality was higher in those requiring dialysis at entry (48.5 vs. 23.1%, p<0.05). No significant difference in mortality was found between those on and no PE (39.1 vs. 30.8%, p=0.3).

Conclusions: Severe AH was more commonly associated with PR-3 than MPO-ANCA or anti-GBM and strongly correlated with renal vasculitis. Current treatment of severe AH with combined immunosuppression (with or without PE) helps to overcome the acute period in most patients but long-term mortality remains high. Concurrent renal failure was associated with worse outcome. Supported by ERA-EDTA Fellowship.

Funding: Private Foundation Support.

FR-PO1885
Outcomes of Renal Vasculitis in Preston, UK: 2005-2010
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Department of Renal Medicine, Royal Preston Hospital, Lancashire Teaching Hospitals, Preston, United Kingdom.

Background: We report outcomes of renal vasculitis in a group of patients treated with standard induction therapy containing pulse cyclophosphamide plus prednisolone followed by Azathioprine and prednisolone for maintenance treatment.

Methods: All new patients diagnosed with renal vasculitis at our centre from 2005 until 2010 were evaluated from presentation until last visit or death.

Results: 79 patients received standard treatment. Median age was 63 ± 14 years. 36 (45%) patients were PR-3-ANCA positive, 31 (40%) MPO-ANCA positive and 12 (15%) ANCA negative. Presenting CreatinineCr was 448±592 mmol/l and mean follow up period was 26 ± 13 months. All patients started on pulse intravenous (i.v.) cyclophosphamide dose as per the Cyclops protocol. 30% patients received oral pulses later during induction while the rest continued with iv pulse. 28 (35%) patients needed renal replacement therapy (RRT) at diagnosis. Of these 28 patients, 10 died, 12 recovered renal function and 6 patients were RRT dependent. Overall 8 (10%) patients reached end stage renal disease (ESRD) and 15 (19%) died. At 2 years, 85% of survivors were dialysis independent. In the ANCA positive group, 1 year and 5 year survival rates were 88% and 82% respectively.

Conclusions: In renal vasculitis with less cumulative dose of cyclophosphamide we report survival rates comparable to other treatment regimens and a low relapse rate.

FR-PO1886
Increased Incidence of Venous Thromboembolism in ANCA Associated Vasculitis
Sehrshar Noor, Issama Oozereally, Olumide Ogundare, Ajay Prabhakar Dhagyue. Renal Medicine, Royal Preston Hospital, Preston, United Kingdom.

Background: ANCA associated systemic vasculitis (AASV) is a heterogeneous chronic inflammatory relapsing autoimmune disease. Dutch and American studies have suggested that there is an increased incidence (1.8 to 7 per 100 person-years) of venous thromboembolism (VTE) in AASV. Recently it has been shown that there is an increased prevalence of anti-plasminogen antibodies and also anti tissue plasminogen activator antibodies in AASV patients and these antibodies were associated with increased severity of renal involvement. In this retrospective study we analysed incidence of VTE in 165 British patients with AASV and compared it with incidence of VTE in the general population.

Methods: Retrospective analysis of case records of 165 patients with AASV was performed to identify patients with evidence of VTE (deep vein thrombosis and/ or pulmonary embolism).

Results: One hundred sixty-five patients were followed for 3.35 person-years. Sixty three patients experienced 19 events of VTE; three of these events predicted the diagnosis of AASV by at least 12 months and were consequently excluded. The overall incidence of VTE in AASV was 2.9 events per 100 person years. Nine patients had active vasculitis and one patient was in partial remission at the time of diagnosis of VTE. VTE occurred between 0 months to 95 months from the diagnosis or relapse of AASV but 10 out of 16 VTE occurred within 6 months of active disease. Age at the time of diagnosis of vasculitis was slightly higher (65.8 years) in patients with VTE compared with non VTE patients (59.9 years). Nine patients with VTE were PR-3 positive.

Conclusions: This study has confirmed previous published findings of increased incidence of VTE in AASV in British population. In general population incidence of VTE is 0.05 per 100 person years but the risk increases with age to 0.2 per 100 person years for age group of 70-79 years. Increased incidence is likely to be multifactorial, related to immunologic, endothelial dysfunction, pro-coagulant state, presence of anti plasminogen antibodies in AASV and chemotherapy. This study has strengthened the association between AAV and VTE and may help the clinicians treating AAV.

FR-PO1887
Serum Polyclonal Free Light Chain Levels in Patients with Vasculitis
Lakhvir Assi,1 Andrew McClean,2 Gemma Webb,1 Lorraine Harper,3 Colin A. Hutchinson.2 The Binding Site Group Ltd, United Kingdom; 2 Renal Institute of Birmingham, United Kingdom.

Background: Serum concentrations of polyclonal free light chains (FLCs) have been shown to be elevated in autoimmune conditions (Sjogrens syndrome and systemic lupus erythematosus). In these diseases, FLCs correlated with disease activity and clinical outcomes. The purpose of this study was to evaluate FLCs in patients with vasculitis and to determine if their levels correlated with established markers of disease activity.

Methods: 42 patients were assessed; their average age was 55 years and median GFR was 36 ml/min/1.73m2. 38% were female and 90% were white. The following laboratory assessments were made at presentation: FLCs (FreeliteTM assay, normal range: k 1.7-3.19mg/L, λ: 0.5-7.1mg/L), high sensitivity CRP (hsCRP), systatin C (cys C) and immunoglobulins (Igs). One month follow up (FU) samples were also analysed (N=38). Patients predominately received induction therapy consisting of steroids and cyclophosphamide.

Results: Total FLCs (TFLCs) were elevated in the cohort (median: 39.2mg/L, range 3.75-343.5mg/L).

<table>
<thead>
<tr>
<th>Disease</th>
<th>N=42</th>
<th>TFLCs (mg/L) median</th>
<th>TFLC/cys C median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wegener's granulomatosis</td>
<td>23</td>
<td>36.9</td>
<td>18.8</td>
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<tr>
<td>Churg strauss</td>
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<td>39.3</td>
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<tr>
<td>ANCA associated vasculitis</td>
<td>2</td>
<td>72.6</td>
<td>25.4</td>
</tr>
<tr>
<td>ANCA -ve vasculitis</td>
<td>2</td>
<td>33.7</td>
<td>20.1</td>
</tr>
<tr>
<td>Mucocutaneous polyangiitis</td>
<td>10</td>
<td>71.9</td>
<td>32.8</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
<td>51.9</td>
<td>41.9</td>
</tr>
</tbody>
</table>

To determine rates of FLC production, TFLCs were corrected for renal function using cys C (TFLC/cys C median: 19.7mg/L, range: 4.6-94.6mg/L). There was a weak association between TFLCs and total Igs (GAM): Spearman’s ρ=0.32, p<0.001. As expected when TFLCs/cys C were correlated with lgs (r=0.72, p<0.001), TFLCs also correlated with CRP (r=0.47, p<0.002) although there was no association with autoantibodies (MPO/ PR3) (r=0.09, p=0.59). TFLCs increased with decreasing GFR (r=-0.03) and increasing age (p<0.01). TFLCs were higher at baseline: 39.21mg/L vs 26.3mg/ml (p=0.007). When analysing patient outcomes, there was no difference between TFLCs in patients admitted to hospital over 12 months (p=0.39), nor was there a difference in patients who had suffered an infection (p=0.88).

Conclusions: Further FU samples will be analysed to further elucidate the role of TFLCs in vasculitis.

Funding: Pharmaceutical Company Support.

FR-PO1888
Clinical Outcomes of Japanese Myeloperoxidase (MPO) Anti-Neutrophil Cytoplasmic Antibody (ANCA) Related Nephritis: Significance of Initial Renal Death for Survival
Kimio Watanabe, Yoshihiro Tani, Kenichi Tanaka, Yoshimitsu Hayashi, Koichi Asahi, Masaki Nakayama, Tsuyoshi Watanabe. Nephrology and Hypertension, Fukushima Medical University School of Medicine, Fukushima, Japan.

Background: MPO-ANCA related vasculitis constitutes 60% of the rapidly progressive glomerulonephritis that occurs among Japanese. The reported 1-year survival rate of such patients is >80%, but the long-term prognosis remains unknown. Therefore, we investigated the prognosis and the clinical factors affecting the survival of patients with MPO-ANCA related nephritis.

Methods: We retrospectively investigated 44 patients (female, n = 26; mean age, 70.2 ± 11.4 years) who were diagnosed with MPO-ANCA related nephritis between 2000 and 2010 at our hospital.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
Underline represents presenting author.

550A
Results: The serum creatinine value was 3.7 ± 2.6 mg/dl, the MPO-ANCA titer was 56 ± 17 EU and the Birmingham Vasculitis Activity Score (BVAS) was 17.1 ± 4.4. At the initial therapy, 38 (86.4%) patients achieved remission (non-renal death group: NRD), whereas the remaining 6 (13.6%) did not and required permanent dialysis therapy (renal death group: RD). After 8 weeks of initial prednisolone therapy, six patients relapsed and four developed kidney failure progression in the NRD group, whereas two patients relapsed in the RD group. Eleven (25.0%; NRD, n = 5; RD, n = 6) patients died during a mean observation period of 36.6 ± 33.7 months due to infection (n = 4), recurrent vasculitis (n = 4), cancer (n = 1) and other causes. The 1- and 2-year survival rates were 100.0% and 96.3%, respectively, in the NRD group and 33.3% and 0.0%, respectively, in the RD group. Multiple logistic analysis revealed that renal death at the initial phase was the only significant risk factor for all-cause death (OR, 29.0; 2.4 - 351.8), BVAS at the initial visit for the relapse (OR, 1.52; 1.09 - 2.14) and BV AS at week 4 for death (OR, 2.33; 1.02 - 5.37).

Conclusions: Renal death at the initial phase was a powerful risk factor for all-cause death in patients with MPO-ANCA related nephritis. Patients at high risk of recurrence and death could be stratified according to BVAS during initial therapy.

FR-PO1889

Outcomes of Two Different Cyclophosphamide Regimes for Treatment of ANCA Associated Vasculitis Issaam Oozeerally,1 Ajay Prabhakar Dharghaye,1 Edmond O’Riordan.2 1Renal Medicine, Royal Preston Hospital, Preston, United Kingdom; 2Renal Medicine, Salford Royal Hospital, Salford, United Kingdom.

Background: Cyclophosphamide (Cyp) treatment has transformed ANCA associated systemic vasculitis (AASV) from a potentially fatal condition to a chronic remitting, relapsing disease though it is associated with significant mortality and morbidity. Recently published meta-analysis of outcome of 535 AASV patients from four different clinical trials suggested that 48% of the mortality was due to infections in the first year and 19% mortality due to active vasculitis. RAVE and RITUXIVAS trials showed that Cyp therapy is as effective as Rituximab therapy in majority of the patients. Pulsed arm of CYCLOPS trial protocol recommends 10 pulses of cyp given over 6 months, initially at 2 weekly and subsequently 3weekly intervals. Lupus nephritis regimes consists of 6 pulses given at monthly intervals. In North-west of England, clinicians have used these two regimes for the treatment of AASV and in this retrospective study we analysed the outcomes of these two different regimes. 

Methods: Data was collected for 103 AASV patients treated with pulse cyp, regarding cumulative cyp dose, rate of major infections, rate of relapses and renal outcomes. All the patients had at least 2 years follow up.

Results: Seventy-nine patients were treated with CYCLOPS protocol and 24 patients were treated with monthly (lupus nephritis) protocol. Cumulative cyp dose was high in CYCLOPS regime (7.14 grams) compared to monthly regime (4.63 grams). Outcomes are presented in Table-1.

Conclusions: These results suggests that CYCLOPS regimes is associated with- 1) Higher cumulative dose 2) Significant improvement in renal function 3) Increased rate of infections though statistically not significant. Limitations: 1) Retrospective study 2) Small sample size for monthly regime patients which may explain the lower rate of relapse in this group in spite of low cumulative dose of Cyp.

FR-PO1890

Outcome of Anti GBM Disease: A Single Center Review Arvind Ponnumasu, Edmond O’Riordan. Renal, Salford Royal NHS Trust Foundation, United Kingdom.

Background: The incidence of anti-GBM disease is reported to be 0.5 per million per annum. It is associated with significant morbidity and mortality. 

Methods: We retrospectively reviewed the case notes of all the patients (n= 19) who were diagnosed with anti-G BM disease over a period of 10 years (1999 – 2009). Creatinine and proteinuria of the crescents at presentation were co-related to outcome (End stage Renal Disease and Death)

Results: The average age at diagnosis was 61 with bimodal peaks at decade 30-40 and those over 60 years of age. Male to female ratio was 10.9:18 out of the 19 had a serum creatinine above 500 umol/l (5.6 mg/dl). 7 (36.84%) of them were dialysis dependent at presentation. Only 2 had clinical and radiological evidence of pulmonary haemorrhage. 4 (22.2%) patients were ‘double positive’ for PANCA (MPO) and anti GBM. Renal biopsy was performed in 14 patients (73.7%) 7 of those had crescentic glomerulonephritis in 100% of glomeruli seen. At 6 months 15 patients (78.9%) were dialysis dependent, 4 patients were dialysis independent with a mean creatinine of 265umol/l (1.3 mg/dl)/µl[underline]117 [1.3]. At last follow up mortality was 52.63%. Causes for death include pulmonary haemorrhage, sepsis, and cardiac related. The mortality figures were better for patients who were double positive for anti GBM and PANCA compared to patients who had positive anti GBM and negative ANCA (25% vs 60%).

Conclusions: Our data confirms previous studies in which creatinine more than 500umol/l and presence of 100% crescent were associated high mortality and poor renal survival. Patients with double-positive (ANCA and anti GBM) appear to have better patient survival rate although the renal survival appear to be same in both the double the positive group and anti GBM group. Only 16% of our patients with creatinine >500 had renal survival at 6 months underlying the potential risk-benefit balance of aggressive therapy in this group.

FR-PO1891

Anti-Glomerular Basement Membrane Disease; a Review of the Experience in an Irish Centre over 21 Years Yvonne C. Ryan,1 Thomas J. McEnery,1 Mary T. Keogon,1 Peter J. Conlon,1 Anthony M. Dorman,1 Mark Donald Denton.1 1Nephrology, Beaumont Hospital, Dublin, Ireland; 1Histopathology, Beaumont Hospital, Dublin, Ireland.

Background: We present a series of 64 patients with anti-G BM disease managed at Beaumont Hospital in Dublin, Ireland.

Methods: The biopsy records from January 1st 1990 to April 1st 2011 were reviewed and patients with biopsy proven anti-GBM disease identified. Patients with anti-GBM who did not get a renal biopsy were identified using a database of immunology sera. We conducted a chart review to ascertain demographic data, presenting features, anti-G BM titre, treatment, renal replacement therapy and outcome. Statistical package used was Stata (version 10, College Station, Texas).

Results: 56% of patients were female. The mean age of males and females was 46 and 54 years respectively. 17% had concurrently positive ANCA at presentation. 22% had lung involvement and the mean anti-G BM titre was 265 iu/ml, ref <10iu/ml (range 0-1000) and 26% patients were anuric at presentation. 62/64 patients had a renal biopsy. Mean crescents on biopsy 80% (SD 27), mean percentage fibrosis was 52% (SD 30). Three patients (4.7%) died during the initial admission.

Patient survival was 78%, 67%, 60% and 46% at 1, 5, 10 and 15 years respectively. Age at presentation HR 1.09 (CI 1.04-1.14) and log serum creatinine at presentation HR 5.59 (CI 1.81-17.25) were significantly associated with survival. The renal survival was 26%, 21%, 16% and 16% at 1, 5, 10 and 15 years respectively. Four patients had recurrence within 1 year of presentation (2 lung involvement, 2 renal involvement). One patient had a renal recurrence 9 years after initial presentation. Thirty two percent of patients had at least one renal allograft during the study period. None had recurrent disease post-transplant. Two patients had anti-GBM disease on biopsy for work up of microscopic haematuria with normal creatinine and no lung disease. Neither progressed.

Conclusions: This is a large series of anti-GBM disease with long term follow up. This series emphasises the devastating consequences of anti-GBM disease on patient and renal survival. We also describe an indolent form of anti-GBM disease which is not well understood.

FR-PO1892

Laser Capture Microdissection (LCM) Followed by Tissue Proteomics Identifies Differences in Protein Expression in Biopsies from Class IV and V Lupus Nephritis (LN) Brad H. Rovin,1 Samir Parikh,2 Michael A. Freitas,3 Anjali A. Satoorsk,2 John P. Shapireo,2 Tibor Nadasy,2 Lee A. Hebert.1 Internal Medicine, Ohio State University; 1Pathology, Ohio State University; 2Molecular Virology, Immunology and Medical Genetics, Ohio State University.

Background: We postulated LCM of kidney biopsies followed by proteomics of the captured tissues can identify disease-specific protein expression that will inform diagnostic biomarker development and provide a better understanding of disease pathogenesis.
Random Spot Urine P/C Ratio Is Unreliable in Estimating 24-h Proteinuria Magnitude in Both SLE GN Patients and CKD Patients, However the Type of Inaccuracies Is Different Between These Conditions. Ganesh B. Shidham, Daniel J. Birmingham, Brad H. Rovin, Lee A. Hebert. Internal Medicine/ Nephrology, Ohio State University Medical Center, Columbus, OH.

Background: We have previously shown that random spot P/C ratio (spot P/C) is highly inaccurate in estimating 24-h proteinuria in individual SLE GN patients (pts), particularly in the sub-nephrotic range. Here we extend this work to CKD pts and compare the results to those of SLE GN pts.

Methods: For the SLE GN analysis, we added a third cohort to our two previously reported cohorts. The individual data were obtained from our own database and from a previously published work. For the CKD analysis we identified from the published literature three CKD studies of spot P/C and 24-h proteinuria in which the data were presented on linear plots. The individual data were extracted by computer graphic analysis. For all the data, only those with sub-nephrotic proteinuria (<3.5 g/d), the most common level of proteinuria encountered clinically, are included in this analysis.

Results: The SLE GN pts (N = 93) showed a mean (spot P/C)/(24-h proteinuria) ratio of 0.86 ± 0.48 (p < 0.001) compared to 1.0. The CKD pts (N = 162) showed a mean (spot P/C)/(24-h proteinuria) of 1.21 (p = 0.021) compared to 1.0. The difference in the ratios was significant (p < 0.0001). The figure below shows a calibration plot of the individual data.

Conclusion: Spot P/C is unreliable in estimating 24-h proteinuria in the sub-nephrotic range. In addition, in SLE GN it tends to underestimate and in CKD it tends to overestimate 24-h proteinuria. The mechanism and pathophysiological significance of this difference is unclear.

Funding: NIDDK Support

FR-PO1895

The Impact of Partial Remission on Renal and Patient's Survival in Severe Lupus Nephritis

Mamduh N. Albaqumi, Lutfi Alkorbi, Dania Alkhafaji. Research Institute of Nephrology, Ohio State University Medical Center, Columbus, OH.

Background: Few studies have addressed the value of partial remission on long term renal and patient’s survival. In this study, we describe the effect of partial remission (PR) in comparison to complete remission (CR), and treatment failure (TF) on long term renal outcome in a cohort of patients with severe lupus nephritis.

Methods: We retrospectively reviewed patients’ archived files and identified all patients with the diagnosis of lupus nephritis from 2003 until 2008. All renal biopsies were reviewed blindly and reclassified according to ISN/RPS 2003 classifications.

Results: A total of 63 patients with Lupus Nephritis class IV were identified. 31 patients (49%) achieved PR, while 13 (21%) had CR, and 19 (30%) had TF. Serum creatinine at presentation was higher in patients with TF (178.0 ± 126.0 umol/L) compared to PR (128.4 ± 97.0 umol/L), however, when GFR is estimated, there was no statistical difference between PR and TF groups at presentation (PR: 94.4 ± 84.5 ml/min, TF: 57.8 ± 39.3 ml/min, P = 0.117). Proteinuria was also similar between PR and TF groups (PR: 4.9 ± 3.9 mg/mgcrea, TF: 5.4 ± 4.9 mg/mgcrea, P = 0.126). Renal survival over the next four years was significantly higher in the CR and PR groups compared to TF (CR: 77%, PR: 55%, TF: 29%, P = 0.033).

Conclusion: Despite having a similar GFR and proteinuria at presentation to TF, PR group had a better renal survival in four years, highlighting the favorable impact of partial remission in severe lupus nephritis.

FR-PO1894

Efficacy and Safety of Double Filtration Plasmapheresis Therapy in Severe Lupus Nephritis

Hai-Tao Zhang, Wei-Xin Hu, Zheng-Zhao Liu, Ying-Hua Chen, Dehua Gong, Daxi Ji, Zhi-Hong Liu. Research Institute of Nephrology, Nanjing, China.

Background: To investigate the clinical efficacy and safety of double filtration plasmapheresis (DFPP) accompanied with corticosteroid in patients with severe lupus nephritis (LN).

Methods: 8 patients (5 females and 3 males, average age 23.9±10.3) with severe LN including class IV (n=5),DLN+IV and V+III were studied. Among them, 7 cases showed rapidly progressive glomerulonephritis (RPGN) with elevated serum creatinine(SCr) (3.9±2.8mg/dl), and 3 of them needed renal replacement therapy. DFPP was performed with two-fold plasma volume on each session using membrane type plasma component separator (EC20W and EC50W, Asahi Kasei Kuraray, Japan).

Results: 1)Clinical efficacy: DFPP treated 2.8 times(±2.3) for each patient. SLE-DAI was reduced significantly from 18.9±3.3 to 10.5±2.2(P < 0.01) and SCR decreased from 3.9±2.8 to 2.3±2.2mg/dl (P<0.05) after DFPP. 2)Patients got off dialysis by 1 and 2 weeks, respectively. 3)Laboratory parameters: Serum IgG decreased from 10.3±5.4 to 6.8±3.1g/L(P<0.01).

The titers of anti-dsDNA and anti C1q antibody were significantly declined after DFPP, 2.3±2.2mg/dl (P=0.13) after DEPP. 2 patients got off dialysis by 1 and 2 weeks, respectively. Serum creatinine at presentation was higher in patients with TF (178.0 ± 126.0 vs 118.4 ± 97.0 mg/dl, P = 0.015). Proteinuria was also similar between PR and TF groups at presentation (PR: 94.4 ± 84.5 mg/mgcrea, TF: 57.8 ± 39.3 mg/mgcrea, P = 0.117). Proteinuria was also similar between PR and TF groups at presentation (PR: 94.4 ± 84.5 mg/mgcrea, TF: 57.8 ± 39.3 mg/mgcrea, P = 0.117). Proteinuria was also similar between PR and TF groups at presentation (PR: 94.4 ± 84.5 mg/mgcrea, TF: 57.8 ± 39.3 mg/mgcrea, P = 0.117). Proteinuria was also similar between PR and TF groups at presentation (PR: 94.4 ± 84.5 mg/mgcrea, TF: 57.8 ± 39.3 mg/mgcrea, P = 0.117).

Conclusion: DFPP can rapidly and effectively clear autoantibodies in severe LN, thus improving renal function and protecting the endothelial cells. DFPP accompanied with corticosteroid is an effective therapeutic method for LN.

FR-PO1896

Urine Neutrophil Gelatinase Associated Lipocalin Is Increased during Active Lupus Nephritis

Li Ping Teng, Sue Kim Lim, Tee Chau Keng, Yap Boon Chong, Wan Ahmad Hafiz Wan Md Adnan, Kok Peng Ng. Department of Medicine, Division of Nephrology, University of Malaya Medical Center, Kuala Lumpur, Wilayah Persekutuan, Malaysia.

Background: Urine Neutrophil Gelatinase Associated Lipocalin (NGAL) is a novel marker of acute kidney injury. While its expression is upregulated chiefly in response to ischemic reperfusion injury, it can also be produced by glomerular mesangial cells and inflammatory cells. Patients with systemic lupus erythematosus (SLE) are often complicated by lupus nephritis, during which the glomeruli can be infiltrated by inflammatory cells. We postulate that urine NGAL levels will rise in patients with active lupus nephritis (LN).

Methods: A cross sectional study was performed over a period of 8 months. Urine NGAL levels were compared between patients with biopsy proven active ILN and SLE patients without LN. Normal healthy volunteers served as a negative control group. Patients were excluded if they had a glomerular filtration rate of <30ml/min/1.73m2.

Results: A total of 72 subjects were recruited. 12 with biopsy proven active lupus nephritis (LN), 27 with SLE but without LN and 33 healthy controls. 67% of the LN group had significantly higher levels of urine NGAL (median 21.95 ng/ml IQR 6.35–52.2) compared to SLE patients without LN (median 3.4 ng/ml IQR 1.75–10.85) (p < 0.001) and the healthy controls (median 8.5 ng/ml IQR 3.5–20.4) (p < 0.005). Correcting for the serum creatinine also produced similar results. With the exception of ESR (r = 0.268 p < 0.05), no correlation difference is unclear.

Funding: NIDDK Support
Results: The concentration of plasma complement in patients with C3GN without MPGN was shown in Table 1.

| C3(g/L) | C4(g/L) | Factor B(µg/ml) | Factor D(µg/ml) | hC3P(µg/ml) | MCP1(ng/ml) | NGAL(ng/ml) | Table 1
|---------|---------|----------------|----------------|-------------|-------------|-------------|---------
| 0.84±0.41 | 0.80±0.23 | 6.78±0.53 | 110.9±54.2 | 0.53±0.23 | 3.6±0.3 | 103.3±57.3 | 6.59±6.98 | 2.62±0.02

The analysis between concentration of plasma complement and proteinuria, plasma creatinine was shown in Table 1. Glomerular C4d was positive in 8 biopsies in mesangial areas and glomerular capillary wall, and negative in 2 biopsies. MAC was detected in 8 biopsies in a mesangial pattern and negative in 2 biopsies (one patient was negative for C4d, and the other one was positive for C4d).

Conclusions: Alternative pathway in circulation might play an important role in the pathogenesis of C3GN without MPGN. There might also be complement activation via both the lectin and alternative pathways in glomeruli, which needs further investigations.

FR-PO18999

The Terminal Complement Complex (TCC) – A Potential Biomarker for Disease Activity in Patients with Membranoproliferative Glomerulonephritis

Magdalena Riedl, 1 Alejandro Rosales, 1 Verena Jeller Jeller, 1 Johannes Hofer, 1 Umdila Podracka, 2 Christoph Rudin, 1 Henry Fehenbach, 4 Heiko Billing, 4 Reinhard Würzner, 7 Therese C. Jungraithmayr, 1 Medical University, Innsbruck, Austria; 1 University Children’s Hospital, Kosice, Slovakia (Slovak Republic); 3 University Children’s Hospital, Basel, Switzerland; 5 Children’s Hospital, Memminger, Germany; 6 University Children’s Hospital, Heidelberg, Germany.

Background: The role of complement (C) in membranoproliferative glomerulonephritis (MPGN) has been investigated in more detail lately. Mutations in C regulators genes, antibodies against C proteins and a common persistent hypocomplementemia (low C3) in many patients support the role of C in the pathogenesis. No biomarker for monitoring disease activity is available yet.

Methods: Here we present data on the concentration of the soluble terminal complement complex (TCC, sc5b-9) in 11 pediatric patients with MPGN. Seven patients were classified as MPGN type I and 4 as DDD (MPGN II) by their renal biopsies. 6/11 were tested positive for C3Nef. In 3 patients multiple tests were performed. 98 healthy adult blood donors were used as controls. The measurement of the TCC, generated as a potentially lytic end product of C activation, was performed by a sandwich ELISA technique. Mann-Whitney-U-test was used for statistical evaluation.

Results: Patients with MPGN showed a statistically significant higher TCC concentration in plasma (3.9±2.4 µg/ml vs 1.5± 1.2 µg/ml, p<0.01) than controls. Patients with an active disease (hematuria, gross proteinuria, arterial hypertension) had a significant increased TCC value (4.8±3.3 µg/ml) compared to patients in remission (1.9±0.6 µg/ml, p<0.03). No significant difference of C levels during active disease (37.2±16.1mg/dl) and patients in remission (30.0±14.7mg/dl, p=0.15) was discovered.

Conclusions: This work emphasises the role of C activation in DDD as well as in MPGN I. Thus, complement inhibition therapies targeting the terminal C cascade, such as Eculizumab, should also be considered in patients with MPGN and elevated TCC. The TCC concentration may represent a good biomarker for monitoring disease activity in patients with MPGN.

Funding: Government Support - Non-U.S.

FR-PO1900

Membranoproliferative Glomerulonephritis and Mixed Cryoglobulinemia after Hepatitis C Virus Infection Secondary to Glomerular NS3 Viral Antigen Deposits

Stanislas Batisse, Bertrand Dussol, Nephrology, Hôpital de la Conception, Marseille, France.

Background: Hepatitis C virus infection is the main etiology of type I membranoproliferative glomerulonephritis. We report on three cases of type I membranoproliferative glomerulonephritis associated with type II cryoglobulin in patients with hepatitis C virus (HCV) antibodies but with a negative viral load.

Methods: We searched for occult HCV infection in B cells or kidney glomeruli using PCR assays and immunohistochemistry.

Results: Hepatitis C virus infection-associated lymphoma was excluded by computed tomospectrometry, bone marrow phenotyping and histology but indirect features of B cell proliferation were present in the three patients. Using ultrasensitive PCR assays, we did not evidence occult hepatitis C infection in peripheral blood mononuclear and bone marrow cells, and in the cryoprecipitates but found HCV-NS3 antigen in the kidney tissue tested using immunohistochemistry 6years after viral PCR was negative. Liver tissue specimens were not available. Remission had occurred spontaneously in one patient and after rituximab treatment in one patient. The third patient was lost for follow up.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Persistence of viral antigen HCV-NS3 at least in kidney, even many years after HCV PCR is negative, may explain immunological stimulation and persistence of cryoglobulinemia with a persistent non-responding state.

FR-PO1901
Eculizumab for Dense Deposit Disease (DDD) and C3 Glomerulonephritis (C3GN) Andrew S. Bomback,1 Jai Radakrishnan,1 Pietro A. Canetta,1 Yuhou Zhang,1 Carla Nishimura,2 Nicole Meyer,3 Kathy Fees,3 Michael Jones,3 Richard J. Smith,3 Gerald B. Appel.1 1 Columbia University, University of Iowa.

Background: The principle immune defect in DDD and C3GN is excessive alternative complement activation. Eculizumab, a monoclonal Ab to C5 that prevents formation of the membrane attack complex, may provide targeted therapy for DDD and C3GN.

Methods: We present a 6-month interim analysis of an open label, proof-of-concept efficacy trial of eculizumab in DDD (NCT02212181). Six pts treated with eculizumab 1200 mg IV every other week for 1 yr. All had proteinuria ≥ 2 g/day and/or AKI (serum creatinine ≥150% baseline) at enrollment. All underwent biopsy at enrollment and will have repeat biopsies at 1 yr. Additional testing included: mutation screening of CFH, CFI, MCP, CFRH5, and TFH; autoAb screening for C3 nephritic factors and factor H autoAbs; and functional analysis of complement activity.

Results: We enrolled 3 pts with DDD (2 native, 1 recurrent) and 3 pts with C3GN (1 native, 2 recurrent). One pt (DDD) carried a CFH mutation; 4 pts were C3NEf (≥2) (DDD, 2 C3GN), and 2 pts (1 DDD, 1 C3GN) had elevated sMAC. Total complement levels declined to 0-1 CAE units by week 4 in 5/6 pts (in 1 pt, level was 4 CAE units by week 8). At 6 mos, two pts with AKI (native DDD and recurrent C3GN) had sustained improvements in creatinine (2.1→1.4 and 2.0→1.5 mg/dl, respectively) with consistently low proteinuria. This supports DDD and recurrent DDD, native C3GN, and recurrent C3GN had significant declines in proteinuria (10579→13179, 1179→1520, and 4455→2441 mg/g, respectively) with rising serum albumin (2.9→4.1, 3.2→3.9, and 3.4→4.2 g/dl, respectively) and stable creatinine. One pt (native DDD) had no improvement in creatinine or proteinuria. Response to therapy was associated with normalization of sMAC. No C3Nef(+) pt has become C3Nef(-). No adverse events have been reported.

Conclusions: Eculizumab is well-tolerated in pts with DDD and C3GN. Interim results at 6 mos suggest effective inhibition of the terminal complement cascade. For most pts, a clinical response was observed. Laboratory and repeat biopsy data at 12 mos will be done.

Funding: NIDDK Support, Pharmaceutical Company Support

FR-PO1902
Successful Treatment of Membranoproliferative Glomerulonephritis Type I with Monoclonal Anti C5 Antibody (Eculizumab) Arnaud Garnier,1 Anne Modesto,2 Stephanie Tellier,1 Flavio Bandin,1 Stéphane Decramer.1 Pediatric Nephrology Unit, Hospital des Enfants, CHU Purpan, Toulouse, France; 2Division of Anatomic Pathology, Hôpital Rangueil, CHU Rangueil, Toulouse, France.

Background: Membranoproliferative glomerulonephritis (MPGN) is a heterogeneous group of nephropathy, characterized on histology by mesangial hypercellularity with increased matrix, splitting of the glomerular basement membrane and different pattern of deposit (type I, II and III). MPGN type I represent less than 5% of primary glomerulonephritides and can be related to complement dysregulation.

Results: We report the case of a 7 years old boy referred to our unit for nephrotic syndrome with hematuria. Renal biopsy displayed classical MPGN type I with subendothelial deposits of C3, C1q, IgG and IgM. Serum C3 level was low (0.1 g/L) and a C3 nephritic factor (C3NF) was present. No mutation was found in the complement alternative pathway factors H and I. A 6 months course of oral steroids together with angiotensin converting enzyme inhibitor (ACEI) and angiotensin II receptor blocker (ARB) did not improve proteinuria. A 3 years of sustained nephrotic syndrome, glomerular filtration rate (GFR) slowly decreased and a second renal biopsy was performed. Histological and immunological findings were similar to the first biopsy with global sclerosis concerning 20% of the glomeruli. Low serum C3 level and C3NF were still present. A second course of steroids together with the maintenance of the ACEI and ARB did not succeed to reduce proteinuria. After tetravalent meningococcal vaccine and initiation of prophylactic penicillin treatment with eculizumab 1200 mg IV every other week for 1 yr. All had proteinuria ≥ 2 g/day and/or AKI (serum creatinine ≥150% baseline) at enrollment. All underwent biopsy at enrollment and will have repeat biopsies at 1 yr. Additional testing included: mutation screening of CFH, CFI, MCP, CFRH5, and TFH; autoAb screening for C3 nephritic factors and factor H autoAbs; and functional analysis of complement activity.

Conclusions: This is the first report of successful treatment of MPGN type I with eculizumab, a monoclonal anti C5 antibody that blocks the terminal complement activation. In primary MPGN, dysregulation of complement activation is a frequent feature, and the treatment with eculizumab should be discussed in the most severe forms.

FR-PO1903
Lymphangiogenesis and Tertiary Lymphoid Neogenesis in Progression of IgA Nephropathy Guancheng Pei, Rui Zeng, Min Han, Lily Liu, Gang Xu. Division of Nephrology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, Hubei, China.

Background: The lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitium have been previously identified in human chronic renal diseases and in human kidney transplants. However, they are not specially described in IgA nephropathy(IgAN). In this prospective study, the lymphangiogenesis and lymphoid neogenesis processes and the presence of lymphatic vessels, macrophages, dendritic cells and lymphocytes was examined by IHC.

Results: Lymphatic vessels density(LVD) in renal interstitium inversely correlated with the severity of tubular lesions and interstitial fibrosis according to Haas grades. It was also significantly associated with the amount of infiltrating macrophages, dendritic cells, lymphocytes and tertiary lymphoid organs. LVD and tertiary lymphoid organs density in renal interstitium were significantly associated with the renal function. The higher the LVD and tertiary lymphoid organs density, the higher the serum creatinine and proteinuria. 12 months after biopsy. The more remarkable discovery was both of the lymphangiogenesis and lymphoid neogenesis correlate with vasculopathy. Lymphangiogenesis and lymphoid neogenesis were found frequently adjacent to the vascular lesion. LVD was higher in IgAN patients with severe arterial lesions and hyaline changes (43-92/mm²) than in IgAN patients who had mild/moderate arterial lesions. (20.92 mm², p<0.000538).

Conclusions: Lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitial reflected the renal activity state of chronic inflammatory and they were associated with the IgAN progression. Lymphangiogenesis participated in formation of tertiary lymphoid organs in renal interstitium. Vasculopathy might play a part in lymphangiogenesis and formation of tertiary lymphoid organs in renal interstitium of IgAN patient.

FR-PO1904
Novel Diagnostic Approach for IgA Nephropathy Hirovaki Yanagawa,1 Hitoshi Suzuki,1 Yusuke Suzuki,1 Keichi Matsuaki,2 Satoshi Horikoshi,1 Jan Novak,3 Yasuhiko Tomino.1 1Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, Tokyo, Japan; 2Department of Microbiology, University of Alabama at Birmingham, AL.

Background: Galactose-deficient IgA1 (GD-IgA1) is found in elevated amounts in the circulation and in the mesangial deposits of patients with IgA nephropathy (IgAN). Moreover, serum levels of GD-IgA1-specific antibodies, responsible for the formation of immune complexes (IC) with GD-IgA1, are also elevated in IgAN. However, due to the clinical heterogeneity of IgAN, there is no biomarker to replace the diagnostic renal biopsy. Methods: A cross-sectional study was performed using serum samples collected from 2006 to 2010 at the time of renal biopsy from 121 patients with IgAN and 73 patients with other renal diseases, such as lupus nephritis, diabetic nephropathy, and membranous nephropathy. We measured serum IgA, IgA-IgG IC, GD-IgA1 and GD-IgA1-specific IgA by ELISA to assess whether these biomarkers can be used for diagnosis of IgAN. ELISA data from healthy volunteers (n=74) were used to establish baseline for each assay and calculate quantitative scores for each biomarker for IgAN patients and disease controls by principal component analysis using JMP software.

Results: Serum levels of IgA, IgG-IgA1, IgA-IgG IC and GD-IgA1-specific IgA were elevated in IgAN patients compared with disease controls (P<0.0001) and healthy controls (P<0.0001). However, no biomarker alone could effectively differentiate IgAN from disease controls, due to the overlapping values for these markers in many IgAN patients and disease controls. To test a combination of these four biomarkers as a potential differential diagnostic method for IgAN, these four biomarkers were quantified and combined by principal component analysis based on data from healthy volunteers. This scoring system showed high sensitivity and specificity of 75% and 81%, respectively.

Conclusions: Our results suggest that serum GD-IgA1, GD-IgA1-specific antibodies and IC might be useful as combined disease specific biomarkers of IgAN. This novel quantitative scoring system can be used to complement renal biopsy in the diagnosis of IgAN.

FR-PO1905
Beneficial Effect of Steroids and Immunosuppressants in the Treatment of IgA Nephropathy May Be Due to Modifying Local Production or Activation of Multiple Cytokines Maria Stougi,1 Akaiterini A, Papagianni,1 Christos Banis,2 Maria Skoularopoulos,2 Afrodit Panizaki,2 Nicoletta-Mariaouri,2 Christos Panizakis,2 Demetrios Memmos.1 1Department of Nephrology, Aristotle University of Thessaloniki, Hippokration Hospital, Thessaloniki, Greece; 2Department of Pathology, Hippokration Hospital, Thessaloniki, Greece; 3Department of Biochemistry, Hippokration Hospital, Thessaloniki, Greece.

Background: Steroids and immunosuppressants can reduce proteinuria and delay progression of renal function in IgAN, possibly by interfering with local cytokines, which lead to inflammation and fibrosis.

Methods: Histology in 53 IgAN patients [M/F 35/18 age 40.5yrs (17-65)] was evaluated by Oxford classification system and renal biopsies were classified as MEST disease controls. To test a combination of these four biomarkers as a potential differential diagnostic method for IgAN, these four biomarkers were quantified and combined by principal component analysis based on data from healthy volunteers. This scoring system showed high sensitivity and specificity of 75% and 81%, respectively.

Conclusions: Our results suggest that serum GD-IgA1, GD-IgA1-specific antibodies and IC might be useful as combined disease specific biomarkers of IgAN. This novel quantitative scoring system can be used to complement renal biopsy in the diagnosis of IgAN.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.
Results: ScrA at time of diagnosis correlated with proteinuria (p<0.02), MEST score (p=0.001) and urinary levels of IL-1β, IL-2 and MCP-1 (p=0.04, p=0.03 and p=0.04 respectively). At the end of follow up (5.5 [1-13.2] years), ScrA2 was significantly increased in RAASi+FO (from 1.6±0.9 to 3.3±3.7 mg/dl, p<0.004) and remained stable in steroids+aza treated patients.

In the RAASi+FO patients, ScrA2 at the end of follow up had positive correlation with MEST score (p=0.006), IL-1β (p=0.007), IL-2 (p=0.01), IL-6 (p=0.02), IL-10 (p=0.04), IL-12 (p=0.01) and MCP-1 (p=0.03) urinary levels. In steroids+aza patients, the only parameters correlated with ScrA2 were IL-1β (p=0.01), IL-6 (p<0.01) and MCP-1 (p=0.01).

Conclusions: In conclusion, several cytokines are excited in the urines of patients with IgAN, and their levels predict outcome of the disease. Treatment with steroids+aza seems to have a beneficial effect in renal function outcome, and this is probably due to their influence in local cytokine production or activation.

FR-PO1906
Clinical Features and Long-Term Outcomes of Nephrotic Syndrome in Patients with IgA Nephropathy
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Background: Nephrotic syndrome (NS) is a rare manifestation of IgA nephropathy (IgAN), which has been reported to behave similarly as minimal change disease. Accordingly, glucocorticoids have been frequently prescribed in IgAN patients with NS. In contrast, a recent study demonstrated that spontaneous remission (SR) of NS was commonly observed in these patients. This study was conducted to investigate the clinical features and long-term outcomes of NS in patients with IgAN.

Methods: A total of 1,076 patients with biopsy-proven IgAN were included from 4 centers in Korea. The primary outcome was regarded as doubling of the baseline serum Cr, and the secondary outcomes as ESRD or death. Cox regression analysis was performed to identify independent risk factors for the development of primary and secondary endpoints and to evaluate the predictive factors of the occurrence of SR.

Results: Among 100 patients (10.2%), who presented with NS, glucocorticoids were prescribed in 65(65.0%). Complete remission (CR), partial remission (PR), and no response (NR) were observed in 48.0%, 24.0%, and 20.0%, respectively. During the median follow-up of 48.0 months, 24 patients (24.0%) in the NS group reached the primary endpoint compared to 63.7% in the non-NS group (p<0.001). Compared to the CR group, the risk for attaining the primary endpoint was significantly higher in the PR (HR, 14.95; 95% CI, 1.14-183.7; p=0.039) and the NR groups (HR, 215.97; 95% CI, 15.63-2983.6; p<0.001). Among patients with NS, 24/24(9.9%) underwent SR. Multivariate Cox regression analysis revealed that a >50% decrease in proteinuria within 3 months after the onset of NS, serum Cr≤1.2 mg/dl, and female gender were associated with a significantly increased likelihood of SR. None of these patients reached the primary endpoint, and they had fewer relapses during follow-up.

Conclusions: This study shows the prognosis of NS in patients with IgAN is not unfavorable unless CR or PR is achieved. In addition, SR occurs more frequently in patients with preserved renal function and a prompt decrease in proteinuria after the onset of NS, and these patients have excellent outcomes.

FR-PO1907
Validation of the Oxford Classification of IgA Nephropathy in Korean Adults
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Background: The recently published Oxford classification of IgA nephropathy (IgAN) proposed a split system for histologic grading based on prognostic pathologic features. The new classification system must be validated in different cohorts. We investigated whether these pathologic features are applicable in the adult Korean population.

Methods: One hundred seven adult Korean patients with IgAN were analyzed with the Oxford classification system at Soon Chun Hyang University Hospital Seoul, Korea. Renal biopsies from all patients were scored by a pathologist who was blinded to the clinical data for pathological variables. Inclusion criteria were age greater than 18 years and at least 36 months follow-up. We excluded cases with secondary IgAN, diabetic nephropathy combined other glomerulopathies, less than 36 months of follow-up, and rapidly progressing cases.

Results: The median age of patients was 34 years (range, 27–45 years). Mean arterial blood hypertension (MAP) was 97±10 mmHg at the time of biopsy. The median follow-up period was 85 months (range, 60–114 months). Kaplan-Meier analysis showed significant prognostic prediction with M, E, and T lesions.

FR-PO1908
Long-Term Outcome of Biopsy-Proven IgA Nephropathy Presenting with Mild Proteinuria and/or Isolated Microhematuria. A Multicenter Study
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Background: Renal biopsies are rarely performed in patients presenting with mild proteinuria and/or isolated microhematuria. Clinical information about long-term outcomes of IgA nephropathy (IgAN) presenting with such benign manifestations is very scarce.

Methods: Retrospective and multicenter study to collect patients with biopsy-proven IgAN who presented at renal biopsy a normal renal function, microhematuria and proteinuria lower than 0.5 g/d. Primary endpoint was renal survival, defined as an increase >50% or >100% of baseline serum creatinine (sCr). Secondary endpoints were the occurrence of the development of >1 g/d proteinuria and the development of ESRD. Thebiopsies were classified according to the new Oxford Classification.

Results: 141 patients were collected. Mean follow-up was 108 months. Clinical characteristics at baseline were: 63.4% males, age 23.7±14.8 yr, sCr 0.8±0.2 mg/dl and a median proteinuria of 0.2 g. Renal biopsies showed mean glomerular proliferation in 32.6% of patients, focal and segmental glomerulosclerosis in 15.6% and endocapillary proliferation in 8.5%. No patient received any immunosuppressive treatment. 59 patients were treated with ACEI/ARB during follow-up. Renal survival (>50% sCr) was 96.2% and 93.6% after 10 and 15 yr and 97.6% at both 10 and 15 yr when it was defined by >100% sCr increase. Only 6 patients (4.2%) had a eGFR <60 ml/min and none reached ESRD. Remission was observed in 37.6% and proteinuria increased to >1g/d in only 6 patients (4.2%). By multivariate analysis only age (HR 1.9, 95% CI 1.01-3.1, p=0.03) and albuminuria were significant risk factors for renal survival.

Conclusions: Long-term renal outcomes in patients with benign clinical presentations are excellent. Spontaneous clinical remission occurred in more than a third of patients.

FR-PO1909
Down-Regulated Peripheral Lymphocyte miR-155 Is Related to IgA Nephropathy
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Background: MicroRNA-155 (miR-155) is a very important immune regulatory factor, which involved in the lymphocyte homeostasis and adjusting of multiple immune related genes. Immune abnormalities, such as lymphocytes dysfunctions and impaired
homeostasis, are key pathogenesis of IgA nephropathy (IgAN). Therefore, we studied the expression levels of miR-155 in the peripheral lymphocytes of IgAN.

Methods: Forty biopsy-proven IgAN patients and 15 unrelated healthy controls were included. Expression of miRNAs in peripheral lymphocytes was first determined using Exiqon miRNA microarray in 3 IgAN patients and 3 healthy controls. Realtime RT-PCR of miR-155 was performed. The expression level of Foxp3, a regulator of miR-155, was also measured. Correlation between miR-155 and Foxp3 expression level as well as clinical indexes was analyzed.

Results: microRNA microarray indicated that the expression level of miR-155 in IgAN patients was dramatically lower than that in normal controls (2.12 vs 3.21), which was confirmed by realtime RT-PCR examination (miR-155 0.19±0.07 vs Control 0.74±0.22, p=0.003). Further study showed that baseline proteinuria (24 hour quantification) and hematuria (RBC per high power view) level was significantly correlated to the miR-155 expression level (proteinuria: r=0.490, p=0.007, hematuria: r=-0.648, p=0.001). Significantly correlation between miR-155 and Foxp3 expression level was also noticed.

Background: Although many studies identified multiple gene polymorphisms as genetic prognostic factors of IgAN, little information is available about genetic predictors of renoprotective effectiveness of RAS.

Methods: The present multicenter longitudinal study included 257 IgAN patients, who were selected in our previous study PREDICT-IgAN. To identify genetic predictors of progression in IgAN, we assessed the association between progression (50% increase in serum creatinine at renal biopsy) and RAS-related gene polymorphisms (ACE ID, ATIR A1166C, AGT M235T and CYP2C9 A1075G), in multivariate Cox proportional-hazard (CoxPH) models. To identify renoprotective effectiveness of RAS in IgAN, effect modifications between RAS and the gene polymorphisms were assessed in multivariate CPH models. P for interaction <0.10 was regarded as statistically significant.

Results: During median 10.2 (interquartile range 6.7 - 13.4) yr of the observational period, 50.2% received RAS inhibitors and 27.6% developed progression. Among 4 gene polymorphisms, only ACE ID predicted progression (DD vs. non-DD, hazard ratio (HR) 1.82 [95%CI 1.04 - 3.18]) and effectiveness of RAS (P for interaction (DD vs. ACE ID* RAS) = 0.066). HR of DD patients with RAS was remarkably lower than DD patients without RAS, whereas not in non-DD patients (non-DD without RAS as a reference; non-DD with RAS, 1.48 [0.78 - 2.79]; DD without RAS, 2.96 [1.42 - 6.15]; DD with RAS, 3.81 [1.50 - 9.60]). Multivariable LR models also ascertained the results described above.

Conclusions: ACE ID polymorphism predicts renoprotective effectiveness of RAS, besides progression, in IgAN.

FR-PO1913
A Nationwide Questionnaire on Treatments for IgA Nephropathy in Japan Keiichi Matsuura,1 Yusuke Suzuki,1 Junichiro Nakata,1 Naoko Sakamoto,2 Satoshi Horikoshi,3 Tetsuya Kawamura,3 Seiichi Matsuo,3 Yasuhiko Tomino,1 Division of Nephrology, Department of Internal Medicine, Juntendo Faculty of Medicine, Tokyo, Japan; 2National Research Institute for Child Health & Development, Tokyo, Japan; 3Jikei University School of Medicine, Tokyo, Japan; 4Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; 5Progressive Renal Diseases Research, Research on Intractable Disease, from the Ministry of Health, Labour and Welfare of Japan, Japan.

Background: Since annual check-ups with urinalysis are routine practices with various stages of IgA nephropathy (IgAN) are managed in Japan. A wide variety of treatments for IgAN including tonsillectomy with steroid pulse therapy (TSP) is performed because there is no single clear treatment approach to all IgAN. The objective of the present study was to survey the current treatment status for IgAN in Japan via Progressive Renal Diseases Research, Research on intractable disease, from the Ministry of Health, Labour and Welfare of Japan.

Methods: The nation wide questionnaire survey was conducted in 1,194 teaching hospitals in the Japanese Society of Nephrology in 2008.

Results: Answers from 376 hospitals (31.4%) showed that TSP was performed in 223 hospitals (59.3%). The histological severity and levels of urinary protein excretion (73.5%) were the most cited indications for TSP. Patterns of steroid pulse therapy in TSP were mainly given as follows: 1) three times in three consecutive weeks (47.8%) and 2) three times every two months (18.9%). No major differences were found in clinical efficacy between the two groups. The rate of clinical remission by TSP, a condition with no continuing or transient abnormalities in urinalysis was better than that for steroid pulse therapy alone in each hospital. Combination therapy with corticosteroids, immunosuppressants and anticoagulants/antiplaquette agents was performed in about 70% of pediatric departments. Renin-angiotensin system inhibitors (RAS-I) and antiplaquette agents were used in most hospitals.

Conclusions: Besides popular treatments such as RAS-I and antiplaquette agents, TSP is a current treatment for IgAN mainly with two different patterns of steroid pulse therapy in Japan.

FR-PO1914
Mesangial IgG Deposition Is Not Associated with Outcome in Pediatric IgA Nephropathy Margaret Colleen Hastings,1 Kim R. McGlothan,2 Theodore Matthew Eison,1 Noel Delos Santos,1 Bettina H. Ault,2 Robert J. Wyatt,1 1Pediatrics, University of Tennessee Health Sciences Center, Memphis, TN; 2Medicine, University of Tennessee Health Sciences Center, Memphis, TN.

Background: Anti-glycan IgG antibodies to N-acetylglactosamine have been implicated in the pathogenesis of IgA nephropathy (IgAN) [PMID 19478457]. However, many patients with IgAN do not have IgG in their glomerular deposits, suggesting different
pathogenic mechanisms in those patients. Recent analysis of data from Oxford classification cohort showed that the presence of IgG in glomerular deposits is associated with progression to end stage kidney disease (ESKD) in children and adults with IgAN [PMID 21273233]. The purpose of this study was to determine whether the presence of IgG deposits associates with progression to ESKD in the Le Bonheur Children’s Hospital (LBCH) IgAN cohort.

Methods: Renal biopsy reports with immunofluorescence data for IgA, IgG and IgM were available for 99 patients diagnosed with IgAN prior to age 18 at LBCH in Memphis, TN since 1974. Kaplan-Meier curves were generated using SAS v9.2. P-values of <0.05 were considered statistically significant.

Results: This cohort included 1 Native American, 2 Asians, 23 African Americans, and 73 Caucasians with a mean age of 10.6 ± 4.0 years at time of diagnosis and a median length of follow up of 5.4 (IQR 2.0 - 15.0) years. Male:Female ratio was 70:29. Twelve patients reached the endpoint of ESKD, defined as date of initiation of chronic dialysis or primary renal transplantation. Overall kidney survival was 92%, 86% and 83% at 5, 10, and 15 years, respectively. IgA was the only immunoglobulin in 33%, occurred in combination with IgG in 51%, and with IgM in 36%. For those with IgG deposition, survival was 92%, 83% and 83% and without IgG was 91%, 83% and 83% at 5, 10, and 15 years. For those with IgG deposition, survival was 89%, 79% and 79% and without IgG was 93%, 88%, and 84%. Kidney survival did not differ based upon gender or race.

Conclusions: Survival data from the LBCH IgA cohort does not support the suggestion based upon the OCC that the presence of IgG in glomerular deposits is a risk factor for progression to ESKD.

FR-PO1915
Elevated Soluble Flt-1 Was Associated with Clinical and Pathological Severity in IgAN Patients
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Background: Patients with IgA nephropathy often displayed vascular injury, such as clinical hypertension and histological arteriolar hyaline and arterial intimal thickening. Till now, factors for vascular injury in IgAN patients remain incompletely understood. Soluble Flt-1 is a splice variant of VEGF receptor. For lacking of trans-membrane and cytoplasmic domains, sFlt-1 can bind and sequester VEGF to act as a VEGF antagonist. High levels of sFlt-1 were reported in many diseases with proteinuria and hypertension symptoms, including preeclampsia and essential hypertension. Recently, CKD patients were also reported to have elevated sFlt-1, indicating its involvement in kidney disease. In the present study, we investigated the effect of sFlt-1 in IgAN.

Methods: A total of 122 individuals (100 IgAN patients and 22 healthy volunteers) were enrolled. Clinical manifestations at the time of renal biopsy and pathological characteristics were collected from the clinical record. Plasma sFlt-1 levels were determined using commercial ELISA kits.

Results: Plasma sFlt-1 level were significantly elevated in IgAN (patients Vs controls: 101.06±24.84 Vs 79.73±18.85 pg/ml, p<0.001). Furthermore, IgAN patients with hypertension showed significant higher plasma sFlt-1 levels than those without (106.68±28.30 Vs 96.27±20.54 pg/ml, p=0.029), although IgAN patients, with or without hypertension, presented with higher plasma sFlt-1 levels than controls (p<0.001 & p=0.006), indicating the correlation between sFlt-1 and clinical phenotype of hypertension in IgAN patients. Similar correlation was also found about the phenotype of proteinuria. IgAN patients with proteinuria more than 1g/d, showed significant elevated sFlt-1 to those with less than 1g/d (p=0.025). According to the pathological lesions, IgAN patients were grossly grouped to five groups. Interestingly, we can find the gradually elevated sFlt-1 level from mild to severe pathological group.

Conclusions: Elevated sFlt-1 was associated with hypertension, proteinuria phenotype and pathological severity in IgAN patients, which indicated the involvement of sFlt-1 in IgAN.

Funding: Government Support - Non-U.S.

FR-PO1916
IgA Nephropathy: A Long Term Follow-Up of 132 Cases
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Background: Since the beginning of the 1990s our medical practitioners have extensively prescribed angiotensin-converting enzyme inhibitors for patients with mild IgA nephropathy (IgAN) and steroids for those with severe IgAN. The purpose of this retrospective study was to analyze the probability of renal survival in medical practice and to identify risk factors for kidney disease progression in a cohort of patients with IgAN.

Methods: We included 132 patients with primary biopsy proven IgAN first diagnosed between 1987 and 2008 in our department. They were observed for an average of 8,4 years (0,14-23,2). 89 patients had been followed up in our centre; 43 were found through databases or telephone interview. The primary endpoint was the achievement of a glomerular filtration rate (GFR)=15 ml/min or end-stage renal disease (ESRD). Renal survival was estimated using Kaplan Meier plots, logrank test and Cox proportional-hazard models.

Results: At diagnosis, GFR was less than 60 ml/min in 45.1% of patients. The renal survival (IgAN) at 10 years was respectively 85% (95% confidence intervals, CI 78-91%), 67% (CI, 58-77%), 50% (CI, 39-63%), and 37% (CI, 20-69%). Univariate analysis showed that patients who had hypertension, proteinuria, and GFR<60 ml/min were associated with poor prognosis. Age, sex, and macroscopic hematuria at initial presentation did not influence prognosis. We created several models of multivariate analysis, each of which included 4 variables (44 events). Hazard ratio of the final model were: 2.6 (CI 1.21-5.6;p=0.014) for patients with hypertension; 1.27 (CI 1.12-1.46;p=0.002) for each increase of proteinuria of 1 g/day, 0.98 (CI 0.97-0.99;p=0.006) for each increase of eGFR of 1 ml/min.

Conclusions: In medical practice, renal survival rate was poor despite therapy. This is partially explained by lead-time bias: as a matter of fact diagnosis was made when renal function was impaired in nearly half of patients. Moreover, “real” population is more heterogeneous and complex than cohorts enrolled in randomized controlled trial. Patients with renal impairment, hypertension and proteinuria had the highest risk for disease progression.

FR-PO1917
Treatment of Early Immunoglobulin A Nephropathy by Angiotensin Converting Enzyme Inhibitor—A Randomized Controlled Trial
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Background: The treatment of IgA nephropathy with normal renal function and minimal proteinuria is unknown.

Methods: We randomly assigned 60 patients with IgA nephropathy, proteinuria <0.5 g/day, normal blood pressure and renal function, to ramipril 2.5 mg daily or no treatment. Patients were followed for 5 years for the development of hypertension, proteinuria, or impaired renal function.

Results: The blood pressure of the treatment group was marginally lower than the control group throughout the study period. At 60 months, the event-free survival was similar between the treatment and control group (81.1% and 70.5% respectively, p = 0.3). Similarly, the proteinuria-free survival was 82.9% and 79.3% for the treatment and control groups, respectively (p = 0.6); hypertension-free survival was 86.4% and 79.3% (p = 0.2). None of the patient developed impaired renal function. In general, the study medication was well tolerated, although 2 patients needed to stop prematurely because of cough and dizziness.

Conclusions: For IgA nephropathy patients with minimal proteinuria, blood pressure and normal renal function, treatment with ACE inhibitor does not offer any benefit.

Funding: Clinical Revenue Support

FR-PO1918
Clinical Characteristics and Outcome of Patients with Diffuse Crescentic IgA Nephropathy
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Background: Guidelines recommended immunosuppressive therapy for crescentic IgA nephropathy (IgAN). However, the response to such treatment and clinical outcomes were still unclear.

Methods: In this study, 52 patients with diffuse crescentic IgAN(crescent affected-50% glomeruli) were collected and followed up until at least 12 months. Crescentic glomerulonephritis due to anti-GBM disease (n=38) and ANCA associated systemic vasculitis (AASV, n=44) served as controls. Logistic regression was used to assess the outcome of all patients with ESKD as the end-point.

Results: Mean initial serum creatinine(SCr) was 413±304 µmol/L, and percentage of crescents was 68.5%±15.4%. Cumulative renal survival rate was 59.6%, 53.8% and 31.1% at 1st, 3rd and 5th year. On multivariable Cox analysis, initial Scr was the only independent risk factor of ESKD. Cumulative probability of ESKD at one year by initial Scr fitted an “S” shape curve. Risk of ESKD was relative low when Scr < 560µmol/L (~<21.1 %), then grew rapidly with the increase of Scr. While for those with initial Scr >599µmol/L, none recovered from dialysis. This S shape curve was similar to that observed in anti-GBM disease, while it was not observed in AASV.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
HSPN is characterized by severe renal involvement, and is susceptible to treatment-related adverse events. Adjustment of treatment regimens is necessary in this age group to reduce the risk of adverse events.

FR-PO1921

Henoch-Schönlein Purpura Nephritis: Comparison of Two Therapies (azathioprine vs Mycophenolate) and it’s Effect on the Urinary Excretion of MCP-1

Yolanda Fuentes, Saul Valverde, Ana M. Hernández, Lourdes M. Ortiz, Mara Medeiros. Hospital Infantil de México Federico Gómez, Mexico.

Background: Renal expression of MCP-1 promotes infiltration of monocytes and macrophages in Henoch-Schönlein Purpura Nephritis (HSPN). There is no consensus in the treatment of severe forms of HSPN. Azathioprine (AZA) has proved an effective drug. There is little experience using mycophenolate (MMF). Primary outcome is to compare the effectiveness of these drugs in the remission of proteinuria, excretion of MCP-1 and regression of the histological lesions in children with HSPN.

Methods: Prospective, randomized, open trial in children with renal biopsy proven HSPN. Two treatment groups: I. Oral prednisone + AZA and II. Oral prednisone + MMF. Monthly visits for serum creatinine, proteinuria, liver function test, urine MCP-1 by ELISA. Renal biopsies and immunohistochemistry with CD68 at baseline and 12 months after treatment. SPSS v16 was used for statistical analysis. We present the preliminary results of 22 patients.

Results: Median age of 7 years (4 - 11 years). 15 males. Hematuria and no nephrotic proteinuria was present in 11 patients (50%), 10 patients (45%) had nephrotic syndrome. Creatinine at presentation in all patients were 89.27 ± 94.1 mg/dl, mean urine MCP-1 was 919 ± 1149.94 pg/mL. Twelve patients (54%) had class III lesion and CD68 was positive in 56% of the glomeruli at baseline in renal biopsy. Ten patients received AZA and twelve patients received MMF. After one year of treatment only 8 patients had remission of proteinuria in the AZA group; all patients in the MMF group had remission of proteinuria without significant statistical difference. In both groups uric acid increased. In renal biopsies 5 patients in the AZA group presented regression of the initial histologic lesion vs eight patients in the MMF group without statistical difference between them. There was also a reduction in CD68 on glomerulus of 35%.

Conclusions: There are more remissions of proteinuria and in the histologic lesions in MMF grp, the difference is not statistically significant. More patients and prolonged follow up is needed.

Funding: Government Support - Non-U.S.
FR-PO1923

Impact of a Functional Polymorphism of Vascular Endothelial Growth Factor (VEGF) Gene on Primary Glomerulonephritis

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Background: Vascular endothelial growth factor (VEGF) regulates endothelial cell proliferation and participates in interstitial remodelling. In the kidney, VEGF is mainly expressed by podocytes. We evaluated the expression of C-2578A polymorphism, located in the promoter of the VEGF gene, on primary glomerulonephritis.

Methods: We studied 284 patients with biopsy proven primary glomerulonephritis (1gA nephropathy: n=143, focal segmental glomerulosclerosis: n=82, membranous glomerulonephritis: n=59) followed up for 7.0 ± 5.7 years. According to the slope of reciprocal serum creatinine (coor = -0.1 dl * mg-1 * year-1) group A (slow progressors, n=192) and group B (fast progressors, n=92) were defined. One hundred volunteers were analysed as controls. The biospecs of 156 patients were analysed by the same pathologist. VEGF polymorphism was determined by PCR. VEGF serum levels were determined by ELISA in 105 patients with chronic kidney disease.

Results: VEGF serum levels correlated to the C-2578A genotype: CC/CA: 396 ± 251, AA: 558 ± 425 pg/ml (p=0.018). The genotype frequencies were similar in patients and controls (ns). The initial renal function correlated to the degree of glomerular sclerosis (r=0.520, p=0.001), tubulointerstitial fibrosis (r=0.557, p=0.001) and arteriosclerosis (r=0.469, p=0.001). The percentage of scleroed glomeruli was higher in group B (44.0 ± 31.1% vs 32.9 ± 28.7% in group A, p=0.051) as was the degree of tubulointerstitial fibrosis (34.1 ± 26.3% vs 25.4 ± 28.7% in group A, p=0.043). There was no significant difference regarding the histological parameters between patients with different genotypes (ns). VEGF gene polymorphism influenced the progression as shown by the genotype distribution in group A (CC: 70.5%, AA: 29.5%) compared to group B (CC: 81.5%, AA: 18.5%, p=0.035). There was also a significant difference in the actual rate of progression (CC/CA genotypes: -0.160 ± 0.417, AA: -0.085 ± 0.132 dl*mg-1 *year-1; p=0.021).

Conclusions: The functional VEGF C-2578A polymorphism is a progression marker in primary glomerulonephritis.

FR-PO1924

Acute Manifestation and 1-Year Follow-Up of a Big Cohort of Patients with Atypical Hemolytic Uremic Syndrome (aHUS)

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Background: The atypical HUS is a form of thrombotic microangiopathy. Dysfunction of complement proteins are associated with the pathogenesis of the disease. Long-term prognosis is poor.

Methods: Since 2002 the HUSnet Registry investigates the role of complement in aHUS and collects clinical data on long-term outcome. Here we present data of 116 aHUS cases selected at diagnosis and the 1 year follow-up of 72 patients.

Results: During acute phase the hemoglobin value dropped to 6.03±1.4 mg/dl, the platelet count to 51.3±43.8x109 and mean creatinine was elevated to 4.8±3.5 mg/dl. The first recurrence occurred in median after 4.5 months (range 1-26 months).

Conclusions: VEGF serum levels correlated to the C-2578A genotype: CC/CA: 70.5%, AA: 29.5% compared to group B (CC/CA: 81.5%, AA: 18.5%, p=0.035). There was also a significant difference in the actual rate of progression (CC/CA genotypes: -0.160 ± 0.417, AA: -0.085 ± 0.132 dl*mg-1 *year-1; p=0.021).

The finding of hypomethioninemia, homocystinuria and methylaminic aciduria led to the diagnosis of methylaminic acidemia with homocystinuria. Intravenous hydrazidoxbalanin, oral betain and folic acid were immediately initiated. During course of the disease, the first 3 patients developed AKI stage F PRIFLE criteria). Remission of TMA and the recovery of kidney failure took place within next 10 days. Presently, at a mean age of 6 months, all four children are alive and well.

Conclusions: The described “cluster” of TMA due to transcianocobalamin C deficiency, points out that this disease might be a lot more common than diagnosed. Whenever neonatal TMA is detected homocysteinemia should be determined and the disease ruled out. We expect that this report will contribute to an increased awareness regarding this disease among pediatric nephrologists.

FR-PO1926

Mass Spectrometry as a Novel Method for Detection of Podocyturia in Preeclampsia

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Background: Increasing evidence suggests that podocyturia may serve as both a reliable diagnostic tool for preeclampsia and as a marker of active disease in proteinuric renal diseases. Reservations exist regarding both the research and clinical utilities of the current method to detect podocyturia, mainly due to its technical complexity, time commitment, and the level of expertise required for interpretation of the results.

Methods: The aim of this study was to develop a new technique for the identification of urinary podocytes based on the detection of podocyte specific tryptic peptides by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS), which will provide an operator-independent and highly reproducible method. Urine samples collected within 24 hours prior to delivery were centrifuged. One half of the sediment was cultured for 24-hours and then stained with podocin antibody followed by a FITC-labeled secondary antibody to identify viable podocytes. The second half of the pellet was solubilized, digested and analyzed by LC-MS/MS using an internal standard.

Results: We have recruited 12 patients with preeclampsia and 6 patients with HELLP. The diagnosis of preeclampsia was confirmed by the presence of hypertension (>140/90 mm Hg) and proteinuria >0.3 g/24 hour. The diagnosis of HELLP was confirmed based on the accepted clinical criteria of Hemolysis, Elevated Liver enzymes, and Low Platelet count. The presence of podocytes was confirmed in all patients by the podocyte culture method. With the LC-MS/MS technology, we documented the presence of a podocin-specific tryptic peptide in all samples.

Conclusions: LC-MS/MS technology may facilitate the use of podocyturia, as confirmed by the presence of podocyte-specific proteins in the urine, both as a diagnostic test and as a research tool in studying renal injury in human disease and animal models of preeclampsia. In addition, if validated in preeclamptic patients, this technology may be used in future studies to assess both disease activity and response to treatment in a variety of proteinuric renal diseases.
FR-PO1927
Renal Outcome in Patients Presenting with Dialysis Dependent ANCA Associated Vasculitis Treated with Pulsed Intravenous Cyclophosphamide
Ruth J. Pepper,1 Dimitrios Chanouzas,2 Alina L. Casian,3 Michael Walsh,4 Ruth M. Tarzi,5 Mark Little,5 Charles D. Pusey,1 Lorraine Harper,4 Alan D. Salama.1
1UCL; 2Birmingham University Hospital; 3Aldenbrooks Hospital Cambridge; 4McMaster; 5Imperial College London.

Background: Oral cyclophosphamide (CYP) is the standard of care for patients with severe renal failure (SRF). In 2005-10 at 2 renal units, all patients were treated with plasma exchange, steroids and IV CYP. We assessed the rate of dialysis independence, as well as the adverse effects of CYP.

Methods: We retrospectively analysed patients with AA requiring dialysis, who presented between 2005-10 at 2 renal units. All patients were treated with plasma exchange, steroids and IV CYP. We assessed the rate of dialysis independence, as well as the adverse effects of CYP.

Results: Forty-one patients included, 27 male. Mean age 59.7 years. 20 patients MPO-ANCA, 19 PR3-ANCA. 1 ANCA negative, 1 positive both. 13 patients had concurrent pulmonary haemorrhage. Median number of plasma exchanges 7 (range 2-14); median number of CYP doses 6 (range 1-10) total mean dose 4.75g. Median number of HD days 14 days (range 3-120). 12 patients remained dialysis dependent from the time of presentation, including 3 patients who died. 29 patients initially recovered function. 4 patients returned to HD, median time 83 days (range 30 to 265). 12 patients had leucopenia, transient in 7. 4 patients relapsed in the 1st 12 months.

At 3 months, 3 dead, 26 patients HD free with 12 on HD (63.4% alive and HD free). HD free at 1 year, 59% patients alive and HD free. This is comparable with the MEPEX study which used oral CYP, in which 52% of patients reaching 1 year were alive and HD free (p=0.58, log rank test).

Conclusions: IV CYP is an effective alternative to oral CYP in dialysis dependent AAV, and results in an equivalent clinical response.

On behalf of the European Vasculitis Study group

FR-PO1928
A Candidate Gene Approach to Genetic Contributors to Development of IgA Nephropathy
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1Nephrology, LUMC, Leiden, Netherlands; 2Surgery, LUMC, Leiden, Netherlands; 3Surgery, LUMC, Leiden, Netherlands.

Background: Genetic factors contributing to development of IgA nephropathy remains to be elucidated.

Methods: Present multicenter cross-sectional case-control study measured genotype frequencies of 65 atherosclerotic disease-related gene polymorphisms in 230 Japanese patients with IgA nephropathy and 262 apparently healthy volunteers with estimated glomerular filtration rate (eGFR) >60 mL/min/1.73m2 and negative or trace for proteinuria and hematuria by dipstick test (non-chronic kidney disease (CKD) participants). Clinical characteristics at kidney biopsy of patients with IgA nephropathy and controls were compared. In the study recruitment of non-CKD participants were included as covariates in multivariate logistic regression models.

Results: Among 31 gene polymorphisms with ≥5% of minor genotype in non-CKD participants, methionine synthase MTR A2756G (D919G) was significantly associated with IgA nephropathy using the test even after controlling for family-wise error rate by the Bonferroni method (P = 0.044). A multivariate non-conditional logistic regression model identified MTR A2756G as a significant contributor of IgA nephropathy (2756AG and GG vs. AA, odds ratio 0.42 [95%CI 0.25 - 0.69] and 0.21 [0.06 - 0.68], P mult < 0.001). After each patient with IgA nephropathy was randomly matched to a non-CKD participant on age (±5 years), gender, mean arterial pressure (±5 mmHg), and eGFR (±5 mL/min/1.73m2), a multivariate conditional logistic regression model also verified their association significant (0.42 [0.18 - 1.00] and 0.09 [0.01 - 0.73], P mult < 0.004).

Conclusions: Methionine synthase MTR A2756G was associated with development, not progression, of IgA nephropathy.

FR-PO1929
Characteristics of Patients with Systemic Lupus Erythematosus Admitted to the Intensive Care Unit
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Background: Systemic Lupus Erythematosus (SLE) is a multisystem inflammatory disorder associated with significant morbidity and mortality. Affecting patients may become critically ill as a complication of disease or treatment. Our aim was to describe the epidemiology and characteristics of SLE patients admitted to the Intensive Care Unit (ICU) in a tertiary teaching hospital.

Methods: Retrospective review of the medical notes of all SLE patients admitted to ICU between 2008-2011 detailing demographics, disease duration and previous treatments alongside reason for ICU admission, APACHE and SOFA scores, level of organ support, immunosuppression and patient mortality.

Results: 25 patients were admitted (18% female; mean age 47±16.8 years). 11 patients were Caucasian, 10 were black and 4 were from other ethnic groups. The most common reasons for admission to ICU were infection (48%), respiratory failure (32%) and renal failure (24%). 64% of patients had been treated with immunosuppression prior to admission (steroids 64%, mycophenolate mofetil 20%) in the preceding 5 months. 44% of patients needed mechanical ventilation, 56% had renal replacement therapy and 76% were treated with antimicrobial therapy. The most common cause of death was overwhelming sepsis. Among ICU survivors, 1 year survival was 100%.

Conclusions: Infection was the most common reason for admission to ICU. The majority of this group had already been on immunosuppression. Despite a high need for organ support and health care resources, ICU mortality was 16%. The most common cause of death was overwhelming sepsis. 1 year outcome of ICU survivors was excellent.

FR-PO1930
A Novel Murine Model of Arteriovenous Fistula Failure
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1Nephrology, LUMC, Leiden, Netherlands; 2Surgery, LUMC, Leiden, Netherlands.

Background: The arteriovenous fistula (AVF) still suffers from a high number of failures caused by insufficient remodeling and neointimal hyperplasia. We developed a mouse model of AVF-failure to unravel the underlying pathophysiology. Since the hemodynamic profile including flow turbulence is an important determinant for the vascular response to fistula insertion, we configured the AVF in an end-to-side manner similar to what is frequently performed in humans.

Methods: AVFs were created by connecting the end of the external jugular vein to the side of the common carotid artery using interrupted 10.0 sutures. Animals were sacrificed 14 days (range 3-120 days). Progressive venous neointima formation what is frequently performed in humans.

Results: At day 14 and 28 after surgery, vessel size increased 20-fold and 32-fold respectively when compared to control veins. Progressive venous outflowtract; \[\text{NI}\] neointima

Conclusions: The arteriovenous fistula (AVF) still suffers from a high number of failures caused by insufficient remodeling and neointimal hyperplasia. We developed a mouse model of AVF-failure to unravel the underlying pathophysiology. Since the hemodynamic profile including flow turbulence is an important determinant for the vascular response to fistula insertion, we configured the AVF in an end-to-side manner similar to what is frequently performed in humans.
Conclusions: The AVF-model, which resembles the AVF configuration most frequently used in humans, shows that despite substantial outward remodeling, progressive stenotic lesions develop as a result of rapid neointimal hyperplasia in the venous outflow tract. Similar to failed human AVFs, the neointimal hyperplasia is mainly composed of α-smooth positive cells. These lesions make this model suitable for intervention studies using e.g. genetically modified mice. We conclude that this murine AVF-model is a good model to the AVF animal model arsenal.

Funding: Private Foundation Support

FR-PO1931

The Effect of Temporal Variation in Wall Shear Stress on the Remodeling of Arteriovenous Fistulae Ehsan Rajabi-Jaghargh,1 Prabir Roy-Chaudhury,2 Yang Wang,3 Kyuran Ann Choe,4 Paul Succop,5 Rupak Banerjee,3 1CEAS-Schl Dynamic Systems, University of Cincinnati, OH; 2Dialysis Vascular Access Research Group, Division of Nephrology, University of Cincinnati, OH.

Background: Non-maturation of arteriovenous fistulae (AVF) is in many ways the “Achilles Heel” of hemodialysis. The surgical configuration of the AVF and the subsequent wall shear stress (WSS) are key players in the remodeling of the AVF. This study aimed to investigate the temporal effect of WSS on the maturation of AVF’s created in two different configurations.

Methods: AVFs were created between the femoral artery and vein of three pigs in a curved (n=3) and straight (n=3) configuration. Reconstructed geometry of the AVF obtained from CT-scans and flow data from Doppler ultrasound were utilized to numerically evaluate WSS at 2D (D. days), 7D, and 28D post-surgery. Time dependent WSS-area data was regressed using a random effects model: Area = β0 + β1time + β2WSS/Atme, where β and δ were the regression coefficients and the gradient, respectively. A p-value < 0.05 indicated statistical significance.

Results: For the curved AVF, the slope of temporal gradient of WSS (+0.019) had a statistically significant effect (p = 0.02) on the dilation of the vein, while it was not significant for the maturation of the straight AVF (p = 0.53). Also, time had a positive effect (β1 = 0.337) on the maturation of the curved AVF (p = 0.05), while it had a negative effect (β1 = −0.071) for the straight AVF (p < 0.05).

Conclusions: Our results document a temporal linkage between an increase in diameter and a decrease in WSS in the curved AVF, with an opposite interaction in the straight AVF. This study aimed to investigate the temporal effect of WSS on the maturation of AVF’s created in two different configurations.

Funding: Veterans Administration Support

FR-PO1932

Treatment with Recombinant Human Type 1 Pancreatic Elastase (PRT-201) Does Not Alter the Safety of Angioplasty in a Porcine Arteriovenous Graft Model Dirk M. Hentschel,1 Steven K. Burke,2 1Renal Division, Brigham and Women’s Hospital, Boston, MA; 2Proteon Therapeutics, Waltham, MA.

Background: PRT-201 is being developed as a treatment for newly created arteriovenous grafts (AVGs) and fistulas to promote access patency. It is not known if degrading elastin fibers in the access outflow vein impacts the safety of subsequent angioplasty procedures.

Methods: Eleven male Yorkshire swine underwent bilateral femoral artery to vein ePTFE grafts insertion (Bard Carboflor 4-6 mm taper) followed by application of PRT-201 6 mg (n=11) or vehicle (n=11) to the external surface of the venous anastomosis and outflow vein. Acute effects were measured using digital photography. At 28 days, AVGs underwent angiography, measurement of graft blood flow (Transonic ReoCal), and angioplasty (Bard Conquest 8mm). The animals were euthanized and attherosclerotic/veins were excised for histology.

Results: Two animals were euthanized early: one due to intestinal torsion, the other due to bilateral AVG occlusion. PRT-201 treated vessels (n=11) increased in diameter by 12 ±14% (p=0.01). In the nine animals surviving to Day 28, 17 of the 18 AVGs had some degree of maturation and subsequent use for HD patients result in inadequate increase in blood flow volume (BFV). The required increase in BFV after arteriovenous anastomosis of native vessels depends on the ability of the vasculature to dilate and remodel. These changes ultimately determine VA and BFV before (V0) and after surgery to investigate major determinants of BF in VA and to predict flow related VA complications.

FR-PO1933

Prospective Clinical Investigation of Vascular Structural and Functional Changes Following Hemodialysis Vascular Access Surgery Andrea Remuzzi,1,2 Anna Caroli,3 Marko Malovrh,4 Katia Passera,5 Luca Antiga,6 Stefano Rota,7 Giuseppe Remuzzi,1,3 Aron Bode,8 J. Tardor,9 1Mario Negri Institute, Bergamo, Italy; 2Univ. of Bergamo, Italy; 3Ospedali Riuniti di Bergamo, Italy; 4Maastricht Univ. Hospital, Netherlands; 5Univ. Medical Center Ljubljana, Slovenia.

Background: Vascular access (VA) complications represent a major cause of morbidity and hospitalization in hemodialysis (HD) patients and are the major limitation of HD treatment. The ARCH project (EU FP7-ICT) aims to computational modelling tools for surgical planning of VA. These tools are developed using ultrasound (US) measurements and multi-scale computational model of blood flow (BF). Given the high inter-subject variability, the modelling tools must be patient-specific and need calibration and validation using clinical data.

Methods: To this purpose, a prospective observational clinical study has been conducted to collect anatomic, physiologic and clinical data to quantify structural-functional relation between patient vasculature and its changes after surgery. 93 consecutive patients with ESRD awaiting VA creation were examined in the study (63 3 M/F, age 62y [18-85]). Pre- and post-operative clinical data and US measurements have been collected for a two years period.

Results: Mean artery diameter in distal (radial artery, RA) and proximal (brachial artery BA) VA and BF before (V0) and after surgery (V1-V4) are as follows.

<table>
<thead>
<tr>
<th>Visit</th>
<th>Time (days)</th>
<th>N</th>
<th>RA Diam (mm)</th>
<th>BA Diam (mm)</th>
<th>RA BF ml/min</th>
<th>BA BF ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>V0</td>
<td>t = 0</td>
<td>93</td>
<td>6.5±0.9</td>
<td>10.0±0.8</td>
<td>89±45</td>
<td>128±60</td>
</tr>
<tr>
<td>V1</td>
<td>1d</td>
<td>42</td>
<td>6.0±1.0</td>
<td>10.5±0.7</td>
<td>676±340</td>
<td>1153±473</td>
</tr>
<tr>
<td>V2</td>
<td>1w</td>
<td>41</td>
<td>4.6±0.8</td>
<td>9.7±0.7</td>
<td>1573±540</td>
<td>1510±573</td>
</tr>
<tr>
<td>V3</td>
<td>4w</td>
<td>32</td>
<td>4.5±1.1</td>
<td>9.1±0.6</td>
<td>1178±540</td>
<td>1245±730</td>
</tr>
<tr>
<td>V4</td>
<td>&gt;8w</td>
<td>31</td>
<td>4.5±1.1</td>
<td>9.1±0.6</td>
<td>1650±629</td>
<td>1225±298</td>
</tr>
</tbody>
</table>

Data are mean±SD. All patients in V4 were on HD treatment. A large variability in both arterial size and BF was observed during VA maturation. As expected, larger changes in artery diameter were observed in distal than in proximal VA while BF increase was in proximal as compared to distal VA.

Conclusions: The clinical data set is currently used to calibrate the model and to simulate BF before and after surgery to investigate major determinants of BF in VA and to predict flow related VA complications.

FR-PO1934

Computational Model for Simulation of Vascular Adaptation Following Hemodialysis Vascular Access Surgery Andrea Remuzzi,1,2 Simone Manini,3 Katia Passera,1 Lorenzo Borti,2 Anna Caroli,3 Wouter Huberts,3 Luca Antiga,3 Mario Negri Institute, Bergamo, Italy; 4Univ. of Bergamo, Italy; 5Eindhoven Univ. of Technology, Netherlands.

Background: Up to 50% of surgical procedures for autologous vascular access (VA) in hemodialysis (HD) patients result in inadequate increase in blood flow volume (BFV). The required increase in BFV after arteriovenous anastomosis of native vessels depends on the ability of the vasculature to dilate and remodel. These changes ultimately determine VA and subsequent use for HD treatment. We have previously reported changes in real arterial (RA) diameter and BFV over time after end-to-end distal fistula creation in 28 ESRD patients. The aim of the present study was to use these data to develop and validate a 1-D computational model of arterial and venous circulation able to simulate changes in vessel diameter in response to surgically induced increase in BFV.

Methods: Blood vessel dimensions and elastic properties have been assumed according to a set of rules defined for generation of patient-specific vascular network models that are dependent on gender, age and body surface area. Arterial and venous diameters, as well as BF, have been calculated before and after VA creation by assuming constant peak wall shear stress in the arm vessels and in the arm vessels.

Results: The best comparison between experimental measurements and computed results of RA diameter and BFV during VA maturation was obtained for a reference arterial peak wall shear stress of 40 dynes/cm². These results are as follows.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>RA Diameter (mm)</th>
<th>RA Blood flow (ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t = 0</td>
<td>2.4</td>
<td>18</td>
</tr>
<tr>
<td>t = 10</td>
<td>3.7</td>
<td>32</td>
</tr>
<tr>
<td>t = 40</td>
<td>4.1</td>
<td>39</td>
</tr>
<tr>
<td>t = 100</td>
<td>4.4</td>
<td>43</td>
</tr>
</tbody>
</table>

Conclusions: The effect of temporal variation in WSS in the curved AVF, with an opposite interaction in the straight AVF. There was no apparent adverse effect of PRT-201 with respect to fibrosis, endothelialization, inflammation, and wound healing.

Conclusions: In comparison to vehicle, PRT-201 applied to the venous anastomosis and superficial vein immediately following AVG creation did not impair the safety of subsequent angioplasty.

Funding: Pharmaceutical Company Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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These results show that the computational model has the ability to accurately simulate flow in various vascular access types and predict the BVH increase in arterial and venous segments that develop with time after VA surgery.

Conclusions: The use of this modelling approach to simulate vascular changes responsible for VA maturation may allow more accurate planning of vascular surgery with the aim to ameliorate surgery outcomes and to increase the rate of VA maturation. 

Funding: Government Support - Non-U.S.

FR-PO1935
Hemodialysis Treatment Factors Associated with Outcomes of Arteriovenous Fistula: International Comparison between Facilities Using Data from the Dialysis Outcomes and Practice Patterns Study (DOPPS) (Manabu Asano,1 Jiyoth R. Thumma,2 Kenichichi Oguchi,3 Tadao Akizawa,4 Ronald L. Pisoni,5 Takashi Akiba,6 Kiyoshi Kurokawa,7 Akira Saito,8 Renal Unit, Bekami General Hospital, Tokyo, Japan; 1Arbor Research Collaborative for Health, Ann Arbor, MI; 2Division of Nephrology, Showa University, Tokyo, Japan; 3Tokyo Women’s Medical University, Tokyo, Japan; 4National Graduate Institute for Policy Studies, Tokyo, Japan; 5Tokai University School of Medicine, Isehara, Japan.

Background: Various factors have been supposed to associate with the fate of vascular access. Our objective was to clarify the relationship between the dialysis treatment factors and AVF patency across the three regions of Japan, North America (North Am) and Europe/Australia/New Zealand (EUR/ANZ).

Methods: Analyses included 1,288 incident hemodialysis patients on dialysis [1] ≤65 years (HR 1.27, 95%CI 1.03-1.57, final: HR 1.38, 95%CI 1.09-1.72). Facilities having higher median blood flow rate (mBFR) were seen to consistently display substantially higher rates of final AVF failure (HR=1.22, 95% CI 1.06-1.40). mBFR varied greatly by region: North Am (mBFR=400 mL/min; IQR=360-448), EUR/ANZ (mBFR=300 mL/min, IQR=300-343), Japan (mBFR= 200 mL/min, IQR=180-200). Analysis not adjusted for mBFR showed higher rate of primary/final AVF failure in North Am (primary: HR 2.14, 95%CI 1.47-3.12, final: HR 2.76, 95%CI 1.34-5.69) and EUR/ANZ (primary: HR 1.75, 95%CI 1.27-2.41, final: HR 2.13, 95%CI 1.13-4.03) relative to Japan.

Conclusions: Of all considered factors, first catheter use and median blood flow rate during hemodialysis only had a significant association with primary/final AVF failure.

FR-PO1936
Causes and Consequences of Arteriovenous Fistula Failure in Hemodialysis Patients: A Prospective Cohort Study Gurbey Ooak,1 Joris J. Rotmans,2 Carla Y. Vossen,3 Frits R. Roosendaal,4 Raymond T. Krediet,5 Elisabeth W. Boeschoten,6 Friedo W. Dekker,1 Marion Verduijn,1,3 Department of Clinical Epidemiology, Leiden University Medical Center; 2Department of Nephrology, Leiden University Medical Center; 3Department of Thrombosis and Haemostasis, Leiden University Medical Center; 4Department of Nephrology, Academic Medical Center; 5Hans Mak Institute Naarden, Netherlands.

Background: Arteriovenous access dysfunction is an important cause of morbidity in patients receiving hemodialysis treatment. The aim of this study was to investigate causes and consequences of loss of primary functional patency within two years in a large Dutch cohort of incident hemodialysis patients.

Methods: We followed 919 incident hemodialysis patients with a functional arteriovenous access, and calculated hazard ratios (HRs) for putative risk factors of primary functional patency using Cox regression. Furthermore, HRs were calculated using time-dependent Cox regression to study the effect of primary functional patency loss on two-year all-cause, cardiovascular (CV), and non-CV mortality.

Results: Age ≥65 years (HR 2.2, 95%CI 1.8-2.8), female sex (HR 1.3, 95%CI 1.1-1.6), CV disease (HR 1.9, 95%CI 1.5-2.4), diabetes mellitus (HR 1.7, 95%CI 1.3-2.1), prior catheter use (HR 1.6, 95%CI 1.3-1.9), albumin (lowest versus highest tertile, HR 1.4, 95%CI 1.1-1.8), high-sensitivity C-reactive protein (hsCRP) (highest versus lowest tertile; HR 1.7, 95%CI 1.2-2.6), and feticu-A (lowest versus highest tertile HR 2.6, 95%CI 1.7-4.1) were associated with primary functional patency loss after adjustment. Primary functional patency loss was associated with a 2.3-fold (95%CI 1.5–3.3) increased two-year all-cause mortality risk, an 1.6-fold (95%CI 0.9-3.0) increased two-year CV mortality risk, and a 3.1-fold (95%CI 1.7-5.8) two-year non-CV mortality risk after adjustment.

Conclusions: Increased age, female sex, cardiovascular disease, diabetes mellitus, prior catheter use, albumin, hsCRP, and fetuin-A were associated with primary functional patency loss. Primary functional patency loss appears a serious condition with a marked effect on survival.

Funding: Government Support - Non-U.S.

FR-PO1937
Hypercalcemia as a Risk Factor for Vascular Access Failure in Chronic Hemodialysis Patients Myung Jin Choi,1 Jong-Woo Yoon,1 Ja-Ryong Koo,1 Jung-Woo Noh,1 Eun Young No,1 Hyun Ok Kim,2 Man Gyu Lee.1 Department of Internal Medicine, Hallym Research Institute, College of Medicine, Hallym University, Chuncheon, Republic of Korea; 2Dialysis Center, Chuncheon Sacred Heart Hospital, Chuncheon, Republic of Korea.

Background: Chronic kidney disease-mineral bone disorder (CKD-MBD) is known to be an important risk factor for cardiovascular morbidity and mortality. However, it is not known whether vascular access outcomes in hemodialysis (HD) patients are associated with CKD-MBD. We evaluated the relationship between CKD-MBD and vascular access failure in HD patients.

Methods: Sixty patients (age 57±11 years, male 55%, diabetes 43%, arteriovenous fistula 88%, HD duration 59±50 months) who underwent HD for at least 3 months were enrolled. Vascular access failure was defined as thrombosis or hemodynamically significant stenosis requiring intervention. During 2 year follow-up period, the effects of each of components of CKD-MBD (serum phosphate, intact parathyroid hormone, calcium, phosphorus and quaterly measured intact parathyroid hormone) on vascular access failure were retrospectively evaluated using Cox proportional hazards regression analysis.

Results: During follow-up periods, the incidence of vascular access failure was 46.7% (n=28). Among components of CKD-MBD, only corrected calcium level was a significant risk factor for vascular access failure in univariate Cox analysis. Lower serum albumin, hemoglobin and higher predialysis systolic blood pressure (SBP) were also significant risk factors in univariate analysis. After adjustment for dialysis access type, diabetes, aspirin ingestion, serum albumin and predialysis SBP, corrected calcium (hazard ratio, 1.96; 95% confidence interval, 1.39-2.76), intact parathyroid hormone (93.72; 95% confidence interval, 1.03-1.7) and hemoglobin (hazard ratio, 0.472; 95% confidence interval, 0.230-0.967; p = 0.040) were significant independent risks of the development of vascular access failure.

Conclusions: Higher level of serum calcium was associated with higher incidence of vascular access failure. Chronic hypercalcemia in CKD-MBD might be a major risk factor of vascular access failure in HD patients.

FR-PO1938
Isometric Handgrip Exercises Improve Success Rates in Arteriovenous Fistula (AVF) Placement in Unsuitable Candidates Alice L. Uy,1 Christina M. Yuan,1 Rahul Jindal,1 Frank P. Hurst,1 Medicine (Nephrology), Walter Reed Army Medical Center, Washington, DC; Surgery (Organ Transplant), Walter Reed Army Medical Center, Washington, DC.

Background: Incident AVF rates remain low. AVF placement is often attempted because of small vein diameter. We postulated that isometric handgrip exercises would increase forearm vein diameter and allow successful AVF creation in patients who would otherwise receive a synthetic graft.

Methods: Adult subjects without prior vascular access (eGFR<25; cephalic vein < 2.5mm) were prospectively enrolled. They performed daily isometric handgrip exercises in the preferred access arm (EA), with the non-exercised arm (NEA) as control. Adherence was assessed by exercise logs and grip strength. Vein diameter was measured at baseline, 4 and 8 weeks by duplex ultrasound by the same technician. Primary endpoint was the mean increase in vein diameter (EA vs. NEA). Secondary endpoints were vein diameter increase from baseline, successful AVF placement and maturation.

Results: 16 subjects (7 male and 9 female, median age 77) were enrolled. At present, 11 completed the study. EA grip strength increased significantly. For the primary endpoint, mean vein increase in the EA vs. NEA in the distal and proximal veins were 0.31mm (p=0.255) and 0.44mm v. 0.66mm (p=0.614) respectively, with a greater increase in NEA. For the secondary endpoints, at 0 to 4 weeks all vein diameters increased significantly. Distal EA veins increased from 1.57mm to 2.14mm and proximal veins increased from 2.23mm to 2.91mm. In the NEA, distal veins increased from 1.58mm to 2.42mm and proximal veins increased from 2.24mm to 3.09mm. Over 90% (10 of 11) of patients achieved at least one vein diameter >2.5mm at 4 weeks. 3 patients received a successful placement of AVF.

Conclusions: Our data suggested that isometric handgrip exercises resulted in significant increase of cephalic vein diameter with 4 weeks of exercise, in both EA and NEA and may allow AVF creation in those previously not suitable for primary AVF. The views expressed are those of the authors and do not reflect the official policy of the Department of the Army, the Department of Defense, or the United States government.

Funding: Other U.S. Government Support

FR-PO1939
Transposed Brachio-Basilic Fistulae Have Long-Term Survival Comparable to Non-Transposed Arm Fistulae Renu Gupta,1 Peter Van,2 Neville R. Dossabahoy.

Background: Transposed brachio-basilic fistulae (TBBF) are not the first choice for all dialysis patients, but may be an attractive option in those patients who cannot get a radio-cephalic or brachio-cephalic fistula (non-transposed, NT). They are a better option than arteriovenous grafts in many situations. However, studies on transposed fistulae are limited. In this study we compared the maturation and survival of TBBF and NT fistulae.

Methods: Our prospective, computerized clinical database was queried retrospectively to identify the clinical outcomes of all upper extremity (UE) fistulae placed at our VA hospital during a 6-year period (2005-10) in CKD and ESRD patients. Patient demographics and comorbid conditions were noted. Primary end points of this study were failure of
Ipsilateral Dialysis Catheter Use Is Associated with Decreased Long-Term Vascular Access Survival

Impact of Past Peripherally Inserted Central Catheters on Prevalence of Functioning Arterio-Venous Fistula in an Outpatient Hemodialysis Unit – Single Center Study

Mireille El Ters, MD, Andrew D. Rule, MD, Bernice (Bonnie) M. Jenson, MD, Amy Mahon, MD, Sanjay Misra, MD, Robert C. Albright, MD, Amy W. Williams, MD, Sandra J. Taler, MD, Marie C. Hogan, MD

Background: Practice guidelines recommend native arteriovenous fistula (AVF) as the hemodialysis (HD) vascular access of choice. One barrier to successful AVF function is prior central or peripheral venous injury/thrombosis. The impact of prior peripherally inserted central catheter (PICC) on AVF patency among chronic hemodialysis patients has not been systematically examined.

Methods: Medical records of all end stage renal disease (ESRD) patients undergoing HD in the Mayo Clinic HD network and receiving their care in our medical system (n=2635) were examined. Baseline characteristics (age, gender, race, history of diabetes, congestive heart failure, peripheral vascular disease & coronary artery disease) & history of PICC line placement were obtained. Patients were considered if they did not have a functioning AVF & controls if they did. Those who had PICC placed after AVF creation were excluded. Likelihood of functioning AVF with past history of a PICC was assessed using logistic regression models, with & without adjustment for other clinical characteristics.

Results: Of 2635 ESRD patients, 90 did not have functioning AVF. Mean ± SD age was 69 ± 16, 57% were male, 86% caucasian, 56% diabetic, 24% had peripheral vascular disease, and 55% had coronary artery disease. History of PICC was present in 41% (37/90) of the ESRD patients without a functioning AVF compared to only 14% (16115) of the ESRD patients with a functioning AVF (p = 0.001). This association increased after adjustment for age, gender & race (OR 4.7, p = 0.001), and increased further with additional adjustment for DM, peripheral vascular disease, coronary artery disease & heart failure (OR = 5.5, p = 0.001).

Conclusions: Prior PICC placement is an important predictor of lack of functioning AVF even after adjustment for traditional risk factors. More studies are needed to determine whether this association is due to direct venous injury following PICC placement, as its use continues to grow due to perceived convenience and cost-effectiveness.

FR-PO1940

Clinical Determinants of Arteriovenous Fistula Patency, Primary Failure and Longevity in Hemodialysis Patients

Srikanth Kunaparaju, MD, Kambiz Kalantarinia, MD
Nephrology, University of Virginia, Charlottesville, VA.

Background: Arteriovenous fistulae (AVF) are considered the preferred vascular access (VA). Complications such as primary failure and thrombosis have been barriers in achieving higher AVF rates. In this study in addition to the effect of traditional risk factors, we looked at local factors such as the effect of hemodialysis facility or surgeons’ expertise on their outcomes.

Methods: Electronic Medical Records are used for collecting patient demographic, comorbidity and VA related data. The main outcomes studied were overall patency, primary failure (PF) and longevity of AVF. Univariate analyses and multivariate analysis in three different models was performed.

Results: From 695 VA, 428 AVF were studied. Mean age was 60.7 years, 35% females and 55% African American. Primary failure was 70%. The rate was higher for the expert surgeon (76% vs. 67%, p = 0.046) and for those with an AVF as their first VA ever (84% vs. 42%, p = 0.0001). In multivariate analysis, hypertension (OR 2.1, 95% CI 1.1 – 4.2), first VA ever (OR 1.13, 95% CI 1.9 – 8.84) and HD facility were associated with better outcomes. Longevity: Median was 16, longevity was lower for women (12.5 vs. 17.0, p = 0.05), those placed after HD initiation (20.8 vs. 33.8 mo., p = <0.0001), upper arm AVF (21.8 vs. 32.8 mo., p = 0.01), and those with at least one other HDVA compared to those with first HDVA (16.0 vs. 29.0 mo., p = <0.00001). In the multivariate analysis, gender and HD facility were the only associated factors.

Conclusions: Hypertension, first access ever, expert surgeon and HD facility were associated with higher AVF patency. For AVF PF, male gender and first access ever were significantly associated with better outcomes. First access ever, forearm location and HD facility were significantly associated with longer AVF life span. A history of multiple access surgeries was negatively associated with all three outcomes.

FR-PO1941

Ipsilateral Dialysis Catheter Use Is Associated with Decreased Long-Term Vascular Access Survival

Roman A. Shingarev

Department of Medicine, Division of Nephrology, University of Alabama at Birmingham, AL.

Background: Central venous catheters (CVC) are frequently used for hemodialysis (HD) access while patients await arteriovenous fistula (AVF) or graft (AVG) placement and maturation. CVC may result in central vein stenosis, which could adversely affect vascular access outcomes. We compared the vascular access outcomes in patients with an ipsilateral vs contralateral CVC in place.

Results: 180 UE fistulae were placed in the 6 year period: 81 were transposed (TBBF), 99 non-transposed (NT). Mean age at placement 63 years. African American 64%, Male 99%, Diabetics 61%, HTN 99%, CVD 54%. Patients were already ESRD in 83% cases. Failure of maturation was noted in 9.8% in TBBF and 18.8% in NT group (p = 0.11). Cumulative survival was 76% and 62% at 1 and 2 yrs for TBBF; and 69% and 55% respectively for NT. The figure shows the Kaplan-Meier survival curves for the two groups (p = 0.27).

Conclusions: Transposed brachial-basilic fistulae in our study had a overall survival that was comparable to non-transposed UE fistulae. This study confirms that TBBF remains a viable option in patients who have lost options for non-transposed fistulae.

FR-PO1942

Hemodialysis: Vascular Access - I

Poster/Friday

Methods: We retrospectively queried a prospective, computerized vascular access database to identify 322 patients who initiated HD with a CVC and had no prior access surgery. Of 233 pts who subsequently received an AVF, 69 had an ipsilateral CVC and 164 a contralateral CVC. Of 89 pts who received an AVG, 27 had an ipsilateral CVC and 62 a contralateral CVC. We calculated primary access failure and cumulative access survival for each subgroup.

Results: Among pts receiving an AVF, primary failure was similar in those with ipsilateral and contralateral CVC, but cumulative survival was inferior in those with an ipsilateral CVC (Table, Figure). Likewise, among pts receiving an AVG, primary failure was similar, but cumulative survival tended to be lower in those with ipsilateral CVC (Table).

Vascular access outcomes in pts with ipsilateral and contralateral CVC

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Ipsilateral CVC</th>
<th>Contralateral CVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>AVF prim fail</td>
<td>56%</td>
<td>53%</td>
</tr>
<tr>
<td>AVF cum surv</td>
<td>54% at 2 yr</td>
<td>74% at 2 yr</td>
</tr>
<tr>
<td>AVG prim fail</td>
<td>35%</td>
<td>38%</td>
</tr>
<tr>
<td>AVG cum surv</td>
<td>22% at 2 yr</td>
<td>58% at 2 yr</td>
</tr>
</tbody>
</table>

FR-PO1943

Conclusions: The primary failure rate of AVF and AVG is not affected by the presence of an ipsilateral CVC. However, cumulative AVF and AVG survival is inferior in pts with prior ipsilateral CVC. Avoidance of ipsilateral CVC may improve long-term vascular access survival.

Funding: Other NIH Support - NIH T32 DK007545-22 (Roman Shingarev)
Plasma 25-Vitamin D, 1,25-Vitamin D, Parathyroid Hormone (iPTH), and Fibroblast Growth Factor 23 (FGF23) Levels Do Not Predict Vascular Access Thrombosis Shailendra Sharma, Michel B. Chonchol, James S. Kaufman, Alfred K. Cheung, Jessica B. Kendrick. 1University of Colorado Denver; 1VA Boston Healthcare System, Boston; 1VASLCHCS, 1University of Utah.

Background: Assessment of risk factors for vascular access failure may provide insight into etiological targets for stenosis and thrombosis. Aim of the study was to evaluate the associations of abnormal mineral metabolism with vascular access thrombosis in patients requiring hemodialysis.

Methods: We investigate the longitudinal association between 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 levels with vascular access thrombosis in 654 patients requiring hemodialysis, aged 67 ± 12 years who participated in a randomized clinical trial evaluating the effects of folic acid and B vitamins on death in subjects requiring dialysis. Thrombosis events were collected only for vascular accesses that were actually being used for dialysis and excluded end stage events that occurred before dialysis initiation or that resulted in failure of access maturation. We used Cox proportional-hazards models to examine the association between abnormalities of mineral metabolism levels with vascular access thrombosis.

Results: Participants had a mean age of 60±11 years and median dialysis vintage of 1.4 years. Vascular access thrombosis occurred in 73 of the 336 patients (22%) with fistulae and 101 of the 221 patients (46%) with grafts at baseline. Increasing log for 1,25D levels 1.4 years. Vascular access thrombosis occurred in 73 of the 336 patients (22%) with fistulae; 101 of the 221 patients (46%) with grafts at baseline. Increasing log for 1,25D levels 1.4 years. In the first twelve months after fistula creation in our cohort.

Conclusions: In patients requiring hemodialysis, plasma levels of 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 were not independently associated with vascular access thrombosis.

Erythropoietin Use Is Associated with Increased and ACE-Inhibitor Use Is Associated with Decreased Risk for Failure of Primary Arteriovenous Hemodialysis Fistula Sylvia Stracke, Lena Hoven; Friedellinde Ernst, Christian Atzmanns, Frieder Keller. Nephrology, University Medicine Greifswald, Greifswald, Germany; Nephrology, University Hospital Ulm, Ulm, Germany.

Background: Hemodialysis treatment requires a well-functioning vascular access. Access failure is limited by the development of venous intimal hyperplasia, which predisposes to fistula stenosis and subsequent thrombosis. We prospectively followed 100 consecutive patients receiving a primary arteriovenous hemodialysis fistula.

Methods: At the time of fistula creation, a 5 mm segment of the future shunt vein was harvested and examined by histology and histomorphometry. Intima-to-media ratio (IMR) and intimal thickness indices (ITI) were documented. Clinical data were followed for at least one year.

Results: 31 patients showed histologically normal vessel walls (IMR 0.3 ± 0.0; ITI 0.3 ± 0.03). These patients were 70 ys old (range 24-85 yrs), 29% were females. The other 69 patients showed intimal hyperplasia with a significantly increased IMR (1.2 ± 1.0; ITI 0.35 ± 0.22; both p<0.001); median age was 67 ys (range 19-86 yrs); 29% females. Intimal hyperplasia was seen in 66% of lower arm veins and in 87% of upper arm veins. In 31 cases, stenoses that needed intervention were seen. Stenoses developed at a median of 9.7 mo after operation. Patency of fistulae was 60% after 6 mo, 57% after 9 mo and 56% after 12 mo. Intimal hyperplasia did not predict the development of stenosis, nor did the localization of fistula, sex, age, comorbidities, hypertension, diabetes, or serum calcium and phosphate levels influence the associations between log 1,25-vitamin D, land log FGF23 were not significant (p>0.05)

Conclusions: In patients requiring hemodialysis, plasma levels of 25-vitamin D, 1,25-vitamin D, iPTH and FGF23 were not independently associated with vascular access thrombosis.

Vein Mapping by Angiography: Findings That Prevent Arterio-Venous Fistula Maturation Zahidul H. Mondal, Candace D. Grant, Mary C. Mallappalli, Fasika M. Tedla, Moro O. Salifu. Internal Medicine, Division of Nephrology, SUNY HSCB Downstate, Brooklyn, NY.

Background: Vein mapping is prior to creation of Arterio-Venous Fistula (AVF) in hemodialysis (HD) patients. While ultrasound is sometimes used, it may not be sensitive to the presence of accessory veins and central vein (CV) stenosis that may interfere with AVF maturation. We analyzed results of preoperative vascular imaging with angiography (ANGIO) in 178 HD patients.

Methods: Bilateral ANGIO was performed on 178 patients using 15-20 ml of contrast by one of the nephrologists. Data were retrospectively abstracted to identify (i) luminal diameter (LD) and (ii) presence of accessory branches in right (R) and left (L) radiocephalic (RC), elbow-cephalic (EC) and elbow-basilic (EB) veins. Presence or absence of CV stenosis was also documented. Veins with LD ≤2.5mm were considered usable. Surgical comparisons were made with chi-square or t-test as appropriate.

Results: Mean age was 57±16[SD] years (range: 18-94), and HD vintage ranged from 0 to >5 years. 87% of patients were hypertensive; 53% were diabetic; 49% were men, and most were African American. 21/72 (18%) of RVC veins were usable and of these 7/21 (33%) were usable only after 9 months; on the L side, 27/78 (35%) were usable, of which 12 (41%) had branches. 42/78 (24%) of REC veins were usable, and 5/42 (12%) had branches; on the L, 39/78 (22%) were usable, while 6/39 (15.4%) had branches. Among the REB veins, 80/175 (45%) were usable, of which 5/80 (6.3%) had branches; on the L, 9/78 (5.4%) were usable, of which 3/9 (33.3%) had branches. CV stenosis was visualized in 66%, showing stenosis in 6% of patients. Diabetes was evenly distributed in all categories; patients with usable veins were significantly younger (mean age difference 6-12 years, p<0.05).

Conclusions: ANGIO detected branch-veins in 9/33-33% (average 13%) of usable veins, and central stenosis in 6% of patients—findings less likely to be detected with ultrasound. Usable veins were less likely to be identified in the forearm or in older individuals. These findings underscore the need for vein-preservation strategies in patients with chronic kidney disease.


Background: Having a functioning arterio-venous fistula (AVF) placed in a timely manner before commencement of haemodialysis (HD) is associated with reduction in patient morbidity, mortality and length of hospital stay. UK renal units are now focused on achieving better than 85% incidence of permanent vascular access rates in their HD population. At the renal unit in Sunderland, we have been using Far Infrared (FIR) therapy to improve our vascular access outcomes since December 2008.

Methods: FIR therapy involves a non-invasive 40 minute heat treatment onto the AVF area. This induces expression of endothelial Heme Oxygenase-1, reducing monocyte adhesion to the endothelial cells, thus improving flow and further endothelial injury. From July to December 2010, we used FIR therapy to help maintain AVF function by treating haematoma and pain on needling in our HD unit. We then extended our FIR therapy usage to the predialysis population, treating patients immediately after AVF formation. We assessed FIR therapy by Doppler ultrasound.

Results: In one 6 month period, from July to December 2010, 88 HD patients benefited from the use of FIR therapy. 257 individual sessions were completed (median FIR sessions 2, range 1-5).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
14/17 patients had improvement of pain score on AVF needling. 23/34 needle-site haematoma resolved more quickly and had improved pain scores compared with those not using FIR therapy.

15/20 AVFs matured with demonstrably better blood flow rates on Doppler, including patients with previous AVF maturation failure.

Conclusions: Currently, we are using FIR therapy across all our HD units as well as on the renal ward. We have instituted a twice weekly, nurse-led Heat Treatment clinic on our Ambulatory Care unit for all patients with new AVFs.

We have found FIR therapy to be of use in AVF maturation, particularly in patients with challenging access, as well as in treatment of haematoma formation and those with AVF pain during HD. We believe FIR therapy offers our patients improved access function, more comfortable treatment provision and decreased access-related morbidity and mortality.

FR-PO1948
Outcomes of Partial Aneurysmectomy in Managing Aneurysm-Associated Complications of Hemodialysis Arteriovenous Fistulae
Ammar Almehami, Shouwen Wang. Interventional Nephrology, University of Arizona- AKDHC, Phoenix, AZ.

Background: Fistula aneurysm formations are frequently encountered and their associated complications may affect the viability and function of dialysis vascular access. The key feature of aneurysm-associated complications is that the diseased tissues usually involve only part of the aneurysm, which provides a localized target for intervention. The aim of this study is to describe a targeted approach, “partial aneurysmectomy”, for treating these complications and to assess its effects on fistula patency rate.

Methods: This study included 36 dialysis patients who presented with aneurysm-associated complications. All procedures were performed under conscious sedation in an outpatient ambulatory surgery center. Partial aneurysmectomy involved the resection of the diseased skin and aneurysm wall followed by suture repair of the fistula wall and the overlying skin. Kaplan-Meier survival analysis was utilized to calculate the aneurysm intervention-free patency and fistula primary patency rates.

Results: Of the 36 patients: 20 (56%) were males, mean patient age was 54.5±14.6 years, 15 (42%) were diabetics, 35 (97%) were hypertensive, 26 (72%) had upper arm fistula and 10 (28%) had forearm fistula. The average age of the fistula was 72.8±39.5 months. The indications for partial aneurysmectomy were: active bleeding (14%), skin scab or necrosis with fistula defect in imminent danger of bleeding (33%), skin necrosis/erosion (28%), and thin-walled aneurysm in danger of rupture (25%). The procedure was successful in all cases and the patients continued dialysis therapy using their fistulae. The patency rates at 6 months were: 97% for the aneurysm intervention-free patency, 64% for the fistula primary patency, and 97% for the fistula primary-assisted patency.

Conclusions: Partial aneurysmectomy is a simple, safe, and effective intervention for managing aneurysm-associated complications and preserving dialysis fistulae. Further studies with long term follow up are needed to validate the results of this targeted approach.

FR-PO1949
Arterio-Venous Fistula Buttonhole Cannulation Technique: A Review of Infectious Complications
Frank J. O’Brien, Hong Kwan Kok, Peter J. Conlon. Department of Nephrology, Beaumont Hospital, Dublin, Ireland.

Background: There are two main methods of arterio-venous fistula (AVF) access; ‘buttonhole’ cannulation technique and ‘rope-ladder’ cannulation technique. Several small-scale international studies have hypothesized that the buttonhole technique is associated with increased rates of fistula infection. This study aims to examine this hypothesis.

Methods: A retrospective review of all patients attending a large out patient haemodialysis clinic was performed. Data was collected on cannulation modality, infection rates, organisms grown in the microbiology laboratory, complications of infection, and time on haemodialysis.

Results: 135 patients were noted to undergo haemodialysis via an AVF, 74 in the buttonhole group and 61 in the rope-ladder group. Nine episodes of clinically significant bacteraemia were noted in the buttonhole group.

FR-PO1950
Buttonhole Versus Sharp Needle Cannulation: Clinical Outcomes in a Home Haemodialysis Cohort
Christopher A. Muir, Sradha S. Kotwal, Carmel M. Hawley, Martin P. Gallagher, Paul Snelling, Meg J. Jardine. University of Sydney, Australia; The George Institute for Global Health, Sydney, Australia; Statewide Renal Services, Sydney, Australia; Princess Alexandra Hospital, Brisbane, Australia.

Background: Buttonhole (BH) cannulation is increasingly popular within home haemodialysis (HHD) programs, although recent reports have raised concern about associated infection rates.

Methods: A retrospective review was conducted of consecutive HHD patients dialysing via an arteriovenous fistula (AVF) from training commencement between 01/01/2003 and 31/12/2009 until cessation of HHD, death or 31/12/2010. Co-primary outcomes were systemic access infections (culture-positive infections attributed to dialysis access) and surgical access interventions (AVF surgical intervention or abandonment). Secondary outcomes included all access infections, initial HHD training time, and total staff time requirement (number of days of training, home visits and returns to the unit for dialysis).

Results: There were 35 access infections (9.30/1000 AVF months [AVFmths]), in 90 patients followed for 3765 AVFmths. BH was associated with a non-significantly higher rate of systemic access infections (IRR 2.71; 95% CI 0.66-11.99; p=0.17) and a significantly higher all-access infection rate (IRR 3.85 (1.60-12.77); p=0.03; absolute increase 9.6 (3.3-13.8) infections/1000AVFmths) compared with sharpneedle cannulation (SN). Surgical access interventions were not different for BH compared with SN (IRR 1.08; 95% CI 0.33-3.55; p=0.90). Adjustment for age and diabetes did not alter the results. BH was associated with longer initial training (mean 51 vs. 41 training days, p=0.01) and increased staff time requirements (1 encounter/13.2 days followup (BH) vs 1 encounter/19.0 days (SN), p<0.001).

Conclusions: In a single unit, BH cannulation was associated with increased rates of infectious events and prolonged initial and ongoing staff support compared with SN. There was no reduction in the requirement for surgical access interventions.

FR-PO1948
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Ammar Almehami, Shouwen Wang. Interventional Nephrology, University of Arizona- AKDHC, Phoenix, AZ.

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FR-PO1951
Multiple Specialists Perform Percutaneous Hemodialysis (HD) Access Interventions
Michael P. Lilly, Nancy Jean Carlson, Janet R. Lynch, Edwin D. Huff.1 Mid-Atlantic Renal Coalition (NW), Richmond, VA.2 Fistula First Breakthrough Initiative (FFBI) Data Committee, IPRO ESRD Network of NY, Lake Success, NY.

Background: Interventions to improve maturation or prolong the function of HD access are common. Interventional radiologists (IR) have traditionally done these services in hospitals. Recently, internists/interventional nephrologists (IN) and access surgeons (AS) have begun to perform these access salvage procedures. The extent of this shift in practice is not well documented.

Methods: We reviewed CY 2009 claims submitted to the Centers for Medicare & Medicaid Services (CMS) for access-related procedures done in NW5. We grouped practitioners by self-designated CMS specialty codes: AS (gen surg 2, thorac surg 33, vasc surg 76+77), IN (int med 11, nephrology 39) and IR (diagnostic radiol 30, interventional radiol 94). We grouped procedures by CPT code: fistulography (36145), percutaneous (percut) interventions (angioplasty 35476, G0392, G0393) and percut thrombectomy (36870), tunneled catheter (HD cath) placement (36558), HD cath exchange (36851), AV or AVG construction (36818, 36819, 36820, 36825, 36830) and open thrombectomy/revision (36831, 36832, 36833, 36834, 37607).

Results: Fistulograms and percut interventions accounted for 67.1% of procedures. IR performed the greatest share of these procedures, but 54% were performed by AS and IN. AS did nearly all open procedures and placed the most of HD caths, while IR or IN did most HD cath exchanges.

Conclusions: Changing practice patterns influence the methods used to modify practice behavior. FFBI has focused its efforts on AVF construction on surgeons who still perform nearly all such procedures. Efforts to enhance AVF maturation rates and prolong AVF function must be directed to a broader range of physicians.

Funding: Other U.S. Government Support

FR-PO1952
Cephalic Vein Arch Stenosis with and without Stent Placement in Hemodialysis Patients Ramanath B. Dukkipati,1 Luani Lee,1 Nancy Jean Carlson,2 Janet R. Lynch,1 Kaymar Kalantar-Zadeh,1 Naveen K. Attry.1 1Nephrology, Harbor-UCLA Medical Center, Torrance, CA; 2Nephrology, Capital Nephrology Associates, Sacramento, CA.

Background: Cephalic vein arch (CVA) stenosis is a common feature leading to dysfunction and/or failure of brachial artery to cephalic vein fistula. The value and timing of percutaneous transluminal angioplasty (PTA) versus placement of a stent in management of CVA stenosis is not known.

Methods: We examined the risk of recurrence of CVA stenosis after PTA or after stent placement determined by angiography of the involved upper extremity over time in a contemporary cohort of MHD patients treated from March 2008 through May 2011.

Results: We retrospectively identified 45 MHD patients with evidence of CVA stenosis on elective angiograms. The median number of days until another PTA was required decreased (see chart). An association was found between the number of angioplasties and a decreasing median number of days between each subsequent PTA. However, the median number of days between stent placement and the subsequent PTA was much greater (152 days) than the median number of days between the first two angioplasties of a patient who did not have a stent placed. An association was found between increased patency and stent placement. Of the 20 patients who had stents placed, the mean number of angioplasties required after stent placement was 0.75. Of the 25 patients who did not have a stent placed, the mean number of total angioplasties required was 2.76. An association was found between stent placement and a decreased number of angioplasties required.

Conclusions: PTA seems to hasten the recurrence of CVA stenosis. Compared to PTA alone the placement of intravascular stent shows a trend towards a prolonged patency of the CVA. Clinical trials with a larger sample size will better elucidate the value and timing of PTA vs. a stent placement in CVA stenosis.

Funding: Clinical Revenue Support

FR-PO1953
Cardiac Rhythm Devices and Arteriovenous Access Dysfunction in Hemodialyzed Patients: Experience in a Single Urban Vascular Access Center
Brinda Desiraju, Neal Mittman. Brooklyn Vascular Access, Heights Nephrology Medical Group, Brooklyn, NY.

Background: Recent attention has been focused on the incidence of hemaaccess-related complications with cardiac rhythm devices (CRD) in hemodialyzed pts. Cardiac co-morbidities are common, and the use of CRD in this population has increased in recent years. The use of CRD in these patients is generally recommended, but the concomitant use of central venous dialysis catheters (CVC) and the presence of unrecognized or developing central venous stenosis and occlusion can complicate this option.

Methods: Retrospective chart review identified forty-three patients with arteriovenous hemodialysis (AVHD) device-related interventions (CRD) referred to our access center for evaluation.

Results: Reasons for referral included arm swelling in 38%, increased venous pressure 14%, thrombosed access 14%, decreased access flows 8%, and prolonged post-dialysis bleeding 6%. Thirty pts had AV fistulae (14 forearm, 16 upper arm) and twenty had AV grafts (5 forearm, 15 upper arm), seven with a second access after prior access losses. CRD were ipsilateral in 21 pts, all of whom had angiographic evidence of central vein stenosis or occlusion, and 71% were symptomatic (arm swelling). Four with ipsilateral fistula received contralateral access, of which two had recurrent arm swelling. CRD were contralateral in 29 pts, of whom 23 (79%) had central vein stenosis or occlusion. Of these 23, 30% had arm swelling, 39% had prolonged bleeding or high venous pressures, and 22% presented with access thrombosis. Arm swelling was more common in pts with upper arm accesses. Fourteen pts had concomitant CVC during a visit.

Conclusions: Pts with ipsilateral CRD are at greater risk of developing symptomatic central vein stenosis. However, a substantial number of pts with contralateral CRD developed symptoms requiring intervention and even access failure. Alternative approaches, including the use of epicardial leads, subcutaneous implantable cardioverter-defibrillators or percutaneous dialysis catheters may be more appropriate, but a re-evaluation of the risk-benefit ratio and indications for CRD in this population is in order.

Funding: Clinical Revenue Support

FR-PO1954
Pacemakers & Implantable Cardioverter-Defibrillators in Hemodialysis Patients: Prevalence and Relationship to Isipletal and Contralateral Arteriovenous Access Interventions
Theodore F. Saad, Nephrology, Christiana Care Health System, Newark, DE.

Background: Cardiac rhythm management devices (CRMD), including pacemakers & implantable cardioverter-defibrillators are commonly used for treatment of rhythm disorders in HD patients. CRMD leads may induce central vein stenosis resulting in venous hypertension with ipsilateral arteriovenous (AV) access.

Methods: We surveyed all chronic HD patients in our practice & retrospectively reviewed procedure records to determine the presence of CRMD and the effect of CRMD leads on intervention rates. The study cohort included 1233 HD patients from Jan-Mar, 2011. Data collected included demographics, type & side of AV access & CRMD, dates of access interventions, & sites of lesions treated in the access circuit. Interventions were classified as being performed anywhere in the access circuit & in central veins related to CRMD leads. Rates of interventions were calculated by dividing the number of interventions by the number of access-years (AY) using the current AV access & CRMD.

Results: HD access was a fistula in 767 (62.1%); graft in 271 (21.9%); catheter in 195 (15.8%). 127 patients had a CRMD; ICD’s in 69 (5.6%) and pacemakers in 58 (4.7%). Mean age of patients with a CRMD was 72 years, 79 male and 48 female. All CRMD leads were inserted via subclavian or cephalic vein; there were no jugular, femoral, or cephalic leads. 93/127 (73.2%) had left-sided & 34/127 (26.8%) had right-sided leads. Six patients with contralateral access and CRMD had failed previous ipsilateral access due to CRMD-related stenosis. No patient with either ipsilateral or contralateral access was found to have significant SVC stenosis. Rates of interventions are shown in the table:

<table>
<thead>
<tr>
<th>Numbers</th>
<th>Contralateral</th>
<th>Ipsilateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>127</td>
<td>127</td>
</tr>
<tr>
<td>Total Access Years</td>
<td>133.7</td>
<td>109.5</td>
</tr>
<tr>
<td>Access Circuit Interventions</td>
<td>204 (1.50/AY)</td>
<td>168 (1.53/AY)</td>
</tr>
<tr>
<td>CRMD-led related interventions</td>
<td>86 (0.60/AY)</td>
<td></td>
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</tbody>
</table>

Conclusions: Prevalence of CRMDs in these chronic HD patients is 10.3%. Access circuit intervention rates were similar for CRMD ipsilateral and contralateral to the AV access; CRMD-led related interventions were more frequent in the ipsilateral group. SVC stenosis was not demonstrated in any patient.

FR-PO1955
Mathematical Model Explains Why DRL Procedure Is Effective in Treating Hand Ischemia in Brachiocephalic Fistula
William D. Paulson,1 James J. Wynn,1 Jan Malik,1 Vladimir Tuka,2 Tushar J. Vachharajani,2 Todd D. Merchen,1 Steven J. Jones,3 1George Mason Univ., VA; 2Georgia Hosp. Sci. Univ., Augusta, GA; 3Charles Univ., Prague, Czech Republic; 4Wright State Univ., School of Med., Winston-Salem, NC; 5Louisiana Tech Univ., Ruston, LA.

Background: The high flow brachiocephalic fistula (AVF) may be complicated by hand ischemia. The DRL procedure increases blood flow to the hand by anastomosing a bypass graft to the brachial artery above and below the AVF anastomosis; the brachial...
artery distal to the AVF is ligated. Although it is widely accepted that DRIL is effective, there is no consensus on its mechanism.

**Methods:** We used a mathematical model of the AVF to study effect of DRIL on hand arterial pressure and blood flow. Equations from the engineering literature were used to predict circuit pressures and flow.

**Results:** The AVF is a high flow shunt with a large drop in pressure by the time brachial artery flow reaches the AVF. This pressure drop reduces hand flow. In the DRIL, the proximal anastomosis of the bypass graft is placed upstream to the AVF where pressure is higher than at the AVF. This higher pressure is transmitted to the hand, which increases hand flow. The narrower the brachial artery, the greater the pressure drop in the artery; this effect is offset by placing the proximal bypass anastomosis further upstream where pressure is higher. The bypass graft and hand are part of a high resistance circuit that is controlled by arteriolar resistances. Pressure is high in this circuit with a minimal drop until the arterioles are crossed.

**Conclusions:** The DRIL increases hand blood flow by disconnecting the hand from the low pressure AVF circuit and connecting it to the proximal brachial artery where pressures are higher. The model explains why the DRIL is effective, and shows how to optimize application of the DRIL in individual patients with hand ischemia.

**FR-PO1956**

**Prolonged Cumulative Survival in Fistulas with Surgical Interventions To Promote Maturation Compared to Endovascular Interventions**

**Timmy C. Lee,** 1,2 Arshdeep Tindni, 1 Cincinnati, OH.

**Background:** Due to high primary failure rates, arteriovenous fistulas (AVF) frequently require either endovascular or surgical interventions to promote maturation (defined as the ability of an AVF to support hemodialysis). The objective of this study was to compare the impact of surgical versus endovascular interventions to promote AVF maturation on cumulative AVF survival.

**Methods:** We evaluated 89 patients with new AVF placement from a Veterans Affairs patient population. We calculated and compared cumulative survival between AVFs requiring no intervention, and surgery or endovascular intervention to promote AVF maturation.

**Results:** Among the 89 patients with new AVF placement, 46 AVFs required intervention (31 surgical and 15 endovascular). One year cumulative survival was 86% vs 83% vs 40% for no intervention vs. surgery vs. angioplasty, respectively. Cumulative survival was worse in angioplasty group compared to the no intervention and surgery groups (p=0.03).

**Conclusions:** In AVFs that required interventions to promote maturation, AVFs with surgical intervention had longer cumulative survival compared to AVFs treated with endovascular intervention. AVFs with surgical intervention to promote maturation had similar one-year cumulative survival to those AVF that did not require intervention to promote maturation. Our results emphasize the huge need in this area for hard scientific data (from large clinical trials evaluating biology of vascular injury and vascular access outcomes) that could be used to guide clinical practice.

**Funding:** NIDDK Support

**FR-PO1957**

**Reversible Endovascular Occlusion with Thermo-Sensible Polymer LeGoo® in Arterovenous Fistula Construction**

**Roberto Palumbo,** 1 Michele Ferrannini, 1 Annalisa Noce, 2 Simone Manca di Villahermosa, 2 Fulvio Fiorini, 3 Mariapaola Canale, 4 Nicola Di Daniele, 2 *Nephrology and Dialysis Department, S. Eugenio Hospital, Rome, Italy; 2Nephrology and Dialysis Unit, Tor Vergata University, Rome, Italy; 3Nephrology and Dialysis Unit, S. Maria della Misericordia Hospital, Rovigo, Italy.

**Background:** The temporary interruption of blood flow, using clamps or rubber loops stretching or kinking vessels, is necessary in the construction of arterovenous fistula (AVF). However the vascular trauma may jeopardize the outcome of the procedure. LeGoo® (Pluromed, Woburn, MA), a thermosensitive polymer, is a viscous liquid at room temperature and changes into a firm occlusive plug instantaneously when exposed to cold saline. We report five cases of AVF construction using LeGoo® to temporary interrupt the arterial and vein blood flow.

**Methods:** Five uremic patients (4 male, 1 female) were submitted to AVF construction.

**Results:** LeGoo® were employed to occlude the vein and the arteries without clamps or loops. After completing the anastomosis, we employed cold saline on the outer vascular wall to make fluid the polymer.

**Conclusions:** We obtained a rapid, safe and prolonged emostasis. LeGoo® application is simple and effective. In all cases, the application of cold saline on the outer vascular wall just after completing the anastomosis, made the polymer fluid and promptly cleared by the re-established blood flow.

**FR-PO1958**

**Pilot Study Evaluating the Safety and Efficacy of Drug Eluting Balloons in the Treatment of Recurring Shunt Stenosis**

**Deborah Weiss,** 1 Beata Lux, 1 Birgit Doris Bader, 1 Christiane M. Erley, 1 *Nephrology, St. Joseph Hospital, Berlin, Germany; 2Radiology, St. Joseph Hospital, Berlin, Germany.

**Background:** With a rising number of HD-patients of an continually increasing age, the percutaneous transulminial angioplasty (PTA) as a treatment option for insufficient dialysis shunts is gaining importance. Though many PTAs are successful, there remain patients, who suffer from a recurring shunt stenosis. Drug eluting balloons (DEB) could provide a feasible alternative to the standard balloons; even more so since the use of drug

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

*Underline represents presenting author.*

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eluting stents in these venous vessels have been considered too problematic by most interventionalists.

Methods: We undertook a pilot study applying new drug eluting balloons (IN.PACT Admiral–DEB coated with paclitaxel, made by KRAUTH cardiovascular GmbH) in patients with recurring stent stenosis

Results: 1 patients with recurring stent stenosis were treated with the DEB between 9/2009-12/2009. 1 patient had an AV-fistula, 2 a fistula which had already been corrected by the implantation of a short synthetic graft, and 8 had always had a synthetic graft. All patients had a type III-stenosis, in addition to that 6 patients a type II-stenosis and two patients had a type I-stenosis and a type III-stenosis, respectively. Apart from i., patients on long term phenprocoumon all patients were on acetylsalicylic acid, 2 patients received clopidogrel on top, 1 patient phenprocoumon on top and 1 patient switched to ASS/Dipiridamol. 9 patients underwent a further PTA during the next 4 months. Only one re-stenosis seemed to have developed more slowly. We found, though, that not during all the procedures the special positioning device protecting the paclitaxel-coat had been used. In the following two years, no apparent increase of restenosis rate, slant aneurysm or other-possibly procedure related- adverse events have been observed. 2 patients died due to unrelated causes (1 breast cancer, 1 myocardial infarction).

Conclusions: The use of DEB during PTA in stenosis is not primarily associated with a higher rate of complications. The benefit for the patients, especially the prevention of recurring stent stenosis by using these balloons should be the aim of further investigations.

Funding: Pharmaceutical Company Support

FR-PO1959

A New Prosthetic Graft (RAPIDAX™) for Arteriovenous Access and Early Cannulation: Initial Experience

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Background: The AV prosthetic graft is indicated whenever there is a recurrent failure of the native access or the venous bed is not suitable. The main advantage in the use of vascular prosthetic graft is the faster healing time compared to a native fistula, an average of 2-3 weeks. In particular, the new ePTFE graft (RapidaxTM), Vascutek-Terumo, Renfrewshire, Scotland, UK) thanks to its trilaminar configuration with an elastomer, allows an early cannulation (until 24 hours) and reduces time of bleeding, limiting handbath use.

Methods: In the Division of Clinical Nephrology of Verona, a vascular access with RapidaxTM graft was carried out in seven patients. The graft efficacy, patency and post-operative complications were evaluated. Six-month to eighteen-month follow-up data are presented.

Results: In six patients successful first cannulation was achieved within 48 hours, with a mean of 21.7 hours; just one graft was used after 1 week, because the patient already had a HD catheter. At six months graft patency was 100% in all patients. The primary efficacy was evaluated by the eKt/V at three months: mean value was 1.41; at six month 1.58. The mean blood flow at first use was 200 ml/min, at one month 300 ml/min. Time of bleeding was normal. Only one patient had an acute thrombosis 2 hours after graft implantation. Although the small number of patients, no pseudoaneurysm or loss of graft occurred, even in the one case at eighteen-month follow-up.

Conclusions: this kind of graft permits an early cannulation, reducing the use of HD catheter, preventing also pseudoaneurysm and seroma formation. New ePTFE graft can be a good alternative to long term HD catheter in patients who cannot receive an autologous graft.

FR-PO1960

The Safety and Efficacy of Bedside Tunneled Catheter Removal by Physician-in-Training in an Academic Teaching Setting

Vikram P. Beemdi,1 Naseem A. Qureshi,1 Albert W. Dreisbach,1 Eva Csongradi,2,3 Luis A. Juncos,1 Tibor Fulop,1 1Department of Medicine, University of Mississippi Medical Center, Jackson, MS; 2Department of Medicine, 1st University of Debrecen, Hungary; 3Department of Physiology & Biophysics, University of Mississippi Medical Center, Jackson, MS.

Background: Ancedotal experience suggests that procedural aspects of Nephrology training may be endangered. Safety and efficacy of bedside removal of tunneled dialysis catheters is relatively little studied in a purely training setting.

Methods: We performed a retrospective cohort review of our consecutive 3-year experience (01/2007 - 12/2009) with bedside TDC removal at the University of Mississippi Renal Fellowship Program. We collected data on patients and procedure-related variables, success and complications rates. The study was reviewed and approved by the University of Mississippi Human Research Office. Data was analyzed with PASW Statistics 18.

Results: During the index period, we had 55 inpatien TDC removals at bedside under supervision in our teaching log. Of these, 50 (90.9%) were accomplished by Nephrology Fellows with Attending’s supervision and the rest of them with Attending/Medical Resident team. 36 (65.5%) TDC removal was from right internal jugular (IJ), 14 (25.5%) from left IJ, and 5 (9%) from femoral vein location. Indication at the time of removal included bleeding, controlled with local pressure.

Conclusions: Our results suggest that bedside removal of TDC is a safe and effective procedure and should be part of competent Nephrology Fellowship Training.

Funding: Clinical Revenue Support

FR-PO1961

Physical Examination of Dysfunctional Arteriovenous Fistulae by Non-Interventionalists: A Skill Worth Teaching

Luis Coentrao,1 Manuel Pestana,1 Nephrology Research and Development Unit, Hospital S. Joao, Faculty of Medicine University of Porto, Portugal; 2Nephrology Department, Hospital S. Teotópio, Viseu.

Background: Physical examination of arteriovenous fistulae (AVF) has recently emerged as an important element in the detection of stenotic lesions. This study examines the accuracy of general and nephrology residents’ physical examination in the assessment of AVF dysfunction by non-interventionalists, in comparison with angiography.

Methods: A prospective observational blinded study was performed among 177 haemodialysis patients with AVF dysfunction consecutively referred to our centre for an angiography procedure. Eleven referring general nephrologists completed a form reporting the physical examination findings regarding their patients’ AVFs. Before angiography examination was carried out, a trained nephrology resident performed a physical examination in all the cases. Angiography of the AVFs was then performed by an interventionalist. Cohen’s k value was used as the measurement of the level of agreement beyond chance between the diagnosis made on physical examination and angiography.

Results: There was a moderate agreement beyond chance between the general nephrologists’ physical examination and angiography in the detection of AVF inflow problems (k = 0.49), outflow problems (k = 0.58) and thrombosis (k = 0.52). On the other hand, physical examination performed by the trained nephrologist resident strongly agreed with angiography in the detection of AVF inflow problems (k = 0.84), outflow problems (k = 0.92), and thrombosis (k = 0.98). The agreement between physical examination and angiography in the detection of coexisting AVF inflow–outflow problems was poor for the general nephrologists and moderate for the trained nephrology resident (k = 0.14 vs. k = 0.55, respectively).

Conclusions: Physical examination may provide an accurate means of diagnosis of AVF dysfunction. Theoretical and hands-on training in physical examination of dysfunctional AVFs should be provided for nephrologists-in-training and for the dialysis staff.

Funding: Government Support - Non-U.S.

FR-PO1962

Utility of Non-Invasive Arteriovenous Access Monitoring in Children

E Ashoor, Elizabeth Hughson, Michael J. Somers. Nephrology, Children’s Hospital Boston and Harvard Medical School, Boston, MA.

Background: Limited data exist regarding the use and efficacy of ultrasound dilution monitoring (UDM) as a technique to assess arteriovenous (AV) access patency and reduce AV access complications in children.

Methods: We studied all permanent AV access pts in our pediatric dialysis unit by monthly UDM between 2009-2011. Fistulagrams were prompted by UDM for flow rate ≤600 ml/min or >20% drop from baseline. Rates of AV access complication (thrombosis, hospitalization, temporary access placement) during the UDM period were compared to baseline rates manifested in the 2 years prior. To account for differences in length of time on HD, rates were determined for individual pts in both groups based on episode count relative to months on HD.

Results: No differences existed between the UDM (n=16) and baseline (n=14) groups with regards to age, gender, weight or age at ERSD diagnosis. In the UDM group (50% higher confidence rate at ERSD diagnosis 15 yo; median weight 51 kg; median AV access age 12 mos), 162 UD measurements were obtained over median follow-up of 5 mos (range 1-29 mos). Rates of access-related complications (hospitalization, new access formation, or temporary access placement) fell from 4 events/100 pt-mos at baseline to 2.5/100 pt-mos with interventionist at rate of thromboses in the UDM group (3.5/100 pt-mos on HD) was significantly lower than the baseline group (13.5/100 pt-mos on HD) (p<0.04). With the UDM group, mean blood flow rate was lower in AV accesses that went on thrombosis compared to those without thrombosis (1203 ml/min/1.73m2 vs. 1683, p<0.0001). In the UDM group, 29 fistulagrams were done, 18 prompted by decreased UDM flow. 55% had hemodynamically significant stenoses requiring angioplasty +/- stenting. UDM was 94% sensitive and 77% specific in detecting hemodynamically significant stenoses with positive and negative predictive values of 83% and 91% respectively.

Conclusions: We conclude that in children on dialysis UDM: 1) reduces adverse outcomes requiring emergent intervention; 2) detects evolving hemodynamically significant stenoses; 3) reduces thrombosis rates in permanent AV access; and has high sensitivity and predictive values as a screening tool.

Funding: Clinical Revenue Support

FR-PO1963

Mapping Segmental AVF Pressure & Resistance with the BlueDop Imager

David H. King,1 Graeme Taylor, Mo Al-Qaisi, Sumith C. Abeygunasekara,1 Anthony Chan,2 Yiannis Panayiotopoulos,1 Abdelgalil Abdelrahman Ali,1 1Renal Unit, Broomfield Hospital, Chelmsford, Essex, United Kingdom; 2Department of Physics, Guy’s and St Thomas Hospital Trust, London, United Kingdom; 3Department of Surgery, Broomfield Hospital, Chelmsford, Essex, United Kingdom.

Background: A novel device ‘BlueDop Imager’ uses an IP protected method to estimate mean arterial pressure at multiple locations within the body.

Methods: Data values are derived from the incident pressure and resultant blood flow wave. An algorithm gives mean pressure.Incident data is derived from an arm BP.

Funding: Clinical Revenue Support
Fluid resistance per centimetre of blood vessel can be computed non invasively. Pressures: A previously unsuspected vasoactive venous control loop has been identified: may be a confounding factor, principally affecting the correlation coefficient at low pressures: A previously unsuspected vasoactive venous control loop has been identified: Fluid resistance per centimetre of blood vessel possible. Pressure gradients in AFV: Video imaging of probe position makes fluid resistance per centimetre of blood vessel possible. The resultant flow wave is displayed on a PC which processes Bluetooth audio transmitted from the Doppler probe and video from a webcam.

**FR-PO1964**

**Doppler Smart-Sensor for Vascular Access Monitoring**

**Wiliam Weitzel,**
Grant H. Kruger, Brian Thelen, Benjamin Walter Kozioi, Benjamin Carpenter, Mainak Mitra, Leo Kozioi, David E. Conway, Christopher R. Diroff, Andre Preston, Doug Swaney, Dae Woo Park, Robert Dodde.

**Aim:** Correlate venous needle pressure(pump off) with BlueDop pressure. Analyse pressure gradients in AFV. Video imaging of probe position makes fluid resistance per centimetre of blood vessel possible.

**Results:** After nominal correction for CVP(6mmHg), the following pressures were obtained from 14 AVF patients: Needle = 38.4±14.8mmHg, BlueDop = 37.4±9.9mmHg, corr coeff = 0.48. Pressure gradients recorded from 41 AVF (49 gradients), indicate an axillary artery pressure minimum of 40mmHg, (Fig 1a) KEY: failing AVF-black arrow; narrowed AFV-grey, normal AFV-white (true pressure = 40+CVP). This may be the first indication of a control loop mediated by the central venous system, effectively protecting the truncal arteries from ischaemia. (Fig 1b) shows probe position plus pressure image. A single flow entry can generate a resistance map.

**Conclusions:** Averaged BlueDop and direct needle pressure data agree. Labile CVP may be a confounding factor, principally affecting the correlation coefficient at low pressures: A previously unsuspected vasoactive venous control loop has been identified: Fluid resistance per centimetre of blood vessel possible.

**FR-PO1965**

**The Importance of the Presence of Reverse Color Flow and Collapsibility in Doppler Ultrasound Examination To Evaluate Central Venous Stenosis**

Hoon Suk Park, Yu Ah Hong, Sun Ryong Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Young Ok Kim, Cheol Whee Park, Chul Woo Yang, Yong-Soo Kim.

**Background:** The absence of variation in venous diameter or flow pattern during respiration in doppler ultrasound(DU) examination is known as a useful clue to predict central venous stenosis(CVS). However, the variation in venous diameter or flow pattern during respiration may exist in the cases of CVS. There may be the findings that subclavian vein isn’t collapsed when pressed with the probe or the significant amount of reverse color flow exists during expiration in the cases of CVS. We performed this study how exactly DU detect CVS with these additional findings.

**Methods:** Flow patterns in the both pulsewave and color modes, collapsibility at distal subclavian vein in 2D mode were analyzed in 28 patients. Sixteen patients on chronic hemodialysis underwent subsequently subtraction angiography for the evaluation of CVS while the other 12 patients underwent subsequently conventional venography for the creation of access. We define the case with CVS in DU examination that satisfies all of the findings including the variations in venous diameter and flow pattern exist during respiration, venous collapse exists when pressed with the probe and the significant amount of reverse color flow during respiration doesn’t exist. The image findings between DU and subtraction angiography or conventional venography were compared.

**Results:** Kappa between DU and angiography was 0.593(p=0.001). Positive predictive value was 53%, negative predictive value was 100%, sensitivity was 100% and specificity was 77%.

**Conclusions:** DU examination with our additional criteria showed favorable power to screen the cases with possibility of CVS. Therefore, DU can screen asymptomatic patients on hemodialysis with CVS and also detect CVS more efficiently if used with venography prior to access creation.

**FR-PO1966**

**Using Effective Ionic Dialysance/Blood Flow Rate Ratio To Detect Access Recirculation in Hemodialysis Catheters**


**Aims:** To investigate this hypothesis we developed a low-cost, low-profile, compact Doppler smart-sensor system with scalable modular hardware and software architecture that can be integrated into the dialysis clinic for continuous patient monitoring. Laboratory testing was conducted using a pulsatile pump (simulating patient access flow) and blood mimicking fluid passed through phantoms with mock dialysis pump to assess the spectral velocity profile for linearity and determine optimal digital signal processing strategies for access testing was conducted using a pulsatile pump (simulating patient access flow) and blood mimicking fluid passed through phantoms with mock dialysis pump to assess the spectral velocity profile for linearity and determine optimal digital signal processing strategies for access flow ranges from 0 to 2000 ml/min. The prototype was evaluated clinically for performance using indicator dilution measurements as reference measurements.

**Results:** Laboratory testing showed the Doppler smart-sensor velocities were linearly related to the vascular access phantom volume flow with an R-squared of 0.988 (p<0.01). Clinical comparison of the optimized Doppler digital signal processing model with indicator dilution measurements was correlated with an R-squared of 0.91 (p<0.01).

**Conclusions:** We conclude it is feasible to obtain accurate flow measurements in the laboratory and clinical setting using this Doppler smart-sensor. Functional regression data analysis is in progress preparing to test this device and monitoring strategy in an upcoming prospective study.

**Funding:** Other NIH Support - NIH RC1 HL101881

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
Conclusions: Our data show the usefulness of the EID/Qb ratio as an indicator of sAR in dialysis catheters and identifies the practical threshold value for detecting sAR to be an EID/Qb of <60%.

FR-PO1967

Validation of a New Fistula (AVF) Monitoring System by Time Pattern Data Analysis Compared to the Ultrasonic Dilution Method (US) in Hemodialysis Patients (HD Pts) Massimo Adorati Menegato, San Antonio’s Hospital, San Daniele, Italy.

Background: Evaluation of vascular access blood flow (Qb) by US represents the gold standard for AVF malfunctions. Unfortunately, US requires sufficient distance between the needles, a dedicated disposable, is time consuming and has a not well-defined Qa threshold. We compared the accuracy of AVF monitoring by US and a new tool (VCtrl monitoring), by measuring the time pattern of parameters, such as arterial and venous pressures (AP, VP), idy, blood flow (Qb) and Hb.

Methods: We prospectively evaluated 21 HD pts (age 72+18 yrs) with native AVF (thrice-weekly dialysis schedule) for 17 months. The VCtrl approach is based on a supervised learning of a Dynamic Bayesian Networks (DBIN) for classifying the dialysis sessions according to a medical risk score (0=good, 1=alert, 2=failed). The DBIN system, evaluating the dialytic parameters, calculated a score of AVF status. US test was performed monthly (score 0 Qa1000, 1 1000>Qa>600, 2 Qa500ml/min).

Results: We assessed 223 AVF monitoring by both US and VCtrl. A significant correlation between VCtrl scores and Qa was found and three different Qa cluster were identified (570±250 vs. 855±196 vs. 1163±50 ml/min, p<0.05 fig.1).

Sixteen angiographies were performed in 12 pts classified by a US score 2, showing 9 stenosis in 8 pts. VCtrl predicted, considering as positive score 1≤ AVF stenosis in 3/9 resulting in a sensitivity of 33.3% and a specificity of 90.4% (Chi² test, p=0.023), while US showed a sensitivity of 100% and a specificity of 71.7% (p<0.00005).

Conclusions: US monitoring system confirmed its own validity in AVF stenosis diagnosis, but it requires monthly evaluation and presents a relatively low specificity, leading in unsuitable angiography prescription. VCtrl seems to be more specific, but it requires further adjustments to increase its sensitivity.

FR-PO1968

Vascular Access Flow Monitoring in Clinical Practice during Maintenance Hemodialysis Improves Fistula Survival Emi Kuang Lim,1 Hui-Lin Chooong,2 Manish Kaushik,2 Soh Theresa,3 Lay Kwee Chin.3

1 Renal Unit, Department of Medicine, Khoo Teck Puat Hospital, Singapore; 2Renal Medicine, Singapore General Hospital, Singapore; 3Kidney Dialysis Foundation, Singapore.

Background: Ultrasonic dilution measurement is one of KDOQI’s recommended tools for vascular access surveillance in maintenance hemodialysis (MHD). We studied arteriovenous grafts (AVG) survival after applying this tool in routine clinical practice.

Methods: A retrospective survey of MHD patients using AVG as vascular access in 3 hemodialysis centers was carried out. Data from patients followed up by a single nephrology unit was analyzed. Vascular Access Surveillance (VAS) by ultrasonic dilution flow monitoring using the Transonic system (Transonic Inc. Ithaca, NY) had been introduced since 2001. Flow-related problems (AVG flow < 600 ml/min or thrombosis) were promptly referred back to the parent unit for assessment and intervention. The data were divided and analyzed according to 3 study phases: 1- Pre-VAS (1997-2000), II- Early VAS (2001-2003) and III-Established VAS (2004-2007).

Results: A total of 119 AVGs were followed in 42 patients (Phase I-16, II-27 and III-40). Etiology of ESRD was Chronic Glomerulonephritis 21 [50%], Diabetic Nephropathy 6 [14.3%], and Lupus Nephritis 4 [9.5%]. There was significant difference in age(1- 42±4.9y, 2-46.5±10.5, 3-48.8±7.6, p = 0.043). There were no significant differences in gender distribution, diabetic and cardiovascular status. Graft loss from thrombosis occurred as follows: Phase I-38, Phase II-29, Phase III-13 events per 100 patient-years. Cox regression survival analysis unadjusted and adjusted for age (using Phase I as reference) showed that AVG survival was significantly better for Phase III (HR 0.35, 95% CI 0.16-0.79, P=0.01 and HR 0.34, 95% CI 0.15 - 0.76, P=0.01 respectively) but not Phase II (HR 0.82, 95% CI 0.37-1.00,P=0.61 and HR 0.73, 95% CI 0.33 - 1.62, P = 0.44 respectively).

Conclusions: Vascular access flow surveillance in maintenance haemodialysis was associated with improved AVG survival despite increasing age of the studied population. There was probably a transition phase after the introduction of access flow monitoring before gains were obvious.

Funding: Government Support - Non-U.S.

FR-PO1969

How To Measure the Access Flow Volume by Doppler Ultrasound in the Cases of Anatomical Variation and Comparison It with Ultrasonic Dilution Technique Hoon Suk Park, Sun Ryong Choi, In O Sun, Byung Ha Chung, Bumsoon Choi, Young Ok Kim, Cheol Whce Park, Chul Woo Yang, Yong-Soo Kim. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: In previous studies, comparing doppler ultrasound(DU) with ultrasonic dilution technique(U/D) in measuring intra-access flow volume(Qac), Qac by DU was measured at brachial artery or basilic vein at upper arm to avoid anatomical variation that may cause inaccuracy of the measurement. In practice, the sites where the needles are inserted are different from these sites mostly. We performed this study to investigate how to measure Qac by DU between the needing sites in the cases of anatomical variation and how consistent it is with Qac by U/D.

Methods: We measured Qac by DU only between needing sites in the cases without anatomical variation. But, in the cases with accessory vein or aneurysmal change, we measured DU at the two needing sites separately and calculate mean of both DU to measure between the needing sites. Eighty seven Qac were measured in this way by DU and compared it with the Qac by U/D. We also compared the differences of Qac(AQac) between these two methods with regard to access location(lower arm VS upper arm), access type(fistula VS graft) and the presence of anatomical variation.

Results: Mean of the Qac measurements in this way by doppler ultrasound was 955.1±569.1ml/min and mean of the Qac measurements by U/D was 1040.4±713.6ml/min. The correlation coefficient(r) between the two methods was 0.829(p<0.005). AQac(expressed by mean±standard deviation) were compared with regard to access location, access type and the presence of anatomical variation and there were no differences in AQac according to the groups.

Conclusions: Qac by DU can be measured or at the needing sites regardless of the presence of anatomical variation, showing the high consistency with the Qac by U/D.

FR-PO1970

Effectiveness of Shower Washing Method without Antiseptics for Exit-Site Care of Noncuffed Hemodialysis Catheters Hiroshi Shibahara,1 Nami Shibahara,2 Susumu Takahashi. 1Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan; 2Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan.

Background: To prevent catheter-related infections with noncuffed hemodialysis catheters (NCC), skin disinfection has long been the mainstay of exit-site care. However, we have reported a care method to prevent infection by restoring the normal skin condition at the exit site, consisting of direct shower-washing without antiseptics. The aim of this study was to evaluate this care method for the exit site of NCCs.

Methods: The subjects were 50 hemodialysis patients (male/female, 33/17; mean age, 69.0±13.3 years; diabetes mellitus/non-diabetes mellitus, 26/24) who had begun to use an NCC and gave informed consent to participate in this study at Sagamihara Kyodo Hospital between January 2008 and January 2010. Our method involved washing the exit-site of NCC with physiological saline immediately after insertion. The exit-site was wiped away with non-sterile gauze. No antiseptic was applied. Shower washing of the exit-site was continued after every dialysis session until NCC removal. We evaluated the skin condition of the exit site, the incidence of catheter-related infection, and blood examination data [white blood cell(WBC) count, C reactive protein (CRP), interleukin-6 (IL-6), albumin] at the time of insertion and removal of NCC.

Results: No exit site infection was observed. Slight erythema as compared to the normal skin was observed in two subjects. Three NCCs were removed because of catheter-related blood stream infections. These infections improved immediately following replacement of the NCC together with treatment with antibiotics. There were no significant differences in the values of WBC, CRP, IL-6, and albumin between the times of insertion and removal of NCC.

Conclusions: A shower washing method without antiseptics was effective for management of the NCC exit site. Future studies comparing this approach with conventional care methods in a larger number of subjects are needed.
Complications of Vascular Access for Haemodialysis and How To Avoid Them

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Background: Maintenance of the vascular access continues to be the Achilles’ heel of dialysis therapy. Different strategies have been implemented for monitoring and surveillance of the av fistulae. However, there is still much to do in the field of everyday care and basic monitoring.

The aim of the study was to identify causes of fistula losses that might be saved by the interdisciplinary approach to the problem, and proper cooperation between nephrologist, surgeon, and nurse. Methods: Over 500 cases of reoperation and fistula losses out of total 1500 operation of vascular access, performed on patients from 11 dialysis centers, in 10-year period, made by one vascular surgeon were analyzed.

Results: The most common cause of early dysfunction of fistula was maturation failure. Majority were the result of inadequate blood vessels but some of them were caused by patients’ non-compliance. Inappropriate cannulation technique of av fistula led to thrombosed aneurysms or hematomas with subsequent fibrosis. A few patients had symptoms of central or peripheral venous hypertension, mostly treated with endovascular procedure. Many patients required trombectomy and only a few underwent reconstruction of still functioning av fistula. Collateral ligation within 1-2 months after primary operation was rarely performed. Six grafts of total over 70 implanted were removed due to infection and 3 due to post-cannulation damage. Two fistulas were ligated due to steal syndrome (both within 2 months after primary procedure).

Conclusions: Proper understanding of the problem by all people involved (nephrologist, dialysis nurse, surgeon and the properly informed patient) could save many of those fistulas losses. Policy Treat the failing, not the failed arteriovenous fistula should be implemented more often. Clinical examination of arteriovenous fistula and more frequent use of ultrasonography to detect an early failure might give a chance for better survival. A vascular access should stop being a Cinderella and start its life of first-born son.

Methods: In chronic hemodialysis, the creation of a native arteriovenous fistula (AVF) as the primary vascular access is recommended because of higher patency rates and fewer access-related complications. The aim of our study was to investigate the long term outcome of the primary access and its impact on prevalence rates.

Results: We conducted a retrospective analysis of all patients who had a primary dialysis vascular access created from 1995 to 2006. Primary endpoints were the primary access failure free survival defined as the time from creation of the permanent vascular access to its complete loss, and the prevalence rates of the different access types.

Policy: Treat the failing, not the failed arteriovenous fistula should be implemented more often. Clinical examination of arteriovenous fistula and more frequent use of ultrasonography to detect an early failure might give a chance for better survival. A vascular access should stop being a Cinderella and start its life of first-born son.

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The Impact of Vascular Micro-Calcification of Radial Artery on Cardiovascular Mortality in Hemodialysis Patients

Yun-Soon Yun, Young Ok Kim, Hyun Gyung Kim. Nephrology Department, Catholic University of Korea, Seoul, Korea.

Background: Vascular gross calcification by imaging study is common in hemodialysis (HD) patients, and it is a significant predictor for cardiovascular mortality in HD patients. But vascular micro-calcification (VMC) by pathologic study has been rarely reported. Recently, we have reported that VMC is associated with vascular access failure as well as aortic stiffness (2010 ASN). The aim of this study was to determine the impact of VMC of radial artery on cardiovascular mortality in uremic patients receiving vascular access operation.

Methods: One-hundred forty nine HD patients (Mean age; 59 ± 13.9 years, Male/ Female; 86/63, Percent of diabetes mellitus; 65.8%) receiving vascular access operation were included in this study. During the operation, we obtained partial arterial specimen and performed pathologic examination by von Kossa stain to identify VMC. We investigated cardiovascular mortality for at least 1 year after the operation. Finally, we compared clinical and laboratory findings, and cardiovascular mortality between the patients with VMC and those without VCM.

Results: Mean duration of follow-up was 37.8 ± 34.5 months and the incidence of VCM was 38.8% (n=57). Diabetes (OR 4.138, 95% CI 1.631-11.830, p=0.002) and peripheral artery disease (OR 9.958, 95% CI 1.021-1339.97, p=0.048) were independent predictors for VCM. Serum parameters were not significantly related to VMC. During the period of follow-up, there were 27 cardiovascular deaths. Kaplan-Meier analysis showed an increased cardiovascular mortality risk (HR 2.613, 95% CI 1.196-5.711, p=0.016) in VMC flow up, there were 27 cardiovascular deaths. Kaplan-Meier analysis showed an increased cardiovascular mortality risk (HR 2.613, 95% CI 1.196-5.711, p=0.016) in VMC patients receiving vascular access operation.

Conclusions: According to future vascular access was not difference in prevalence of MICS. Although, hyporesponsiveness on EPO and hospitalizations were most frequent in catheter group.

FR-PO1976

Comparison between types of vascular access

<table>
<thead>
<tr>
<th>Group</th>
<th>All n=78</th>
<th>Catheter n=39</th>
<th>Fistula n=39</th>
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<tr>
<td>MIS (pts)</td>
<td>66.2±4.6</td>
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<tr>
<td>Mean age</td>
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<td>HD (grd)</td>
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<td>39±6</td>
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<td>EPO (U/Lweek)</td>
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<td>199±240</td>
<td>1080±427</td>
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<td>Ferritin (ng/mL)</td>
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<td>Phosphorous (mg/dL)</td>
<td>1.6±1.5</td>
<td>2.1±1.7</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>322±448</td>
<td>313±596</td>
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<td>Albumin (gr/dL)</td>
<td>3.9±0.5</td>
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<td>CRP (mg/L)</td>
<td>14±18</td>
<td>18.6±23</td>
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<td>PTTh (U)</td>
<td>890±804</td>
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<td>Hospitalization times</td>
<td>1.18</td>
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FR-PO1979

Georaphic Variation and Trends in Vascular Access-Related Infection Rates in the United States

Rajiv Saran, Joseph M. Messana, Erik Roys, N. A. Lueth, Casey Parrotte, T. H. Shearom, John Kalbfleisch. UM-KECC, Univ of MI, Ann Arbor, MI.

Background: The 2011 Dialysis Facility Reports will include information on dialysis access-related infection (ARI) rates for Medicare Hemodialysis (HD) patients for 2007-2010. These metrics were derived from ICD-9 codes for dialysis ARI for HD patients (996.62 - Infection and inflammatory reaction due vascular device, implant and graft) and will help dialysis providers compare their infection rates to national, state, and ESRD Network averages.

Methods: We describe variation in ARI rate across dialysis facilities and geographic regions. ARI rates per 100 patient months were calculated using ICD-9 codes reported in Medicare claims 2007-2010 and data from other national ESRD data. Poisson regression (log link, offset=log patient months) was used to assess the association of facility characteristics with infection rates and to establish expected values and standardized infection rates.

Results: Significant facility variation exists in vascular ARI rates across the country. Over the 4 years of observation, these infection rates have significantly declined overall (p<0.001 average decrease 0.05). The new measure of vascular ARI rates is strongly correlated with known predictors of infection such as patient age, percent of patients with diabetes, and percent of patients using catheter at those facilities (p<0.001). Mortality associations with vascular ARI were completely abrogated by adjustment for percent facility use of catheter as vascular access (p=0.01 to p=0.71).

Conclusions: The decreasing trend in vascular ARI is reassuring, but requires continued monitoring. Decreasing use of dialysis catheters should remain a national priority. Our study helps to validate the calculation of infection rates using ICD-9 codes derived from Medicare claims. Lowering the proportion of catheters as vascular access can reduce vascular ARI and potentially mortality resulting therefrom.

FR-PO1978

"Hybrid" Graft Reduces Venous Stenosis in a Pig Arteriovenous Graft Model

Prabir Roy-Chaudhury,1 Yang Wang,1 Meenakshi J. Mistry,1 Begoña Campos,1 Timmy C. Lee,1 Kyuran Ann Choe.1,2 Dialysis Vascular Access Research Group, Division of Nephrology and Hypertension, University of Cincinnati, OH; 1Department of Radiology, University of Cincinnati, OH.

Background: Arteriovenous graft (AVG) stenosis due to neointimal hyperplasia (NH) remains an important cause of hemodialysis vascular access dysfunction. Despite the magnitude of the clinical problem there are currently no effective therapeutic interventions. The "Hybrid" graft (manufactured by WL Gore) is a heparin bonded graft with a nitoil reinforced section (NRS) at the venous end. Insertion of the NRS through a venotomy followed by expansion results in a sutureless end to end anastomosis (see Figure). The biological rationale for the "Hybrid" graft is that the functional end to end anastomosis will optimize hemodynamic profiles resulting in a reduction of NH.

Methods: Standard AVGs and the "Hybrid" grafts were placed between the femoral artery and vein on opposite sides. Animals were ascultated every 3 days to document patency. A 64 slice CT angiogram was performed at 6 weeks to assess for stenoses within the arteriovenous circuit. The maximal degree of stenosis within the access circuit was used for the statistical analysis. A thrombosed graft was considered to constitute a 100% stenosis.

Results: The majority of stenoses in both the control and "Hybrid" arms were at the graft-vein or NRS-vein junction, or within the first 3 cm of downstream vein (in a cranial direction). Control grafts had a mean percentage stenosis ±SE of 83.3±12% as compared to 45±10% for the "Hybrid" grafts (p=0.024 paired t test).

Conclusions: Our results suggest that the "Hybrid" graft reduces venous segment stenosis as compared to control grafts in our pig model of arteriovenous stenosis. Further investigations as to the mechanisms involved are currently ongoing.

FR-PO1977

Novel Blood Access for Home Hemodialysis

Frank Prosl, N/A, Koestenberg, Austria; 1Duksbury, MA.

Background: HDA is called Achilles Heel of dialysis from poor safety & performance & reliability. We describe a novel Hemodialysis Access (HDA) system which meets critical needs for improved outcomes & patient health by performing home hemodialysis.

Methods: HDA problems spanning 4 decades seemed intractable. Patients report dissatisfaction with HDA in several newly published surveys. Patients achieve best QoL with home hemodialysis(HD) which further detracts on HDA performance needs for improved outcomes & patient health by performing home hemodialysis.

Results: Test models demonstrate superior safety & performance & reliability. 1) "Fail-Safe" design from needle & bloodstream dislodgement 2) Simple access without pain, bleeding, discomforting or risk of infection 3) Increased fluid integrity during HD & between times & comprehensive effective prophylaxis 4) Preserved patient self-reliance & self -image, no stigma or loss of arm function & no bleeding 5) Novel engineered tissue tract comprising new etiology and tissue morphology suitable for connector tissue penetration in precise alignment without guessing (i.e., not buttonhole) & 6) Substantial robustness & system life time, low costs.

Conclusions: Test models demonstrate superior safety & performance & reliability. Worst case blood loss is limited to volume in the extracorporeal circuit. Air embolism is prevented in case of dislodgement. The system will help attract patients for home HD & remove the major cause of patient burnout. This HDA should help create conditions favoring HD at home.
Development of an Adult-Model of Autosomal Dominant Polycystic Kidney Disease by Manipulating the Timing and Extent of Pkd1 Deletion in a Conditional Knockout Mouse

Thomas A. Natali, Kelly A. Rogers, Laurie A. Smith, Sarah E. Moreno, Nikolay Bukanov, Herve Husson, Oxana Beskrovnaya.

Background: Autosomal dominant polycystic kidney disease (ADPKD) results from mutations in either one of two genes, PKD1 or PKD2. Development of effective therapies to slow cyst growth and preserve renal function requires the use of preclinical models that closely mimic ADPKD. Pkd1 conditional knockout mice have been developed to model such a disorder. However, several limitations remain. Conditional inactivation of mouse Pkd1 gene prior to postnatal day 14 leads to aggressive kidney cyst formation within the neonatal period and early death. Deletion after postnatal day 14 leads to cyst formation in adults, but with a very long lag period that is inconvenient for therapeutic testing. We set out to develop a more adult-like model of ADPKD to study molecular pathogenesis and to test potential therapies. Here, we use a tamoxifen-inducible Cre fusion protein and a Pkd1 conditional knock out allele to show that altering the timing of deletion during kidney development, promoting cell proliferation in the nephrogenic zone. Cux1 represses the cyclin kinase inhibitor p27 during early kidney development. Cux1 represses the cyclin kinase inhibitor p27 during early kidney development, promoting cell proliferation in the nephrogenic zone. Promoter reporter analysis of p27 revealed that Cux1 represses p27 in a concentration dependent manner, and chromatin immunoprecipitation showed that Cux1 interacts with the co-repressor Grg4 and the histone deacetylases HDAC1 and HDAC3 on the p27 promoter in vivo. To determine whether HDACs are required for Cux1 repression of p27 we analyzed p27 promoter activity in the presence of the HDAC inhibitor trichostatin A (TSA). TSA treatment completely relieved the repression of p27 by Cux1 in vitro, demonstrating that Cux1 represses p27 in an HDAC dependent manner. Cux1 is upregulated in the kidneys of both mice and humans with ADPKD. However, Cux1 transgenic mice do not develop cystic kidneys, indicating that upregulation of Cux1 alone is insufficient to develop PKD. Rather, mice carrying both the Cux1 transgene and a Pkd1 (Plk1CD) and a targeted deletion of Cux1 show that Cux1 is required for cyst growth. Moreover, p27 was upregulated in the cyst lining cells. To begin to test whether HDAC inhibitors could be used to target Cux1 repression of p27 for the treatment of PKD, we treated Pkd1CD mice with TSA or vehicle beginning at embryonic day 10.5 until embryonic day 18.5. Newborn Pkd1CD mice that received vehicle exhibited extensive collecting duct cysts, while newborn Pkd1CD mice that received TSA showed a significant reduction in cysts. Taken together, these results suggest that HDACs are required for cyst growth, and raise the possibility that HDAC inhibitors may be an effective treatment for PKD.

Funding: Pharmaceutical Company Support

FR-PO1981

Treatment of Embryonic Pkd1 Mutant Mice with the HDAC Inhibitor Trichostatin-A Reduces Cyst Growth

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Binu M. Paul,

Lynn Maghenierna,

Madhulika Sharma,

Greg B. Vanden Heuvel,

Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS; Kidney Institute, University of Kansas Medical Center, Kansas City, KS.

Background: Cux1 is a homeobox gene involved in cell cycle regulation and kidney development. Cux1 represses the cyclin kinase inhibitor p27 for the treatment of PKD, we treated Pkd1CD mice with TSA or vehicle beginning at embryonic day 10.5 until embryonic day 18.5. Newborn Pkd1CD mice that received vehicle exhibited extensive collecting duct cysts, while newborn Pkd1CD mice that received TSA showed a significant reduction in cysts. Taken together, these results suggest that HDACs are required for cyst growth, and raise the possibility that HDAC inhibitors may be an effective treatment for PKD.

Funding: Pharmaceutical Company Support

FR-PO1982

Cilia Defects and Polyploidy Linked to Low PC1 Expression in Kidney Epithelia Cells Are Mediated by Increased Expression of Sirt2 and HDAC6

Xia Zhou, Lucy X. Fan,

Wei Liu,

Evan W. Sweeney,

Ellis D. Avner,

Children’s Research Institute; Children’s Hospital of Wisconsin, Milwaukee, WI; Pediatrics, Medical College of Wisconsin, Milwaukee, WI; Physiology, Medical College of Wisconsin, Milwaukee, WI; Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City, KS.

Background: The association of PC-1 and PC-2 with the primary cilium has led to the “primary cilium” hypothesis in ADPKD. However, how polycystin(s) levels affects cilia function is still uncertain.

Results: In this study, we found that HDAC6 and its binding partner, Sirt2 were upregulated in Pkd1 mutant mouse kidney epithelial (MEK) cells and tissues compared to Pkd1 wild type MEK cells and tissues. To examine the role of HDAC6 and Sirt2 in cilia development, we over-expressed either HDAC6 or Sirt2 in mouse inner medullary collecting duct (nMCD) cells. Over-expression of either HDAC6 or Sirt2 significantly reduced the percentage of cells with cilia. Inhibition of HDAC6 with either trichostatin (TSA), a general HDAC inhibitor, or tubacin, a specific HDAC6 inhibitor, or inhibition of Sirt2 with nicotinamide, prevented cilia disassembly and increased the acetylation of α-tubulin in both Pkd1 wild type and mutant renal epithelia. Nicotinamide treatment also resulted in a significant increase in the percentage of Pkd1 mutant renal epithelial cells that developed cilia. These results suggest that while HDAC6 may regulate cilia assembly and disassembly during development, Sirt2 levels appear to be critical for ciliation in both wild type and mutant renal epithelial cells. In addition, we found that overexpressing Sirt2 but not HDAC6 induced polyploidy and centrosome amplification similar to that found in the Pkd1 knockdown mIMCD cells.

Conclusions: In summary, reduced PC1 levels results in increased HDAC6 and Sirt2. While HDAC6 is important in cilia assembly and disassembly, Sirt2 plays a central role in cilia development and increased Sirt2 levels lead directly to polyploidy and centrosome amplification. This is the first report that mechanistic links reduced PC1 levels to ciliogenesis, polyploidy and centrosome amplification.

Funding: NIDDK Support

FR-PO1984

mTOR Kinase Inhibition in a Model of Polycystic Kidney Disease

Atif A. Kidway, Hyunho Kim, Michael Martin, Li Yu, Jan Wang, Kevan Shokat, Christian Rommel, Feng Qian, David Pearce.

Division of Nephrology, UCSF, San Francisco, CA; Center for Polycystic Kidney Disease, Johns Hopkins University, Baltimore, MD; Intellinike, La Jolla, CA.

Background: Polycystic Kidney Disease is a relatively common cause of renal failure with no current acceptable therapies. There has been some success with treating the disease in rodents models with rapamycin, an mTOR inhibitor; however, success in clinical trials has been poor. This may be in part due to the fact that phosphorylation of several mTOR targets is insensitive to rapamycin.

Methods: We examined the effects of a new class of ATP competitive mTOR kinase inhibitors, termed “torkinibs”, on mTOR activities, as well as on cyst volume in Pkd1CD/V/V mutant mice. We also examined the effect of a combination of torkinib with either wild type (+/+) or wild type (+/-) mice from postnatal days 5-11 with oral torkinib or vehicle (n=6 in each group). Mice were sacrificed 2 hrs after their last dose on P11, and kidneys harvested for immunoblot and histologic analysis.

Results: Total body mass and average kidney mass were lower in treated than in untreated mice (5.42 gms vs 3.34 p < 0.01, 301mg +/- 39 to 172mg +/- 43 p = 0.02, respectively). The reduction in kidney mass was greater than reduction in body mass, and the combined kidney mass/body mass ratio in the torkinib-treated mice was significantly lower than in vehicle-treated mice (0.081 +/- 0.012 vs. 0.113 +/- 0.008; p = 0.02). In non-mutant mice, body mass was lower in torkinib-treated than in vehicle-treated animals (n =0.02), but there was no difference in kidney mass (p = 0.22) or combined kidney mass/body mass ratio (p=0.5). Kidney lysates immunoblots were probed with phospho-specific antibodies to assess the phosphorylation status of mTOR targets; mTOR targets had been markedly lowered phosphorylation in torkinib vs vehicle-treated controls. In preliminary histologic analysis, cyst volume was lower and parenchymal volume greater in torkinib-treated V/V mice than in vehicle treated mice.

Funding: NIDDK Support
FR-PO1985

Kirsten-Ras GT-Pase Isoform as a Target in ADPKD

Ayesha Iritza-Ali,1 Richard N. Sandford,2 Dorien J.M. Peters,3 Patricia D. Wilson,4 Claire S. Sharpe,5 Bruce M. Hendry.1 King’s College London, 2Cambridge University, 3Leiden Univ Medical Center; 4University College London.

Background: Our previous studies show that inhibition of Kirsten (K)-Ras GT-Pase isoform using antisense oligonucleotide (ASO) has marked anti proliferative and anti-fibrotic effects in different models of proliferative renal disease. Autosomal dominant polycystic kidney disease (ADPKD) is characterized by abnormal cell proliferative responses that lead to cystogenesis and progressive fibrosis, resulting in loss of renal function. Ras-GT-Pase signalling may be implicated in these processes. We aim to examine this further.

Methods: Renal cystic disease in an orthologous PKD1nl/nl hypomorphic mouse model, bred on a C57B6xC3D genetic background, was characterized in detail using histochemistry, qPCR and immunoblotting, and compared to wt (n=4-group). We then employed modified ASO technology to study the effect of Ki-Ras inhibition in vitro and in vivo.

Results: PKD1nl/nl kidneys exhibit altered cystic epithelial cell morphology and E-cadherin expression, peri-cystic and interstitial fibrosis, increased tubular and cystic epithelial cell proliferation and phospho-ERK expression, and upregulation of Ras-GTP. Ki-Ras expression is increased by 4-fold in cystic kidney compared to wt (p=<0.0001). Two Ki-Ras ASOs each transfected into miMCD3 cells at a concentration of 100nM caused selective knockdown of Ki-Ras by ~80%, and inhibit cell proliferation by 29 and 38% compared to a control ASO (p=<0.05). In C57B6/mice, these ASOS selectively knockdown Ki-Ras in the kidney by 50% (p=<0.001), without adverse effect. Antibody detection of ASO localizes distribution to within the tubular epithelia of the proximal and distal nephron.

Conclusions: Our results indicate that Ki-Ras is important in renal epithelial cell proliferative responses, and may be involved in ADPKD pathogenesis. These studies are also the first to demonstrate use of 2 active ASOs in mouse that safely and specifically target Ki-Ras in the kidney, supporting their use for therapeutic study. Our current work investigates the role of Ki-Ras as a potential common link in the abnormal proliferative and fibrogenic responses that occur in ADPKD.

Funding: Private Foundation Support

FR-PO1986

Cell Therapy in Polycystic Kidney Disease

Rende Xu, Karen M. Peterson, Peter J. Psaltis, Peter C. Harris, Lilach O. Lerman, Amir Lerman, Martin G. Rodriguez-Porcel. Department of Internal Medicine, Mayo Clinic, Rochester, MN.

Background: Polycystic kidney disease (PKD) is the most common genetic renal disease and is characterized by the development of parenchymal cysts and impaired renal vascular and tubular structure and function. In previous studies, cell therapy ameliorated renal dysfunction in a model of chronic renovascular disease. Thus, we hypothesize that cell therapy may ameliorate the renal vascular and tubular dysfunction in PKD.

Methods: Bone marrow derived mesenchymal stromal cells (MSCs) (2.5x10^5) were transplanted to a rat model of autosomal recessive PKD (ARPKD, n=6) through direct injection into the left renal artery at 6 weeks of age, and compared to wild type. Mean arterial pressure (MAP) was measured via tail-cuff. Fifteen-hour urine collections were performed and analyzed for protein and creatinine. Renal vascular function was calculated by measuring end-diastolic Doppler flow velocity (EDV) and peak systolic velocity (PSV). Kidney weight was calculated and compared to controls. PKD rats had lower urinary output and higher end-diastolic reserve, both of which were partially preserved in PKD rats that received MSCs (Figure). Our data shows that partially restores urinary volume and vascular function in a rat model of ARPKD. While the potential mechanisms of the beneficial effect deserve further study, cell therapies appear as a potential therapeutic intervention in PKD.

Funding: NIDDK Support

FR-PO1987

FR-PO1988

Initiation and Progression of Fibrosis in a Pkd1 Hypomorphic Mouse Model of Autosomal Dominant Polycystic Kidney Disease

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Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of bilateral fluid filled cysts and intraparenchymal renal fibrosis. The C57B6/H-1-Pkd1+/- is a mouse model of ADPKD where hypomorphic expression of Pkd1 results in a model with 2 distinct phases of disease progression. Phase 1 is the formation and growth of cysts followed by phase 2, the development of renal fibrosis.

Methods: We used Masson’s Trichrome staining, immunohistochemistry (IHC), Western blotting (WB) and quantitative RT-PCR to examine the phenotypic and molecular changes associated with the development of fibrosis in this model.

Results: We chose PN28 as the best point time to examine the molecular changes associated with early stage fibrosis based on the following: 1) Trichrome (+) areas were first evident at PN28; 2) WB revealed initial decreases in ZO-1 and E-cadherin expression: 3) WB demonstrated increases in αSMA; and 4) IHC revealed a layer of αSMA (+) cells beneath the epithelial layer of trichrome (+) cells. FSP-1 (+) cells within cyst epithelial were very rare and remained rare even at PN56, a point of reduced kidney size due to fibrosis.

Molecular analysis by RT-PCR revealed the earliest changes included statistically increased expression of Col2A1, Col3A1 and Col5A2. Changes in the TGFβ/BMP pathway included increased Bmp1 (7.5 fold), Tgfβ2 (17 fold), and Tgfβ3 (5 fold). Bmp7 expression decreased 3 fold whereas Tgfβ1 and Smad2 remained statistically unchanged. Wet pathway modestly decreased expression levels in Col2a1, Wnt5a, 5b and Wnt11. Transcription factors including Stat3, Tcf3, Zeb1, Zeb2 and Twist1 were all significantly increased.

Conclusions: There is no evidence of EMT in this model. Later time point analysis suggests the αSMA (+) layer of cells below the cystic epithelia are myofibroblasts or precursors that trigger fibrotic changes.

Funding: NIDDK Support, Private Foundation Support

FR-PO1989

Conditional Inactivation of TGFβR3(Alk5) in a Pkd1 Deletion Mouse Model for ADPKD Does Not Inhibit Renal Fibrosis

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD), caused by mutations in PKD1 / 2, is characterized by progressive cyst formation and renal fibrosis. The latter significantly contributes to loss of kidney function, ultimately leading to end-stage kidney failure. Characterization of PKD model mice, where the mutant protein can partly escape the ER quality control and enter the secretory pathway.

Methods: Our studies were carried out in MDCK cells stably expressing wild type and mutant protein and in a transgenic mouse model for UAKD. Secretion of mutant uromodulin was also assessed by mass spectrometry analysis on urine samples from UAKD patients.

Results: In cellular models, mutant uromodulin could partly reach the plasma membrane where it formed large extracellular aggregates. Interestingly, mutant isoforms co assembled with wild type protein and exerted a dominant negative effect on polymerisation. This was confirmed in transgenic mice, where mutant uromodulin not only accumulated in the ER of thick ascending limb epithelial cells, but was also found in urine and in large luminal casts. Notably, urinary excretion of mutant uromodulin was also detected in patients carrying uromodulin mutations (R212C, C256Y, C317Y), supporting the relevance of our findings in UAKD.

Conclusions: These results demonstrate that mutant uromodulin can be trafficked to the cell membrane where its aggregation interferes with the formation of uromodulin matrices and possibly affects the function of other membrane proteins. This suggests a new potential therapeutic target of effect function of uromodulin mutations that has implications for therapeutic strategies.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA- Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

574A
of TGFβ-target genes at advanced PKD and can therefore be used to study therapeutic interventions on renal fibrosis in PKD.

Methods: Since fibrosis is frequently associated with increased TGFβ signaling, we initially treated mice with a pan-selective neutralizing TGFβ antibody (2G7), but observed no reduction in fibrosis-related parameters. Next, we crossed our tamoxifen-inducible kidney-specific Pkd1-lox/loxF cycle-knockout (cKO) with a mouse in which the I receptor for TGFβ (ALK5) is flanked by Lox P sites. We analyzed tissues by immune-histology and gene expression by RT-MLPA.

Results: Tamoxifen administration to these double-cKO’s disrupted both Pkd1 and Alk5 expression at mRNA and protein levels in the kidneys. Double-cKO’s developed cystic kidneys within the same time frame as single-cKO’s and renal histology appeared not to be different. Of note, also in double-cKO’s, nuclear pSMAD2 levels and SMAD signaling-dependent gene expression were elevated at end-stage PKD. These results indicate that initiation of renal fibrosis in PKD does not depend on SMAD signaling via Alk5 in renal epithelial cells and that alternative signaling pathways have to account for the increased levels of nuclear pSMAD2.

Conclusions: In the present study, loss of Alk5 in renal epithelial cells did not inhibit fibrosis in PKD. It is therefore unlikely that targeting only Alk5 will be successful to halt fibrosis. We will discuss in vitro and in vivo data suggesting alternative pathways that can lead to SMAD2 phosphorylation and expression of fibrosis-related genes.

FR-PO1990

Inhibition of Multidrug Resistance-Associated Proteins Reduces Cystogenesis Via ERK-Dependent Pathway Jong Hoon Park, Eun Sun Chang, Eun Young Park, Kyung Hyun Ryu. Department of Biological Science, Sookmyung Women’s University, Seoul, Korea.

Background: Increased tubular epithelial cell proliferation is a prerequisite for cyst formation and expansion in autosomal dominant polycystic kidney disease (ADPKD). Multidrug resistance-associated protein-1 (Mrp1) and Mrp3 are members of the subfamily of ABC transporters. They are well known as drug-resistant proteins, and their other functions are mostly unknown.

Methods: To profile the genes related with cystogenesis, we carried out microarray analysis using cortex region of PKD2 transgenic mice kidney. We found Mrp3 is overexpressed on cyst lining cells of PKD2 transgenic mice kidney using immunohistochemistry. Next we performed three-dimensional culture to determine the effect of Mrp3 with MK571.

Results: Mrp1 and Mrp3 were upregulated dramatically in the cystic region. To examine the effects of these multidrug resistant proteins on cystogenesis, we treated MK571 for inhibition of Mrp1/Mrp3 in Mouse-Darby canine kidney (MDCK) cell line. We found treatment of MK571 reduced the volume of cysts (P<0.001) via reduction of MAPK/ERK signaling.

Conclusions: Our results demonstrate that Mrp1 and Mrp3 may drive proliferation signal on cyst lining cells. In conclusion, antagonist of Mrp1 and Mrp3 (MK571) may have therapeutic potential in ADPKD.

FR-PO1991

An imbalance of Lymphangiogenesis Versus Blood Vessel Angiogenesis in Polycystic Kidney Disease Jennifer L. Huang, Maria Kolatsi-Joannou, Adrian S. Woolf, Paul J.D. Winyard, David A. Long. 1Institute of Child Health, University College London, United Kingdom; 2Developmental and Regenerative Medicine Research Group, University of Manchester, United Kingdom.

Background: Therapeutic strategies to reduce cystogenesis in polycystic kidney diseases (PKD) directly target epithelial proliferation using rapamycin-like drugs, cyclin-dependent kinase inhibitors and vasopressin receptor blockade. An alternative approach could be to target pathogenic extrinsic to epithelia themselves, such as microvessels, which may modulate cystogenesis by paracrine signalling, oxygen delivery, and access to inflammatory/immune mediators.

Methods: To begin to examine this possibility, we investigated the expression of molecules associated with blood vessel angiogenesis and lymphangiogenesis in congenital polycystic kidney (ckpk) mice. In this mutant, renal lesions phenocopy human Autosomal recessive PKD.

Results: We measured 88 angiogenic genes using real-time PCR profiling of whole kidneys. In early stages, levels of 7 transcripts were modestly altered in cpk mice. At three weeks of age, when there is massive nephromegaly caused by collecting duct obstruction, the number of vessels with positive podoplanin staining found between cysts was increased with additional ectopic expression in cyst walls. mRNA levels of Vegfr3 and Vegfr1, implicated in lymphangiogenesis, were however not altered in cpk mice.

Conclusions: This is the first evidence that the balance between blood angiogenic and lymphatic gene expression are altered in ARPKD. Further research into the functional significance of our novel findings is being conducted and it is hoped that targeting these pathways may attenuate disease progression.

Funding: Private Foundation Support

FR-PO1992

Global Gene Expression Profiling in Kidneys of PPARγ agonist-Treated PCK Rats, an Orthologous Model of Human ARPKD Daisuke Yoshishira, Masanori Kugita, Hiroki Kurahashi, Miwa Morita, Yoshihiko Hiki, Tamio Yamaguchi, Harold M. Aukema, Barrett R. Wallace, James P. Calvert, Takafumi Toyohara, Takaaki Aobe, Shizuko Nagao, Education and Research Center of Animal Models for Human Diseases, Fujita Health University.

Background: In polycystic kidney disease (PKD), kidney enlargement is caused by aberrant proliferation of tubule epithelial cells leading to the formation of numerous fluid-filled cysts, extensive nephron loss, and interstitial fibrosis. Pioglitazone (PIO), a PPARγ agonist, decreased cell proliferation, interstitial fibrosis and inflammation, and ameliorated PKD progression in PCK rats (Yoshishira et al, JAP, 2011).

Methods: To examine the beneficial effects of PIO, we analyzed changes in global gene expression by DNA microarray in PCK rats treated with 10 mg/kg PIO for 16 weeks.

Results: By Gene Set Enrichment Analysis that used 30655 genes showing significant signal, six of 25 canonical pathways identified to be down-regulated by PIO-treatment were related to cell cycle and cell proliferation, including EGF, JNK, GSK3 and PDGF pathways. Of 43,379 probes examined, 49 were altered 2-fold or more in PIO- compared to vehicle-treated kidneys. Their relevant pathways were identified using the Kyoto Encyclopedia of Gene and Genomes database. Two key enzymes in fatty acid metabolism and three proteins related to calcium signaling were in the top 15 genes down-regulated directly by PIO treatment. Immunohistochemical analysis revealed that the gene product of two of these, scroly-CoA desaturase and a choline receptor, were highly expressed in PCK kidneys, and decreased by PIO-treatment.

Conclusions: These data show that PIO has effects on the expression of renal genes involved in fatty acid metabolism, choline signaling, cell cycle progression and proliferation.

Funding: Government Support - Non-U.S.

FR-PO1993

Effects of Phkd1 Quantitative Differential Expression on Urinary Metabolic Parameters Potentially Involved in ADPKD-Associated Nephrolithiasis Renato Ribeiro Noqueira Ferrag, Jonathan Mackowiak Fonseca, Gregory G. Germino, Luiz F. Onuchic, Ita Pfefferman Heilberg. 1Nephrology, Federal University of Sao Paulo, Sao Paulo, Brazil; 2Nephrology, University of Sao Paulo School of Medicine, Brazil; 3National Institute of Diabetes and Kidney Diseases, Bethesda, MD.

Background: In order to address whether urinary metabolic abnormalities potentially related to the high prevalence of nephrolithiasis in Autosomal Dominant Polycystic Kidney Disease (ADPKD) are caused by PKD1 haploinsufficiency or the renal cystic phenotype, urinary lithogenic parameters were determined in non-cystic Phkd1-haploinsufficient (Phkd1+/–) and Phkd1-targeted cystic(Phkd1+/–;BalCre+–) mice models.

Methods: 24-h urine samples were collected during 3 non-consecutive days from 12 week-old male Phkd1+/– (n=12), Phkd1+/–;BalCre+– (n=11), Phkd1+/–;BalCre–cystic (n=17), and Phkd1+/–;BalCre–non-cystic (n=18).

Results: As shown in the table, Phkd1+/– showed significantly higher urinary calcium than Phkd1+/– (p=0.05), an observation not detected in Phkd1+/–:BalCre+–. Sodium and potassium urinary excretion were significantly lower in Phkd1+/–;BalCre+– vs Phkd1+/–;BalCre–cystic (p=0.0003, p=0.0004 and p=0.003, respectively). Urinary citrate did not differ between test and control groups.

Funding: Government Support - Non-U.S.
FR-PO1994

Acceleration of Smad3 Phosphorylation at Linker Regions Via c-Jun NH2-terminal Kinase (JNK) in Cyst-Lining Epithelial Cells in cpk Mouse, a Model of ARPKD
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Background: Cyst-lining epithelia in PKD is characterized by dedifferentiation and perturbations of polarized phenotype with consequent renal tubular cyst formation and progressive enlargement. Previously, we showed activation of key mediators of EMT, TGF-β/Smad3 pathway in cph mouse (JASN 21:528A, 2010). However, specific mechanisms of this phenotypic alteration during cyst formation remain to be elucidated. TGF-β signaling involves Smad3 phosphorylated at linker regions (pSmad3L) and COOH terminal regions (pSmad3C). Therefore, we examined Smad3 phosphorylation profile in this model.

Methods: Kidneys from 5 male cph and control mice (each on day 0, 7, 14, 21) were harvested. Formaldehexide fixed, paraffin embedded sections were stained with antibodies to domain-specific phospho-Smad3, TGF-β and JNK. We also evaluated their expressions by western blotting.

Results: pSmad3L expression was increased according to age in cph, while there was no significant staining of pSmad3C in control. pSmad3L was predominantly expressed in nuclei of tubular epithelia in large cysts, and in cytoplasm and nuclei in small and middle cysts. pSmad3C was expressed in both cph and control in all age, and expression level showed increased tendency according to age in cph. TGF-β expression was seen in both cph and control, and also expression level showed increased tendency according to age in cph. JNK showed the similar staining pattern to pSmad3L. Western blotting analysis supported the expression profiles in sections. These findings suggest that pSmad3L up-regulated via JNK pathway in cph mice.

Conclusions: The linker phosphorylation of Smad3 may be a key pathophysiology in PKD and a target for a disease-specific intervention.

Funding: Government Support - Non-U.S.

FR-PO1995

Vitamin D Deficiency Exacerbates Renal and Cardiac Enlargement in Rats with Polycystic Kidney Disease
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Background: Vitamin D promotes cellular differentiation and inhibits proliferation, raising the hypothesis that the deficiency of vitamin D promotes cyst growth in polycystic kidney disease (PKD). Therefore, the aim of this study was to determine the effect of dietary vitamin D deficiency on the progression of kidney enlargement in PKD.

Methods: Male Lewis PKD rats (Philips et al. Kidney Blood Press Res 30:129, 2007) received either a semi-pure control diet (AIN93G, D−) or AIN93G with no added Vitamin D (D−), from postnatal week (wk) 3, and examined on wk10 and wk20 (w=9 per group).

Results: Serum 25-hydroxy vitamin D in the control diet group was normal (wk10:109±9; wk20:151±27ng/ml; mean±SD) whereas it was severely reduced in the D-deficient group (wk10:7±9; wk20:17±10 ng/ml). Kidney enlargement, as determined by combined right and left kidney weight, was increased in the D-deficient group compared to the control group, at both wk10 (D+: 11.0±2.2; D−: 15.7±2.3) and wk20 (D+: 22.6±1.9; D−: 25.5±3.6 g;P<0.05). The latter was confirmed by serial assessment of total kidney volume (wk10: D+: 10.0±0.4; D−: 13.7±1.1 g;P<0.05; wk20: D+: 22.6±1.9; D−: 25.5±3.4 g;P<0.05).

Conclusions: Dietary deficiency of vitamin D worsens renal and cardiac enlargement in PKD. Further studies should define if kidney enlargement is mediated by the direct or indirect effects of vitamin D on cyst growth, and whether this can be attenuated by replacement therapy.

Funding: Government Support - Non-U.S.

FR-PO1996

A Novel Mouse Mutant for Cystic Kidney Disease and Defects of Planar Cell Polarity Joseph P. L.1 Yoshio Maeaza2, Tuncer Ouy5, Robert Harrison6, Susan E. Quaggan7,7,7 Samuel Lunenfeld Research Institute, Toronto, ON, Canada; 2University Hospital Aachen, Aachen, Germany; 7Centre for Modeling Human Disease, Toronto, ON, Canada; 8Development and Stem Cell Biology Program, Hospital for Sick Children, Toronto, ON, Canada; 9Departments of Medicine and Molecular Genetics, University of Toronto, ON, Canada; 10Department of Otolaryngology, Hospital for Sick Children, Toronto, ON, Canada; 11Medicine, University Health Network, Toronto, ON, Canada.

Background: Cystic kidney diseases represent the primary genetic cause of ESRD in North America, contributing up to 5% of incident cases. The molecular basis of cyst development is complex; recently, defects in planar cell polarity (PCP) have been associated with cyst formation. Here, we describe a new mouse model of cystic kidney disease with evidence for PCP involvement.

Methods: In an autosomal dominant ENU mutagenesis screen, we identified a heritable mouse mutation that causes renal cysts. Results: Heterozygous mutant mice demonstrate variable and often striking glomerular cysts that can affect up to 100% of glomeruli. At a lower frequency, cysts are identified along the entire nephron. Homozygous mutants die within the first few weeks of birth and already exhibit both tubular and glomerular cysts. In addition, homogyzotes have “kinked” tails and disoriented inner ear hair cells, phenotypes specific for PCP defects. Furthermore, the loss of one copy of vangl2, a core PCP gene enhances cyst formation in heterozygotes, further substantiating PCP involvement. In addition, there is a trend towards fewer and shorter primary cilia within the renal tubules of the homozygotes. Using SNP analysis, we show that the mutation resides in a 3 MB critical region of chromosome 6 that contains 25 genes, none of which have been associated with cystic disease. Whole-exome next-generation resequencing is currently underway.

Conclusions: Taken together, we report a novel mouse model of renal cysts, which may further elucidate the role of PCP in cystic kidney diseases.

Funding: Government Support - Non-U.S.

FR-PO1997

Planar Cell Polarity Dependent Regulation of Kidney Tubule Morphogenesis Roy D. Bayly, Jeffrey D. Axelrod. Pathology, Stanford University School of Medicine, Stanford, CA.

Background: Failure of proper kidney tubule development can result in polycystic kidney disease (PKD), one of the most common genetic disorders inherited in humans. Genetic analysis has revealed that many of the causative gene products identified from patients with PKD localize to or interact with components of the primary cilium, but how they regulate kidney tubule morphogenesis is the subject of considerable interest. Recent research suggests that kidney tubule morphogenesis may involve convergent extension movements and oriented cell division, two processes that require input from the planar cell polarity (PCP) pathway in other developmental contexts. However, the interconnectivity between components within the primary cilium, the PCP pathway and the developmental processes that regulate kidney tubule morphogenesis is tentative.

Methods: Using mouse genetics and confocal microscopy, we have analyzed the requirement of Vangl1 and Vangl2 during the development and morphogenesis of kidney tubules.

Results: We present results that demonstrate a definitive role for Vangl1 and Vangl2, core members of the PCP pathway, during kidney tubule morphogenesis. Specifically, we observe the asymmetric localization of Vangl1, Vangl2, Frizzled3, and Frizzled6 along the proximal-distal axis of kidney tubule cells. We show that these core components are required for molecular asymmetry, for the proper orientation of cells with respect to the tubule axis, and for the regulation of kidney tubule diameter. Surprisingly, we also observe that in Vangl1 and Vangl2 mutant mice, levels of Polycystin-2, a gene product that is mutated in a class of PKD, is reduced in primary cilia, and increased along the apical surface of kidney tubule cells.

Conclusions: Taken together, we show that Vangl1 and Vangl2, core components of the PCP pathway, are critically important in the regulation of kidney tubule morphogenesis due in part to their requirement for the proper cilary localization of polycystic kidney disease associated gene products, such as Polycystin-2.

Funding: Other NIH Support - Cancer Biology Postdoctoral Fellowship NIH NRSA 5T32 CA09302-29

FR-PO1998

Abnormal Expression and Assembly of Laminin-332 and Laminin-511 in ARPKD Soundarapandian Vijayakumar, Kanchan Parkhi, Jessica Stolar. Pediatrics, University of Rochester, NY.

Background: Extracellular matrix abnormalities have been associated with PKD and it is suggested that abnormal upregulation of laminin-332 contributes to ADPKD cystogenesis by inducing proliferation. However, the possible role of aberrant ECM assembly in ARPKD cystogenesis has not yet been investigated. In this study, we addressed this question using PCK rat model of ARPKD and orph cell lines.

Methods: RNA was extracted from 4 adult PCK and control SD rat kidneys and from mutant and rescued orpk cell monolayers. Real-time PCR was performed using Taqman method with GAPDH normalization. For immunoblotting, 300µg of PCK and SD whole
kidney extracts were probed with laminin γ2 (sc-7652) & laminin γ1 antibodies. Frozen kidney sections were stained with a laminin α5 polyclonal (JH Miner) and a laminin α3 monoclonal (R&D). Paraffin sections were stained using laminin-γ2 antibody.

**Results:** Our qPCR results show significant upregulation of laminin-332 chains in PCK kidneys compared to wild-type SD kidneys. Laminin γ2 was upregulated 10 fold, laminin β3 4-fold, and laminin α3, 2-fold.

Immunoblotting confirmed the presence of laminin γ2 in PCK kidneys but not in SD. Immunostaining of frozen and paraffin sections using two different laminin-332 antibodies clearly demonstrated the abnormal expression of laminin-332 in PCK kidney cysts. We also observed a 2 to 4 fold increase in the expression of laminin-332 chains in the cilia defective orpk cells. Also, qPCR studies show a modest (1.5-1.8 fold) increase in the expression of laminin-511 chains in PCK kidneys. Preliminary staining results show a weaker laminin α5 staining in PCK cysts but increased staining in nephron tubules.

**Conclusions:** The abnormal expression and assembly of laminin-332 (and possibly laminin-511) in various ARPKD model systems makes a strong case for further investigation into the role of aberrant laminin assembly in ARPKD cystogenesis.

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**MicroRNA Regulation of Angiogenesis in Autosomal Dominant Polycystic Kidney Disease**

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**Background:** It is apparent that vascular changes including expansion and remodeling must occur in order to support the massive growth of cysts in patients with autosomal dominant polycystic kidney disease (ADPKD). Evidence for angiogenesis in the form of renal cysts with attendant compression of the renal vasculature results in ischaemia within the kidney. We hypothesized that microRNAs (miRs) may play a role in up-regulation of angiogenesis in the ADPKD kidneys.

**Methods:** Total RNA was extracted from 5 normal and 6 ADPKD kidneys and cDNA prepared. Renal expression of miR-107, miR-126, and miR-132 was measured by quantitative PCR and compared to expression of the housekeeping genes RNU-6 and SNORD 48.

**Results:** Expression of miR-107 was significantly decreased (P = 0.0066) by 19 fold in the ADPKD kidneys compared to control kidney. There was no significant change in miR-126 expression, while variable increase in expression of miR-132 occurred (45 fold increase, P < 0.03) in ADPKD kidneys compared to control kidney.

**Conclusions:** miR-107 has previously been shown to inhibit hypoxia signaling pathways and inhibit tumor angiogenesis, while decreased miR-107 expression increases HIF-1β, hypoxia signaling and angiogenesis. miR-132 has been shown to facilitate pathological angiogenesis by targeting p120RasGAP in the endothelium. Thus, the observed changes in ADPKD kidney are consistent with upregulation of angiogenesis in response to hypoxia.

**Funding:** Private Foundation Support

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**Dose-Expression of Exogenous Polycystin-2 Can Significantly Ameliorate the Phenotypic Severity of Pkd2 Knock-Out Mice**

Guanglei Wu, 1 Medicine, Vanderbilt University, Nashville, TN, ‘State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China.

**Background:** Loss of polycystin-2 (PC2) in mice (Pkd2−/−) results in total body edema, focal hemorrhage, structural cardiac defects, abnormal left-right axis, hepatorenal and pancreatic cysts, and embryonic lethality. To study PC2 functions in vivo, we have produced a Pkd2 transgenic mouse (Pkd2tg7) to test if ectopic overexpression of Pkd2 is able to rescue its disease phenotypes.

**Methods:** The full-length Pkd2 ORF cDNA was constructed under the control of a CMV immediate early enhancer, a chicken β-actin promoter, and a rabbit β-globin poly A signal downstream of the native termination codon (pCAGGS expression vector). Four founders (Tg7, 9, 10, 14) were found to overexpress PC2, determined by Western blotting using an internal antisense hybridization probe or Mabs to E7 as our founder (Pkd2tg7) because these mice have been most extensively studied.

**Funding:** National Institutes of Health (NIH) grant (DK-58429) to L.L. Futoran and R.C. King.

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**Transgenic Expression of Epitope Tagged Pkd2 from Bacterial Artificial Chromosomes Provide a Model for Structure-Function Studies of Polycystin-2 In Vivo**

Yiqiang Cai,1 Ming Ma,1 Xin Tian,1 Yuehong Wang,1 Rachel Gallagher,1 Sorin V. Fedele,1 Stefan Solmos.1,2 Internal Medicine, Yale University School of Medicine, New Haven, CT; 2Genetics, Yale University School of Medicine, New Haven, CT.

**Background:** Transgenic mice overexpressing Pkd1 or Pkd2 have been reported to develop renal cystic phenotypes. Our recent studies on dual epitope-tagged Pkd1/FH-BAC mouse lines showed that Bacterial Artificial Chromosome (BAC) transgenic expression of PC1 with eight or less copies of the transgene does not cause cystic phenotypes. This observation suggests that low copy BAC-transgenic mouse models are appropriate for the study of polycystin function.

**Methods:** We modified a BAC containing Pkd2 by homologous recombination in E. coli to introduce a triple-HA epitope tag before the stop codon in the last exon of Pkd2. Transgenic mouse lines were produced using this Pkd2-BAC transgene. Immunoblotting analysis using anti-HA antibodies showed that transgenic PC2 is expressed in all tissues tested in a pattern and at levels comparable to that of native PC2. Immunofluorescent staining of transgenic PC2 expression by anti-HA in kidney tissue demonstrated a cystic staining pattern in vivo. Histological examination of tissues from five-month old kidneys of Pkd2-BAC transgenic mice showed normal tubular structure without microscopic cysts. These observations suggest that the BAC-transgenic HA-tagged PC2 recapitulates the expression pattern of native PC2 in vivo. Studies on subcellular localization of transgenically expressed PC2 are ongoing.

**Conclusions:** The HA epitope tagged Pkd2-BAC can be further modified with site-specific mutation and transgenic mice produced from these BACs will serve as a platform for studying structure-function relationships in PC2 in vivo.

**Funding:** NIDDK Support

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**The Phenotypic Severity of Pkd2 Knock-Out Mice**

Guanglei Wu, 1 Medicine, Vanderbilt University, Nashville, TN, ‘State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China.

**Background:** Loss of polycystin-2 (PC2) in mice (Pkd2−/−) results in total body edema, focal hemorrhage, structural cardiac defects, abnormal left-right axis, hepatorenal and pancreatic cysts, and embryonic lethality. To study PC2 functions in vivo, we have produced a Pkd2 transgenic mouse (Pkd2tg7) to test if ectopic overexpression of Pkd2 is able to rescue its disease phenotypes.

**Methods:** The full-length Pkd2 ORF cDNA was constructed under the control of a CMV immediate early enhancer, a chicken β-actin promoter, and a rabbit β-globin poly A signal downstream of the native termination codon (pCAGGS expression vector). Four founders (Tg7, 9, 10, 14) were found to overexpress PC2, determined by Western blotting using an internal antisense hybridization probe or Mabs to E7 as our founder (Pkd2tg7) because these mice have been most extensively studied.
Results: Using a mating strategy, PKD2g7 mice were crossmated with Pkd2 +/- mice to generate PKD2g7/Pkd2 +/- and PKD2g7g7/Pkd2g7mice. Our results showed that PKD2g7/Pkd2 +/- and PKD2g7g7/Pkd2g7mice can overcome the embryonic lethality seen in PKD2g7mice. However, PKD2g7/Pkd2g7mice were born at a frequency of 10%, much lower than 21% in PKD2g7/Pkd2g7mice with the double Tg7 allele (PKD2g7g7/Pkd2g7). In addition, the PKD2g7g7/Pkd2g7mice exhibited significantly decreased cystic number and volume in the pancreas, liver and kidneys compared to PKD2g7g7/Pkd2g7 littermates, suggesting that an increased expression level of PC2 can ameliorate ADPKD disease phenotypes seen in PKD2g7/-/- mouse models. Kaplan and Meier survival analysis also indicated that the PKD2g7g7/Pkd2g7mice can survive much longer than the PKD2g7mice with a single Tg7 allele.

Conclusions: This finding indicates that the functional restoration of PKD2 gene product can rescue disease phenotypes in PKD2g7/-/- mice and sufficient expression of PC2 can potentially ameliorate PKD2-mutant ADPKD.

Funding: NIDDK Support

FR-PO2003

Expression of Bicc1 Protein during the Mouse Development Guangning Wu 1 Medicine, Vanderbilt University, Nashville, TN; 2State Key Laboratory of Molecular Oncology, Cancer Hospital and Institute, Chinese Academy of Medical Sciences, Beijing, China; 3Pathology, Yale University, New Haven, CT.

Background: Bicc1 is a mouse homologue of Drosophila Bicaudal-C (Bic-C). Orthologs of Bic-C can be identified in many species, from C.elegans to human being. The Bic-C1 mutant mouse models, jcpk and bpk, both exhibit cystic phenotype in the kidney that are very similar to human polycystic kidney disease. Yet, the developmental profiles of these mouse models remain uncharacterized.

Methods: We have therefore generated two polyclonal antibodies against different portions of Bicc1 to examine its spatial and temporal expression patterns during mouse embryogenesis and organogenesis. Two mouse Bicc1-fusion proteins encoding residues from E11.5 to A199 (mBic-N), and Q711 to D858 (mBic-C) of Bicc1, were used to produce rabbit polyclonal antisera.

Results: Our study demonstrated that Bic-C can be detected in epithelial cells of the neural tube as early as embryonic day 8.5 (E8.5) and positive staining appeared in the myocardial wall of the heart at E10.5. By E11.5, the immature hepatocytes and epithelial cells of the primordial gut, main bronchi and aorta exhibit positive staining at their cytosol. In the kidneys, Bic-C expression is seen in the epithelia of early ureteric bud and mesonephric tubules, as the bud penetrates into the metanephrogenic mesenchyme during E11.5. Significant staining continues in the renal comma-shaped body and the S-shaped body until E12.5. By 1-month of age, increased Bic-C staining extended to the renal juxta-medullary region. At this stage, no significant staining was seen in the medullary and papillary regions and relatively weak Bic-C staining were present in the cortical region of the kidneys. By co-staining adult kidney with Bic-C antibody and renal tubular segment markers, we found that Bic-C is predominantly stained at proximal convoluted tubules in the kidneys.

Conclusions: These results indicate that Bic-C is developmentally regulated and Bic-C exerts an important role in tubulogenesis and organogenesis during the renal development.

Funding: NIDDK Support

FR-PO2004

Targets and Binding Motif of the Polycystic Kidney Disease-Associated RNA Binding Protein BICC1 Iddo Zeev Ben-Dov, Thomas Tusche, Laboratory of RNA Molecular Biology, Rockefeller University, New York, NY.

Background: Mutations in protein bicaudal C homolog 1 (BICC1) have been shown to cause polycystic kidney disease in mice. We aimed to systematically explore the RNA binding characteristics of BICC1, which is the mouse homologue of Drosophila bicoid, a maternal effect gene.

Methods: BICC1 was cloned from human podocyte cDNA. Photoactivatable ribonucleoside-enhanced crosslinking and immunoprecipitation (PAR-CLIP) was performed on FLAG-BICC1 expressing HEK293 cells. Recovered small RNA was sequenced (Solexa), genome-aligned and annotated.

Results: Two BICC1 splice isoforms were (inadvertently) cloned from human podocyte cDNA (panel A). Upon expression, both showed cyttoplasmic localization in HEK293 cells (panel B). Ample in vivo crosslinking of BICC1 to RNA was noted with or without the photoactivatable nucleoside (panel C). PAR-CLIP evidence for BICC1 crosslinking to mRNA was widespread, and sporadic associations were noticed with noncoding RNA (lRNA, mRNA). High confidence mRNA clusters were >90% exonic (both isoforms). Of these, 59% (isoform 1) and 82% (isoform 2) mapped to 3'UTRs, while 36% and 17% (tRNA, miRNA). High confidence mRNA clusters were >90% exonic (both isoforms).

Conclusions: PAR-CLIP robustly reveals the in-vivo RNA binding characteristics of BICC1. Nucleotide-level information from these experiments can directly further biochemical studies, while transcript-level data may identify targets involved in the pathogenesis of human polycystic kidney disease.

FR-PO2005

Tissue Engineering Models for Human ADPKD Wei-Che C. Ko, Balaji Karthick Subramanian, Tessa Desrochers, David Kaplan. Department of Biomedical Engineering, Tufts University, Medford, MA.

Background: Modeling cystic diseases in vitro presents a unique challenge as cyst morphogenesis, in addition to complex intercellular interactions, is also governed by synergistic spatial, mechanical and temporal effects. We report the development of kidney-like tissue structures for normal and diseased (cystic) states using commercially available human kidney cells.

Methods: Gene silencing is used to simulate autonomic dominant polycystic kidney disease, as inactivating mutations in polycystins -1 and/or -2 are responsible for the disease in vivo. Our system utilizes extracellular-matrix molecules infused in slow degrading porous silk scaffolds, which provides a 3D microenvironment for proper cell polarization (ECM), while exhibiting structural robustness and tension (silk scaffold).

Results: Our results indicate development of cyst-like structures in a 3D environment, while also demonstrating the respective normal and altered phenotypes concurrent with normal tissue and patient-derived ADPKD tissue. The structural and functional features of kidney-like tissue structures were further characterized based on distribution of E-cadherin, N-cadherin, transport phenomena of 6-carboxyfluorescein, and cell-matrix interactions through integrin signaling.

Conclusions: Importantly, this 3D in vitro model may be further extended via perfusion reactor for long term studies of ADPKD or other renal cystic diseases, and may have beneficial use as a therapeutic drug screening tool.

Funding: Other NIH Support - NIBIB, Pharmaceutical Company Support

FR-PO2006

Lodinamide Reduces Cyst Size in Polycystic Kidney Disease Brenda S. Magenheimer, Shirin Sundar, Sumanth Mulamalla, Monica K. Johnson, Gail Reit, Darren P. Wallace, James P. Calvet. Kidney Institute, Univ of Kansas Medical Center, Kansas City, KS.

Background: Currently, there is no effective treatment to slow kidney enlargement in ADPKD. A lead therapeutic compound for ADPKD should effectively block both abnormal cell proliferation and cyst-forming fluid secretion in addition to having favorable safety/ toxicity and biodistribution profiles. Lodinamide, an indazole carboxylic acid derivative, was originally developed to inhibit tumor growth. It is approved for the treatment of prostate, breast, and head and neck cancers and is being tested as a combination therapy with other conventional chemotherapeutic agents. Lodinamide inhibits hexokinase activity, which is often important in the anaerobic glycolysis typical of abnormally growing neoplastic cells; and it inhibits CFTR channel activity. We showed that lodinamide inhibits ADPKD cell proliferation with an LD50 of 5.7 µM, as shown by MTT assay; and at 5 µM, lodinamide inhibits forskolin-induced chloride secretion in M-1 cells as measured by short-circuit current in the presence of benzamid.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Methods: Cell culture, organ culture, and mouse models of PKD. Results: We determined whether lonidamine alters the cyst-forming process in response to cAMP, kidney from Pkd1+/–,–/–, and –/– mice were placed in metanephric culture at E15.5 and treated with 100 μM 8-BrcAMP with or without lonidamine for 4 days. Lonidamine did not appear to be toxic to the kidneys at these doses, yet it significantly inhibited cyst formation. Lonidamine was applied for 4 days and significantly prevented cyst formation, while 10 μM lonidamine resulted in dramatic cyst reduction in Pkd1–/– kidneys. Lonidamine was also shown to significantly inhibit wound closure in a cell motility scratch assay at 25 μM and 50 μM. Cyst Nephph (pcy) mice treated with 50 mg/kg lonidamine for 8 weeks showed a decrease in kidney weight to body weight ratio and cystic index upon quantification of mid sagittal sections. The drug appeared to be well-tolerated.

Conclusions: These studies suggest that lonidamine or its derivatives may be effective therapeutic agents for long-term use in ADPKD.

Funding: NIDDK Support, Government Support - Non-U.S.

FR-PO2007
Therapeutic Potential of Phospholipase D Inhibitor in Autosomal Dominant Polycystic Kidney Disease Yang Liu,1 Rudolf P. Wuthrich,2 Andreas L. Serra. 1 Institute of Physiology, University of Zürich, Zürich, Switzerland; 2Division of Nephrology, University Hospital, Zürich, Switzerland.

Background: The mTOR pathway is activated in tubular epithelial cells lining cysts in animal and human polycystic kidneys. Phospholipase D (PLD) and the metabolite phosphatidic acid (PA) regulate mTOR activity which is competitive with the classic mTORC1 inhibitor rapamycin. We explored novel and highly specific PLD inhibitors as new therapeutic agents for PKD.

Methods: The cellular responses and mechanisms to the PLD1/2 inhibitor Honokiol, a natural compound purified from Magnolia, as well as to the highly specific PLD1, PLD2 and dual PLD1/2 inhibitors were evaluated in primary renal tubular epithelial cells either derived from human SPRD rats (Cy+) or obtained from human ADPKD kidney (HAK).

Results: PLD2 inhibitor decreased cell proliferation of Cy+ TECs and HAK more effective compared with either PLD1 inhibitor or dual PLD1/2 inhibitor. Honokiol reduced total cell numbers, cell viability and DNA synthesis of Cy+ TECs with inducing complete inhibition at 40m. Cleavage fragments of caspase 3 could be detected. Phosphorylation of PLD (P-LPD Ser561) decreased upon Honokiol treatment in a dose-dependent way. The phosphorylation of the upstream regulators (AktThr308) and downstream effectors of mTORC1 (4EBPThr43/47, S6KThr221/Ser242, S6Ser235/236) was prevented at a concentration of 30m. Honokiol suppressed phosphorylation of Akt at Ser473 site, the readout of mTORC2 activity. Phosphorylation of the MAPK (ERK1/2Thr202/204) decreased at the concentration of 40m.

Conclusions: The PLD2 inhibitor halted primary tubular epithelial cell proliferation derived from human and animal polycystic kidneys, at least in part by inducing apoptosis. The signaling through PLD and mTORC1/2, and MAPK pathways was blocked by Honokiol. PLD2 but not PLD1 controls proliferation of tubular epithelial cells. PLD2 is a critical regulator of mTOR activity which has been largely overlooked in the rapamycin-based treatment strategies in PKD. Targeting PLD may offer a new therapeutic target for PKD.

FR-PO2008
Persistin, a Soluble Extracellular Molecule, Activates Focal Adhesion Kinase and Cytoskeleton Reorganization of Human ADPKD Cells Gail Reif, Emily Nivens, Cibele S. Pinto, Corey White, Stephen C. Farnell, Maodong Liu, Darren P. Wallace. Internal Medicine, University of Kansas Medical Center, Kansas City, KS.

Background: In ADPKD, abnormal cell proliferation, apoptosis and matrix production suggest that persistent cellular and extracellular matrix are poorly differentiated, possibly involving an aberrant repair mechanism. Persistin, a soluble extracellular matrix (ECM)-related protein involved in tissue development and repair, is highly over expressed in ADPKD cells and accumulates within the matrix adjacent to cysts. Persistin binds α(v)integrins and activates ADPKD cell proliferation. Focal adhesions are sites of integrin clustering and formation of protein complexes involved in ECM communication with intracellular signaling molecules including focal adhesion kinase (FAK). Rho and FAK regulate the assembly and disassembly of focal adhesions by activating pathways that lead to actin polymerization and contraction. We tested whether persistin expression induced cell migration, while the focal adhesion related Rho and FAK dependent changes in the cytoskeleton and cell adhesions which contribute to a less differentiated phenotypes.

Methods: Human ADPKD cells were treated with recombinant 100-250 ng/ml persistin and levels of phospholipase (Rho) and FAK (P-FAK) were measured by immunoblot analysis. To determine the effect of persistin on the cytoskeleton, actin filaments were stained with Phalloidin-FITC.

Results: Persistin increased P-Rho and P-FAK levels within 30 min; however these increases were not maintained at later time points. Persistin increased the expression of α(v)integrins and α6-integrins within this time period, consistent with reciprocal activation of Rho and Fak during cell migration. Staining indicating persistin caused bundling of actin filaments into stress fibers, whereas FAK inhibition either in the absence or presence of persistin caused broad lamellipodia.

Conclusions: Persistin binding to integrins at focal adhesions causes activation of Rho and FAK, leading to cytoskeletal reorganization. These effects are consistent with persistin-induced changes in cell adhesion which may play a role in aberrant regulation of cell morphogenesis, repair, and matrix deposition.

Funding: NIDDK Support

FR-PO2009
Ouabain Regulates Expression of Adhesion Proteins and Cell-Cell Interaction in ADPKD Cells Madhulika Sharma,1 Elsa Bello-Reuss,1 Gustavo Blanco. 1Molecular and Integrative Physiology and Kidney Institute, University of Kansas Medical Center, Kansas City, KS; 2Internal Medicine, Division of Nephrology and Hypertension, Texas Tech University Health Science Center, Lubbock, TX.

Background: Epithelial cell proliferation is one of the hallmarks of the development of new cysts in autosomal dominant polycystic kidney disease (ADPKD). Cell proliferation requires regulation of the contact between neighboring cells and cell junctions. Cell junction function is controlled by regulation of the expression of adhesion proteins. We have previously shown that the hormone ouabain, at physiological concentrations, stimulates proliferation of cultured human ADPKD cells.

Methods: In this work, we studied the effect of ouabain on adhesion proteins of tight and adherens junctions of ADPKD cells. ADPKD cells were incubated in the absence or presence of 3nM ouabain for 24hrs and the expression levels of a series of adhesion molecules were determined in the whole cells and in plasma membrane fractions by immunoblot and immunochemistry.

Results: Ouabain differentially affected tight junction protein expression. While claudin1 and ZO-1 were not significantly modified, occludin amounts were elevated in ouabain-treated ADPKD cells. Ouabain also regulated expression of zonula-adeherens proteins in a specific manner, increasing the amounts of vinculin, reducing the levels of E-cadherin and maintaining β-catenin unchanged. In addition, ouabain modulated cell-cell interaction, reducing the ability of ADPKD cells to adhere to each other. In contrast, permeability of ADPKD cell monolayers to dextran showed no significant changes after ouabain treatment.

Conclusions: These results show that in ADPKD cells, ouabain regulates the expression of adhesion proteins in a complex manner to concomitantly favor relaxation of cell-cell junctions while still preserving the permeability of the paracellular pathway of the ADPKD epithelium. These effects are in line with contributing to the overall action of ouabain of promoting proliferation of ADPKD cells.

Funding: NIDDK Support

FR-PO2100
Curcumin Analog 2a Inhibits In Vitro Proliferation of Autosomal Dominant Polycystic Kidney Disease Cyst Cells Beatrix Adriana Veliz,1 Laura Parra,2 Moses Lee,2 Albert C. Ong,3 Madhulika Sharma,1 1Molecular and Integrative Physiology and Kidney Institute, University of Kansas Medical Center, Kansas City, KS; 2Internal Medicine, Division of Nephrology and Hypertension, Texas Tech University Health Science Center, Lubbock, TX; 3Hoepe College, Holland, MI; 4Academic Nephrology Unit, University of Sheffield Med School, United Kingdom.

Background: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by progressive renal cyst development and destruction of the renal parenchyma or tubules due to the kidney failure. Curcumin inhibits proliferation pathways activated in cancer that are common to ADPKD cyst cells. However no therapeutic levels are achieved by oral administration. Analogue 2a, a curcumin derivative lacking curcuminoid structures has improved intestinal absorption and is a more potent cytotoxic agent to cancer cell lines. Thus, it may be of value for treatment of ADPKD. Since cyst cells exhibit proliferative pathways not present in normal kidney cells, Analogue 2a may have a predominant inhibitory effect on cyst cell proliferation. We addressed the effects of Analogue 2a on proliferation of cyst cells, normal kidney cells and cyst cell lines.

Methods: We used the ADPKD-cyst cell lines OX161 and SKI-001, ADPKD-cyst cells in primary cultures, normal cortical tubule cells in primary cultures, and the normal kidney cell line UCL93/9. Cell survival was measured at concentrations between 0 and 3µM. The half-maximal inhibitory concentrations (IC50) of Analogue 2a were determined by data fitting to a 4 parameter logistic curve.

Results: Analogue 2a was cytotoxic to cyst cells at lower concentrations than to normal kidney cells with a selectivity index larger than 3. The IC50 (mean ± SD) were: ADPKD-cyst-cell lines, 0.98 ± 0.13 µM (n=3); cyst-cell primary cultures, 0.73 ± 0.32 µM (n=4); control cell line, 0.87 µM ± 0.09, not different from cyst cells; normal kidney primary cultures were unaffected by Analogue 2a at 3 µM. P < 0.001 vs cyst cells.

Conclusions: 1. ADPKD cell lines and primary cultures of ADPKD cells have high and similar sensitivities to Analogue 2a. 2. Normal kidney cells, in primary cultures, are less resistant than ADPKD cells to Analogue 2a. 3. Immortalized cells from normal kidney are sensitive to Analogue 2a, and hence are not an appropriate control system. These results indicate that Analogue 2a has potential for the treatment of ADPKD.

Funding: Private Foundation Support

FR-PO2101
Bidirectional Regulation between Polycystin-2 and Cellular Stress Jungwoo Yang,1 Qian Wang,1 Wang Zheng,1 Carlos Lara,1 Zuocheng Wang,1 Guanqing Wu,2 Xing-Zhen Chen. 1Physiol, Univ of Alberta, Edmonton, AB; Canada; 2Medicine, Vanderbilt Univ, Nashville, TN.

Background: ADPKD is associated with several cellular abnormalities such as cell over-proliferation and apoptosis. Mutations in polycystin-2 (PC2), a Ca2+-permeable channel present in the plasma membrane, plasma membrane and cilia, account for ~10% of ADPKD cases. How PC2 expression is regulated and how it regulates processes is not yet known. We recently reported that PC2 down-regulates cell proliferation and protein synthesis through promoting the phosphorylation of eukaryotic initiation factor eIF2α by PERK.

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Results: Here we found that endogenous PC2 protein expression is up-regulated in human renal cystic fibroblasts and endoplasmic reticulum (ER) stress conditions, including ER stress, oxidative stress and virus infection-induced stress, which all increase the phosphorylated eIF2α (P-eIF2α). Increased P-eIF2α triggers a number of downstream processes, including global inhibition of translation and proliferation, and regulation of gene expression and apoptosis via transcriptional up-regulation of transcription factor ATF4. Inhibition and stimulation of the activity of protein phosphatase of eIF2α by salubrin and Gadd34 over-expression respectively up- and down-regulated P-eIF2α expression in cultured cells or live mice. RT-PCR assays indicated that the Pkd2 mRNA level is not affected by stress, whereas the 5' upstream ORF (uORF) of Pkd2 mRNA abolishes the regulation of P-c2 by P-eIF2α. These data together indicate that P-eIF2α translationally up-regulates P-eIF2α expression through uORF. We found that P2C binds ER chaperone and ER stress marker GRP-78 and that the binding is substantially reduced by proteasome inhibition. Furthermore, removal of the 5' upstream ORF of Pkd2 mRNA abolishes the regulation of P-c2 by P-eIF2α. This effect was toward a cytotoxic endpoint.

Conclusions: In conclusion, PC2 is not only up-regulated by P-eIF2α under stress conditions but is also an important mediator of cellular responses to ER stress.

Funding: Funding Government Support - Non-U.S.

FR-PO2012

Endoplasmic Reticulum Stress in the Pathogenesis and Treatment of TSC

Renal Cystic Disease

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Background: Tuberculosis spleen complex (TSC) is an inherited tumor predisposition syndrome in which TSC1 or TSC2 mutations lead to deregulated mammalian target of rapamycin complex 1 (mTORC1) signaling. Nearly 50% of TSC patients develop renal cysts, and these patients are at the highest risk to progress to end stage renal failure. However, it has been demonstrated that rapamycin is highly effective in reducing renal cystogenesis. However, mTOR activity in PC2 mutants has not been studied thus far.

Methods: We developed conditionally immortalized human proximal tubule epithelial cell models of ADPKD, we demonstrate that mTORC1 activity seemed not to be affected under stress conditions or P-eIF2α. Further, PC2 also interacted with calnexin, an ER chaperone assisting protein folding, but the binding was increased by ER stress. Finally, using mouse collecting duct cells with Pkd2 knockout and HeLa cells with PC2 knockdown, we found that PC2 is critical for the up-regulation of GRP-78 by ER stress.

Results: In the HK-2 human proximal tubule cell line, RNAi-induced knockdown of TSC2 increased expression of BiP, IRE1α, and CCAAT/enhancer-binding protein homologous protein (CHOP), a pro-apoptotic transcription factor. ER stress exacerbation by proteasome inhibition with polymerase (PARP) in EKT2 cells, indicating pro-apoptotic signaling. This effect was toward a cytotoxic endpoint.

Conclusions: In conclusion, PC2 is not only up-regulated by P-eIF2α under stress conditions but is also an important mediator of cellular responses to ER stress.

Funding: Funding Government Support - Non-U.S.

FR-PO2015

Reduced Proteoglycans in the Kidney Causes Both Tubule and Glomerular Abnormalities

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Background: Most cells produce some form of proteoglycan(s). The initial assembly of proteoglycans on the core protein requires the transfer of a xylose to a designated serine residue. A sulfotransferase responsible for this is xylosyltransferase II (XylT2), which remains elusive, possibly due to the highly polymeric nature of proteoglycans. Xylosyltransferase 2 (XylT2) is ubiquitously expressed in many organs suggesting a significant role in the generation of glycosaminoglycans (GAGs) and other GAG-dependent proteoglycans. Several animal studies have shown a significant role of XylT2 dependent proteoglycans in those organs. In our XylT2 knockout mice (Xylt2−/− mice), considerable XylT activity remains in the kidney due to the existence of another isoform of the enzyme, XylT1.

Methods: We developed Xylt2-/- mice, which have a much poorer long term prognosis. Since the cause of the proteinuria in PKD patients is unclear, there is no specific therapeutic intervention. Our analyses in the Xylt2-/- mice established that proteoglycans are important in cyst development in the liver. The additional findings in the kidney indicate that reduced glycosaminoglycan assembly onto core proteins due to XylT2 deficiency has a pathologic impact as well. Seventy percent of PKD patients develop liver cysts as well as renal cysts. Overall our findings suggest that reduced proteoglycans may have a genetic modifying role in inherited polycystic kidney disease (PKD) the fourth leading cause of renal failure in the United States. PKD patients that develop proteinuria have a much poorer long term prognosis. Since the cause of the proteinuria in PKD patients is unclear, there is no specific therapeutic intervention. Our analyses in the Xylt2−/− mice suggest that one source of the proteinuria is reduced renal proteoglycans.

Results: The results provide evidence for an increased rate of cystogenesis in the disease system, initiated by abnormal pericytic ECM interactions between matrix molecules and integrin subunit proteins. In addition, molecular signaling analysis showed abnormalities in cyclin proteins and cell-cycle progression. Very importantly, we show that inhibiting the pericytic interaction by double silencing the integrin proteins reverses the abnormalities and reduces the rate of cystogenesis and may be considered for ADPKD therapeutic interventions.

Conclusions: We provide evidence for an autocrine signaling involving abnormal matrix interactions, to regulate the rate of cystogenesis and could be targeted for ADPKD therapeutics.

Funding: Other NIH Support - NIBIB, Pharmaceutical Company Support

FR-PO2016

A Metabolic Approach Reveals a Metabolic Switch in PKD

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Background: Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a very large plasma membrane receptor. It has been described to regulate several cell signaling pathways, in

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particular the mTORC1 and Akt pathways, major actors in energy sensing and cellular metabolism and regulation.

**Results:** To investigate the role of PC-1 in regulating metabolism, we performed metabolomic profiling of the extracellular medium of Pkd1-/- and Pkd1+/+ MEF (murine embryonic fibroblast) cells. NMR Spectra were superimposed and showed differences between Pkd1-/- and Pkd1+/+ cells. In particular, Pkd1-/- cells presented an increase in glucose uptake and lactate production, suggesting an increased anaerobic glycolysis (Warburg’s effect).

Consistent with this, intracellular ATP content was enhanced in the mutant cells compared to the wild type. Measurement of the mitochondrial potential has shown that it was normal in Pkd1-/- cells. There was no increased ATP anymore in Pkd1-/- cells compared to the Pkd1+/+ cells after glucose starvation showing that ATP content was exclusively caused by an increased glycolysis. In line with these results, Pkd1-/- cells were hypersensitive to glucose starvation, with a ramapycin sensitive increased apoptosis. In the same conditions, autophagy was induced in Pkd1+/+ cells as expected while Pkd1-/- cells failed to undergo autophagy in line with the increased mTORC1 activity in these cells.

Next we monitored the activation of the energy sensor AMPK, which was downregulated in Pkd1+/+ cells. Next, we looked if a similar glycolytic switch could be detected in vivo, we found that ATP content was increased and AMPK downregulated in the cystic kidneys of a Pkd1flox-/-:Ksp-Cre mouse model. Preliminary results suggest an increase in glucose uptake in these kidneys in vivo.

**Conclusions:** Our data uncover a previously unrecognized role for the Pkd1 gene in regulation of cellular energy balance.

**Funding:** Private Foundation Support

**FR-PO2017**

**Renal and Cardiovascular Phenotypes in Mice with Pkd2-/-: Mice Evidence for Nephrogenic Diabetes Insipidus and Impaired Urinary Acidification but Normal Glomerular Filtration Rate (GFR)**

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**Background:** Pkd2-/- mice have high levels of cell death and show an increased propensity for osteosclerosis. They have decreased urine output and decreased urinary pH, but it is not clear if they exhibit diabetes insipidus. In this study we wanted to evaluate the renal and cardiovascular phenotypes in these mice.

**Methods:** We evaluated the renal and cardiovascular phenotypes in male Pkd2-/- mice using direct carotid blood pressure (BP) and echocardiography. The animals were also subjected to a variety of tests, including blood testing and histological analysis.

**Results:** The results show that Pkd2-/- mice have increased blood pressure and decreased kidney function. The mice also exhibit decreased urine output and decreased urinary pH, consistent with diabetes insipidus. The decrease in urine output is accompanied by a decrease in renal mass, consistent with kidney failure. The decrease in urinary pH is consistent with the decreased renal function. The increased blood pressure is consistent with the decreased kidney function.

**Conclusions:** The results suggest that Pkd2-/- mice have diabetes insipidus and impaired urinary acidification. The decrease in kidney mass and decreased urinary pH are consistent with kidney failure. The increased blood pressure is consistent with the decreased kidney function.

**Funding:** Private Foundation Support

**FR-PO2019**

**Projection Structure of the Polycystin-1 Membrane Domains**

Brian D. Adair, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA.

**Background:** In the majority of cases, polycystic kidney disease arises from a mutation in either of the genes PKD1 or PKD2. PKD2 encodes a nonspecific cation channel (polycystin-2 or PC-2) which possesses six transmembrane spans and is believed to act as a tetramer. In contrast, PKD1 encodes an extremely large membrane protein (polycystin-1 or PC-1) with eleven transmembrane domains and an extensive extracellular N-terminal region possessing numerous structural motifs for binding cell matrix and membrane proteins. The two proteins interact in vivo and are believed to participate in the same signaling pathway with PC-1 modulating the channel activity of PC-2. The nature of the interaction remains elusive, although the region of PC-1 containing the six terminal transmembrane spans shows sequence homology with the transmembrane region of PC-2.

**Methods:** A region of the PKD1 gene encoding the eleven transmembrane domains has been cloned and expressed in the yeast Pichia pastoris. The construct begins at the GPS domain prior to the first transmembrane span and includes the complete cytosolic C-terminal domain. The recombinant protein forms 2D crystalline arrays in membranes. Electron cryoelectron microscopy has been employed determine the projection structure of the membrane embedded protein.

**Results:** The 2D crystals have a plane group symmetry of p4 with unit cell parameters a=b=159 Å and γ=90°. The projection structure derived from electron cryoelectron microscopy reveals that a unit cell is composed of four PC-1 dimers related by four-fold rotation symmetry. The dimers are closely associated with direct, protein-protein interactions, while the packing between dimers in the unit cell is much looser with the dimers clearly separated by lipid molecules.

**Conclusions:** The sequence and topology homology between the transmembrane domains of PC-2 and the terminal transmembrane domains of PC-1 had suggested that the latter might form an equivalent, tetrameric channel. The structure reveals that this is not the case and PC-1 cannot form a channel on its own. However, the dimeric organization suggests that PC-1 might form a heterotetrameric channel with the homologous domains of PC-2, where opposed dimers of PC-1 and PC-2 would form a pseudo-four-fold channel.

**Funding:** Private Foundation Support

**FR-PO2020**

**Mitochondrial Defects and Lifespan Extension in Yeast Model of XPNPEP3 Deletion**

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**Background:** XPNPEP3 is a nuclear gene that encodes a mitochondrial aminopeptidase and mutations have been identified in individuals with heritable kidney failure with tubular atrophy and interstitial fibrosis. The Saccharomyces cerevisiae ortholog, ICP55, localizes to mitochondria and stabilizes its target proteins. Thus, we used yeast as a genetic model of XPNPEP3 to investigate the role of ICP55 in yeast lifespan extension.

**Methods:** Yeast strains were generated with PCR based homologous recombination and grown using standard culture media and conditions. Oxygen consumption was measured with a Clarke-type oxygen electrode. Chronological lifespan (CLS) and resistance to H2O2 were determined with trypan blue staining and plating serial dilutions. Reactive oxygen species (ROS) were measured with dihydrothiobarbituric staining and flow cytometry.

**Results:** ICP55 deletion strains are viable and respiratory competent. Growth in glucose media is comparable to parental strains, but mitochondrial oxygen consumption is significantly decreased. As mitochondrial defects often lead to decrements in lifespan we examined CLS. Surprisingly, ICP55 deletion increased the CLS. Since TOR1 is known to affect mitochondrial respiration and lifespan, we treated deletion strains with rapamycin, which restored the mitochondrial oxygen consumption to wild-type levels. Deletion of TOR1 also increased the CLS, but interestingly, combined deletion of TOR1 and ICP55 increased CLS additively, suggesting independent mechanisms of CLS extension. Deletion strains were also more resistant to H2O2 treatment than parental strains and had lower levels of ROS.

**Conclusions:** These results suggest that deletion of XPNPEP3/ICP55 results in mitochondrial stress possibly related to dysregulation of mitochondrial protein degradation pathways. The stress response pathways activated in the yeast model may include TOR1 activation, but the resistance to oxidative stress and CLS extension likely occurs through mechanisms that are independent of that observed with TOR1 deletion. Further characterization of yeast mitochondrial stress pathways should define mechanisms underlying the renal tubular atrophy and interstitial fibrosis observed in patients.

**Funding:** NIDDK Support

**FR-PO2018**

**Diabetes Accelerates Cystogenesis and Results in Glomerular and Tubular Damage in the Adult Conditional ift88 Knockout Mouse**

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**Background:** Diabetes, the leading cause of end stage renal disease, results in structural and functional hypertrophy in the kidney. We have previously found that renal hypertrophy accelerates cystogenesis in the glomeruli and collecting ducts of diabetic mice (1). The goal of this study was to determine if the presence of diabetes would modify the time course and degree of cyst formation in the adult mouse with deletion of cilia.

**Methods:** To examine the role of cilia in diabetes, we utilized a conditional floxed allele (ICP55/C1198flox2) to produce cilia (+) or cilia (-) adult mice. Mice were administered streptozotocin at 50 mg/kg for 5 days to induce diabetes. After 6 weeks, mice underwent MRTI to determine cystic burden and kidney weights were removed after 6 or 12 weeks for histological analysis.

**Results:** Blood glucose concentrations were elevated but not significantly different between groups. MRI and histological analyses identified accelerated cystogenesis mainly in the glomeruli and collecting ducts of diabetic cilia (-) mice. Additionally, diabetic cilia (-) mice exhibited abnormalities in non-cystic regions with signs of tubular stress and glomerular atrophy. These changes were accompanied by primary renal tubules, mesangial expansion, and prominent podocytes. These abnormalities did not occur in cilia (+) mice. There was also a marked increase in lymphocytic infiltration and enhanced mTOR, AKT, and B-catenin signaling, as well as decreased E-cadherin and RIKP expression in kidneys from the diabetic cilia (-) mice.

**Conclusions:** Diabetes may be a significant risk factor for accelerated cyst formation and renal failure in PKD, and cilia may be nero-protective in diabetes.

**Funding:** NIDDK Support, Veterans Administration Support
Lymphocyte Subpopulations during Hemodialysis and Hemodiafiltration

Dietel H. Kriet1, Sebastian Seidel,1 Karin Merget,1 Horst-Dieter Lemke,2,3 Christoph Wanner,1 Nephrology, University Hospital, Würzburg; 2ExCerLab GmbH, Ohmburg; 3Membrana GmbH, Wuppertal, Germany.

Background: Lymphocytes are central to the adaptive immune response and their function is impaired in maintenance dialysis patients increasing the susceptibility to infection. The effects of different dialysis modes on the course of lymphocyte subpopulations are largely unknown.

Methods: In a prospective, randomized, controlled, cross-over trial, 10 maintenance dialysis patients were subjected to 4 weeks of each low-flux HD (LF-HD), high-flux HD (HF-HD), low- and high-efficiency postdilution hemodiafiltration (Lo-HDF and Hi-HDF, respectively), and ultrafiltration with filtration rate 500 ml/min (UF). Immunohistological, highly biocompatible synthetic dialysis membranes and ultrafiltrate were used in all treatment forms (LF-HD, PUREMA® L, 1.8 m²; HF-HD, Lo-HDF and Hi-HDF, PUREMA® H, 1.9 m²). Counts of total lymphocytes and lymphocyte subsets (T-lymphocytes (CD3+), T-inducer cells (CD3+/CD4+), cytotoxic T-cells (CD3+/CD8+), B-lymphocytes (CD45+/CD19+/CD3−), natural killer cells (CD45+/CD16+56/CD3+) were determined during individual treatments as well as at baseline and at the end of each 4-week period.

Results: At 10 min, total lymphocytes (between 79±12% (LF-HD; P<0.05) to 89±11% (HF-HD; P<0.05) to 90±9% (HF-HD of baseline), CD3−/CD3+(86±11% (LF-HD; P<0.05) to 90±9% (HF-HD of baseline), CD3−/CD4+(91±10% (LF-HD; P<0.05) to 104±11% (HF-HD of baseline), CD3−/CD8+(76±10% (LF-HD; P<0.05) to 89±15% (HF-HD of baseline) and CD45+/CD19+/CD3−(82±17% (LF-HD; P<0.05) to 90±14% (Hi-HDF of baseline) cells had only slightly and moderately increased. In contrast, CD16+/CD56−/CD3− cells steeply increased to the course of CD3−/CD8− cells, permanently dropped to between 51±14% (LF-HD) to 63±16% (Lo-HDF) of baseline (P<0.01). No differences between treatment modes and the predialysis cell counts at baseline and at the end of the 4-week periods were observed.

Conclusions: Different to other lymphocyte subpopulations, CD45+/CD16−/CD3− cells are strongly activated during extracorporeal dialysis procedures. Enhanced convective toxin removal, such as in HDF, has no additional effect on the biological behavior of lymphocytes.

Funding: Pharmaceutical Company Support

FR-PO2021

Acidic Hemodialysis (HD) Patients, High Dialysate Bicarbonate Concentration Corrects Pre-HD Acidosis but Induces Post-HD Metabolic Alkalosis

David Tovbin,1 Shimon Storch,1 Lone Solling Avnon,1 Amir Abd Eldakar,2 Moshe Zlotnik.1 Nephrology; Pulmonary, Soroka Medical Center; Bi-o-engineering, Ben-Gurion University of the Negev, Beer-Sheva; Nephrology, Bnai-Zion Medical Center, Haifa, Israel.

Background: Acidosis is a common severe problem in HD patients. K/DOQI guideline for pre-dialysis bicarbonate serum level (BIC) (SBIC) ≥ 22 mEq/L is probably insufficient and frequently not achieved on routine dialysis BIC concentration (DBIC) of 33-34 mEq/L. Additionally, highly biocompatible synthetic dialysis membranes and ultrafiltrate may induce intra-dialytic metabolic alkalosis/alkalemia, or due to increased buffering capacity and CO2 production, severe hypercapnia in pulmonary problems or apneic sleep episodes during HD. This study aimed to assess effects of high (40 mEq/L) vs low (33-34 mEq/L) DBIC of pre-HD serum bicarbonate (BIC) level (SBIC) > 22 mEq/L is probably insufficient and resulted in a significant reduction of Treg cells and elevation of Th17 cells. There were significant changes in the number of Treg and Th17 cells by AOPPs in ASCVD compared to non-ASCVD and healthy controls groups.

Conclusions: Treg and Th17 cells from ASCVD patients were more susceptible to AOPPs-mediated alterations. Increased AOPPs have an effect on Th17/Treg imbalance, promote the micro-inflammatory state, and may ultimately contribute to the occurrence of ASCVD in MHD patients.

Funding: Government Support - Non-U.S.

FR-PO2024

Tinzaparin for Anticoagulation in Haemodialysis (HD); A Simple Dosing Algorithm Which Is Safe and Efficacious

Christopher J. Kirwan, Neil Ashman, Zahid Farooq Baig. Royal London Hospital, United Kingdom.

Background: Bleeding complications when using heparin, essential as anticoagulation during HD, are common. Low molecular weight heparins (LMWH) have reduced their use through the bolus Tinzaparin (TP), has a 1/2life of 4 hrs in controls but this is longer in renal failure pts allowing potentially dangerous latent anticoagulation. Plasma anti Xa activity (anti-Xa) is used as a surrogate marker for TP activity. TP can be an effective anticoagulation during HD but dosing regimens vary & end HD anti-Xa has not been fully assessed. We studied anti-Xa in pts receiving TP from a fixed dose HD protocol, assessing efficacy & safety aiming to standardise anticoagulation

Methods: Anti-Xa measured during HD in consecutive pts on a single HD unit. 2500,3500 or 4500iu of TP was given on based length of HD and prior coagulation events. Anti-Xa was measured at T0, 5, 10, 15 & 20 mins & at the end of HD. Anti-Xa<0.2 is considered therapeutically ineffective. Efficacy & safety data was collected from 5 HD sessions spanning the study day.

Results: Anti-Xa 3 pts on HD (3.5 or 4hrs), had anti Xa profiling. 19,18 & 6pts received 2500, 3500 or 4500iu of TP respectively. 3 pts had TP respectively. 3 hrs HD, 17 received 2500iu, 6/2500, 8/3500, 4/4500iu of TP. No pt had detectable anti-Xa at T0. Pts receiving 3500iu weighed more than those for 2500iu (69±56kg; p<0.05) & the greater the dose/kg the higher the peak anti-Xa (p<0.01). Dose/kg did not predict end HD anti-Xa (p=0.07). Higher TP doses correlated to peak anti-Xa (p<0.05). 15pts had anti-Xa >0.2 at the end of HD, 5/2500iu, 8/3500iu & 4/4500iu. Across 260 HD sessions (52 pts) mean AVF compression time post HD was 9 mins & there were 4 incidents of minor bleeding & 1 clotted circuit.

Conclusions: We based dosing of TP in HD does not add to safety & efficacy of anticoagulation. Higher doses lead to higher ant-Xa & end HD anti-Xa is considered inadequate. Anticoagulation can be achieved with a fixed dose of TP in most cases. A higher TP dose carries a significant anti-Xa at the end of HD. We recommend starting TP at 2500iu, increasing to 3500iu if there are signs of circuit coagulation. A single anti-Xa level should be measured at the end of HD guiding dose, if there is a coagulation concern

Funding: None

FR-PO2025

Pharmacokinetics of the Direct Renin Inhibitor Aliskiren in Patients with End-Stage Renal Disease Undergoing Hemodialysis

Dmytro Khadzhynov,1 Torsten Slowinski,1 Ina Licker,2 Diego Albrecht,2 Henk Johan Sterreek,3 Sam Rebelo,1 Harm Peters,1 Department of Nephrology, Charité Campus Mitte, Charité Universitätsmedizin, Berlin, Germany; 2Novartis Institutes for BioMedical Research, Novartis Campus, Basel, Switzerland; 3Novartis Pharmaceuticaels Corporation, East Hanover, NJ.

Background: Aliskiren represents a new class of orally active direct renin inhibitors and is approved for the treatment of hypertension. The present study compares the pharmacokinetics, safety and tolerability of a single dose of aliskiren in patients with End-Stage Renal Disease (ESRD) on hemodialysis (HD)im comparison to matched healthy subjects.

Methods: This open-label, single-sequence study enrolled 6 male ESRD patients undergoing chronic HD and 6 healthy, age and body-weight matched volunteers without HD treatment. The ESRD patients underwent two treatment periods (Period 1: single oral dose of aliskiren 300mg 1 h before the HD session and Period 2: single oral dose of aliskiren 300mg 1 h before the HD session) with a wash out period of 10-14 days between them. HD treatment lasted 4 h (blood flow 300ml/min, dialysate flow 500ml/min). Blood and dialysis samples were taken for up to 96 h post-dose. Serum concentrations of aliskiren were measured using liquid chromatography-tandem mass spectrometry. The mean AUC (area under the curve) of aliskiren 300mg and 9h plasma pharmacokinetics was determined.

Results: In both treatment protocols, dialysis clearance was low (1-2% of oral clearance) and not significantly altered by timing of dialysis. Fraction of aliskiren eliminated by HD was <0.2% in both settings. Compared to the healthy subjects, administration of 300 mg aliskiren was associated with an increase in AUC (+61%, 2031±737ng·h/ml).
and Cmax (+17%, 279±203 mg/mL) in ESRD patients receiving HD at 48 h and increase in AUC (+42%, 1776±1149 mg*h/mL) and decrease in Cmax (-16%, 200±32 mg/mL) in those receiving HD at 1 h.

Conclusions: Only a minor faction of aliksiren is eliminated by an intermittent HD. The aliksiren exposure is not significantly different in ESRD patients receiving a single oral dose of 300 mg either at 1 h or 48 h before HD. No severe adverse reactions occurred in this study.

Funding: Pharmaceutical Company Support

FR-PO2026
Effective Elimination of Dabigatran with Haemodialysis: A Phase I Single Centre Study in Patients with End-Stage Renal Disease Harm Peters,1 Dmytro Khadzhynov,1 Frank-Dietrich H. Wagner,1 Stephan Formella,2 Viktoria Moschett,3 Andreas Clemens,3 1Nephrology, Charité, Campus Mitte, Berlin, Germany; 2Charité Research Organisation GmbH, Berlin, Germany; 3Boehringer Ingelheim Pharma GmbH Co. KG, Ingelheim, Germany.

Background: Dabigatran etexilate (DE) is the pro-drug of dabigatran (D), a synthetic low molecular weight orally active reversible direct thrombin inhibitor. DE is approved for the prevention of stroke and systemic embolism in patients with atrial fibrillation and for the primary prevention of thromboembolic events in the orthopaedic setting. A specific antidote for D has been recently preclinically identified and is not yet available for clinical use in patients. Haemodialysis may be a measure in emergency situations requiring fast elimination of D.

Methods: Dialysis modalities (catheter setting, blood flow rate of 200 ml/min, dialysate flow rate of 700 ml/min) are thought to apply for the majority of patients anticoagulated with DE and having a normal renal function. Six patients with end-stage renal disease (ESRD) received DE once daily over 3 days to achieve steady state (Day 1: 150 mg; Day 2: 110 mg; Day 3: 75 mg).

Results: Plasma trough concentrations at Day 3 were 146±58.1 ng/ml and increased to peak concentrations of 176±60 ng/ml 2 h following the last dose of 75 mg. Dialysis was started 8 h following the last dose to avoid absorption and distribution of the xG (D=150±58.9 ng/ml). After the 4-h dialysis, plasma concentrations had dropped sharply to approx. one half (76±48 ng/ml). The extraction ratio calculated from blood entering and leaving the dialyser was 81±2.0%. The rebound after stopping dialysis was minor representing a 10% increase in plasma concentration.

Conclusions: The high dialysis extraction ratio of 81.3% in patients with ESRD and the minor rebound of plasma concentration after dialysis indicate that haemodialysis is an effective method to remove D from the body. Four-hour dialysis under normal blood flow conditions eliminated about 50% of D. Elimination rates with a high blood flow rate of 400 ml/min will be reported in an updated version of this abstract. Haemodialysis can be employed as an effective measure to eliminate D in emergency situations.

Funding: Pharmaceutical Company Support

FR-PO2027
A Multi-Center, Prospective, Open-Label, 8-Week Study To Investigate the Efficacy, Safety and Pharmacodynamics of Certoparin for Anticoagulation during Maintenance Hemodialysis – The MEMBRANE Study Detlef H. Krieter,1 Oliver Dorsch,1 Horst-Dieter Lenke,1 Nina Melzer,1 Christian Sieder,1 Peter Bramlage,2 Job Harenberg,3 1Nephrology, University Hospital, Würzburg, Germany; 2KfH Renal Center, Kronach; 3EXcorLab, Obernburg, Germany; 4Novartis Pharmaceuticals Corporation, East Hanover, NJ, USA.

Background: Anticoagulation is a prerequisite for hemodialysis (HD) to prevent clotting in the extracorporeal circuit. We provide efficacy, safety and pharmacodynamic data on the LMWH certoparin.

Methods: Multicenter, open-label, prospective, 8-week trial in patients (pts) undergoing HD receiving a single dose of 3,000 IU certoparin i.v. patients with atrial fibrillation and for the primary prevention of thromboembolic events in the orthopaedic setting. A specific antidote for D has been recently preclinically identified and is not yet available for clinical use in patients. Haemodialysis may be a measure in emergency situations requiring fast elimination of D.

Methods: Dialysis modalities (catheter setting, blood flow rate of 200 ml/min, dialysate flow rate of 700 ml/min) are thought to apply for the majority of patients anticoagulated with DE and having a normal renal function. Six patients with end-stage renal disease (ESRD) received DE once daily over 3 days to achieve steady state (Day 1: 150 mg; Day 2: 110 mg; Day 3: 75 mg).

Results: Plasma trough concentrations at Day 3 were 146±58.1 ng/ml and increased to peak concentrations of 176±60 ng/ml 2 h following the last dose of 75 mg. Dialysis was started 8 h following the last dose to avoid absorption and distribution of the xG (D=150±58.9 ng/ml). After the 4-h dialysis, plasma concentrations had dropped sharply to approx. one half (76±48 ng/ml). The extraction ratio calculated from blood entering and leaving the dialyser was 81±2.0%. The rebound after stopping dialysis was minor representing a 10% increase in plasma concentration.

Conclusions: The high dialysis extraction ratio of 81.3% in patients with ESRD and the minor rebound of plasma concentration after dialysis indicate that haemodialysis is an effective method to remove D from the body. Four-hour dialysis under normal blood flow conditions eliminated about 50% of D. Elimination rates with a high blood flow rate of 400 ml/min will be reported in an updated version of this abstract. Haemodialysis can be employed as an effective measure to eliminate D in emergency situations.

Funding: Pharmaceutical Company Support

FR-PO2028
Asymmetric Dimethylarginine Removal in a Hemodiafiltration-Adsorption Technique: Comparison with Bicarbonate Dialysis and Hemodiafiltration Antonio Sidoti,1 Marina Biagoli,1 Donella Borracelli,1 Luisa Sereni,2 Céline PiekarSKI,3 Giuseppe Palladino,2 1Nephrology, Poggibonsi, Italy; 2Bellco, Miranda, Italy.

Background: Asymmetric dimethylarginine (ADMA) whose plasma levels increase with worsening of kidney failure is a strong independent mortality predictor in ESRD. A large part to understand which is best dialysis technique to remove it, has not achieved definitive results. A significant protein binding could affect ADMA dialysance.

Methods: SUPRA provides separately, in the same device, hemodilution, filtration and adsorption with a 0.7 μm super high flux polyphenylene membrane 43000 D cut-off, a diffusive membrane 1.7 μm low-flux polyethylene and a styrenic resin cartridge to regenerate by adsorption ultrafiltrate for so called endogenous reinfusion.

We studied four patients, three men and a woman, mean age 68.5±15.5, dialytic age 243±119 mo.s, none with residual renal function, each had a 240’ SUPRA, on-line HDFaHemodiafiltration(HDF)(1.8 mm polysulfone(PS) hi-flux and bicarbonate dialysis (BD) 1.8 mm PS low-flux treatment as first session of the week. Blood flow was 330±35 ml/min, infusion liters were 130±1.9 on SUPRA and 12.9±1.19 on HDF. We used ELISA ADLA is plasma samples obtained at 0’ and 240’.

Results: ADMA at 0’ and 240’ were 1.92±0.48 and 0.27±0.09 for SUPRA, 1.7±0.355 and 0.625±0.075 for BD, 0.945±0.63 0.497±0.063 for HDF. Reduction Ratio Percent(ER) was 75.9±6.14 for SUPRA, 62.5±5.7 for BD, 47.8±2.4 for HDF. Differences by pairs were from start to end 0.917±0.48(0.25-1.58) p<0.022 for SUPRA, 1.075±0.321(0.95 0.56-1.53) p<0.007 for BD, 0.447±0.099 for HDF(95% 0.28-0.60) p=0.003. Two a way ANOVA indicate a significant effect due to techniques [F=44.1; p<0.01].

Conclusions: SUPRA demonstrated a better RR than BD and HDF most likely due to its ability to ultrafilter a proportion of plasma protein e.g. albumin (6g) during each treatment. Substances bound to proteins but also present in plasma water are liable to adsorption during the passage through the cartridge before ultrafiltrate returns to the patient. Further studies are needed to elucidate effectiveness of this technique in a long follow up i.e. in lowering in a permanent way ADMA levels.

Funding: Government Support - Non-U.S.

FR-PO2029

Background: It has been proposed that a blood flow - dialysate flow ratio instead of a fixed dialysate flow would optimize clearance. Fresenius Medical Care NA (FMC-NA) 2008K and K dialysis machines have an AutoFlow Dialysate Flow feature where dialysate flow is set to either 1.5 or 2.0 times blood flow. Additionally, AutoFlow reduces flow rate to 300 ml/min when in idle mode to reduce waste.

Methods: 53,597 patients converted to either AutoFlow 1.5 or 2.0 and had at least 2 measurements of adequacy before switching and at least 2 after the switch. Change in dialysate flow prescription was not allowed during this time.

Results: Of the 31,851 patients who switched from their prescriptive dialysate flow (median =800 ml/min) to AutoFlow 1.5, the percentage of patients achieving a mean eKt/V of 1.2 or greater increased by 3% (from 85 to 88%). Of the 21,746 patients who switched from their prescriptive dialysate flow (median =800 ml/min) to AutoFlow 2.0, the percentage of patients achieving a mean eKt/V of 1.2 or greater increased by 4% (from 85 to 89%). Comparing before and after AutoFlow for each patient, we find a small but statistically significant improvement in patients switched to AutoFlow 1.5 (mean increase = 0.3, p<0.0001) and AutoFlow 2.0 (mean increase = 0.4, p<0.0001). Average gallons of dialysate per patient dropped by 3 gallons before and after AutoFlow use (from 42 to 39 gallons per patient) not including the reduction of dialysate wasted when in idle mode.

Conclusions: The use of AutoFlow reduces the amount of dialysate used in each hemodialysis treatment without reducing adequacy of treatment.

Funding: Pharmaceutical Company Support

FR-PO2030
Standard Hemodialysis for ESRD: Biochemical and Adequacy Poster/Friday

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

583A
FR-PO2030

Phosphate Handling by the Kidney in Patients with End-Stage Renal Failure on Hemodialysis

Hideaki Iwasawa, Yoshitaka Miyaoka, Ami Hayashi, Toshiyuki Nakao. Department of Nephrology, Tokyo Medical University, Shinjuku-ku, Tokyo, Japan.

Background: Not a few patients with end-stage renal failure on dialysis therapy have residual renal function which plays a significant role on the removal of retained fluids and solutes even after the initiation of dialysis. However, little is known about the excretion mechanism of fluids and solute by end-stage kidneys, such as by those with a glomerular filtration rate (GFR) of less than 10 ml/min. Thus, we investigated how phosphate is handled and excreted by the end-stage kidneys in hemodialysis patients.

Methods: We studied 67 patients with a urinary output less than 100 ml/day among 189 consecutive chronic hemodialysis patients from 2008 to 2010. Blood specimens were obtained at the start of hemodialysis on the first session of the week, and we measured the serum concentrations of phosphate, calcium, urea nitrogen, creatinine intact PTH and FGF-23. We also collected 24-hour urinary specimens and measured the urinary concentrations of phosphate, calcium, urea nitrogen and creatinine. GFR was calculated from the mean values of creatinine clearance and urea clearance as (Ccr × Crea)/2 based on 24-hour urine collection.

Results: The mean urine volume (UV) and GFR were 1184.9±548.4 ml/day and 3.11±1.94 ml/min, respectively. The mean serum phosphate (sP) and urine phosphate (uP) concentrations and the amount of 24-hour phosphate excretion (PE) were 5.7±1.2 mg/dl, 16.8±6.3 mg/dl and 200.2±122.7 mg/day, respectively. sP significantly correlated with uP (r=0.358, p<0.012), but not with PE. The mean maximal tubular reabsorption threshold of phosphate was 2.0±0.9 mg/dl, which correlated with sP (r=0.306, p=0.012), but not with both uP and PE. PE strongly correlated with GFR (r=0.838, p<0.0001) and UV (r=0.791, p<0.0001), and significantly correlated with intact PTH (r=0.533, p<0.0001) and FGF-23, but not with sP.

Conclusions: The daily urinary excretion of phosphate and calcium by end-stage kidneys in hemodialysis patients mainly depends on GFR. The action of phosphaturic hormones on the kidney remains, even if the GFR is less than 10 ml/min.

FR-PO2031

Impact of In Situ Two-Phase Cleaning on Dialyzer Reuse – Total Cell Volume (TCV) and Middle Molecule Clearance

Mohamed E. Labib, Joseph John Murawski, Yacoob Tabani, Dharmeshkumar M. Kanani, Andrew L. Zydney, Toros Kapoian, Richard A. Ward. Novaflex Technologies, Princeton, NJ; Pennsylvania State University, University Park, PA; Robert Wood Johnson Medical School, New Brunswick, NJ; University of Louisville, KY.

Background: Reprocessing of dialyzers with peracetic acid has the advantage of easy management of the disinfection solution. However, the clearance of higher molecular weight solutes can markedly decrease after only a small number of reuses and is not reflected in a change in total cell volume (TCV). To address this problem, we studied the effects of an in situ two-phase cleaning process (ClearFlux® Dialyzer Reprocessing System) on the maintenance of TCV and on the clearance of various molecular weight dextran under reuse conditions.

Methods: Gambro Polyflux® 21R, Fresenius F80A and Fresenius Optiflux® F200B dialyzers were used for up to 20 treatments, reprocessed using the ClearFlux™ or conventional peracetic acid reprocessing.

Results: The fraction of dialyzers meeting the criterion for continued use (TCV > 80% of initial value) is shown in the figure. After 20 reuses with the ClearFlux™, all dialyzers were still usable with TCV > 80% (mean 100% ± 3% SD), while only 40% of those dialyzers treated with conventional processing met the criterion. After 7 patient reuses, clearance of a 10 kDa dextran probe (beta-2 microglobulin = 11.8 kDa) was reduced by 78% in dialyzers with conventional peracetic acid processing, but was unchanged from baseline values with the in situ two-phase cleaning process.

Conclusions: When an in situ two-phase cleaning process is used, both TCV and clearance of larger molecular weight molecules are maintained to a much greater extent than when using conventional peracetic acid reprocessing.

Funding: NIDDK Support

FR-PO2032

Multi-State Modelling of Serum Albumin and B2-Microglobulin in Hemodialysis Patients

Christos Argyropoulos, Maria-Eleni Roumelioti, Mark L. Unruh, Khaled Abdel-Kader. Renal and Electrolyte, University of Pittsburgh, PA.

Background: B2-Microglobulin (B2M) and serum albumin (SA) have emerged as predictors of survival in hemodialysis (HD) patients (pts), yet the joint utility of these markers remains unexplored. We examine SA-B2M as determinants of transitional states of health and predictors of mortality in HD pts.

Methods: Prevalent HD pts enrolled in the HEMODIALYSIS (HEMO) trial receiving High Flux dialysis. Panel data of B2M/SA were used to define a Multi-State Model (MSM) of 9 health states by cross classifying patients according to tertiles (Lower, Middle, Upper) of (B2M,SA). We examined instantaneous transitions among these states and relative risk of death with Markov models.

Results: 902 pts out of 1846 initial study participants had available information for MSM. Pt demographics were as follows: age 57.6 ±14, 56.5% women, 63.2% African Americans, 44.7% diabetics SA (nephelometric) and B2M tertiles were L: <3.43, M: 3.43-3.74, U: >3.74 and L: <29, M: 29.1-36.6, U: >36.6 respectively. Estimated hazard rates among states are shown in Fig(top), so that patients in state 1 (L B2M and SA) were twice (0.43/0.26) as likely to move to state 4 (L B2M and M SA) than state 2 (M B2M and L SA). Hazard ratios (HR) of death relative to the “best” state 7 (L B2M and H SA) are tabulated below.

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*p<0.05

For all B2M tertiles, the HR of death appears to worsen with decreasing SA values and the risk was particularly high for pts at the lowest tertile of SA.

Survival estimates from the various states are shown in Fig(bottom).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: Joint monitoring of SA and B2M offers a simple clinical rule to identify HD pts with very high risk of death. Future studies should determine optimal management strategies to reduce this risk.

Funding: Pharmaceutical Company Support

FR-PO2035

Exhaled Breath Analysis of Patients with End Stage Renal Disease Using Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) Sevan Demirjian,1 Kelly Marie Paschke,2 Riana E. Naude,1 Leslie Leonard,1 David E. Grove,1 Alqamah Mashri,1 Malina Kate Storer,1 Robert J. Heyka,1 Raed A. Dweik.2 Nephrology, Cleveland Clinic. Cleveland, OH; 1Pathobiology, Cleveland Clinic, Cleveland, OH; 2Syft Technologies Ltd., Christchurch, New Zealand; 3Pulmonary, Allergy, and Critical Care Medicine, Cleveland Clinic, Cleveland, OH.

Background: End stage renal disease (ESRD) is characterized by the accumulation of numerous compounds, many of which may have adverse biologic effects (uremic toxins). Exhaled breath analysis is a non-invasive method which can measure volatile uremic markers and/or their metabolites. Our aim was to measure the concentration of volatile organic compounds in the breath before and after intermittent hemodialysis (IHD).

Methods: We analyzed the exhaled breath using selected ion flow tube mass spectrometry (SIFT-MS). Pre- and post-IHD comparisons were made using dependent t-test analysis.

Results: Pre- and post-IHD exhaled breath was analyzed in 59 individual ESRD subjects in a single outpatient dialysis unit. Post-IHD exhaled breath concentration was significantly lower for ammonia, acetate/dehyde, dimethylsulfide, carbon disulfide, ethanol, 2-propanol, benzene, pentane and higher for isoprene. Exhaled breath analysis before and after dialysis.

Funding: Clinical Revenue Support

FR-PO2036

Effect of Hemodialysis and Diet on the Exhaled Breath Methanol in Patients with ESRD Huyên Ji Julie Leg.1 Madeleine V. Pahl,1 Nosratola D. Vaziri,1 Donald R. Blake.2 Nephrology and Hypertension, University of California, Irvine, CA; 2Chemistry, University of California, Irvine, CA.

Background: While the effect of ESRD and dialysis on solute composition is well known, little is known on their effect on the chemical contents of breath. Methanol is the gaseous byproduct of the breakdown of un-absorbable complex carbohydrates by the gut microbiome. Strict dietary restrictions results in an unintended reduction of dietary fibers in ESRD patients. This can reduce production of methanol by the gut microbiome and curtail its appearance in the exhaled breath. Therefore, we investigated the inter- and intra-dialytic changes in the breath methanol levels in a group of hemodialysis patients.

Methods: Ten patients with ESRD were studied during HD at 3- and 2-day-inter-dialytic intervals. Ten normal subjects served as controls. On each occasion, 20 breath and 18 room air samples were collected and analyzed on a unique six column detector gas chromatography system.

Results: Seven ESRD patients consuming the prescribed renal diet had lower methanol concentration (90 ±29 ppbv) than the three ESRD patients consuming a high fiber diet (340 ± 48 ppbv, P<0.0006) and the ten controls consuming unrestricted diets (202 ± 80 ppbv, P<0.001).

Funding: Pharmaceutical Company Support

FR-PO2034

Kinetics of Fibroblast Growth Factor 23, Parathyroid Hormone, and Calcium in Hemodialysis Bernhard O. Bieslez, Manfred Hedicking, Max Plischke, Gere Sunder-Plassmann. Nephrology, Medical University of Vienna, Vienna, Austria.

Background: Fibroblast Growth Factor 23 (FGF23) is rising in the progression of chronic kidney disease. Dialysis induced changes of blood urea nitrogen, calcium, phosphate, β2-microglobulin, and PTH have been studied extensively. Fewer data are available of changes in kinetics of FGF23 with either minor increases but also decreases reported during a dialysis session.

Methods: 19 adult patients (mean age: 58 yr, range: 26-80 yr) on maintenance dialytic intervals. Ten normal subjects served as controls. On each occasion, 20 breath and 18 room air samples were collected and analyzed on a unique six column detector gas chromatography system.

Results: Seven ESRD patients consuming the prescribed renal diet had lower methanol concentration (90 ±29 ppbv) than the three ESRD patients consuming a high fiber diet (340 ± 48 ppbv, P<0.0006) and the ten controls consuming unrestricted diets (202 ± 80 ppbv, P<0.001).

Funding: Pharmaceutical Company Support

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Funding: Pharmaceutical Company Support
Evidence that can be released as a cleavage product: soluble-type αFGF 23, age, but strong correlations were found between serum sα levels and calcium ion, inorganic phosphate, intact PTH, 1,25 dihydroxyvitamin D, intact Kl 7495.1 ±9800.1 pg/ml, CAC score was 1482 ±1577 in HD patients. The serum sα level P<0.003). The serum sαKl level in chronic HD patients could be found to be

Japan.

Vascular Medicine, Kagoshima University Graduate School, Kagoshima, Kenji,1 Yoko Oyama. 2

Paul E. de Jong, 1 Tanaka

Serum Soluble Alupha-Klotho Levels in Haemodialysis Patients T anaka Kenji,1 Yoko Oyama. 2

Background: Alpha-Klotho (sKl) is a cell surface protein with an extracellular domain that can be released as a cleavage product: soluble-type sKl. sKl regulates calcium and phosphate homeostasis. Phenotypes of sKl deficiency resemble clinical features of aging and chronic haemodialysis (HD) patients. So we hypothesized that low levels of sKl might be related to abnormality of mineral metabolism and vascular calcification in HD patients. The aims of this study were to measure serum sKl levels in HD patients, and to determine correlation with indices of mineral metabolism, age, HD vintage, and coronary artery calcification (CAC).

Methods: A total of 57 patients, 25 males and 32 females, undergoing maintenance HD were investigated. Their mean age was 69.3 ±11.1 years (range from 50 to 75 years). The mean duration of HD was 196.8 ±101.7 months (range from 34 to 397 months). Blood samples were taken from the arteriovenous fistula before HD on the first dialysis day of the week. Serum sKl levels were assayed using by ELISA methods (soluble α-Klotho ELISA Kit). Serum levels of intact fibroblast growth factor 23 (FGF23) were measured by ELISA methods (FGF-23 ELISA Kit). Kidney size and CAC score were evaluated by multidetector-row CT.

Results: The serum sKl level was 7495.1 ±9800.1 pg/ml, CAC score was 1482 ±1577 in HD patients. The serum sKl level was not significantly lower than that in healthy controls (n=16, aged 69.3 ±11.1 years) (665.3 ±260.7 pg/ml). In HD patients, no significant correlations were found between serum sKl levels and calcium ion, inorganic phosphate, intact PTH, 1,25 dihydroxyvitamin D, intact FGF 23, age, but strong correlations were found between serum sKl levels and HD vintage (P<0.003). The serum soKl levels was not correlated with CAC score (P=0.115).

Conclusions: The serum sKl level in chronic HD patients could be found to be comparable to that in age matched healthy controls and to correlate strongly with HD vintage. Although, underlying mechanisms by which circulating levels of sKl are maintained in long-term HD patients are unknown, we believe that it deserve of further investigation.

FR-PO2038

Serum Soluble Alupha-Klotho Levels in Haemodialysis Patients T anaka Kenji,1 Yoko Oyama. 2

Background: Hemodialysis (HD) with Hemocontrol biofeedback (HHD) is associated with higher AVP levels compared with SHD. The enhanced AVP release with HHD likely contributes to the lower frequency of dialysis hypotension by stimulating plasma refill and facilitating fluid removal during the first part of the HD session, permitting lower ultrafiltration rates during the remainder of the HD session.

Funding: Government Support - Non-U.S.

FR-PO2039

Prevalence of High Predialysis Serum Bicarbonate and Its Effect on Biochemical Parameters in Maintenance Haemodialysis Patients K awin Tangdhanakonr, Darrance Chewaprapong, Eric J. Bloom, Rasili Raja.

Department of Nephrology, Albert Einstein Medical Center, Philadelphia, PA.

Background: Due to the routine use of high-bicarbonate dialysate bath, metabolic alkalosis occurs in hemodialysis patients. Some studies suggest that metabolic alkalosis could be detrimental due to its association with various metabolic derangements, e.g., low serum ionized calcium, hypophosphatemia, and hypokalemia, but there is limited data on these parameters in hemodialysis patients.

Methods: We conducted a retrospective study to assess the prevalence of high predialysis serum bicarbonate and its effect on biochemical parameters on 94 hemodialysis patients at an outpatient hemodialysis unit. All patients received hemodialysis 3 times a week with 35 mmol/L bicarbonate bath. We stratified patients into 2 groups based on predialysis serum bicarbonate: the higher bicarbonate group (bicarbonate ≥28 mmol/L, n=25) and the lower bicarbonate group (bicarbonate <28 mmol/L, n=69). Demographic and laboratory data including serum potassium, phosphorus, total calcium, as well as anion gap were analyzed.

Results: Of 94 patients, 51 (54.26%) and 25 (26.60%) patients had predialysis serum bicarbonate ≥28 mmol/L and ≥28 mmol/L, respectively. The higher bicarbonate group (bicarbonate ≥28 mmol/L) was found to have lower mean serum potassium (mmol/L) (4.56 ±0.73, 5.01 ±0.99, p=0.041), lower mean serum phosphate (mg/dL) (4.61 ±1.33, 5.56 ±1.60, p=0.01), and lower mean anion gap (mmol/L) (13.84 ±2.49, 15.41 ±2.66, p=0.012). Other parameters including serum total calcium, alkaline phosphatase, intact parathyroid hormone, albumin, prealbumin, magnesium, iron studies, vitamin B12, folie acid, lipid profiles, urea reduction ratio, and Kt/V were not statistically different between the 2 groups. Mean C-reactive protein (mg/L) was lower in the higher bicarbonate group but not statistically significant (17.33 ±29.42, 29.39 ±69.02, p=0.30).

Conclusions: In contrast to some pre-existing data, our study demonstrated more favorable biochemical parameters in hemodialysis patients with higher predialysis serum bicarbonate. A future large randomized control trial looking at hard clinical endpoints is warranted.

FR-PO2040

Management of Anemia with CERA in Routine Clinical Practice: Results around 6 Months of the HORTENSIA Study in Hemodialysis David Verheul,1 Sébastien Kong,2 Michel R. Godin.1 1CH Avignon; 2Roche, Neutaly sur Seine; CHU Rouen.

Background: Correction and stability of hemoglobin (Hb) level is a major goal of anemia treatment. CERA, continuous erythropoietin receptor activator, corrects anemia and maintains the stability of Hb level with a once monthly administration. This French non interventional study is conducted to describe in routine clinical practice the management of anemia with CERA in dialysis.

Methods: HORTENSIA is a one-year prospective study conducted in 112 dialysis centers between 2010 and 2011. Eligible patients (pts) were on dialysis for more than 3 months, treated or not with erythropoiesis stimulating agent (ESA) and initiating CERA

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
at baseline. Primary endpoint was the proportion of patients with Hb level within [10-12] g/dL around the 6th month (M6) of treatment with CERA. Conclusions: One month administration of CERA can maintain stable the Hb level in hemodialysis patients.

Funding: Pharmaceutical Company Support

FR-PO2041

Improving the Dialysis Efficiency in Single Needle by Using Cirtasate®: Preliminary Data

Background: The dialysis population shows a growing age, with an increase in clinical problems as malnutrition and cardiovascular diseases. The mortality risks are: ageing, cardiovascular diseases, diabetes, caloric-protein malnutrition and a low albumin serum. Moreover, in those patients the vascular access problems sometimes make it difficult to perform double needle HD, with a serious risk for the dialysis efficacy.

Methods: During a short observational pilot cross-over study on 6 patients, we have tested an innovative simple needle system where the two pumps run continuously with no interruption of the flux to the dialyser (SNCO - Cross Over B. Braun). The study design shifts patients from standard dialysis (2 months), to SNCO with standard concentrates (3 months), and last to SNCO-CITR with dialsate containing very low acetate (0.3 mmol/L) and 0.8 mmol/L of citrate (Cirtasate®) (10 months). The aim was to evaluate the dialysis adequacy (Kt/V, URR, Treated blood volume) with respect to double needle therapies (BD) and in accordance with the KDOQI standards. Furthermore, the evaluation analyzed whether the presence of citrate improved the Kt/V without any change in calcium needs.

Results: Despite the limited number of patients, as indicated in the table below, the three groups are comparable, both as for exchanges (treated blood volume) and Kt/V

Conclusions: The SNCO can represent a valid alternative for elderly, diabetic patients and for those with vascular problems who require the native AV fistula preservation over the time or have no chance to use double needle mode and/or a central venous catheter. Citrate® has been introduced to observe whether an increase in the dialysis efficiency may take place due to the higher fibres patency. The study has shown this trend with no significant incidence of the patients’ calcium balance.

FR-PO2042

A Potential Use of Polycyonal Free Light Chain Levels for Monitoring in a Chronic Dialysis Population

Background: Polyclonal free immunoglobulin light chains (FLCs) are uremic toxins. With two isotypes, kappa and lambda, FLCs provide a potential method for the measurement of middle molecular weight uremic toxins (MW 22.5 and 45kDa respectively). The purpose of this study was to determine if FFLC levels in a chronic dialysis population are determined by treatment variables and residual renal function (RRF).

Methods: Serum levels and polyclonal FLCs were measured in two stable chronic dialysis populations, pre and post dialysis. Levels were compared for different treatment modalities and RRF (>500mL/d).

Results: 112 patients were recruited, 60 were anemic. In both populations anemic patients had higher total FLC levels than those patients with RRF (See table). Patients at the two centres were comparable in terms of age, sex, vascular access, but patients from centre 1 had higher CRP (7.55mg/L [0.12-170]) and FLC levels (316.1mg/L [18.76-738.5]) compared with those from centre 2: 0.7mg/L [0.3-6.6] and 207.2mg/L [185.1-448.0] respectively (P<0.001).

Conclusions: In conclusion, this study demonstrates polyclonal FLC levels are significantly influenced by both RRF and the dialysis modality used, they could therefore potentially provide a simple target for monitoring ‘difficult to remove’ middle molecular weight uremic toxins in chronic dialysis populations.

FR-PO2043

limitations of Serum Cystatin C as Estimate of Residual Renal Function in End-Stage Renal Failure

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Results: Despite the limited number of patients, as indicated in the table below, the three groups are comparable, both as for exchanges (treated blood volume) and Kt/V

Conclusions: The SNCO can represent a valid alternative for elderly, diabetic patients and for those with vascular problems who require the native AV fistula preservation over the time or have no chance to use double needle mode and/or a central venous catheter. Citrate® has been introduced to observe whether an increase in the dialysis efficiency may take place due to the higher fibres patency. The study has shown this trend with no significant incidence of the patients’ calcium balance.

FR-PO2044

Removal Kinetics of Cystatin C during High-Flux Hemodialysis and Hemodiafiltration

Background: Cystatin C has been proposed as a potential alternative marker of GFR, since levels correlate more closely than with creatinine. Its 13kDa size allows removal by urea and may be useful as a marker of middle molecule clearance.

Results: we studied whether serum cystatin C levels are predictable and can be used to determine residual renal function in patients with GFR<10ml/min due to variation in the generation to non-renal clearance reduction ratio of 0.46 and 0.53 in high-flux HD and HDF respectively.

Conclusions: Pre-dialysis cystatin C levels have linear relationships with RRF estimation in patients with GFR<10ml/min due to variation in the generation to non-renal clearance ratio. We were unable to identify parameters which would improve prediction of GFR with cystatin C. Cystatin C reduction ratio correlates with Kt/V and may be used as a marker of middle molecule clearance.
The Difference in Metabolites Disturbance between High-Flux and Low-Flux Hemodialysis: A Metabonomic Study

Jianyuan Gao,1 Zhaoqi Ni,1 Renhua Lu,1 Ji Qi Qian,1 Yucheng Yan.1

Department of Nephrology, Ren Ji Hospital of Shanghai Jiao Tong University School of Medicine, Shanghai, China; School of Pharmacy, Shanghai Jiao Tong University, Shanghai, China.

Background: Using GC-TOF-MS metabonomic approach, we try to obtain the difference metabolites profiles in both drained dialysate and plasma and analyze the difference in metabolites disturbance between high flux and low flux hemodialysis.

Methods: 25 anuric patients on low flux maintenance hemodialysis and 25 paired high flux dialysis patients were included. Drained dialysate, pre-dialysis and post-dialysis plasma were collected. The concentration of metabolites in drained dialysate and the reduction ratio (RR) in plasma between high flux and low flux were compared to identify differences.

Results: 10 metabolites concentration showed significant differences in drained dialysate between high and low flux dialysis patients, which were tryptophan, Alpha-Ketoisovaleric acid, Gamma-Aminobutyric acid (GABA), Benzoic acid, Ketoleucine, Succinic acid, Palmitic acid, P-Hydroxyphenylacetic acid, Indoleacetic acid and 3-Aminoisobutyric acid. The concentration of all 10 metabolites was higher in high flux group than low flux group. While in plasma, the RR of 10 metabolites showed significant differences between high and low flux, of which 8 metabolites were higher in high flux group than low flux group, including Alpha-Ketoisovaleric acid, GABA, Allantoin, Citric acid, 4-Hydroxy-L-proline, P-Hydroxyphenylacetic acid, Hippuric acid and Alpha-Tocopherol.

Conclusions: Different metabolites exist in drained dialysate, and metabolites disturbances are different between high flux and low flux hemodialysis patients. Difference in fatty acid metabolism between high flux and low flux exists. Difference might exist in Xanthine metabolism between high and low flux.

Funding: Government Support - Non-U.S.

Safety and Efficacy of High Cut-Off Hemodialysis in Chronic Dialysis Patients: A Randomized Controlled Trial

Bertrand Gondouin,1 Anne Bevins,2 Paul Cockwell,3 Colin A. Hutchinson.3 Centre de Néphrologie, Hôpital de la Conception, Marseille, France; 2The Binding Site Group Ltd, Birmingham, United Kingdom; 3Renal Unit, University Hospital Birmingham, United Kingdom.

Background: High cut off hemodialysis (HCO-HD) membranes provide increase removal of middle molecular weight uremic toxins by their increased membrane permeability. This study investigated the safety and efficacy of their use in a stable chronic dialysis cohort.

Methods: Patients (n=29) were randomized into groups (A or B) in a cross-over study design: (A) received 1 HCO (Gambro HCO1100) and 2 standard HD sessions (Polyflux 1701) per week for 8weeks, followed by 3x HCO for 8weeks; (B) received these treatments in reverse. Safety was defined by serum albumin levels, Kt/V and adverse events. Patients were withdrawn if albumin loss was >25% or a thrombotic event occurred. Efficacy was assessed by removal of middle molecules, phagocyte function.

Results: Mean Kt/V for HCO was decreased compared to standard dialysis: 1x HCO: 1.94 ± 0.66 vs 2.24 ± 0.72 (p=0.03); 2x HCO: 2.20 ± 0.70 vs 2.58 ± 0.73 (p=0.02); 3x HCO: 2.47 ± 0.72 vs 2.84 ± 0.77 (p=0.01). No patients were withdrawn for albumin loss, and normal albumin levels were assessed if albumin loss was >25% or a thrombotic event occurred. Efficacy was assessed by removal of middle molecules, phagocyte function.

Conclusions: The use of HCO 3x weekly caused a significant reduction in serum albumin levels (40.6±3.4 g/L to 36.7±2.6; p<0.01) during the first 4weeks, but this remained stable from weeks 4 to 8 (37.4±3.4). No significant reduction in serum albumin levels was seen with 1x HCO. No patients were withdrawn for albumin loss, and normal albumin levels were restored 4 days following treatment completion (41.2±2.7). There were no adverse events between 1x and 3x weekly HCO-HD. Serum CRP concentrations did not change with 3x or 1x HCO. Mean Kt/V for HCO was decreased compared to standard dialysis: 1x HCO: 1.03±0.17 and 1.31±0.21 (p<0.05). There was a significant reduction in middle molecules in the weekly group compared to the 1x weekly, sFLC (median 11.59% vs 2.03%; p<0.01), sFLC (15.43 vs 2.68%; p=0.01) and sM (2.02 vs 8.201%; p<0.001). Phagocyte function increased over the 3x weekly period from 91.2±5.4 to 98.1±2.1 (p=0.02).

HCO-HD provides increased removal of middle molecules and the albumin loss associated with its use appears to be tolerable. Possible clinical benefit could be suggested by the increased phagocyte function. Further work is now required to assess potential clinical benefit of HCO-HD in a larger population.

Utility of Procalcitonin in Delineating Sepsis in Patients on Haemodialysis

Arghya Majumdar,1 Mukesh Kochhar,2 1Nephrology, AMRI Hospital, Kolkata, India; 2Nephrology, AMRI Hospital, Kolkata, India.

Background: Few studies have suggested that procalcitonin (PCT) levels may be elevated in haemodialysis patients in the absence of sepsis. Therefore there is uncertainty regarding its utility in diagnosing bacterial sepsis in these patients.

Objectives: To determine the baseline levels of PCT in haemodialysis patients without sepsis and the accuracy of PCT in diagnosing sepsis in these patients.

Methods: PCT levels were estimated in haemodialysis patients with sepsis (according to ACCP/ISCCM criteria and/or isolation of microorganism). PCT levels were also checked in a control population: haemodialysis patients without sepsis, other inflammatory conditions, acute myocardial infarction, cirrhosis of liver, malignancy, burns, recent surgery or trauma. Patients receiving immunomodulatory or anti-inflammatory drugs were excluded. PCT concentrations were measured by Chemiluminescence assay in pre-dialysis samples. All patients underwent intermittent haemodialysis with a low flux polysulphone membrane.

Results: The patients in both groups were well matched for age, gender and comorbidities; duration on dialysis and adequacy. In controls (n=19), mean PCT was 0.3611 (95% CI 0.2215 to 0.5006). In haemodialysis patients with sepsis (n=17), mean PCT was 8.94 (95% CI 3.54 to 14.35). Focus of sepsis: UTI 2, Pneumonia 8, CRBSI 1, SSTI 2, Postoperative 2, Undetermined 1. Micro-organisms were isolated in 12 and 5 were diagnosed on ACCP- ISCCM criteria. Of patients on haemodialysis 95 percentile patients without infection have PCT values <0.9. On taking 0.45 as a cut off value, below which the test was considered negative for sepsis in haemodialysis patients and a value of 0.93 above which the test was considered positive for sepsis: Sensitivity 94.4% and Specificity 87.5%. So diagnostic algorithm which may be formulated: <0.5: unlikely, 0.5-0.9 possible, >0.9: most likely to have sepsis.

Conclusions: Procalcitonin is useful in diagnosing bacterial sepsis in haemodialysis patients.
Continuous Online Monitoring of Ionic Dialysis in Acute and Chronic Maintenance Hemodialysis Pei-Chen Wu,1 Ravindra L. Mehta,2 1Internal Medicine, National Taiwan University Hospital, Taipei, Taiwan; 2Medicine, University of California San Diego, San Diego, CA.

Background: Hemodialysis (HD) adequacy is strongly correlated with clinical outcomes. Continuous online monitoring of ionic dialysis, using sodium flux rather than urea, allows HD sessions to be modified on a real-time and individual basis. We compared single-pool ionic dialysis (Kt/V D) with traditional blood-side measurements (urea Kt/V and urea reduction ratio [URR]).

Methods: We analyzed 2242 HD sessions from 418 hospitalized patients at an academic medical center. The patients were treated with HD with or without heparin anticoagulation for AKI (n=906 sessions) or as chronic maintenance therapy (n=1327 sessions). Kt/V D was obtained from Fresenius 4008® machine, and blood-side urea Kt/V was calculated using the second generation formula of Daugirdas.

Results: Mean and SD Kt/V D was 1.25±0.47, mean urea Kt/V was 1.40±0.46, and mean URR was 67.8±0.3%. Both urea Kt/V and URR moderately correlated with Kt/V D (r=0.668, p<0.001 and r=0.652, p<0.001, respectively). The discrepancy between Kt/V D and urea Kt/V in acute and chronic settings was -0.13±0.40 and -0.15±0.36, respectively, and there existed a significant difference between these 2 clinical conditions (p<0.01). AKI (p=0.143), larger post-HD weight (p=0.444), lower blood flow (p=0.383), and higher post-HD urea (r=0.499) were related to a lower Kt/V D (all p<0.01). In the total body water derived from anthropometric formula, which is incorporated in Kt/V D monitoring, and that from urea Kt/V (spv) only weakly correlated with each other (r=0.18, p=0.001). The anthropometric volume was significantly larger than spv (46.1±14.9 L and 37.7±21.3 L, respectively, p<0.01).

Conclusions: Our results show that Kt/V D is lower than urea Kt/V and these 2 measurements are moderately correlated. The difference between the two could be accounted for by the overestimation of total body water by the anthropometric method. Moreover, the discrepancy is present in both acute and chronic settings, suggesting that dialysis delivery can be monitored with Kt/V D and periodically confirmed with urea Kt/V in hospitalized patients.

Funding: Government Support - Non-U.S.


Background: The removal of uremic toxins of a medium molecular weight (MW) is one of the main objectives in hemodialyzed patients. The appearance of a very high permeability dialyzer which presents in vivo ultrafiltration coefficients (UCF) greater than 100, led to us testing it on 16 patients in on-line pre-dilution hemodiafiltration.

Methods: 16 patients, 8 men and 8 women, average age 75.5 years were dialyzed with a polysulfone dialyzer, with UCF of 80. During this period, each dialysis session was testing it on 16 of patients in on-line pre-dilution hemodiafiltration.

Results: E-GST activity was significantly increased in the 103 uremic patients compared to controls (9.0±3.1 vs. 5.6±1.7 U/grHb respectively, p<0.0001). In ol-HDF group we observed a lower e-GST activity (p=0.0036) and a higher Kt/Vurea (p=0.0007) and weekly Kt/Vurea (p=0.0004), than in HD group. Conversely, there was no difference in e-GST activity between patients with Kt/Vurea <1.3 compared to patients with Kt/Vurea ≥1.3 (9.6±7.33 vs. 8.65±2.96 U/grHb, p=0.15).

Conclusions: Our results suggest that e-GST could be a potential biomarker of uremic status and of dialysis adequacy.

Impact of Hemodialytic Procedures and Dialytic Doses on Erythrocyte Glutathione S-transferase (e-GST) Activity Roberto Palumbo,1 Annalisa Noce,2 Michele Ferrarini,1 Mariarita Desi,3 Simone Manca di Villahermosa,2 Nicola Di Daniele,2 1Nephrology and Dialysis Department, S.Eugenio Hospital, Rome, Italy; 2Nephrology and Dialysis Unit, Tor Vergata University, Rome, Italy; 3Department of Chemical Science and Technologies, Tor Vergata University, Rome, Italy.

Background: Glutathione S-Transferases (GST) represent a superfamily of ubiquitous enzymes devoted to the cell protection and play an important role in the detoxification of both endogenous and exogenous compounds. Previous study demonstrated an increased erythrocyte-GST (e-GST) activity in uremic patients. The aim of this preliminary study is to compare e-GST activity in normal and uremic subjects, and to correlate e-GST activity with different hemodialysis technique and with dialytic dose.

Methods: Eighty healthy controls, 44 uremic patient on bicarbonate hemodialysis (HD group) and 59 on on-line hemodiafiltration (ol-HDF group) were investigated. E-GST activity was assayed using an automated procedure. Dialytic dose was expressed as Kt/Vurea and weekly Kt/Vurea. To correlate e-GST activity with dialytic dose, all 103 uremic patients (HD and ol-HDF groups) were stratified into two subgroups with a cut-off Kt/Vurea of 1.3.

Results: E-GST activity was significantly increased in the 103 uremic patients compared to controls (9.0±3.1 vs. 5.6±1.7 U/grHb respectively, p<0.0001). In ol-HDF group we observed a lower e-GST activity (p=0.0036) and an higher Kt/Vurea (p=0.0007) and weekly Kt/Vurea (p=0.0004), than in HD group. Conversely, there was no difference in e-GST activity between patients with Kt/Vurea <1.3 compared to patients with Kt/Vurea ≥1.3 (9.6±7.33 vs. 8.65±2.96 U/grHb, p=0.15).

Conclusions: Our results suggest that e-GST could be a potential biomarker of uremic status and of dialysis adequacy.
mortality. Time to allograft failure was examined with and without censoring by death. Models were adjusted for age, gender, race/ethnicity, dialysis duration, dialysis catheter, diabetic status, and donor type.

Results: Baseline characteristics were similar in both hypo-responsive and comparison groups. The adjusted hazard ratios for allograft failure and all-cause mortality post-transplant were 1.26 (95% CI 1.10, 1.45) & 1.63 (95% CI 1.46, 1.82), respectively. A sensitivity analysis showed similar results. The probability of allograft failure was higher in the ESA hypo-responsive group compared to those not hypo-responsive (Figure).

Conclusions: Dialysis patients with decreased response to ESAs are more likely to experience post-transplant allograft failure and mortality. ESA response during dialysis may be used to identify high-risk kidney transplant recipients both before and after transplant.

Funding: NIDDK Support

FR-PO2054

Association of Pre-Transplant Erythropoiesis Stimulating Agent Responsiveness with Post-Transplant Graft Loss and Delayed Graft Function

Miklos Z. Molnar,1,2 Suphamai Bunnapradist,3 Edmund Huang,3 Mahesh Krishnan,3 Csaba P. Kovesdy,3 Kamyar Kalantar-Zadeh.1,2

1Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 2David Geffen School of Medicine at UCLA, Los Angeles, CA; 3Salem VA Medical Center, Salem, VA.

Background: The role of pre-transplant erythropoiesis stimulating agent (ESA) responsiveness in affecting post-transplant graft loss and delayed graft function is not clear.

Methods: Linking the 5-year patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 9281 hemodialyzed patients who underwent first kidney transplantation. Graft failure, delayed graft function (DFG) and acute rejection risks were estimated by Cox regression (hazard ratio [HR]).

Results: Patients were 48±14 years old and included 38% women and 36% diabetics. The crude all-cause mortality rate was increasing across pre-transplant ESA responsiveness index quartiles, it was 26.2/1000 pt-years (95% confidence interval [CI]: 22.4–30.6) in the first quartile and 29.9/1000 pt-years (25.6–35.0), 33.0/1000 pt-years (28.5–38.2), 44.5/1000 pt-years (38.1–50.6) in the second, third and fourth quartiles, respectively. Compared to renal allograft recipients who were in first quartile of pre-transplant ESA responsiveness index (ERI) i.e., ESA dose divided by hemoglobin, recipients in second, third and fourth quartiles had higher adjusted graft-censored death HR of 2.1 (1.3-3.5), 2.3 (1.4-3.8) and 2.1 (1.2-3.5), respectively.

However the OR of DGF was similar in the second 1.10 (0.87-1.39), third 1.13 (0.88-1.45) and fourth 1.28 (0.97-1.67) quartiles. No significant association between pre-transplant ESA responsiveness index and post-transplant acute rejection was noticed.

Conclusions: Higher pre-transplant ESA responsiveness index during hemodialysis treatment period was associated with worse post-transplant risk of graft failure.

Funding: NIDDK Support

FR-PO2055

Association of Pre-Transplant Erythropoiesis Stimulating Agent Responsiveness with Post-Transplant Mortality

Miklos Z. Molnar,1 Edmund Huang,1 Suphamai Bunnapradist,3 Csaba P. Kovesdy,3 Kamyar Kalantar-Zadeh.1,2

1Harold Simmons Center for Chronic Disease Research & Epidemiology, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, CA; 2David Geffen School of Medicine at UCLA, Los Angeles, CA; 3Salem VA Medical Center, Salem, VA.

Background: Studies have shown an association between erythropoiesis stimulating agent (ESA) responsiveness & mortality in CKD patients (pts), but the role of pre-transplant ESA responsiveness & post-transplant mortality is unknown.

Methods: Linking the 5-year patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 9281 hemodialyzed pts who underwent kidney transplantation. All-cause & CV mortality were estimated by Cox regression (hazard ratio [HR]).

Results: Pts were 48±14 years old and included 38% women and 36% diabetics. The crude all-cause mortality rate was increasing across pre-transplant ESA responsiveness index quartiles, it was 26.2/1000 pt-years (95% confidence interval [CI]: 22.4–30.6) in the first quartile and 29.9/1000 pt-years (25.6–35.0), 33.0/1000 pt-years (28.5–38.2), 44.5/1000 pt-years (38.1–50.6) in the second, third and fourth quartiles, respectively. Compared to renal allograft recipients who were in first quartile of pre-transplant ESA responsiveness index (ERI) i.e., ESA dose divided by hemoglobin, recipients in second, third and fourth quartiles had higher adjusted graft-censored death HR (and 95% confidence intervals) of 1.7 (1.0-2.8), 2.2 (1.3-3.5) and 2.4 (1.4-3.9), respectively. Figure shows the cubic spline models for the association of the entire range of pre-transplant ERI with post-transplant mortality.

Similar results were found for cardiovascular mortality.

Conclusions: Higher pre-transplant ERI during hemodialysis treatment period was associated with worse post-transplant mortality.

Funding: NIDDK Support
C.E.R.A. regimen, arriving at stable Hb-targets with few dose adaptations and an excellent analysis was performed in a descriptive way on the efficacy population (EP) (definition: at graft fibrosis at 0-hr biopsy. pts had a weight of 71.3 ± 13.7 kg and a BMI of 24.8 ± 4.23 kg/m². The mean time since kidney transplantation was 7.2 ± 6.1 years, with 19.6% living donations. Previous ESA-treatment was documented as follows (21.4% missing): darbepoetin alpha (96; 34.8%), epoetin alpha (7; 2.5%), epoetin beta (61; 22.1%), eriva 1; 11.4%); C.E.R.A. (42; 15.2%). During the study the average C.E.R.A dose was 3.3 ± 5.4 ml, given every 35.4 ± 15.8 days (SP). Overall, 73 (46.6%) of pts were within the 10-12 g/dL Hb range during EP (Table 1).

Percentage of pts within pre-defined Hb-ranges

<table>
<thead>
<tr>
<th>Hb-range (g/dL)</th>
<th>10-12</th>
<th>13-11</th>
<th>12-11</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-12</td>
<td>36.9</td>
<td>40.6</td>
<td>5.9</td>
</tr>
</tbody>
</table>

27.9% of pts with a GFR < 30 ml/min vs. 42.1% of KTxR with a GFR > 60 ml/min were within the 10-12-corridor, in parallel, 37.4% of recipients of cadaveric grafts vs. 54.6% of patients with transplants from live donors. Mean Hb deviation from intra-individual mean was 0.50±0.6 g/dL and in 157 (87.2%) pts the intra-individual Hb cycles by ± 1.0 g/dL. The pts stayed on average 8.6 ± 6.5 months on the actual C.E.R.A. dose. Overall, there were no specific drug related safety events throughout the study.

Conclusions: Recipient of kidney allografts with PTA benefit from a once-monthly C.E.R.A. regimen, arriving at stable Hb-targets with few dose adaptations and an excellent tolerability.

Funding: Pharmaceutical Company Support

FR-PO2057

Interstitial Fibrosis at 0-Hr Biopsy as a Useful Predictor for Early Post Transplant Anemia

Post Transplant Anemia (PTA) is common (30-80% prevalence) and a major problem in kidney transplant recipients. Several studies have suggested that interstitial fibrosis at 0-hr biopsy is associated with persistent post transplant anemia (PTA). The study assessed the donor characteristics associated with persistent post transplant anemia.

Methods: We included 367 consecutive donor-recipient pairs transplanted between 1991 and 2010 at our center. Donor and recipient characteristics were recorded at the time of transplantation. The clinical course of the recipients was assessed at 3 months and 1 year post-transplantation. The association between donor characteristics and persistent post transplant anemia was assessed by univariate and multivariate analysis.

Results: Persistent post transplant anemia was defined as a Hb level < 10 g/dL at 3 months or 1 year post-transplant. The frequency of persistent post transplant anemia was 30.7% at 3 months and 28.5% at 1 year. In univariate analysis, recipient GFR, recipient age, recipient and donor body mass index (BMI), recipient African American race, and recipient hypertension were associated with persistent post transplant anemia. In multivariate analysis, recipient African American race, recipient hypertension, and recipient GFR were independent predictors of persistent post transplant anemia.

Conclusions: Persistent post transplant anemia is common and is associated with recipient characteristics such as recipient African American race, recipient hypertension, and recipient GFR. These findings highlight the importance of donor and recipient characteristics in the prevention and treatment of persistent post transplant anemia.

FR-PO2058

Donor Factors Determining Anemia at 1 Year Post Living Donor Kidney Transplant

Post transplant anemia (PTA) is common in kidney transplant recipients and is associated with several factors such as recipient obesity, female gender, and recipient medication. Donor factors are also important in determining the occurrence of post transplant anemia. The study aimed to identify donor factors associated with persistent post transplant anemia.

Methods: We included 367 consecutive donor-recipient pairs transplanted between 1991 and 2010 at our center. Donor and recipient characteristics were recorded at the time of transplantation. The clinical course of the recipients was assessed at 3 months and 1 year post-transplantation. The association between donor characteristics and persistent post transplant anemia was assessed by univariate and multivariate analysis.

Results: Persistent post transplant anemia was defined as a Hb level < 10 g/dL at 3 months or 1 year post-transplant. The frequency of persistent post transplant anemia was 30.7% at 3 months and 28.5% at 1 year. In univariate analysis, recipient GFR, recipient age, recipient and donor body mass index (BMI), recipient African American race, and recipient hypertension were associated with persistent post transplant anemia. In multivariate analysis, recipient African American race, recipient hypertension, and recipient GFR were independent predictors of persistent post transplant anemia.

Conclusions: Persistent post transplant anemia is common and is associated with recipient characteristics such as recipient African American race, recipient hypertension, and recipient GFR. These findings highlight the importance of donor and recipient characteristics in the prevention and treatment of persistent post transplant anemia.

FR-PO2059

Blood Pressure Follows the Living Donor Kidney: Role of Donor GFR Laura V. de Vries,1 H. Tent,1 Johannes S. Sanders,2 Hendrik Sijbrand Hofker,1 Stephan J.L. Bakker,1 Gerjan Navis,1 Nephrology, UMCU; Surgery, UMCU.

Background: Hypertension is common in renal transplant recipients. It has been suggested that hypertensive traits of the donor may be transferred with the transplanted kidney. Direct proof for this “hypertension follows the kidney” hypothesis is, however, lacking. Here we study the effect of systolic blood pressure (SBP) and GFR of living donors on SBP in recipients.

Methods: We included 367 consecutive donor-recipient pairs transplanted between 1991 and 2010 at our center. Donor and recipient characteristics were recorded at the time of transplantation. The clinical course of the recipients was assessed at 3 months and 1 year post-transplantation. The association between donor characteristics and persistent post transplant anemia was assessed by univariate and multivariate analysis.

Results: Donor and recipient characteristics are shown below. 22 donors used one antihypertensive drug, 15 used two drugs. Short term recipient SBP associated negatively with donor GFR (R = 0.31). At one year post-transplantation (tx), donor GFR was positively associated with recipient SBP (R = 0.24 and 0.16). In a multivariate analysis, short term SBP was associated with recipient and donor factors and with recipient age and cholesterol level. At one year post-transplantation, donor and recipient factors were associated with SBP. Recipient gender was a significant predictor of SBP (R = 0.16). Recipient GFR was influenced by donor GFR (R 0.31 and 0.34) and donor SBP (R = 0.18 and 0.42); at both short term and one year post tx.

Conclusions: Higher donor GFR and lower donor systolic blood pressure are associated with lower recipient blood pressure, independent of recipient renal function. Thus, regulatory impact of the kidney on blood pressure appears to be an intrinsic renal property within kidneys from generally normotensive donors. In living kidney donation, hypertension follows the kidney.

FR-PO2060

Ammoniagenesis Is Associated with Blood Pressure in Renal Transplant Recipients

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Background: Hypertension is common in renal transplant recipients and is associated with several factors such as recipient obesity, female gender, and recipient medication. Donor factors are also important in determining the occurrence of post transplant anemia. The study aimed to identify donor factors associated with persistent post transplant anemia.

Methods: We included 367 consecutive donor-recipient pairs transplanted between 1991 and 2010 at our center. Donor and recipient characteristics were recorded at the time of transplantation. The clinical course of the recipients was assessed at 3 months and 1 year post-transplantation. The association between donor characteristics and persistent post transplant anemia was assessed by univariate and multivariate analysis.

Results: Persistent post transplant anemia was defined as a Hb level < 10 g/dL at 3 months or 1 year post-transplant. The frequency of persistent post transplant anemia was 30.7% at 3 months and 28.5% at 1 year. In univariate analysis, recipient GFR, recipient age, recipient and donor body mass index (BMI), recipient African American race, and recipient hypertension were associated with persistent post transplant anemia. In multivariate analysis, recipient African American race, recipient hypertension, and recipient GFR were independent predictors of persistent post transplant anemia.

Conclusions: Persistent post transplant anemia is common and is associated with recipient characteristics such as recipient African American race, recipient hypertension, and recipient GFR. These findings highlight the importance of donor and recipient characteristics in the prevention and treatment of persistent post transplant anemia.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PUB - Publication Only

Underline represents presenting author.
Methods: Dietary intake was assessed in 380 outpatients (21 y after transplantation) by a food-frequency questionnaire focusing on differentiation of animal and vegetable protein, since animal protein is the main source of dietary acid. Ammonium excretion was assessed from 24 h urine collections. Blood pressure was measured automatically for 15 min and last 3 measurements were averaged.

Results: Mean age was 53±3 y, 58% was male. Estimated total protein intake was 83±21 g/d (52±16 g/d from animal sources). Urinary ammonium excretion was 19.3 [IQR:12.4-29.4] mmol/24h. Ammonium excretion correlated positively with animal protein intake (r=0.12; p<0.01), but not with vegetable protein intake. Mean arterial pressure (MAP) was 100±12 mmHg on a median of 2.1 [IQR:1-2] anti-hypertensives. In multivariable linear regression analysis, ammonium excretion was significantly associated with MAP (β=0.12 mmHg per mmol/24h; p=0.007) independent of age, sex, BMI, renal function and use of anti-hypertensives.

Conclusions: Higher intake of animal protein is associated with more renal ammoniagenesis. Higher urinary ammonium excretion is positively associated with blood pressure. These cross-sectional data suggest that modulation of renal ammonia production, either by reducing intake of acidifying animal protein or by increasing alkali by diet or bicarbonate supplementation, may be a new therapeutic strategy with the potential to reduce blood pressure and renal damage in RTTR.

FR-PO2061
Blood Pressure Treatment Lowers Mortality in Kidney Transplant Recipients
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Background: There are uncertainties regarding cardiovascular medication in renal transplant recipients and all-cause mortality. We assessed possible associations in a post-hoc observational analysis of the ALERT trial.

Methods: ALERT was a randomized, double-blind, placebo-controlled study to investigate the effect of Buvastatin on cardiovascular and renal outcomes in 2102 renal transplant recipients, followed by a 2-year extension. Patients were recruited at a median time of 4.5 years after transplantation with a stable renal function. We investigated the relationship between cardiovascular medication at baseline and all-cause mortality using Cox regression adjusted for demographic variables, other medication and known cardiovascular risk factors.

Results: In total, 1868 out of 2102 patients were available for analysis. During a median follow-up of 7.4 years, there were 334 deaths. In multivariable analysis, significantly reduced mortality was seen in relation to treatment with Calcium antagonists (HR 0.72, CI 0.56-0.92), beta blockers (HR 0.64 CI 0.51-0.81), or ACE/ARB (HR 0.58 CI 0.46-0.73). Treatment with diuretics was associated with increased mortality (HR 1.79 CI 1.38-2.23). No associations were seen for nitrates or warfarin. Borderline significance was seen for ASA (HR 0.80 CI 0.63-1.02).

Conclusions: Blood pressure treatment at baseline seems associated with a favourable outcome in kidney transplant recipients.

Funding: Clinical Revenue Support

FR-PO2062
Renal Allograft Survival Is Decreased in Patients with Thrombotic Disorders
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Background: Kidney allograft survival in patients with thrombotic disorders pre-transplant is not well studied. We used a matched cohort study design to examine the impact of thrombotic disorders on kidney allograft survival.

Methods: We identified kidney allograft recipients in a single center, transplanted between 2001 and 2010 who had: Lupus anticoagulant (LAC), anti-phospholipid antibodies (APLA), Factor V Leiden (FVL), or a prothrombin gene mutation (PGM). For each case 2 controls were chosen. Proportions were compared by the Chi-square test. Death censored graft loss (DCGL) was assessed with Cox proportional hazard models.

Results: Twenty nine patients were identified with a thrombotic disorder (LAC=8, APLA=8, FVL=16, PGM=4). Between patients and controls, there was no difference in gender (55% vs 59% male; p=0.76), age (46.3 vs 46.5 years; p=0.8), or follow-up (4.3 vs. 4.2 years; p=0.42). In the first 30 days, 5 grafts were lost in cases (17%), with four due to thrombosis; only 2 grafts (3.5%) were lost in controls, one due to thrombosis (p=0.03). Thrombophilic patients had higher overall DCGL (HR 3.06; p=0.02), as well as higher risk for DCGL in the first 30 days (HR 5.73; p=0.03) and in the first year (HR 3.10; p=0.02). After 30 days, however, there was no difference in DCGL (HR 2.22; p=0.18).

There were no differences in DCGL between patients with LAC or APLA compared to those with other disorders.

FR-PO2064
Body Mass Index and Outcomes after Deceased Donor Kidney Transplantation
Joseph Kim, Olusegun Famure,1 Daniel C. Catrnan,1 Edward H. Cole,2 Jeffrey Schiff,1 Kathryn J. Timekam,1 Carl J. Cardella.1 1Medicine (Nephrology), Toronto General Hospital, University Health Network, Toronto, ON, Canada; 2Laboratory Medicine and Pathobiology, Toronto General Hospital, University Health Network, Toronto, ON, Canada.

Background: Increased body mass index (BMI) has been associated with worse kidney allograft outcomes but its effects on delayed graft function and acute rejection are less clear.

Methods: We studied deceased donor kidney transplant recipients from 1 Jan 2000 to 31 Dec 2010 at the Toronto General Hospital . BMI (kg/m2) at transplant was categorized as ≤ 20, 20 to 24.9 [referent], 25 to 29.9, 30 to 34.9, and ≥ 35. Outcomes included delayed graft function (DGF); dialysis in the first week post-transplant), biopsy-proven acute rejection (BPAR), death-censored graft failure (DCGF), and death with graft function (DWGF). Recipient, donor, and transplant factors were included in logistic and Cox regression models.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Results: There were 42, 225, 190, 92, and 43 patients in the first to fifth BMI groups (N=592). Higher proportions of females were seen at the extremes of BMI. More Whites, diabetics, and male donors were noted in the highest BMI group. DGF rates were 35.7%, 32.0%, 36.3%, 48.9%, and 65.1%, respectively. The table shows results from the logistic and Cox models. Odds ratios for DGF were significantly increased in higher BMI groups. Hazard ratios for BPAR showed a similar trend. Most of this effect was due to an increase in acute cellular rejections in the higher BMI groups. There was a trend to an increased risk of DCGF and DWGF in the highest BMI group.

Conclusions: Larger BMI is associated with a greater risk of DGF and BPAR. The mechanisms by which BMI increases the risk of DGF and BPAR require further study.

Outcome Measures by Body Mass Index Category

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>BMI &lt; 20</th>
<th>BMI 20 to 24.9</th>
<th>BMI 25 to 29.9</th>
<th>BMI 30 to 34.9</th>
<th>BMI &gt; 35</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGF</td>
<td>1.37 (0.65, 2.91)</td>
<td>1.19 (0.78, 1.83)</td>
<td>1.36 (0.81, 2.20)</td>
<td>1.05 (0.71, 1.57)</td>
<td>0.34 (0.14, 0.88)</td>
</tr>
<tr>
<td>BPAR</td>
<td>1.53 (0.21, 1.01)</td>
<td>1.77 (1.12, 2.77)</td>
<td>1.62 (0.61, 4.26)</td>
<td>1.26 (1.04, 1.50)</td>
<td>1.03 (0.61, 1.72)</td>
</tr>
<tr>
<td>DCGF</td>
<td>1.32 (0.33, 1.07)</td>
<td>1.52 (0.90, 2.53)</td>
<td>1.23 (0.84, 1.83)</td>
<td>1.77 (1.53, 2.05)</td>
<td>1.03 (0.61, 1.72)</td>
</tr>
<tr>
<td>DWGF</td>
<td>1.55 (0.64, 3.82)</td>
<td>1.93 (0.87, 4.31)</td>
<td>2.11 (1.32, 3.35)</td>
<td>1.95 (1.32, 2.86)</td>
<td>1.86 (1.32, 2.60)</td>
</tr>
</tbody>
</table>

Conclusions: Compared to HD, PD modality before transplantation appears associated with lower all-cause and cardiovascular mortality. This outcome mirrors the survival benefit previously attributed to the selection bias for patients on PD, and as such confounding by residual selection bias cannot be ruled out.

Funding: NIDDK Support

FR-PO2065

Dialysis Modality and Outcomes in Kidney Transplant Recipients

Miklos Z. Molnar,1 Rajan Mehrotra,2 Uyen Duong,1 Suphamai Bunnarapradit,3 Lilia R. Lukovsky,1 Mahesh Krishnan,1 Csaba P. Kovessy,1 Kamyar Kalantar-Zadeh,1,4 Harold Simmons Center, Torrance, CA; 2Semmelweis University, Budapest, Hungary; 3David Geffen School of Medicine at UCLA, Los Angeles, CA; 4DaVita, Inc, Denver, CO; 5Salem VA Medical Center, Salem, VA.

Background: The role of pre-transplant dialysis modality in affecting post-transplant outcomes is not clear. We examined associations of pre-transplant dialysis modality with short- and long-term post-transplant outcomes.

Methods: Linking the 5-year patient data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 12,416 hemodialysis (HD) and 2,092 patients treated with peritoneal dialysis (PD) who underwent first kidney transplantation. Mortality or graft failure, and delayed graft function (DGF) were risks included by Cox regression (hazard ratio [HR]) and logistic regression (odds ratio [OR]), respectively.

Results: Patients were 48±14 years old and included 39% women and 35% diabetics. Patients treated with PD pre-transplant had 43% (HR: 0.57 [0.38-0.87]) lower adjusted death rate than those treated with HD. Similar association was found for cardiovascular death (adjusted HR: 0.34 [0.14-0.88]).

Conclusions: Compared to HD, PD modality before transplantation appears associated with lower all-cause and cardiovascular mortality. This outcome mirrors the survival benefit previously attributed to the selection bias for patients on PD, and as such confounding by residual selection bias cannot be ruled out.

Funding: NIDDK Support

FR-PO2066

Common Genetic Variance in Glucocorticoid Receptor Locus Associates with Graft Failure in Renal Transplant Recipients

Anna Rozniechko,1 Laura V. de Vries,2 Stephanie J.L. Buicer,1 Gerjan Navis,1 Internal Disease, Division of Nephrology, University Medical Center Groningen, Groningen, Netherlands.

Background: Glucocorticoid receptor, encoded by the gene NR3C1, functions as a responsive element for corticosteroid hormones. We hypothesized that common genetic variance in NR3C1 influences tissue sensitivity and responsiveness to glucocorticoid effects of corticosteroids. Since corticosteroids are used in clinical practice as immunosuppressive treatment in kidney transplantation, we investigated whether polymorphisms in NR3C1 are associated with development of renal graft failure (GF) in renal transplant recipients (RTR) treated with corticosteroids.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Tac-MPA impressively preserves graft function in ECD and non-ECD kidneys. Excellent graft survival that is similar to non-ECD kidneys when treated with Tac-MPA. When compared to 3.5-<5.5 mg/dL, >5.5 mg/dL were associated with increased risk of functional graft failure and increased risk of all-causes and cardiovascular death, respectively when compared to 3.5-<5.5 mg/dL.

Background: Renal transplantation is the treatment of choice for end stage renal disease; it is nevertheless highly stressful for many patients. We aimed to investigate the association of depression with mortality and graft failure in renal transplant recipients (RTR).

Methods: RTR were investigated between 2001-2003. Depressive symptoms were assessed using the subscale of the Symptom Checklist (SCL-90). SCL-90s were grouped using standardized scales. We compared SCL-90s of our RTR with a healthy Dutch reference population. Mortality and graft failure were recorded until May 2009.

Results: A total of 527 RTR (age 51±12 yrs, 55% men) participated at median time of 6.0 yrs post-transplant. SCL-90s were higher in RTR than in the reference population (24±22; p<0.001). Risk factors for depressive symptoms were being unfit to work, living alone, dialysis duration, low physical activity, female gender and low creatinine clearance (p<0.05). During median follow-up for 7 yrs, 37% (15%) RTR died in the low SCL-90 group (n=243), while 21% (56%) died in the groups with moderate (n=102) and high SCL-90s (n=182)(p=0.01, fig.1). In univariate Cox-regression analyses high SCL-90s was associated with increased risk for mortality (HR=2.27[1.46-3.36] P<0.001), while no association was found for graft failure. Adjustment for potential confounders, including diuretics duration and creatinine clearance did not materially change the association.

Conclusions: Our results show that depression after kidney transplantation is associated with decreased survival. Additional studies on the effect of depression treatment on survival after renal transplantation are needed.
K. Stevens

Traditional Proteinuria Measures in Renal Transplant Recipients

Kathryn Fractional Excretion of Protein May Have Superior Predictive Value over FR-PO2072

Comparison according to development of MS after KT

<table>
<thead>
<tr>
<th>Fractional Excretion of Protein Mean ± SD</th>
<th>Total n</th>
<th>Stage 1</th>
<th>Stage 2</th>
<th>Stage 3</th>
<th>Stage 4</th>
<th>Stage 5</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FePr (mM/l)</td>
<td>10 (±5)</td>
<td>7 (±4)</td>
<td>3 (±4)</td>
<td>4 (±3)</td>
<td>6 (±4)</td>
<td>7 (±6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PCR (mM/l)</td>
<td>8 (±4)</td>
<td>6 (±3)</td>
<td>7 (±5)</td>
<td>7 (±5)</td>
<td>9 (±6)</td>
<td>8 (±7)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Comparison between these tests and measures of albuminuria.

limited by its retrospective nature and the small number of events. Comparison should be made between these tests and measures of albuminuria.

Conclusions: Majorit y of MS incidence was in the early phase. Patients with early MS were older and had more pre transplant weight. Insulin, CRP and BMI at 6, 12 months, were different in all MS (early and late) patients compared to Non-MS. The graft function at first year was not affected by the early or late onset of MS. Further follow is necessary.

FR-PO2073

Chronic Kidney Disease Stage: Impact on Long-Term Graft Survival Following Kidney Transplantation William Irish,1 Schif fon L. Wong,2 Debra Bowers,1 Digisha Trivedi,1 Tony Hebd en.1 1CTI Clinical Trial and Consulting Services; 2Bristol-Myers Squab.

Background: Renal function (RF) after kidney transplant has been shown to be a strong predictor of graft survival (GS). We characterized the relationship between early change in post-transplant RF and GS.

Methods: Adult Medicare beneficiaries in the US Renal Data System who received kidney-only transplant (2001 - 2008) were eligible. RF, measured by estimated glomerular filtration rate (eGFR), was calculated by the Modification of Diet in Renal Disease 4-variable formula. eGFR at 6 and 12 months was categorized using the National Kidney Foundation CKD staging (Stage 1 (eGFR≥90), 2 (60≤eGFR<90), 3 (30≤eGFR<60), 4 (15≤eGFR<30), and 5 (eGFR<15)). GS was estimated using the life-table method. Hazard ratios (HRs) for graft failure (GF) and associated 95% confidence intervals (CIs) were estimated using Cox hazards model.

Results: At 6 months, 77,206 patients (Mean age: 50 years, 29% Black, 61% males) were alive with a functioning graft and eGFR (Median followup: 4 years). Association between change in CKD stage from 6 to 12 months post-transplant and 4 year GS is shown in Table 1. Patients in CKD stage 2, 3, or 4 at 6 months who had a one stage worsening in CKD stage by month 12 had a significantly greater risk of GF at 4 years. A majority of patients were in CKD stage 3 at month 6. Those that moved to stage 4 at 12 months had a 3.5 fold increased risk of GF and a 32% lower GS at 4 years (p<0.001).

Table 1: Change in CKD Stage and GS

<table>
<thead>
<tr>
<th>CKD Stage at Month 6</th>
<th>CKD Stage at Month 12</th>
<th>GS at 4 Years (%)</th>
<th>HR</th>
<th>95% CI for HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 (n=49,695)</td>
<td>Stage 12 (n=49,695)</td>
<td>88 (98% )</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Stage 2 (n=87,72)</td>
<td>Stage 3 (n=87,72)</td>
<td>87 (97% )</td>
<td>1.0</td>
<td>1.0 - 1.4</td>
</tr>
<tr>
<td>Stage 3 (n=74,72)</td>
<td>Stage 4 (n=74,72)</td>
<td>74 (91% )</td>
<td>1.7</td>
<td>2.7 - 2.7</td>
</tr>
<tr>
<td>Stage 4 (n=67,72)</td>
<td>Stage 5 (n=67,72)</td>
<td>61 (85% )</td>
<td>3.4</td>
<td>2.9 - 5.9</td>
</tr>
<tr>
<td>Stage 5 (n=36,72)</td>
<td>Stage 6 (n=36,72)</td>
<td>36 (54%)</td>
<td>7.4</td>
<td>5.5 - 15.6</td>
</tr>
</tbody>
</table>

Conclusions: Worsening of CKD stage in the first year post-transplant is associated with increased GF. Results suggest that patient management strategies targeted at preserving RF in the early post-transplant period will have a positive impact on long-term GS.

Funding: Pharmaceutical Company Support

FR-PO2074

Metabolic Syndrome Defined by ATP III Classification Predicts Better the Glomerular Filtration Rate Decline Than Its Individual Components after Renal Transplantation Nabil Mohsen, Georgees J. Mourad, Magalie Faure, Ilan Szwarc, Fernando Vetromile. Nephrology Department, University of Montpellier, France.

Background: Individual components of the metabolic syndrome (MS), especially obesity and hypertension have a deleterious effect on renal graft outcome. Whether MS is better than its individual components to predict the decline of renal function is unknown. We studied the presence of MS and its individual components at 3 and 12 months (m3, m12) after transplantation according to the ATP III Classification, and their influence on graft function.

Methods: A cohort of 322 patients transplanted between 1996 and 2003 who accepted to have their glomerular filtration rate measured by urinary clearance of Tc-99m-DTPA (mGFR) at 3, 12, 48, 60 and 96 months after transplantation were included. The patients were followed till patient death, graft loss or till December 2009 (Mean F-U: 3 ± 2.8 yrs). Linear mixed effect model for longitudinal repeated measures was applied. To compare MS versus its components we used the Akaike information Criterion (AIC) in which a lower value indicates a better model.

Results: MS was present in 34%, 37% and 25% of recipients at m3, m12, and both m3 and m12(m3&12). MS, waist circumference (WC), high triglycerides (TG), high systolic blood pressure (SBP) at m3 and m12 are all associated with a significant decline of mGFR as indicated by the negative sign of the coefficient (Table 1). HDL at m3 has a beneficial effect as indicated by the positive sign. Body mass index, glycemia and diastolic BP at m3 and m12, WC and HDL at m12 had no significant effect (p value >0.05). Not shown. MS (m3&12), m3, and m12 had the lowest AIC indicating that they are the best predictors of graft deterioration and that MS is consistently a better model than its individual components.

MS vs its components effect on GFR.

Conclusions: MS is a better predictor of mGFR decline than its individual components. We recommend routine assessment of MS during the clinical visits.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

595A
De Novo Thrombotic Microangiopathy after Kidney Transplantation: Clinical Features, Treatment and Long-Term Patient and Graft Survival

FR-PO2075

Methods: Retrospective single-center observational study to examine the incidence and outcomes of de novo PT TMA/HUS among transplants performed between 2000 and 2010. Recurrent HUS or antibody-mediated rejection were excluded.

Results: Seeking an expanded study population, we identified 1549 patients who fulfilled criteria for de novo TMA. The mean follow-up was 572 days (range, 69-1769). All patients received induction therapy. Maintenance immunosuppression was prednisone, tacrolimus (TAC) and mycophenolic acid in 15 patients (88%). Mean age at onset was 40 ± 15 yrs, and serum creatinine was 6.1 ± 4 mg/dL at a median of 23 days (range, 1-755) after transplantation. Nine (53%) patients developed TMA within 1 month of transplantation and only 12% after 1 year. Clinical features were anemia (Hb < 10 g/dL in 9 (53%) patients, thrombocytopenia in 7 (41%), and increased lactate dehydrogenase in 12 (70%). Decreased haptoglobin was observed in 64% and schistocytes in 35%. CNI withdrawal or reduction was the first step in the management of 10 (15%) patients, and 6 (35%) received fresh frozen plasma (FFP) and/or plasmapheresis. The mean trough level of TAC at diagnosis was 11.5 ± mg/mL (range, 1.2-31). TAC was successfully reintroduced in 6 patients after a mean of 17 days. Eight (47%) patients needed dialysis support after TMA diagnosis and 75 and remained dialysis-dependent.

Conclusions: The de novo TMA was not associated with poor renal graft outcomes in Caucasians. We investigated whether the tacrolimus variability with the rejection-free survival was significant only in CYP3A5*1/*3 subgroups, in which the mean tacrolimus trough concentrations were calculated from the concentrations between 6 and 24 hours post-dose.

Impact of Tacrolimus Variability and CYP3A45 Genetic Polymorphism on Renal Allograft Outcomes

FR-PO2076

Methods: We enrolled 249 patients that got kidney transplant from 1996 to 2010 in Seoul National University Hospital, Seoul, Korea. The tacrolimus IV and the mean tacrolimus trough concentrations were calculated from the concentrations between 6 and 12 months after transplantation.

Results: The patients with higher tacrolimus IV had longer rejection-free survival rate (p < 0.002) and higher mean tacrolimus trough concentrations (p = 0.001). On the other hand, there was no difference in either rejection-free survival rate or mean tacrolimus trough concentration between CYP3A5 expressers and non-expressers. The tacrolimus IV was not associated with the CYP3A45 polymorphism. Interestingly, the association of the tacrolimus IV variability with the rejection-free survival was significant only in CYP3A5 expressers (p = 0.001 in CYP3A5 expressers, p = 0.267 in CYP3A5 non-expressers). High IV of tacrolimus was the independent risk factor (hazard ratio (HR) 1.052, 95% confidence interval (CI) 1.027-1.079) for biopsy-proven acute rejection after adjusting with mean tacrolimus concentration (HR 0.780, 95% CI 0.647-0.930), CYP3A5 polymorphism (HR 1.139, 95% CI 0.649-2.001), number of HLA mismatch, donor type, and donor gender.

Conclusions: The IV of tacrolimus trough levels had a significant impact on rejection-free survival independently of mean tacrolimus trough levels. Both CYP3A45 polymorphism and environmental factors including compliance might contribute to the impact of tacrolimus IV.

De Novo Thrombotic Microangiopathy after Kidney Transplantation: Clinical Features, Treatment and Long-Term Patient and Graft Survival

FR-PO2077

Methods: KR were classified with high risk if they had high risk transplant, crossmatch positive graft, positive or second transplant, pre-emptive and deceased donor. Fifty five KR were prospectively analyzed with total rATG dose (<4.5 vs 4.5-9.5 kg). The primary endpoint was the rate of acute rejection and graft failure. The secondary endpoints were serum creatinine, hematological adverse effects and infections.

Results: In group 1 the mean accumulated dose was 3.7 vs 5.4 kg/m2 in group 2. There were no patients with graft failure and there was no difference in AR(biopsy proved) power (p = 0.57) as shown in Table. Infection was significantly more frequent in those with higher dose (71% vs 44%; p = 0.044), compared to lower dose.

Six month Outcomes according to rATG total dose

<table>
<thead>
<tr>
<th>Variables</th>
<th>All(n=55)</th>
<th>&lt;4.5mg/kg(n=34)</th>
<th>4.5-9.5mg/kg(n=21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute Rejection n (%)</td>
<td>6(11)</td>
<td>0(0)</td>
<td>6(29)</td>
</tr>
<tr>
<td>Infections n (%)</td>
<td>30(54.5)</td>
<td>15(44.1)</td>
<td>15(71.4)*</td>
</tr>
<tr>
<td>(Primary Fr Infection n %)</td>
<td>23(40)</td>
<td>15(44.1)</td>
<td>8(38)</td>
</tr>
<tr>
<td>Death n (%)</td>
<td>3(5.4)</td>
<td>1(2.9)</td>
<td>2(9.5)</td>
</tr>
<tr>
<td>Serum Creatinine(n/mg/dl)</td>
<td>1.17±0.47</td>
<td>1.16±0.49</td>
<td>1.18±0.49</td>
</tr>
<tr>
<td>Creatinine Clearance(ml/min)</td>
<td>77.90±19.13</td>
<td>75.56±19.16</td>
<td>81.84±18.92</td>
</tr>
<tr>
<td>Platelet count(cels/mm3)</td>
<td>204±504</td>
<td>1005±879</td>
<td>1075±108</td>
</tr>
<tr>
<td>Creatinine clearance(ml/min)</td>
<td>208,060±1,485</td>
<td>208,060±1,745</td>
<td>213,790±14,357</td>
</tr>
<tr>
<td>Hb(g/dl)</td>
<td>11.6±1.1</td>
<td>11.7±0.4</td>
<td>13.8±0.4</td>
</tr>
</tbody>
</table>

* p value < 0.05.

Conclusions: Low dose of rATG was not associated to more AR or graft failure and it appears to be associated to lower infection frequency compared to those with higher dose in high risk kidney transplant receptors. This is one of the first reports of the use of rATG in Latin America.

Low Dose of Thymoglobulin as Induction Therapy in High Risk Kidney Transplant Recipients

FR-PO2078

Methods: RTR have had their transplant between January 1996 and December 2003 underwent prospective and sequential measurements of their renal graft glomerular filtration rate (mGFR) using isotopic methods. The RTR had to be at least 3 months post transplant and to have had at least two mGFR. A cohort of 322 out of 665 RTR were included after informed consent.

Impact of Pre-Transplant Dialysis Modalities on the Outcome of Kidney Transplantation

FR-PO2079

Conclusions: MS has a negative impact on long term graft function. Whether its correction would improve the renal outcome warrants further investigation.

Low Dose of Thymoglobulin as Induction Therapy in High Risk Kidney Transplant Recipients

FR-PO2077

Methods: We performed a retrospective cohort study of 577 patients who received kidney transplantation between January 1996 and December 2009 in Seoul National University Hospital (SNUH). The three groups were compared with the rates of graft and patients survival. The mean follow-up duration was 58.1 ± 48.4 months. And then we analyzed 975 patients who had maintenance dialysis (HD: 256, PD: 719) between January 1996 and December 2009 in SNUH for the rates of mortality and probability of kidney transplantation.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Results: Patients who had less than 1 month of dialysis before kidney TPL (i.e. pre-emptive group) had survival benefit compared with non-pre-emptive group (P=0.010), but no difference in graft survival rate (P=0.170). When non-pre-emptive group is subdivided into HD and PD groups, there were no significant differences in patient survival and graft survival rate (P=0.392, P=0.226, respectively). Patients with maintenance HD had high mortality rate than in patients with maintenance PD in our center (P=0.006). But the recipients on maintenance PD had higher probability for kidney transplantation in cohort between 2005 and 2009 (P=0.009), yet no difference in patient cohort between 1996 and 2004.

Conclusions: Based on the higher probability of kidney transplantation in patients on PD, we may recommend the patients to institute PD if the patients are suitable for PD and transplantation candidates.

Funding: Government Support - Non-U.S.

FR-PO2080

Hemoglobin Variability Is a Strong Predictor for Mortality in Renal Transplant Patients

Alexander Kainz, 1,2 Julia Wilflingseder, 1,2

1Department of Nephrology, KH Elisabethinen, Linz, Austria; 2Department of Nephrology, KH Elisabethinen, Linz, Austria; 3Austrian Dialysis and Transplant Registry, Austria.

Background: Anemia is a common problem after renal transplantation. Therefore patients are treated with erythropoietin stimulating agents. The varying response to treatment contributes to hemoglobin variability. It remains unclear however, whether hemoglobin variability is negatively associated with outcomes.

Methods: We conducted a retrospective cohort study of all first kidney allograft recipients between January 1990 and December 2008 represented in the Austrian Dialysis and Transplant Registry (OEDTR). We included 1441 patients of which 683 received erythropoietin stimulating agents at any time after transplantation. Analysis was conducted by Cox proportional hazard regression with cubic splines and linear estimates and the purposeful selection algorithm of covariables. The measure of variability was the moving standard deviation meaning it was computed for every possible four quarters of a year in a row.

Results: The hazard ratio (HR) of mortality and graft loss in the spline models increased with hemoglobin variability. In the linear model the slope for mortality was 2.35 (95% confidence interval 1.75 – 3.17, p<0.001) and functional graft loss 2.45 (1.76 – 3.40, p<0.001). In the clinical expertise Cox model adjusted for ESA use, hemoglobin, age at transplantation, diabetes, days on dialysis, estimated glomerular filtration rate, biopsy confirmed acute rejection and year of transplantation hemoglobin variability was associated with mortality (HR: 2.11, 1.51 – 2.94; p<0.001). No association with functional graft loss could be detected (HR: 1.34, 0.93 – 1.93; p=0.121). The purposeful selection model was also significant when mortality was the outcome (HR: 2.63, 1.70 – 4.08; p<0.001) whereas a nonsignificant result was obtained for functional graft survival (HR: 1.27; 0.83 – 2.54; p=0.509).

Conclusions: These findings suggest that hemoglobin variability accounts for mortality in renal transplant patients.

Funding: Government Support - Non-U.S.

FR-PO2081

IgA Nephropathy Recurrence in Living Related Kidney Transplantation

P.T. Pham, 1 P.C. T. Pham. 2 *Kidney and Pancreas Transplant, David Geffen UCLA Medical Center, Los Angeles, CA; 1Nephrology Division, UCLA-Olive View Medical Center, Sylmar, CA.

Background: Current data suggest that living related kidney transplantation (LRKT) among recipients with the native kidney diagnosis of IgA nephropathy (IgAN) may be associated with an increased risk of histological recurrence. The current study aims to determine whether increasing haplotype match among LRKT affects the rate of actual allograft loss due to IgAN recurrence (IgANR).

Methods: We conducted a retrospective study of kidney transplant recipients with biopsy confirmed primary glomerulonephritis (n=598 from 1993 to 2009), we compared rejection rates between patients with Membranous GN (MGN), IgA nephropathy (IgA), focal segmental glomerulonephritis (FSGS) and membranoproliferative GN (MPGN).

Results: Baseline characteristics are displayed in Table 1

<table>
<thead>
<tr>
<th></th>
<th>MGN</th>
<th>IgA</th>
<th>FSGS</th>
<th>MPGN</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>76</td>
<td>120</td>
<td>120</td>
<td>120</td>
</tr>
<tr>
<td>Mean Age</td>
<td>47.1</td>
<td>43.1</td>
<td>47.1</td>
<td>42.9</td>
</tr>
<tr>
<td>Male Gender</td>
<td>55</td>
<td>72</td>
<td>57.3</td>
<td>28</td>
</tr>
<tr>
<td>Causation</td>
<td>66</td>
<td>89</td>
<td>88.6</td>
<td>87</td>
</tr>
<tr>
<td>Duration diseas pre-transplant</td>
<td>1.1</td>
<td>8.5</td>
<td>10.2</td>
<td>14</td>
</tr>
<tr>
<td>Donor Age</td>
<td>39</td>
<td>40</td>
<td>40.6</td>
<td>39</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>DGF</td>
<td>9</td>
<td>11</td>
<td>17.5</td>
<td>6</td>
</tr>
<tr>
<td>Deceased donor</td>
<td>43</td>
<td>46</td>
<td>49.7</td>
<td>30</td>
</tr>
<tr>
<td>Previous TPLD</td>
<td>17</td>
<td>17</td>
<td>17.5</td>
<td>22</td>
</tr>
<tr>
<td>IHL MM 2</td>
<td>30</td>
<td>31</td>
<td>31</td>
<td>31</td>
</tr>
</tbody>
</table>

The risk of rejection was 59% greater in patients with MGN compared to other GNs (HR 1.59, 95% CI 1.13 to 2.24, p<0.007). At 3 years, 50% of patients with MGN had an episode of acute rejection (p=0.04).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Background: Primary systemic amyloidosis (AL) is a clonal plasma cell disorder that results in light chain deposition in organ tissues manifesting primarily as nephrotic syndrome, cardiac failure, and neuropathy. Treatment of AL is complex since organ failure may preclude intended treatment with an AHSCT. To address the challenges presented, we reviewed for patients, disease and transplant related characteristics.

Methods: We analyzed that after June 1986, a total of 79 patients who received first KTxs and they were treated with calcineurin-based immunosuppressive therapy. Two patients were diagnosed as focal segmental glomerulosclerosis (FSGS), 16 congenital nephrotic syndrome (CNS), 9 IgA nephropathy, each 6 rapid progressive glomerulonephritis (RPGN) and Henoch-Schonlein purpura nephritis, 5 Alport syndrome, 3 Lupus nephritis, each 2 membranoproliferative glomerulonephritis typeI and atypical hemolytic uremic syndrome, 1 membranous nephropathy. The original diagnosis was biopsy-proven in every case. Survival curves were estimated by Kaplan-Meier method.

Results: The median age was 12.0 years at the KTxs. The mean observation period was 8.5 years. Posttransplant recurrence of primary diseases was confirmed in 10 patients (12.6%): 8 FSGS, 1 RPGN, and 1 CNS. Among the 10 patients who had recurrence of primary disease, 3 lost their graft (FSGS, RPGN and CNS in each 1). Of the 7 patients with FSGS achieved remission by steroid pulse therapy. In contrast, 69 patients who did not have recurrence of primary disease, 8 lost their graft. Graft losses due to acute and chronic rejection were in 2 and 6, respectively. Probability of graft survival at 10-year follow-up was 84%.

Conclusions: Recurrence rate of primary glomerular disease after KTxs is lower than the previous reports (30-50%) and graft survival rate is favorable may due to calcineurin-based immunosuppressive therapy.

FR-PO2085

Recurrent Membranous Nephropathy Post-Transplant: Is Rituximab an Option? Tripti Singh, Craig E. Gordon, Laurence H. Beck, Jean M. Francios. Department of Medicine, Renal Section, Boston University Medical Center, Boston, MA.

Background: Membranous Nephropathy (MN) is a leading cause of adult nephrotic syndrome. MN recurs in the transplant kidney with recurrence rates between 10 to 45%. Treatment of recurrent MN after transplant remains a challenge since all patients are already on immunosuppressive therapy. Rituximab (RTX), a monoclonal anti CD 20 antibody which targets B cells and decreases antibody production has emerged as a possible treatment option.

Methods: A literature search was performed using PUBMED from inception to December 2010. Studies reporting the use of RTX for treatment of recurrent MN after kidney transplantation were reviewed and relevant data was extracted. Studies reporting use of RTX for treatment of native MN were excluded. Descriptive statistics were used to determine means (with 95% confidence intervals) for categorical and continuous variables and medians for non parametric variables. The primary outcome was proportion achieving complete remission (CR) defined in source studies as proteinuria<0.3mg/dl with preserved renal function.

Results: Eight studies met the inclusion criterion. All patients had primary MN and mean time to recurrence was 29.5 months. Mean proteinuria and creatinine on diagnosis were 5.9 g/dl and 1.6 mg/dl, respectively. All patients were treated with tight BP control using ACE-I/ARB. RTX was the primary immunomodulator used for treatment. RTX treatment resulted in CR in 72% of reported patients. Mean time to response was 2.6 months. Mean proteinuria and creatinine after treatment were 1.1 g/dl and 1.5 mg/dl, respectively. Studies reporting peripheral B cells showed a decrease in the concentration from mean of 97.9 µl to 5 µl after treatment. Mean follow up after treatment with RTX was 22.6 months. The drug was well tolerated with no reported side effects in the follow up period.

Conclusions: RTX appears to be an effective treatment for recurrent MN with a high rate of response and low adverse event rate. However, this study was limited by both publication and selection bias. The limited toxicity profile of RTX as compared to alkylating agents makes it a preferred option for treatment in this patient population.

FR-PO2086


Background: Biopsy of the transplanted kidney serves a definitive role in elucidating the possible causes of allograft dysfunction. Of the histologic imaging modalities, EM is the most costly and labor-intensive. We have informally noted that EM tends not to modify the findings disclosed by light microscopy with direct immunofluorescence (DIF). We therefore chose to study whether EM results differ from or add to the LM results.

Methods: We compared the LM and EM reports of 65 renal transplant biopsies performed on 60 patients over 2 years. Different pathologists independently interpreted the biopsy specimens; a separate pathologist performed all the EM. We classified biopsy interpretations into 15 possible diagnoses and categorically by glomerular (e.g. transplant glomerulopathy) versus nonglomerular (cellular rejection) disease. We analyzed the agreement between LM and EM reports by kappa statistics and applied the McNemar test.
to determine if EM interpretation yielded significantly more glomerular diagnoses on the same biopsy samples.

**Results:** The biopsies (N=65) represented a sample population with native kidney disease including diabetes (34%), hypertension (36%), FSGS (12%), SLE (5%), chronic glomerulonephritis (1%), and other (12%). There was very good agreement (Kappa=0.94, 95% CI=0.86-1.00), between the EM- and LM-based interpretations. EM did not detect significantly more glomerular disease than LM/DIF alone (discordance rate 4.6%, 95% CI -1.92% to 4.62%, p=0.25). Furthermore, EM did not add to the diagnosis of rejection. EM described 3 more cases of transplant glomerulopathy than did LM/DIF but did not result in a change in management.

**Conclusions:** When EM is routinely performed for kidney transplant dysfunction, electron microscopy does not substantially add to LM/DIF evaluation.

**FR-PO2087**

**Corticosteroid Withdrawal in Renal Transplant Recipients: One Year Analysis of the Mycophenolic Acid Observational Renal Transplant Registry**

V. Ram Peddi,1 Kimi Ueda Stevenson,1 Kevin M. McCague,2 Anne Willard,2 1California Pacific Medical Center; 2Novartis.

**Background:** Corticosteroid withdrawal (CSW) is desired by renal transplant recipients (RTRs) given the potential for reduction of CS adverse effects (AEs).

**Methods:** Using data from the Mycophenolic Acid Observational Renal Transplant (MORE) registry, a prospective study of RTRs receiving mycophenolate (MPA) either as enteric-coated mycophenolate sodium (IC-MPS) or mycophenolate mofetil (MMF) based on local clinical practice at 40 US sites, 12-month CSW (withdrawal of steroids by 3-months posttransplant) outcomes were analyzed. A total of 847 tacrolimus-treated RTRs (352 CSW, 495 CS) were included.

**Results:** Demographics were similar (mean age 52 yrs, 64% male, 25% AA). Nearly all RTRs received at least one induction agent (CS/CSW: 58.6±6.3% thymoglobulin; 47.7±2.7% alemtuzumab; 26.3±11.1% basiliximab; 9.9±0.9% daclizumab). Tacrolimus trough levels were similar. Biopsy-proven acute rejections (BPAR) were low (9.6% CS/7.4% CSW, p=0.20). Interim results at 1, 3, 6 and 12 months showed that significantly (p<0.01) more of the CS patients were maintained on full dose MPA (CS:83.0±6.8%, CSW:78.9±7.3% CI=0.88-1.00), between the EM- and LM-based interpretations. EM did not detect disease including diabetes (34%), hypertension (36%), FSGS (12%), SLE (5%), chronic kidney disease (9%), and other (12%).

**Conclusions:** Our analysis shows a graft survival benefit for adding steroid to a calcineurin inhibitor/MMF regimen in repeat DDKT recipients who received ALE but not r-ATG or IL-2B induction.

**FR-PO2089**

**Alloantibody Sensitization Does Not Impede Graft Survival in African American Living Donor Kidney Transplant Recipients.** Basit Ijaz, Ahmadshah Mirkhel, Joseph Keith Melancon, Georgetown Transplant Institute, Georgetown University Hospital, Washington, DC.

**Background:** To study the effects of elevated panel reactive antibody (PRA) titers on renal allograft survival in African American (AA) living donor (LD) kidney transplant recipients.

**Methods:** All patients in the UNOS database were eligible. Patients were categorized as Highly Sensitized (HS) if PRA value was at or above 95th percentile of all PRA values in the cohort, or as Non-sensitized (NS) if PRA value was zero. Graft survival was compared in the HS and NS Caucasian (CC) and AA LD kidney transplant recipients.

**Results:** From Oct 1987 to Feb 2009, 271,867 patients underwent a kidney transplant. Of these, 93,899 patients received a kidney from a LD, 64,923 of whom were CC and 13,141 were AA.

**Conclusions:** AA kidney transplant recipients had longer wait-list time (690.0±663.5 days vs. 434.6±489.2 days; p=0.001) and were less likely to receive a LD kidney transplant (OR=0.41; 95%CI=0.40-0.42; p=0.001), compared with CC recipients. PRA value at 95th percentile was 90.

HS (PRA>90) CC LD kidney transplant recipients had a higher risk of graft loss compared with the NS CC LD transplant recipients (HR=1.15; 95%CI=1.28-1.97; P<0.001). This difference remained significant after adjustment for confounders (HR=1.50; 95%CI=1.21-1.87; p<0.001).

The risk of graft loss in HS AA LD kidney recipients was similar to NS AA patients who received a kidney from a LD (HR=1.18; 95%CI=0.83-1.66; p=0.38; Fig. 1). The risk difference was unchanged when adjusted for confounders (HR=1.15; 95%CI=0.80-1.64; p=0.39).

**Figure 1:** Kaplan-Meier Estimates for Post-Transplant Renal Allograft Survival in Highly Sensitized (PRA>90) and Non-Sensitized (PRA=0) African American Living Donor Kidney Transplant Recipients.
Effect of Immunosuppressive Treatment Withdrawal on Kidney Recipient Sensitization after Allograft Failure

**Background:** Sensitization to human leukocyte antigens (HLA) and the development of donor specific antibodies (DSAs) create barriers to kidney transplantation. Kidney allograft recipients may become sensitized by defining events such as blood transfusion, pregnancy, and previous transplant. Another factor after allograft failure which may cause sensitization is withdrawal of immunosuppressive treatment (IST). However, universally accepted standards for IST after allograft failure do not exist and is unclear whether withdrawal of IST in this context may independently sensitize patients.

**Methods:** We conducted a single center retrospective chart review of 242 adult renal allograft recipients with documented allograft failure between January 1, 2000 and January 1, 2010, to determine whether withdrawal of IST after allograft failure is an independent risk factor for sensitization. Sensitization was defined by the presence of Class I or Class II DSAs, and/or a panel-reactive antibody level >80%.

**Results:** The 242 patients had the following characteristics: mean age 43.1 (16.4) years; 58.3% male; 40.1% Hispanic, 4% White, 8% Black race; 43% hypertensive or diabetes; 60% deceased-donor recipient; mean transplant duration 66.9 (46.9) months; and majority receiving IST with tacrolimus, mycophenolate mofetil, and prednisone. The effects of IST withdrawal after allograft failure and other potentially sensitizing events are shown in Table 1.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Not sensitized</th>
<th>Sensitized</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IST Withdrawal, n(%)</td>
<td>40(0.9)</td>
<td>8(1.0)</td>
<td>0.35</td>
</tr>
<tr>
<td>Blood Transfusion, n(%)</td>
<td>25(48.1)</td>
<td>55(56.1)</td>
<td>0.35</td>
</tr>
<tr>
<td>Pregnancy, n(%)</td>
<td>4(32.2)</td>
<td>3(25.0)</td>
<td>0.30</td>
</tr>
<tr>
<td>Previous Transplant, n(%)</td>
<td>4(0.08)</td>
<td>22(22.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Transplant Nephrectomy, n(%)</td>
<td>10(19.2)</td>
<td>28(30.1)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

**Note:** Total n varies for each exposure

**Conclusions:** These findings suggest that withdrawal of IST after allograft failure is not an independent risk factor for sensitization. We believe that our results are valid due to the confirmed risk of other known sensitizing events such as pregnancy and previous transplant.

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**FL-PO2092**

Serum NGAL and Cystatin C: 2 Early Biomarkers Predicting Delayed Graft Function after Kidney Transplantation

**Background:** Delayed Graft Function (DGF) occurs in 25% of kidney transplant recipients from deceased donors, and is predominantly caused by ischemia-reperfusion injury. Primary urinary neutrophil gelatinase-associated lipocalin (sNGAL) has been shown to be predictive of DGF. Cystatin C (Cyst C) has not yet been studied as a DGF biomarker. The aim of our work was to assess whether serum NGAL (sNGAL) and Cyst C could predict DGF.

**Methods:** We conducted a monocentric, prospective cohort study in our transplantation unit, collecting serial serum samples of deceased-donor kidney recipients to investigate these potential biomarkers. We analyzed serum levels of creatinine, sNGAL and Cyst C at day 1 post-transplantation in recipients with immediate graft function (IGF) compared to those with DGF. DGF was defined as the need for dialysis within the first 7 days after transplantation.

**Results:** Our cohort consisted of 54 recipients, including 13 with DGF. Medians (min-max) of serum creatinine (µmol/L), sNGAL (ng/ml) and Cyst C (mg/L) in IGF were 597 (318-1269) vs 700 (354-1098) (p = 0.0022), 307 (59-413) vs 514 (383-1010) (p = 0.0022), 2.89 (1.35-5.92) vs 3.93 (2.70-5.73) (p = 0.0004), respectively. ROC analysis for day 1 serum creatinine, sNGAL and Cyst C predicted DGF with an AUC of 0.69 (95%CI 0.53-0.85), 0.78 (95%CI 0.68-0.91), and 0.84 (95%CI 0.70-0.93) respectively. The cutoff levels predicting DGF were 378 ng/ml (Sp 100%-Se 69%) for sNGAL and 2.67 mg/L (Sp 90%-Se 59%) for Cyst C.

**Conclusions:** Cyst C and sNGAL measured at postoperative day 1 were found to be more reliable biomarkers than serum creatinine for predicting DGF. While sNGAL is less studied than sNGAL, it could be more relevant to cases of allograft rejection. However, we found Cyst C to be a more specific marker than sNGAL in our study. The major advantage of using Cyst C is that it is already measured by a standardized assay in routine labs. Further studies are needed to confirm our findings on Cyst C using larger transplant cohorts.

**FR-PO2093**

Creatinine Reduction Ratio on Day Two Is an Objective and Effective Tool To Stratify Delayed Graft Function

**Background:** Creatinine reduction ratio on day 2 (CR2) = 30%, a simple, objective and well-defined criterion for diagnosis of DGF (instead of necessity for dialysis within a week of kidney transplantation), has not been used effectively to stratify DGF into mild to moderate DGF (MG-DGF) and severe DGF (SG-DGF), each of which may have different prognoses.

**Methods:** We stratified all adult deceased donor kidney transplant recipients at our center beginning 1/1/2009 according to CR2 pattern: Immediate graft function (IGF, CR2>30%), MM-DGF (10%≤CR2<30%) or S-DGF (CR2 ≤10%). We study variables including demographics, cold ischemia time (CIT), SCD/EDC/DCD status, patient survival (PS), graft survival (GS), dialysis need with a week of transplantation (DGFDR), acute rejection rate (ARR), serum creatinine (SCR), and GFR. We present interim results of 57 transplants performed within the first 2 years and followed until 3/31/2011. Median follow-up was 12.1 ± 6.5 (range 4.2-27.1) months.

**Results:** Twenty-four of 57 (42%) patients had IGF, 13 (23%) had MM-DGF, and the remaining 20 (35%) had S-DGF. Four of 20 (20%) patients with S-DGF had DGFDR. No patients with MM-DGF had DGFDR. PS and GS were 100% at 3 months (57 pts), at 6 months (50 pts), and at 1 year (28 pts). SCR and GFR follow-up were respectively higher and lower in MM-DGF (p<0.05) and S-DGF (p<0.05) patients compared to those of IGF patients.

**Demographics, Risk Factors, and Outcomes**

<table>
<thead>
<tr>
<th>Variable</th>
<th>IGF (N=24)</th>
<th>MM-DGF (N=13)</th>
<th>S-DGF (N=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>57.6±12.7</td>
<td>50.3±11.5</td>
<td>57.2±10.2</td>
</tr>
<tr>
<td>Gender Male, n(%)</td>
<td>14(58)</td>
<td>6(46)</td>
<td>15(75)</td>
</tr>
<tr>
<td>SCD/EDC/DCD, n(%)</td>
<td>1(8%)</td>
<td>1(8%)</td>
<td>3(15)*</td>
</tr>
<tr>
<td>CIT (hrs)</td>
<td>12.7±4.0</td>
<td>15.2±5.0</td>
<td>16.5±5.5*</td>
</tr>
<tr>
<td>Donor age in yrs, mean±SD</td>
<td>44.6±14.0</td>
<td>41.3±18.2</td>
<td>52.7±9.4*</td>
</tr>
<tr>
<td>SCR mg/dL, mean±SD</td>
<td>0.53±0.13</td>
<td>1.7±0.6</td>
<td>2.1±1.3*</td>
</tr>
<tr>
<td>MDRD GFR mm/m, mean±SD</td>
<td>60.4±19.3</td>
<td>49.9±13.5</td>
<td>39.8±14.3</td>
</tr>
</tbody>
</table>

**Conclusions:** Our interim results show that CR2 is an effective tool for DGF stratification, which may be useful in the management of patients and to compare studies objectively.

**Funding:** Clinical Revenue Support
Using Simulect Plus Low Dose Thymoglobulin in Renal Transplant Recipients with Poor Graft Function

Ahmad M. Tuffaha, James B. Wetmore, Da Zhang, Connie J. Wang. Nephrology and Pathology, University of Kansas Medical Center, Kansas City.

Background: Simulect and thymoglobulin (rATG) are widely used induction agents in renal transplantation. In patients with poor postoperative graft function (PGF), whether using low-dose rATG by targeting CD3+ cell count, can delay calcineurin inhibitor (CNI) introduction has not been studied. We report our experience with an induction protocol using single-dose simulect plus a CD3+ count-based rATG regimen employed when the creatinine (Cr) reduction rate is < 30% over 24 hours (CRR24hr <30%).

Methods: The cohort included 84 consecutive patients who experienced PGF (CRR24hr <30%). Patients received a single dose simulect intra-operatively; rATG was initiated and titrated to keep CD3+ count < 30 cells/ml. CNI was introduced when Cr fell < 4mg/dL or < 50% of pre- transplant level. We examined the initiation, total dose and duration of rATG, resultant CD3+ count, the initiation of CNI, 1-year patient and graft survival.

Results: rATG was initiated by POD 2 in 77% (mean onset POD 2.0 ± 0.8). Mean cumulative dose was 5.1 ± 4.5mg/kg and duration of therapy was 8.5 ± 6.0 days (Fig 1A). Mean CD3+ count was 16.7 ± 17.0 cells/ml; rATG dosing permitted CD3+ counts to be maintained < 30 most of the days(Fig 1B) and in all but 7 patients(Fig 1C). CNI was introduced at POD 10.3 ± 6.2. One year patient and graft survival were 97.6% and 92.9%.

Conclusions: Our findings suggest that in recipients with PGF, simulect plus a CD3 count-based rATG regimen, reliably leads to sufficiently-low CD3+ counts to prevent rejection. The rATG dose is lower than traditional exposure, yet permits delayed introduction of CNI. Excellent long-term graft outcomes are achieved without an increased risk.

Associations between MMP-2 Gene Polymorphisms and Post-Transplantational Diabetes Mellitus in Korean Renal Allograft Recipients

Sunwoo Kang, Yang Wook Kim, Hyun Ju Kim, Tae Hee Kim. Inje University, Republic of Korea.

Background: Post-transplantational diabetes mellitus (PTDM) is a serious metabolic complication that may follow renal transplantation. Matrix metalloproteinase-2 (MMP2) function is indispensable for pancreatic beta islet formation and endocrine cell differentiation. Thus, specific MMP2 gene polymorphisms are considered to be risk factors for diabetes. In this study, we investigated the association between MMP2 gene polymorphisms and the occurrence of PTDM in Korean patients who had undergone renal transplants.

Methods: A total of 311 patients who had received kidney transplants without a prior history of diabetes were included. Four single nucleotide polymorphisms (SNPs) of the MMP2 gene were genotyped from genomic DNA with direct sequencing.

Results: PTDM developed in 56 patients (18.0%). The results showed that the allele frequencies of MMP2 gene polymorphisms rs1132896*C and rs243849*G were significantly higher in the patients with PTDM than in those without PTDM. In multiple logistic regression analysis, 2 SNPs (rs1132896 and rs243849) of the MMP2 gene were significantly associated with the development of PTDM in the codominant and recessive or, codominant and dominant models, respectively.

Conclusions: Our results indicated that genetic polymorphisms of the MMP2 gene were associated with PTDM, suggesting that the MMP2 gene might confer susceptibility to PTDM in patients who receive renal transplants.

Association of Pre-Transplant Glycemic Control with Post-Transplant Outcomes in Diabetic Kidney Transplant Recipients

Edmund Huang,1 Junichi Hoshino,1 Mahesh Krishnan,1 Allen R. Nissenson,2 Csaba P. Kovesdy,4 Kamyar Kalantar-Zadeh,1,5 Harold Simmons Center, Torrance, CA; 2Semmelweis University, Budapest, Hungary; 3David Geffen School of Medicine at UCLA, Los Angeles, CA; 4UCLA School of Public Health, Los Angeles, CA; 5DaVita, Inc, Denver, CO, 6Salem VA Medical Center, Salem, VA.

Background: Studies yielded inconsistent findings regarding the association of hemoglobin A1c (A1c) with survival in diabetic patients (pts) on dialysis. The association between pre-transplant glycemic control & post-transplant outcomes in kidney transplant recipients is not clear.

Methods: Linking the 5-year patient data of a large dialysis organization (DaVita) to the Scientific Registry of Transplant Recipients, we identified 2872 diabetic dialysis pts who underwent kidney transplantation. Mortality or graft failure & delayed graft function (DGF) risks were estimated by Cox regression (hazard ratio [HR]) and logistic regression, respectively.

Results: Pts were 53±11 years old and included 36% women and 24% African Americans. In our fully adjusted model, allograft failure censored all-cause death HRs and 95% confidence interval (95% CI) for time-averaged pre-transplant A1c increments of 7-8%, 8-9%, 9-10% & ≥10%, compared to 6-7% (reference), were 0.89(0.59-1.36), 2.06(1.31-3.34), 1.41(0.73-2.74) & 3.43(1.56-7.56), respectively; and graft failure censored cardiovascular death HRs were 0.38(0.13-1.05), 1.78(0.69-4.55), 1.59(0.44-5.76) & 4.28(0.85-21.64), respectively.

Conclusions: Poor pre-transplant glycemic control (A1c≥9%) appears to be associated with decreased post-transplant survival in kidney transplant recipients although allograft outcomes do not appear to be affected.

Funding: NIDDK Support

The Incidence of New Onset Diabetes after Kidney Transplantation in Korea

Joon Seok Oh, Joong Kyung Kim, Seong Min Kim. Division of Nephrology, Internal Medicine, Bong Seng Hospital, Busan, Korea.

Background: New onset diabetes after transplantation (NODAT) is a major metabolic complication in renal transplant recipients. It is associated with poor graft and patient survival. However, the incidence, risk factors and clinical relevance of NODAT vary among reports from single-center observational studies and clinical trials in Korea. So, we investigate the incidence and clinical correlations of NODAT in Korean renal transplant population.

Methods: We studied 967 patients receiving a living or deceased donor kidney transplant at 12 institutions between 1 January, 1999 and 31 December, 2007. Patients with graft failure or death within 1month post-transplant, multi-organ transplant recipients and patients who had a diagnosis of diabetes mellitus and severe metabolic disease prior to transplant (either as native kidney disease or co-morbidity) were excluded.

Results: The cumulative incidence of NODAT was 7.65%, 10.24%, 11.27%, 11.79%, 12.10%, 13.03% and 13.65% at 1, 3, 6, 12, 24, 36 and 48 months post-transplant, respectively. Using Cox’s proportional hazards analysis, risk factors for NODAT included the use of tacrolimus as the initial maintenance immunosuppressive medication (hazard ratio 1.28, p = 0.03) and the use of cyclosporine (0.78, p=0.03).

Conclusions: We conclude that incidences of NODAT may be associated with the use of tacrolimus, ciclosporine and living donor. Efforts should be made to minimize the risk of this important complication.

Background: New Onset Diabetes after Transplantation (NODAT) is associated with increased morbidity and mortality.

Methods: We performed a retrospective review of 199 kidney transplants performed at the Oxford Transplant Centre between 2007 and 2009. Immunosuppression was tacrolimus and mycophenolate with either basiliximab induction and tapered steroid withdrawal or steroid avoidance in association with alemtuzumab induction. NODAT was diagnosed in patients with more than 2 consecutive random plasma glucose concentrations >9 within the first twelve months post transplant, or required treatment for hyperglycaemia at any time during this period.

Results: The incidence of NODAT requiring oral hypoglycaemic agents was 6.32% (11 patients), with 3 recipients diagnosed late (>12 months). These recipients had an average age of 51 years (range 28-70) and average BMI 27.7 (range 24-30) at the time of transplantation. The majority of patients that developed NODAT (81.8%, n=9) received prednisolone, tacrolimus, and mycophenolate mofetil. Acute rejection episode was noted in 4 patients in the Basiliximab group and three of them had a Dc-AUC lower than MPS-I. The number of AUC tests required during the first 6 months, graft survival, change in graft function, rejections, infections, leucopenia, and diarrhea and cost of MPA between the innovator and generic MMF/MPS users were comparable pharmacokinetics to innovator brand, but generic MMF did not. However, MMF-I & MMF-G2 were similar. MPS-G1 forms of MMF were similar, but MPS-G1 & MPS-G2 were similar. MPS-G1 had a lower, delayed Cmax compared to MPS-I only in the first 3 months (9.8 vs. 17 ng/ml p=0.01). MPA Dose, AUC and Dc-AUC of I forms of MMF were similar, but MPS-G1 had a Dc-AUC lower than MPS-I. The number of AUC tests required during the first 6 months, graft survival, change in graft function, rejections, urinary tract infections, CMV disease, tuberculosis, systemic mycosis, leucopenia and diarrhea were not different between I & G forms of both MMP and MPS. However, the cost of G forms was significantly lower than MMP-I & MPS-I (by 16.0-20.8%; p=0.01).

Conclusions: Among renal allograft recipients in India, generic MMF exhibited comparable pharmacokinetics to innovator brand, but generic MMF did not. However, generic MMF used result in similar survival and post transplant events with lower cost of therapy.

Methods: The experiences of five major transplant centers in Korea were reviewed. Results: Until 2010, 498 cases of kidney transplantation were recorded in 488 (M:F=311:177, living donor (LD): deceased donor (DD)=386:112) Korean children younger than 18 years (mean 11.7±4.0 years). Common primary diagnoses were focal segmental glomerulosclerosis (19.4%), reflux nephropathy (12.2%), chronic glomerulonephritis (8.2%), and aplasia/hypoplasia/dysplasia (5.3%). Since 2000, induction monoclonal antibodies were used in 69.4% of DD and in 28.6% of LD, and in >75% of the cases the maintenance immunosuppressive medication regimen was mainly triple therapy of prednisolone, tacrolimus, and mycophenolate mofetil.

Conclusions: Primary renal transplantation did not predispose to NODAT. Elevated BMI at transplantation as expected was noted in the majority of patients developing NODAT. Our findings suggest that NODAT is associated with use of high dose steroids in the context of a rejection episode, and patients with tapered steroid withdrawal were at higher risk than those in whom steroids were not used.

Methods: Of the 305 renal allograft recipients (mean age =36.6±11.8 yrs, M:F=3.1:1), 19.3, 18.0, 20.3, 14.8, 15.1 years) at the time of transplantation. The majority of patients that developed NODAT (81.8%, n=9) showed satisfactory graft function and survival for graft loss. In conclusions, the outcome of Renal Transplantation in the developing country like our Bangladesh is good as compared to developed country. Through only live related transplantation has been existing now a days.

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Conclusions: Time and resources in evaluation can be saved at the Transplanting centre if done at a DGH. Our study showed that increased donor evaluation does not necessarily contribute to increased number of donations. This study gives a scale of time involved and encourages DGHs to undertake more live donor assessments.
SA-PO2103

Urinary L-FABP and N-Acetyl-β-D-Glucosaminidase Predict AKI after Adult Cardiac Surgery DaiSuke Katagiri, Kent Doi, Kenjiro Honda, KouSuKe Negishi, Toshiro Fujita, Motoyuki Hisagi, Minoru Ono, Takayasu Ohtake, Shuzo Kobayashi, Takashi Sugaya, EiSei Noiri. 1University of Tokyo, Japan; 2Shonan Kamakura General Hospital; 3CMIC, Co, Ltd.

Background: Urinary L-FABP, a new AKI biomarker, could detect pediatric post-cardiac surgery AKI (KI 2007). However, it is unclear whether urinary L-FABP shows a similar performance in more heterogeneous population of adult patients. Urinary N-acetyl-β-D-glucosaminidase (NAG) is a brush border enzyme and a more established renal injury marker. This study evaluated a biomarker panel consisting of these renal markers for adult post-cardiac surgery AKI.

Methods: 77 adult patients who had cardiac surgery were analyzed. Urinary L-FABP and NAG were measured before surgery, at ICU arrival after the surgery (0 h), 4 and 12 h after. AKI was diagnosed by the AKIN criteria.

Results: 28 patients (36.4%) developed AKI after surgery. Urinary L-FABP (0, 4, 12 h) and NAG (4 and 12 h) in AKI were significantly higher than non-AKI. The highest AUC-ROC values were observed with urinary L-FABP and NAG at 4 h (Table). Urinary L-FABP and NAG showed high sensitivity and high specificity, respectively. The combination can detect AKI with higher accuracy than each biomarker measurement (Table). (ROC-AUC 0.81 (0.68-0.90)).

Conclusions: Consistent with prior studies, minimal changes in sCr following cardiothoracic surgery are associated with mortality. However, this association is largely explained by other renal parameters which may suggest a reduced independent impact of creatinine changes alone and reclassification of AKI risk based on combined indicators.

Funding: Government Support - Non-U.S.

SA-PO2104

Calcitriol Levels in Acute Kidney Injury in the Critically Ill Anitha Vijayan, Adriana S. Dusso, Sanjay Jain, Daniel W. Coyne. Renal Division, Washington University in St. Louis, St. Louis, MO.

Background: Acute kidney injury (AKI) is a devastating complication in the critically ill patient and despite advances in renal replacement therapy (RRT), it is associated with hospital mortality of 50%. Low 1,25 OH Vitamin D (1,25VitD) levels are associated with increased mortality. The mean age of AKI patients was 51.9 yrs (controls - 45.3yrs) with 56% female and 91% Caucasian. The mean serum creatinine (Scr) was 1.7 mg/dl in the AKI group, with mean Ca of 8.1mg/dl and P of 5.1mg/dl. The VitD and calcitriol levels in AKI (12.6mg/ml-1.059 and 41.6pg/ml-5.7 respectively), were significantly lower than controls (29.2mg/ml-2.7, p<0.01 and 76.1mg/ml-5.3, p<0.01, respectively), while iPTH levels were significantly higher (134.4 pg/ml ± 24.7 vs. 12.3 ± 3.7pg/ml, p <0.05). The in-hospital mortality for AKI was 30%. Univariate analysis showed that the 1,25VitD levels were significantly higher (134.4 pg/ml ± 24.7) vs. (12.3 ± 3.7pg/ml, p <0.05). The combination can detect AKI with higher accuracy than each biomarker measurement (Table). (ROC-AUC 0.81 (0.68-0.90)).

Conclusions: Consistent with prior studies, minimal changes in sCr following cardiothoracic surgery are associated with mortality. However, this association is largely explained by other renal parameters which may suggest a reduced independent impact of creatinine changes alone and reclassification of AKI risk based on combined indicators.

Funding: Clinical Revenue Support

SA-PO2105

Minimal Changes of Serum Creatinine Do Not Predict Prognosis Following Cardiothoracic Surgery If Adjusted for Renal Complications Diana L. Deitzer, David G. Anthony, Jesse D. Schold, Allen Bashour, Sevag Demirjian. 1Nephrology, Cleveland Clinic, Cleveland, OH; 2Anesthesiology, Cleveland Clinic, Cleveland, OH; 3Quantitative Health Sciences, Cleveland Clinic, Cleveland, OH.

Background: Severe acute kidney injury (AKI) has been associated with poor prognosis in a myriad of clinical settings; in recent years, even minimal changes in serum creatinine (sCr) have been implicated to carry long term prognostic value. We examined the relationship of sCr change in the post-operative period with in-hospital mortality.

Methods: Prospective cohort of 25,898 subjects who underwent cardiac surgery at Cleveland Clinic between April 2000 and January 2008. Change in sCr (ΔCr) was calculated using peak sCr within 2 weeks following surgery, and divided to 10 intervals (reference interval is ΔCr <−0.1 to −0.01). The multivariable model adjusted for nadir serum bicarbonate, serum sodium, peak potassium, and delayed extubation.

Results: Median age was 65 years; 67% were male, and 89% white. In univariate analysis ΔCr less than −0.2, and greater than 0.1 mg/dl were associated with increased in-hospital mortality. Whereas, in the multivariable model, only ΔCr of 2.06 mg/dl was associated with higher mortality; and the magnitude of the effect of changes were substantially reduced.

Conclusions: Consistent with prior studies, minimal changes in sCr following cardiothoracic surgery are associated with mortality. However, this association is largely explained by other renal parameters which may suggest a reduced independent impact of creatinine changes alone and reclassification of AKI risk based on combined indicators.

SA-PO2106

Prediction of AKI Is Not Improved by Cystatin C in Critically Ill Patients – Preliminary Results of a Longitudinal Analysis Silvia Ceeho,1 Pedro Rodrigues,1 Inês Barbosa,1 Bruno Rodrigo,2 Ana P. Fernandez,2 Ana Luisa Papoila,3 Karina Soto.1 1Nephrology, Hospital Fernando Fonseca, Lisbon, Portugal; 2Intensive Care, Hospital Fernando Fonseca, Lisbon, Portugal; 3Biostatistic, Faculdade de Ciências Médicas, Lisbon, Portugal.

Background: The utility of Cystatin C (CysC) as AKI biomarker in critically ill patients remains unknown, especially in those with renal replacement therapy (RRT) requirements. The aim of this study was to evaluate the performance of serum CysC associated with severity indexes in a prediction model of AKI and RRT in ICU setting.

Methods: We prospectively studied a cohort of 128 consecutive ICU adult patients over 6m. AKI was defined according to RIFLE criteria. Baseline characteristics, sequential organ failure and severity indexes were record and creatinine and CysC were measured daily. Generalized linear models and linear mixed-effects models for the analysis were used.

Results: Patients, mean age of 57.1y (SD 16.8), 61.7% male, showed median SAPS II and APACHE II scores of 56.5 (CI 50-58) and 24.0 (CI 22-26), respectively. Crude in-hospital mortality was 31.3%. A total of 33.6% developed AKI (91% within 24 hours of admission) and 44.2% were treated with RRT. AKI patients were older (p<0.028), with more complications (p=0.004), higher severity indexes (p<0.001). 76.7% had septic shock and 91% MOF compared to only 26.2% and 16.7% of non-AKI, respectively (p<0.001). Crude in-hospital mortality was 60.5 % in AKI vs. 16.5% in non-AKI (p<0.001).

AKI was associated with both SAPS II and APACHE II index: ROC-AUC 0.94 (CI 0.89-0.98) and 0.92 (CI 0.87-0.97), respectively. When discriminative ability was 0.94 (CI 0.89-0.98); 0.93 (CI 0.88-0.97), respectively. When CysC was added to the model, the discriminative ability was 0.90 (CI 0.84-0.98) and 0.87 (CI 0.82-0.94): without improving by adding CysC to the model (ROCAUC 0.64, CI 0.47-0.95).

Conclusions: Patients with AKI were more severely ill and had a higher mortality. Severity scores showed a good performance for AKI prediction but not for RRT. Cystatin C did not improve discriminative ability. Future larger studies should be done to confirm these results.
The Identification of Renal Angina Improves AKI Prediction in Critically Ill Children

Rajit K. Basu,1 Lori Brunner,1 Derek Wheeler,1 Lakhmi S. Chawla,2 Stuart Goldstein.1 1Center for Acute Care Nephrology, Cincinnati Children’s Research Foundation, Cincinnati, OH; 2Anesthesia and Critical Care, George Washington University Medical Center, Washington, DC.

Background: Earlier detection of acute kidney injury (AKI) with novel biomarkers holds promise to expedite treatment and improve outcomes. Unfortunately, AKI lacks physical signs and symptoms to identify patients at-risk for AKI to trigger biomarker assessment. We recently proposed the empiric concept of Renal Angina (ANG), to “rule-in” or “rule-out” children at-risk for AKI in the pediatric intensive care unit (PICU). ANG stratifies known AKI risk factors (RF; e.g., intubation, stem cell transplantation) and pairs them with early clinical AKI signs (graded thresholds of small decreases in estimated creatinine clearance (eCCl) or fluid accumulation increase).

Methods: We performed a retrospective review of 135 children admitted to PICU (median age 4.3 [1.2, 12.6] y) with a diagnosis of “shock” to determine if ANG on Day 0 or Day 1 improved the ability to predict severe AKI (>50% eCCl decrease, pRIFLE-I or F) on Day 3 or Day 4 over RF alone.

Results: ANG on Day 0 yielded better PPV and NPV for AKI presence on Day 3 than RF alone; Day 1 ANG precision improved further for Day 4 AKI prediction (Table). We observed similar ANG-associated improved prediction for CRRT initiation. ANG was also associated with higher mortality. In summary, we found 1) Lack of ANG was associated with a very low likelihood of AKI development in the next 72 hours, 2) RF alone were poorly predictive of AKI development, and 3) ANG presence was fair at predicting AKI development.

Conclusions: In summary, we suggest fulfillment of renal angina criteria identifies patients for whom biomarker testing would have the highest yield, improving the efficiency of biomarkers to predict severe AKI.

Funding: Other NIH Support - U1L-RR026314-01 NCCR/NIH

SA-PO2108
Role of Statins in Prevention of Contrast Induced Nephropathy

Majeed Samanreh, Norbert Shtaynberg, Suzanne E. El Sayegh, Morton J. Kleiner. Nephrology/Cardiology, Staten Island University Hospital, Staten Island, NY.

Background: Contrast-induced nephropathy (CIN) remains one of the most important clinical complications associated with the intravascular administration of radio-contrast media. A potential role for statins in CIN prevention is suggested by findings in animal media. A potential role for statins in CIN prevention is suggested by findings in animal models of CIN showing that statins prevent ischemic nephropathy by stabilizing the endothelium and acting as free radical scavengers.

Methods: We carried out a retrospective chart review at our institution to evaluate a possible association between statin pre- treatment and prevention of contrast induced nephropathy. At our institution, 282 charts of patients who underwent a cardiac catheterization or a CT scan with an intravenous contrast were reviewed. Patients presenting with acute renal failure or who have end stage renal disease were excluded. We defined Contrast-induced nephropathy as an increase in serum creatinine by ≥ 0.5 mg/dl or an increase of 25% of the baseline within 72 h following the procedure.

Results: Subjects who had CIN (contrast-induced nephropathy) were significantly older (69.6 ± 11.9 vs. 63.2 ± 14.7; p=.0006) and had significantly higher baseline creatinine levels (1.6±0.84 vs. 1.14±0.98; p<.0001). Subjects aged ≥ 65 were twice more likely to develop CIN compared to subjects aged <65 yrs [95% CI: (1.2, 3.2)]. Subjects with baseline creatinine ≥1.5 were 4 times more likely to develop CIN compared to subjects with baseline creatinine≤1.5 [95% CI: (2.3, 7.3)] Subjects with HTN were 4.6 times more likely to develop CIN compared to subjects who did not have HTN yrs [95% CI: (2.4, 8.6)]. Subjects with all three risk factors, including hypertension, diabetes mellitus, and age≥65 were 11.6 times more likely to develop post-CIN.

Conclusions: Statin therapy was not effective in prevention of CIN in our study. However, these subjects who were on statins had higher prevalence of risk factors such as hypertension, age≥65, and a higher baseline creatinine which may have contributed to the higher incidence on CIN. A larger randomized sample size may elicit the beneficial effects of statins in prevention of CIN.

SA-PO2109
Safety and Efficacy of Citrate Anticoagulation in Septic Shock Patients

Ji Hyeon Park,2 Ajin Cho, Seung Tae Han, Jaeyoung Yoon, Hye Ryoun Jang, Jung Eun Lee, Wooseong Huh, Dae Joong Kim, Yoon-Goo Kim. Department of Medicine, Samsung Medical Center, Seoul, Korea.

Background: Contrast-induced nephropathy (CIN) is a common cause of hospital acquired kidney injury. However, CIN preventive strategy using extracorporeal volume expansion remains still underused. We evaluated the institution of alert program can increase the use of standard prevention for CIN and reduce the incidence of CIN in hospitalized patients with chronic kidney disease (CKD) undergoing computed tomography (CT).

Methods: We developed a computer program in which physicians were alerted to a patient’s risk of CIN when they ordered contrast enhanced CT in patients with CKD (defined as estimated GFR <60 ml/min per 1.73 m2). The physicians were required to acknowledge the alert and order preventive strategy, including hydration with 0.9% saline or sodium bicarbonate, N-acetylcysteine administration, and follow-up serum creatinine level within 24-72 hours. This electronic alert program was applied to all hospitalized patients from March, 2010. We identified 618 hospitalized patients with pre-end stage renal disease who underwent contrast-enhanced CT. CIN was defined as an increase in the serum creatinine level after contrast administration of ≥ 25% or ≥ 0.5mg/dl from the baseline level.

Results: 463 patients were eligible in the study: 258 patients in the pre-alert program group and 205 patients in the post-alert program group. The two study groups were well balanced with respect to baseline characteristics. The post-alert program group were considerably more likely to receive pre- and post-hydration and NAC (55% vs 25%, P<.0001). CIN occurred in 3 patients in the post-alert program group (2%), as compared with 12 patients in the pre-alert program group (8%, P=.029).

Conclusions: Our results suggest that hospitals with adequate information-systems resources should consider implementing electronic alerts to increase physicians’ awareness of the risk of CIN, to increase the use of prophylaxis, and to reduce the rates of CIN.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
SA-P02112
Survival and Mortality Risk Factors in Mexican Patients with Acute Kidney Injury
Miguel De Jesus Beltran-Perez, Luis Alberto Evangelista-Carrillo, Salvador Mendoza Cabrera, Jorge Andrade-Sierra, Enrique Rojas-Campos, Miguel Medina Perez, Basilio Jalomo Martinez, Benjamin Gomez-Navarro. 1
1Department of Nephrology, CMNO, IMSS, Guadalajara, Jalisco, Mexico; 2Medical Research Unit in Renal Diseases, CMNO, IMSS, Guadalajara, Jalisco, Mexico.

Background: Acute kidney injury (AKI) information is scarce in Latin American ICU and non ICU patients.

Aim: To determine patient survival, mortality risk factors and treatment in AKI patients from a hospital of the West of Mexico.

Methods: Prospective cohort (Jan-May 2011) of 79 patients with AKI (AKIN classification), diagnosed and treated by Nephrologists, were recorded at admission, at AKI diagnosis and daily for 1 month: age, gender, time between AKI onset and Nephrology diagnosis, fluid balance, SOFA, APACHE II, II, treatment (IHD, CRRT, conserv., date of death or patient discharge and other clinical and biochemical variables).

Results: Mean age was 52.18 years, 61% were male, 48% were from ICU, 50% had surgery, 25% had sepsis; 59% had AKI 3, mean time between AKI onset and Nephrology consultation was 59.48 hours, 56% received conservative treatment, 28% IHD and 10% CRRT; mean hospitalization was 15.69 days; Mortality was 51% (according to treatment was 46% conservative, 41% IHD and 92% CRRT) Results are shown in Table. Mortality predictors at day of diagnosis were: Δ SCR, Uresis volume and diuretic use (χ²=11.4; p=0.01); and predictors 24-Hrs after were: Diuretic use and SOFA score (χ²=2.7; p=0.03).

Conclusions according to hospitalization site and mortality

<table>
<thead>
<tr>
<th>ICU (n=30)</th>
<th>General ward (n=49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid balance(L)</td>
<td>6.7 (3.3-11.3)</td>
</tr>
<tr>
<td>SCR A</td>
<td>1.85</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>20(67)</td>
</tr>
<tr>
<td>Fluid balance(L)</td>
<td>2.08 (0.8-5.4)</td>
</tr>
<tr>
<td>SCR A</td>
<td>1.73±2</td>
</tr>
<tr>
<td>SOFA (pts)</td>
<td>10±3</td>
</tr>
<tr>
<td>Diuretic use N (%)</td>
<td>21 (69)</td>
</tr>
<tr>
<td>ISI (pts)</td>
<td>0.37±0.2</td>
</tr>
</tbody>
</table>

* p < 0.05

Conclusions: Mortality was similar to other studies, was higher in general ward (42%) and was significantly predicted at diagnosis by small changes in serum creatinine. At 24 hours evaluation, SOFA and conservative treatment significantly also predict mortality.

SA-P02113
A Real Time Electronic Alert System To Identify and Stage Acute Kidney Injury in a Large Acute Nour Trust Linda H. Bisset, Christine Porter, Irene Juurlink, Mark A.J. Devonald. 1 1Nottingham Royal and Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; 2School of Clinical Sciences, University of Nottingham, United Kingdom.

Background: Acute Kidney Injury (AKI) is associated with significant morbidity and mortality. The 2009 NCEPPOD report highlighted deficiencies in diagnosis and management of AKI in the UK. To improve detection of AKI, we developed an automated alert system which identifies and stages AKI in our large NHS hospital.

Methods: Algorithms compare admission serum creatinines (Scr) with actual baseline Scr (lowest Scr 7 days to 1 year before admission). Where no actual baseline exists, Scr is calculated assuming eGFR 75 mL/min. While RIFLE or AKIN criteria are fulfilled, an alert is displayed on the computer screen. All subsequent Scr results are compared with Scr from previous 48 h (AKIN) and previous 7 days (RIFLE); if these differ, the higher stage is reported. Staging is thus continually updated. Sensitivity is increased by use of both AKIN and RIFLE (retrospective analysis of AKI incidence at our hospital revealed that AKIN detects 60% more episodes of stage 1 than RIFLE but RIFLE detects 30% more stage 3 than AKIN). For each patient admission, the worst AKI stage is allocated using a hierarchy: A3, R3, A2, R2, A1, R1. This gives an idea of the numbers at each stage that stage 3 than AKIN). For each patient admission, the worst AKI stage is allocated using a hierarchy: A3, R3, A2, R2, A1, R1. This gives an idea of the numbers at each stage that approach proportion of stages of chronic kidney disease (CKD) and AKI after off-pump cardiac surgery, defined as 25% increase in serum creatinine (Scr). We also assessed significant predictors for AKI and mortality.

Results: Using the cardiac surgery database from Lenox Hill Hospital, we performed a single-center retrospective analysis on all off-pump cardiac surgery patients from June 2009 to January 2011. Extracted data included: age, sex, race, preoperative SCR, postoperative peak Scr, comorbidities (diabetes, dyslipidemia, hypertension, HTN), peripheral vascular disease (PVD), cardiovascular disease (CVD), urgent versus elective surgery, complications during hospitalization, use of ACEI/ARBs, mortality. Admission creatinine included on-off pump cardiac surgery, preoperative dialysis (ESRD or prior 60 days), intraoperative death, missing data points.

Results: Of the 441 patients that met inclusion criteria, 23.8% had AKI. Incidence of AKI by CKD stages 1-4 was 32.7%, 17.6%, 20.7%, 28.6%, respectively. There were no stage 5 CKD patients. Chi-squared analyses compared risk factors between patients with and without AKI. Patients with AKI were more likely to be diabetic (p=0.038) and have postoperative complications (p=0.014), mainly atrial fibrillation and prolonged ventilation. Mortality rates were six times higher when compared to those without kidney injury (p=0.08). Age, sex, dyslipidemia, HTN, PVD, CVD, urgent versus elective surgery, and use of ACEI/ARBs showed no significant difference in predicting risk of kidney injury.

Conclusions: Patients with AKI after off-pump cardiac surgery are more likely to be diabetic and have postoperative complications. They also have higher rates of mortality during hospitalization.

SA-P02116
The Impact of Early Initiation of Continuous Renal Replacement Therapy on Outcomes of Critically Ill Patients with Acute Kidney Injury Hideko Yasuda, Akihiko Kato, Yukitoyo Saka, Naro Ohashi. 1 1Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; 2Division of Blood Purification, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

Background: Acute kidney injury (AKI) requiring continuous renal replacement therapy (CRRT) is a potent risk factor for mortality in critically ill patients. Accumulating evidences suggested that later initiation of CRRT might be associated with worse outcomes in AKI patients. However, it remains to be fully determined as to the appropriate timing to start CRRT therapy in intensive care unit (ICU) patients. The aim of the present study is to clarify the impact of duration from the diagnosis of AKI to CRRT initiation on fluid overload and total mortality in ICU patients.

Methods: We retrospectively collected the data of 67 ICU patients (age: 66.9 ± 19.3, male/female = 47/20) suffering from AKI requiring CRRT in our hospital from 2006 to 2010. We divided all patients into the two groups according to durations until the start of CRRT; the early group (n = 30) that conducted CRRT treatment within 24 hr after the diagnosis of AKI, and the late group (n = 37) that did 24 hr later. We compared clinical parameters at the start of CRRT and prognosis after CRRT therapy between the two groups.

Results: There was a significant difference in serum urea nitrogen (51.5 ± 30.8 vs. 70.1 ± 35.7 mg/dl, p < 0.05) and platelet counts (158 ± 111 x 10³ vs. 98 ± 69 x 10³ /µl, p < 0.05). The late group disclosed a higher rate of total mortality at 30-day (33.3 vs. 73.0%, 60-day (43.3 vs. 75.7%), and in-hospital mortality (43.3 vs. 78.4%). No significant difference was found in fluid balance from admission of ICU to initiation of CRRT (1124 ± 2414 vs 2313 ± 4392 ml) and central venous pressure (8.2 ± 5.7 vs 8.8 ± 5.6 cm) between the both groups.

Conclusions: It follows from these observations that early initiation of CRRT within 24 hr may provide a better prognosis independently of fluid balance in ICU patients with AKI.

SA-P02115
Acute Kidney Injury after Off-Pump Cardiac Surgery Stratified by Stages of Pre-Existing Renal Disease Rachina Sethi Reddy, Michael F. Michielis, Nirav C. Patel, Maria V. DeVita. Lenox Hill Hospital, New York, NY.

Background: Acute Kidney Injury (AKI) is common among hospitalized individuals, particularly those undergoing cardiac surgery. Studies have shown ≥20% drop in glomerular filtration rate, prolonged hospital stay, progression to end stage renal disease (ESRD), and increased mortality following cardiac surgery. However, many were unable to adjust for baseline kidney function or specifically assess off-pump cardiac surgery.

Our study investigates the association between preoperative stages of chronic kidney disease (CKD) and AKI after off-pump cardiac surgery, defined as 25% increase in serum creatinine (Scr). We also assessed significant predictors for AKI and mortality.

Methods: Using the cardiac surgery database from Lenox Hill Hospital, we performed a single-center retrospective analysis on all off-pump cardiac surgery patients from June 2009 to January 2011. Extracted data included: age, sex, race, preoperative SCR, postoperative peak Scr, comorbidities (diabetes, dyslipidemia, hypertension, HTN), peripheral vascular disease (PVD), cardiovascular disease (CVD), urgent versus elective surgery, complications during hospitalization, use of ACEI/ARBs, mortality. Admission creatinine included on-off pump cardiac surgery, preoperative dialysis (ESRD or prior 60 days), intraoperative death, missing data points.

Results: Of the 441 patients that met inclusion criteria, 23.8% had AKI. Incidence of AKI by CKD stages 1-4 was 32.7%, 17.6%, 20.7%, 28.6%, respectively. There were no stage 5 CKD patients. Chi-squared analyses compared risk factors between patients with and without AKI. Patients with AKI were more likely to be diabetic (p=0.038) and have postoperative complications (p=0.014), mainly atrial fibrillation and prolonged ventilation. Mortality rates were six times higher when compared to those without kidney injury (p=0.08). Age, sex, dyslipidemia, HTN, PVD, CVD, urgent versus elective surgery, and use of ACEI/ARBs showed no significant difference in predicting risk of kidney injury.

Conclusions: Patients with AKI after off-pump cardiac surgery are more likely to be diabetic and have postoperative complications. They also have higher rates of mortality during hospitalization.
Cutoff values of sCr to maintain same performance of eGFR decline were determined for each RIFLE stage. It was tested in 260 hospitalized children in a general pediatric hospital. In patients with no baseline Cr, it was estimated considering normal GFR (120ml/ min/1.73m²).

Results: Using mathematical formulas, values required for classification as RIFLE R (1.3 × baseline SCr) and F4 (0.9 × baseline SCr). Patients used to validate this classification were aged 30 days to 12 years and the main diseases were Kala-Azar (56%), pneumonia (14%) and dehydration (8%). Agreement was 100% between pRIFLEcr classified by reduction in eGFR and by increment in sCr.

Distribution of RIFLE according to eGFR and sCr:

<table>
<thead>
<tr>
<th>No AKI</th>
<th>RISE to 1.3 x baseline Scr</th>
<th>RISE to 2.0-3.9 x baseline Scr</th>
<th>RISE &gt; 4 x baseline Scr</th>
<th>sCr increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>No AKI</td>
<td>161</td>
<td>52</td>
<td>38</td>
<td>100%</td>
</tr>
</tbody>
</table>

When the GFR reduced to less than 50ml/min/1.73m², all patients had an increment of at least 5 Scr. The described increments in Scr are different from those suggested by AKIN and by RIFLE for adult patient.

Conclusions: Mathematically, sCr increment was determined to maintain the same performance when compared with eGFR decline. This demonstrates that eGFR decrease magnitude in children is independent from any constant or fixed. Scr increments used for adults are not applicable to children. Moreover, this simplified classification facilitates a prompt AKI diagnosis and makes retrospective studies possible when height is missing.

SA-PO2117
Acute Kidney Injury Following Heart Surgery: A Comparison between Infants and Neonates

Background: Acute kidney injury (AKI) is associated with increased mortality in critically-ill children and adults. Heart surgery is a known risk factor for AKI. The incidence of AKI in children following heart surgery ranges from <1 to 30% depending on various AKI definitions. However, there are no AKI studies specific for infants or neonates undergoing heart surgery. Our objective was to compare the clinical characteristics of post-heart surgery infants (>28 days-1 year) and neonates (>28 days) with AKI, utilizing the AKIN definition.

Methods: Retrospective descriptive study. We included all post-operative heart surgery patients <1 year old who were admitted to our PICU between January 2006 and May 2009 with AKI defined by their maximal AKIN stage. Patient data was collected from a PICU database and medical charts.

Results: 280 infants and 122 neonates had heart surgery in the study period. We identified 125 (45%) infants and 76 (62%) neonates with AKI. The following variables were significantly higher (p<0.05) in neonates: Pediatric Risk of Mortality (PRISM) III scores (median 8 vs 10), ventilation days (median 4 vs 5), cardiopulmonary bypass time (median 102 vs 128 min), need for ECMO (5.6 vs 18.4%), PICU and hospital length of stay (median 6 vs 7.5, 11 vs 18 days respectively). AKI occurred early as 75% of neonates and 76% of infants reached their maximal AKIN stage within 48 hours of admission. The mortality rates of infants and neonates were 7/280 (2.5%) and 11/122 (9%) respectively. Of those who died, 5/7 infants & 11/11 neonates were classified as AKIN stage 3. 15/16 who died in AKIN stage 3 were defined by urine output criteria only. AKI in adults: 3, 15/16 who died in AKIN stage 3 were defined by urine output criteria only. AKI in adults: 3, 15/16 who died in AKIN stage 3 were defined by urine output criteria only.

Conclusions: The incidence of AKI and mortality rate was higher in neonates (62%) vs infants (45%) compared to infants (45%) vs. AKI in adults: 3, 15/16 who died in AKIN stage 3 were defined by urine output criteria only. AKI in adults: 3, 15/16 who died in AKIN stage 3 were defined by urine output criteria only.

SA-PO2118
Acute Kidney Injury Due to Fibrates in the Absence of Rhabdomyolysis. A Common and Occasionally Irreversible Complication

Background: Fibrates are increasingly prescribed for the treatment of hypertriglyceridemia. Several case reports have described acute kidney injury (AKI) in patients treated with fibrates, although most of them were due to rhabdomyolysis. Method: We conducted a prospective cohort study of consecutive patients admitted to critical care units in five Canadian ICUs over a 30-day period. Each patient was followed until hospital discharge or for a maximum of 30 days. The Acute Kidney Injury Network system was used to identify and classify individuals with AKI. We used descriptive statistics to characterize patients with AKI and their outcomes. Among AKI patients, we used multivariable logistic regression to identify predictors of dialysis and the composite outcome of death or discharge.

Results: We identified 603 patients of whom 161 (26.7%) developed AKI. Patients with AKI were more likely to die (29.2% vs. 8.6% of those with no AKI, p<0.001) and the risk of death escalated with AKIN stage (19.2, 37.9 and 51.5% for AKIN stages 1, 2 and 3, respectively). Nineteen (12%) patients received dialysis a median of 1 (1-2) days after AKI diagnosis. Increments in plasma urea and urine output < 400 mL/day on the day of AKI diagnosis accurately predicted the receipt of dialysis (area under the receiver operator curve 0.85). The composite outcome of death or receipt of dialysis occurred in 57 (35.4%) patients with AKI. The combination of plasma urea, urine output <400 mL/day and decrements in serum bicarbonate on the day of AKI diagnosis predicted this occurrence (AUC 0.83).

Conclusions: AKI is a common complication of critical illness in Canadian ICUs. Even mild AKI is associated with a substantial risk of death. At the time of AKI diagnosis, clinical data may be helpful in identifying individuals with a high likelihood of developing progressive disease.

SA-PO2120
Determinants of Nutritional Support in Patients with Acute Kidney Injury

Background: There is limited information on the timing, route, and amount of nutrition required for ICU patients with AKI. We assessed the pattern of nutritional support (NUTS) and utilization of enteral (EN) and parenteral (PN) nutrition in patients enrolled in the MON study (KI, 2004, 66: 1613-1621). We hypothesized that AKI severity and comorbidities would influence the mode of nutrition (MON).

Methods: We analyzed data from 615 of the 618 patients who stayed in ICU >48 hours. We assessed the MON provided in the ICU and its relationship to underlying disease and other factors.

Results: Among 615 patients, 199 ate orally without NUTS (Oral), 183 received EN, 66 PN, 91 EN+PN, and 76 no oral or NUTS (None). A subset of the NUTS patients had oral intake for part of their ICU stay (EN+n=81; PN+n=22; EN+PN+n= 27). Overall, nutrition was provided for 65.4% of the ICU stay (range 2-106; NUTS 69.9 vs Oral 73.2%; p<0.001).

Conclusions: The relationship of nutritional support with AKI is complex and warrants further study.
Control

552±210
0,73±0,79
10±7,6
618±202
5,45±0,64
1,95±0,73
≥7,5±2,6†
χ2
10±6,6
4,82±0,20†

After treat.
3,47±0,33
2,19±0,73

Underline represents presenting author.

608A

SA-PO2121

Urinary Liver-Type Fatty Acid-Binding Protein Predicts Mortality in Critically Ill Patients Eunjung Cho, Sang-Kyung Jo, Won-Yong Cho, Hyoong-Kyu Kim. Department of Internal Medicine, Korea University Hospital, The Institute of Renal Disease, Seoul, Republic of Korea.

Background: Although several urinary biomarkers including neutrophil gelatinase associated lipocalin (NGAL) have been characterized and validated as useful biomarkers for the early detection of acute kidney injury (AKI), their usefulness as outcome predictors is not well established. Here, we determined the diagnostic and prognostic ability of urinary liver-type fatty acid-binding protein (L-FABP), one of the newly recognized candidate biomarkers for kidney injury, in heterogeneous intensive care unit (ICU) patients, comparing with those of NGAL.

Methods: We prospectively collected data of patients admitted to medical and surgical ICUs from July, 2010 to January, 2011, and urine NGAL and L-FABP at the time of admission to ICU were quantitated.

Results: Among 96 patients enrolled, 35 (36.5%) had AKI and 9 patients required renal replacement therapy. Urinary NGAL and L-FABP were significantly higher in patients with AKI compared to non-AKI ICU patients. The diagnostic performance of these biomarkers, assessed by the area under the receiver operating characteristic curve (ROC-AUC), was 0.811 (95%CI 1.0 0.718 - 0.903) for NGAL and 0.796 (95%CI 1.0 0.700 - 0.892) for L-FABP demonstrating their usefulness in diagnosing AKI. In addition, urinary L-FABP was also found to be useful in predicting in-hospital mortality in multivariate analysis along with SAPS II score, whereas urinary NGAL failed to demonstrate it. The ROC-AUC of urinary L-FABP in predicting in-hospital mortality was 0.743 (95% CI 0.635 - 0.851), with a sensitivity of 72% and a specificity of 79% at a cutoff value of 44.5 ng/ml.

Conclusions: L-FABP, an emerging urinary biomarker, seems to be promising both for the diagnosis of AKI and the prediction of prognosis in heterogeneous ICU patients. It needs to be further examined and validated for clinical utility. Discovery of biomarkers to stratify patients at risk of poor prognosis might improve ultimate outcomes in critically ill patients.

SA-PO2122

The Duration of Postoperative Acute Kidney Injury and Risk of Long Term Mortality after Lung Transplantation Edgard I. Wehbe,1 Marie M. Budev,2 Rachel Lauren Brock,1 Sevag Demirjian,1 Martin J. Schreiber,1 Brian R. Stepnany.1 1Nephrology and Hypertension, Glickman Urological and Kidney Institute; 2Pulmonary and Critical care, Cleveland Clinic.

Background: To determine if the duration of Acute kidney injury(AKI)after lung transplantation predicts long term mortality

Methods: We retrospectively evaluated data on 657 patients who underwent lung transplantation from 1997 to 2009. AKI was defined by absolute rise in creatinine by >=0.3 mg/dl and categorized into three stages by the magnitude rise in creatinine according to the AKIN classification and by the duration from baseline to nadir of creatinine (short (less than 5 days), medium (5–10 days) or long (10 days or more)). Outcomes analyzed were all cause mortality.

Results: We identified 424 patients (65%) who had at least one AKI event in the first 2 weeks after transplantation. 115 (17.5%), 184(28%) and 125(19%) experienced short, medium and long duration AKI respectively. After a median follow up of 2.2 year (0.4–6.9), a total of 277 (42%) died. One year patient survival was 84%, 81%, 65% in the short, medium and long duration AKI respectively. The survival curve (figure 1) showed that long-term survival decreased according to the duration of AKI with significant difference between long duration vs medium or short duration AKI. Adjusting for age, gender, race, type and cause of lung transplant, diabetes and hypertension, the hazard ratio for death was 1.6 (95% CI 1.1–2.3), 1.6 (95% CI 1.1–2.2), and 2.7 (95% CI 1.9–3.79) for short, medium and long duration AKI respectively.

Conclusions: This study showed that the duration of AKI is independently associated with long-term mortality and may provide additional prognostic information in patients undergoing lung transplantation.

SA-PO2123

Tubular Dysfunction in American Cutaneous Leishmaniasis: Evolution after Treatment Rodrigo Alves de Oliveira,1 Alexandre Braga Libório,2 Geraldo D. Silva,1 Antonio C. Seguro,1 Elizabeth De Francesco Daher,1 1School of Medicine, UFC; 2UNIFOR; 3School of Medicine, USP.

Background: Leishmaniasis is an infectious, zoonotic disease. Various types of kidney injury have been reported in visceral type. There are few reports of renal lesions in American Cutaneous Leishmaniasis (ACL). The aim of this study was to determine tubular dysfunction in ACL.

Methods: Prospective study, conducted in Brazil. Thirty-seven patients diagnosed with ACL based on histopathological and Montenegro test. Prior and 48 hours after treatment with Glucantime® tubular function was tested and the results were compared with those obtained for 8 control subjects. Urine and plasma osmolality (Uosm and Posm) were tested before and after intranasal DDAVP, Bicarbonate (sBic), urinary pH (UpH) were evaluated before and after acidification test with CaCl2 (acid test). UpH ≥5.5 after CaCl2 test and UpH/Posm ≥2.8 with Uosm≥700mosm/kgH2O after DDAVP were considered abnormal. About acidification, 15 patients presented an abnormal acid test at the diagnosis. From these, six had sBic<22mEq/L. After treatment, six patients (40%) maintained urinary acidification deficit (p<0.01) with three presenting sBic<22mEq/L. Overall, 12 patients had combined tubular dysfunction before treatment and 5 persisted after treatment.

Conclusions: ACL is associated with renal tubular dysfunctions and these are only partially reverted after treatment, especially urinary acidification ability. Glucantime has no detrimental effect.

Renal function before and after treatment vs controls

Diagnosis After treat. Control

CICr (m/m1/m1,73m2) 109,6±31,5 108,4±28,5 116,4±22,7

UpH T4 2,16±0,73 1,95±0,73 5,47±0,31

Uosm T4 618±202 552±210 965±811

UpH/Posm T4 5,45±0,64 5,19±0,60 4,82±0,20

sBic(mEq/L) 1,15±0,64 1,33±1,51 0,73±0,78

FEkC(%) 10,6±6 10,7±6 7,52±6,9

†before and after vs controls, p<0.05; †before vs after treatment, p<0.0066

Funding: Government Support - Non-U.S.

SA-PO2124

Acute Kidney Injury and AKI Biomarkers Are Associated with Fluid Overload in Critically Ill Children Ana Palijan,1 Prasad Devarajan,2 Joseph V. Bonventre,1 Michael R. Bennett,2 Qing Ma,2 Venkata Sabbisetty,1 Michael Zappitelli.1 1Pediatrics, McGill University Health Centre, Montreal, Canada; 2Cincinnati Children’s Hospital Medical Centre, Cincinnati, Ohio; 3Brigham and Women’s Hospital, MA.

Background: Little data exist on fluid overload (FO) of all ICU children and the relation between acute kidney injury (AKI) and FO. We hypothesized that ICU-admitted children with AKI develop worse FO and that urine AKI biomarkers help predict worse FO.

Methods: We prospectively followed 160 non-transplanted children admitted to ICU≥1day. Serum creatinine (SCr) and fluid intake/output were recorded daily. FO% was calculated as fluid in-out (L)/[weightX100]. AKI was defined as $0.50 or 27 umol/L Cr rise from baseline. Risk factors for Day 3 FO were evaluated by multiple linear regression. The association of AKI and Day 3 FO on ICU stay was evaluated by Cox regression. Area under the curve (AUC) was used to evaluate clinical factors to predict presence Day 3 FO≥3% (median FO). In 51 pts with biomarkers measured, first ICU urine neutrophil gelatinase-associated lipocalin (NGAL), interleukin-18 (IL-18) and kidney injury molecule-1 (KIM-1) were added to the clinical model predicting Day 3 FO≥3%, to determine if this increased prediction.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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608A
Results: Mean[SD] age, ICU stay and Day 3 FO% were 4.5[5.7] yrs, 9.3[8.7] d and 4.6[4.2]% respectively. 60% of patients were boys, 4% had sepsis, 65% received pressors, 45% had AKI. Day 3 FO was higher in AKI pts[2.56] vs. [9.33][SD=0.02]. AKI was associated with worse Day 3 FO(>0.03), independent of age, gender, and fluid (ml) per kg in the first 6 ICU hours(<0.001).AKI and higher Day 3 FO predicted longer ICU stay, controlled for age and gender. A clinical model including AKI, age, gender and fluid in first 6 ICU hrs predicted presence of Day 3 FO[3%] with AUC=0.67. When first ICU urine NGAL, IL-18 and KIM-1 were added to the model, AUC increased to 0.76.

Conclusions: When all ICU children are studied, FO is not as severe as previously described, but is associated with presence of AKI and longer ICU stay. Combining clinical risk factors and AKI biomarkers leads to enhanced prediction of more severe FO development and may assist in FO prevention strategies.

SA-PO2125
Fighting Bloody Diarrhea for HUS Prevention and Mitigation: Lombardy Regional HUS Network
Gianluigi Ardissino, Francesca Tel, Sara Testa, Fabio Paglialonga, Stefania Salardi, Silvana Tedeschi, Nicolò Borsa, Rosaria Colombo, Manuela Colosimo, Erminio Torresani, Alberto Edefonti. Center for HUS Control, Fondazione Ca’ Granda Osp Maggiore Policlinico, Milano, Italy.

Background: Typical hemolytic uremic syndrome (HUS), although rare, still represents a major public health problem in industrialized countries caused by a Verotoxin-producing Escherichia coli (VTEC) intestinal infection often presenting with bloody diarrhea.

Methods: In order to identify patients at risk of HUS early in the course of the disease, a network connecting pediatric hospitals in Lombardy Region (10 million s) was developed.

Results: Fifty-three units presently partecipate in the network and since May 28, 2010 (founding day) children with bloody diarrhea were centrally tested for Shigatoxin (Stx) 1 and 2 with a rapid immunochromatographic test and multiplex PCR test. The objectives of the project were: 1. to increase the ability of the surveillance system in identifying the sources of VTEC infection and its spreading; 2. to understand the mechanisms of Stx delivery to target organs endothelia; 3. to test the potential role of overhydration and/or leukoaprexis to prevent or mitigate renal and CNS involvement. So far 248 patients have been tested. Hereafter are the preliminary results concerning the 80 samples for which all the procedures were completed. Ten out of 80 (12.5%) were positive for VTEC. Among negative samples Salmonella (25%) and Campylobacter (11%) were the most common identified bacteria. Among patients with negative culture (47%) 1 patient had Henoch Schoncup purpura, 1 ulcerative colitis and 1 Meckel diverticulum.

Conclusions: In conclusion, our findings point out an unexpected very high frequency of VTEC among bloody diarrhea in children in our region. No conclusion can be anticipated on the remaining objectives.

Acknowledgement: The project is feasible thanks to the collaboration of the members of the Regional HUS Network whose complete list is available at www.centroseu.org.

SA-PO2126
Impact of Acute Kidney Injury (AKI) and Chronic Kidney Disease (CKD) on Re-Admissions in Hospitalized Patients with Congestive Heart Failure (CHF) Charuhas V. Thakar, Pratik Parikh, Yan Liu, Anthony Leonard. Internal Medicine/Nephrology, University of Cincinnati, OH; Medical Service/ Renal Section, Cincinnati VA, Cincinnati, OH; Biomedical, Industrial and Human Factors Engineering, Wright State University, Dayton, OH.

Background: Reducing readmission rate in patients hospitalized with CHF improves quality of care and reduces healthcare costs. Effect of AKI and CKD on readmissions in patients with CHF is not well understood.

Methods: We examined the state inpatient database (SID; derived from AHRQ-HCUP) for the state of Washington. 6,535 patients were discharged with a primary diagnosis of CHF from all hospitals in the state between 1/1/06 and 9/30/06.

The primary endpoint was re-admission within 30 days from the index discharge with either primary or secondary diagnosis of CHF. Patients were classified based on diagnosis of AKI, CKD and other co-morbid diseases based on ICD-9 codes. Logistic regression analysis was adjusted for demographics, co-morbidities, and procedures, and economic indicators.

Results: Patients in the sample had a mean age of 73.8 years (standard deviation, SD, 14 years). 53% were male, 47% were female, 25% had diabetes, 51% had hypertension. Medicare was the payor in 71% of patients. 6.5% of patients developed AKI during the index hospitalization for CHF from all hospitals in the state between 1/1/06 and 9/30/06.

In order to identify patients at risk of tHUS early in the course of the illness, a network connecting pediatric hospitals in Lombardy Region (10 million s) was developed.

Results: Fifty-three units presently partecipate in the network and since May 28, 2010 (founding day) children with bloody diarrhea were centrally tested for Shigatoxin (Stx) 1 and 2 with a rapid immunochromatographic test and multiplex PCR test. The objectives of the project were: 1. to increase the ability of the surveillance system in identifying the sources of VTEC infection and its spreading; 2. to understand the mechanisms of Stx delivery to target organs endothelia; 3. to test the potential role of overhydration and/or leukoaprexis to prevent or mitigate renal and CNS involvement. So far 248 patients have been tested. Hereafter are the preliminary results concerning the 80 samples for which all the procedures were completed. Ten out of 80 (12.5%) were positive for VTEC. Among negative samples Salmonella (25%) and Campylobacter (11%) were the most common identified bacteria. Among patients with negative culture (47%) 1 patient had Henoch Schoncup purpura, 1 ulcerative colitis and 1 Meckel diverticulum.

Conclusions: In conclusion, our findings point out an unexpected very high frequency of VTEC among bloody diarrhea in children in our region. No conclusion can be anticipated on the remaining objectives.

Acknowledgement: The project is feasible thanks to the collaboration of the members of the Regional HUS Network whose complete list is available at www.centroseu.org.

SA-PO2128
Application of the Acute Kidney Injury Network Definition in Post-Heart Surgery Neonates: A Retrospective Study
Abdullah E. Alabbas, Peter Skippen, Andrew I. Campbell, Douglas G. Matsell, Cherry Mammen. BC Children’s Hospital, Vancouver, BC, Canada.

Background: Heart surgery is a known risk factor for acute kidney injury (AKI) in children. The incidence of AKI post-heart surgery ranges from <1 to 30% depending on various AKI definitions and the population of interest. AKI in neonates following cardiac surgery has not been well-studied. Our objectives were: 1) To describe the clinical characteristics of post-heart surgery neonates with AKI, utilizing the AKIN definition 2) To explore potential risk factors for mortality in this population.

Methods: Retrospective, single center observational design. We included all postoperative heart surgery neonates admitted to our PICU between January 2006 and May 2009 with AKI defined by their maximal AKIN stage. Clinical characteristics were compared between patients’ AKIN stages (1.2 and 3). Patient data were collected from a PICU database and medical charts. Multiple logistic regression analyses were performed to evaluate possible risk factors of mortality.

Results: We identified 76 out of 122 (62%) post-heart surgery neonates with AKI during the study period. Overall mean age was 8.4 ± 7.6 days with 90% being term gestation. When compared to stage 1 and stage 2, AKIN stage 3 patients were younger (mean 8.39 ± 6.03 vs 7.8 days for stages 1, 2 and 3 respectively), had higher ventilation days (median 5 vs 4 vs 5 vs 7), PICU length of stay (median 7 vs 6 vs 9 days), and Pediatric Risk of Mortality (PRISM III) scores (mean 9.6 ± 8.68 vs 13.49) in stage 3 compared to 1 and 2. Pediatric Risk of Mortality (PRISM III) scores (mean 9.6 ± 8.68 vs 13.49) in stage 3 compared to 1 and 2. Eleven neonates died after AKI, all with AKIN stage 3. Age <5 days (<OR=25.5, 95%CI=2.3-279) and use of ECMO (OR=53.2, 95%CI=5.4-525.5) were independently associated with mortality.

Conclusions: The incidence of AKI in neonates post-heart surgery is high (62%). Those with severe AKI are younger, require longer ventilatory support, stay longer in the PICU and are more critically ill. The development of severe AKI, age < 5 days and need for ECMO are all potential independent risk factors for mortality in this cardiac population. These findings need to be supported by larger prospective studies.

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Poster/Saturday
SA-PO2129
The Risk Factors on the Prognosis of Acute Kidney Injury under AKIN Definition in Critical Ill Patients Yang Lichuan, Ping Fu. Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan, China.

Background: Despite significant advances in the medical management and therapeutics, acute kidney injury (AKI) is still a familiar and serious complication with enhanced morbidity and mortality in hospitalized patients, especially in intensive care units (ICUs). The primary purpose of this study is to apply the definition proposed by the Acute Kidney Injury Network (AKIN) workgroup to assess the incidence, the short-time (28 days) outcomes and the risk factors on the prognosis of AKI in ICU.

Methods: In this retrospective study, we collected the data from a cohort of 4642 patients admitted in ICUs from Dec 2009 to march 2011. Univariate and multivariate analysis were performed to investigate the risk factors for short-time mortality of AKI (AKI defined by the increase of serum creatinine by 26.4 μmol/L in ICU).

Results: After further exclusion, there were 1036 patients of ICU admissions enrolled in this research. We found AKI occurred in 335 of the 1036 patients (34.1%) under the AKIN criteria and the mortality was 54.4%. The variables that were related to the prognosis of AKI in multivariable analysis were as follows: AKI III (odds ratio (OR), 12.5338), AKI II (OR, 4.6625), SAP (OR, 2.3522), renal replacement therapy (RRT, OR = 2.1113), the timing of AKI in ICU (OR = 1.2351), base serum creatinine (OR = 1.0059),the length of stay in ICU (OR = 1.0546) and age (OR = 1.0223). The area under the receiver operator characteristic (ROC) curve for 28 days mortality was 0.7571 for the AKIN criteria (p < 0.001).

Conclusions: In these ICU patients, the mortality of AKI is correlated with various risk factors, among which the timing of AKI in ICU (OR = 1.2351), base serum creatinine (OR = 1.0059), the length of stay in ICU (OR = 1.0546) and age (OR = 1.0223) are independent risk factors for ICU mortality. Our results support the utility of the AKIN criteria in predicting outcomes for patients with AKI.

SA-PO2130
Acute Kidney Injury and Its Severity Are Independently Associated with 10-Year Survival and End Stage Renal Disease Following Cardiac Surgery, Resembling a Dose-Response Pattern Alejandro Ferreiro, 1 Raul Lombardi, 1 Emma Schwedt, 2 Gonzalez-Bedat Carlota Maria, 3 Nelson Mazzuchi, 1 1National Institute for Cardiac Surgery, Uruguay; 2Uruyayan Dialysis Registry, Uruguay.

Background: Acute kidney injury (AKI) has been associated with long-term mortality and ESRD in large medical claim-based databases. The relationship between multivariate-adjusted and especially AKI II AKI III SAP, the timing of AKI was independent risk factor for ICU mortality. Our results support the utility of the AKIN criteria in predicting outcomes for patients with AKI.

Methods: All adult patients submitted to cardiac surgery between 1/1/2000 and 12/31/2009 (n=7773) were enrolled. Co-morbidities, type of CS procedure, and outcomes were prospectively registered. Long-time survival (up to 10 years) was obtained from a telephone survey and/or National Population Registry. The ESRD status was obtained from the Uruguayan Dialysis Registry which includes all dialysis units in the country. Baseline renal function (eGFR) was assessed by the Cockroft-Gault formula. AKI was defined by a 50% increase in serum creatinine post-operatively. All-cause mortality was determined via Office of National Statistics data.

Results: The incidence of AKI was 10.5%. Patients developing AKI were older (67.06±6.41, p<0.0001), with more previous MIs (53.9 vs 40.5%, p<0.0005), more hypertension (45.5vs28.3%, p<0.0001), more hypotension (86.8vs79.1%, p<0.0012), more PVD (5.03±3.21, p<0.0001). In-hospital mortality was significantly increased in patients with AKI (5.6±3.12, p<0.0001). 5-year survival was also worse among patients with AKI (82.9±0.75%, p<0.0001).

Conclusions: AKI was the strongest independent predictor of long term mortality with an adjusted HR of 3.27 (CI 2.09 to 5.12). Only LV function (adjusted HR 1.07 (CI 1.03 to 1.1)) was also independently associated with 5 year all cause mortality.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only.
Methods: This is a retrospective observational study. Inclusion criteria were adult (age ≥ 18) patients who underwent non-cardiac surgery under general anesthesia from 2007-2009 at our institute. Exclusion criteria were urological surgery, those without creatinine (Cre) values, and who had undergone dialysis preoperatively. Exposure of interests are preoperative use of anti-hypertensives including ACE-I/ARB and diuretics. Outcome variable was postoperative AKI. Kidney injury defined by AKI network (increase in Cre ≥ 0.3 mg/dL or 150 %, or urine output < 0.5 ml/kg/hour for > 6 hours). Multivariable logistic regression was performed. We adjusted for 23 covariates including kinds of surgery. A propensity score (PS) of receiving ACE-I/ARB therapy preoperatively was estimated and PS analyses were performed including PS adjustment and PS matching as sensitivity analyses.

Results: There were 123 AKI (5.0 %) among 2472 subjects. Odds ratio of AKI by multivariable logistic regression analysis

<table>
<thead>
<tr>
<th>Odds ratio</th>
<th>p value</th>
<th>95 % CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE-I/ARB</td>
<td>0.477</td>
<td>0.350-1.379</td>
</tr>
<tr>
<td>Diuretics</td>
<td>5.360</td>
<td>1.288-4.325</td>
</tr>
</tbody>
</table>

PS analyses yielded similar results with regard to ACE-I/ARB therapy. Other independent predictors of AKI included male sex, intrathoracic, intraperitoneal surgery, surgery with large fluid shift, emergent surgery, insulin-dependent diabetes, hypertension, cerebrovascular disease, intraoperative use of pressors.

Conclusions: Not prescription of ACE-I/ARB, but of diuretics was an independent risk factor for postoperative AKI in non-cardiac surgery.

SA-PO2134

The Impact of Early Nephrology Follow-Up among Survivors of Acute Kidney Injury Requiring Dialysis

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Background: Acute kidney injury (AKI) is associated with an increased risk of death and kidney disease progression. However, the optimal management of AKI survivors is unclear. We postulate that early follow-up with a nephrologist following a hospitalization for AKI may modify the risk of death.

Methods: Using linked administrative databases for all of Ontario for the period April 1994 to March 2008, we conducted a cohort study of all adults with AKI who received in-hospital dialysis and remained dialysis free at least 90 days after discharge. The exposure of interest was early nephrology follow-up, defined as at least one nephrologist visit within 90 days of discharge. A propensity score was used to match those who did versus those who did not receive early nephrology follow-up after discharge. A Cox Proportional Hazards model was used to estimate the treatment effect. The primary endpoint was all-cause mortality within 2 years of cohort assembly.

Results: Of the 3877 eligible patients, 1553 (40%) received early nephrology follow-up. We successfully matched 1184 individuals who all received early follow-up to 1184 of those who did not. The incidence of death was 0.02 per 100 person-years among individuals receiving early follow-up compared to 0.03 per 100 person years in those who did not (hazard ratio of 0.76, 95% confidence interval 0.62-0.93).

Conclusions: Early nephrology follow-up after a hospitalization with AKI requiring in-hospital dialysis was associated with improved survival. Whether this is a reflection of better care provided by nephrologists, or a biased tendency to bring less morbid individuals to nephrology care, is unknown. In light of these results, further research is needed to evaluate the processes of care undertaken by nephrologists when following-up patients hospitalized with AKI requiring in-hospital dialysis.

Funding: Government Support - Non-U.S.

SA-PO2135

Acute Kidney Injury Is Associated with Increased Mortality After Cardiac Surgery in Patients with Pre-Existing Renal Impairment

Sean Gallagher, Matt Lovell, Dan A. Jones, Krishnaraj S. Rathod, Akhil Kapur, Andrew Wragg, Magdi Yaqoob, Rakesh Uppal. Cardiac and Renal, Barts and the London NHS Trust, United Kingdom.

Background: Acute kidney injury (AKI) following coronary artery bypass graft surgery(CABG)has been associated with increased mortality. Whether AKI is as prognostically important in patients with pre-existing renal impairment remains unclear. Methods: An analysis of prospectively collected data upon 286 patients with chronic liver disease who were consecutively admitted in an urban tertiary medical center to undergo OLT from June 1st, 2005, to December 31, 2009. AKI score was determined at the moment of admission and immediately before OLT. AKI was defined according to the AKIN criteria as a 50% or greater increase of baseline creatinine level.

Results: From the 286 included patients (52.3±11.7 yrs), 54% had viral-related disease, with MELD of 19.4±9.8, and 21% had diabetes mellitus. AKI at admission and on the day of OLT was observed in 84 (29%) and 73 (26%) subjects, respectively, with the following Odds ratio (OR): 2.2±2.3, OR: 2.3±2.9 at admission; 1.48±2.22, 2.30±3.2 on the day of OLT. RRT was needed in 81 cases (28%). Mortality rate at 28 days was
Renal Function in Patients with Multiple Myeloma Treated with Bortezomib-Based Regimens: A Single-Center Cohort Analysis

Background: Bortezomib is a proteasome inhibitor that blocks several molecular pathways in a cell and may cause cancer cells to die. When added to standard therapy significantly improves time to progression and overall survival in patients with multiple myeloma (MM). The aim of our study was to evaluate the effect of bortezomib-based regimens in renal function in patients with MM.

Methods: In all, 34 patients (mean age: 63 years; 18 male; 30 Caucasian) with MM treated with bortezomib-based regimens were studied retrospectively. Bortezomib was administered at the dose of 1.3 mg/m² on days 1, 4, 8, and 11 of each cycle of 21 days. Twenty patients, eligible to stem cell transplantation (SCT), were treated with bortezomib, doxorubicin (40 mg/m² q4d) and prednisolone (60 mg/m² d). Six patients, not eligible to SCT, were treated with bortezomib, melphalan (9mg/m² d) and prednisolone (60 mg/m² d). Eight patients, with relapsed or refractory MM, were treated with bortezomib and prednisolone (60mg/m² d). Glomerular filtration rate (GFR) was calculated by Modification of Diet in Renal Disease equation. Comparisons between GFR values determined at the beginning of the first cycle and one month after the last cycle were performed by paired-samples T test.

Pre-existing chronic kidney disease (CKD) was considered whenever baseline GFR was lower than 60 ml/min/1.73m². A P value <0.05 was considered statistically significant.

Results: There were no statistically significant differences between GFR values determined at the first cycle and GFR values determined one month after the last cycle, both when evaluating all patients (97±49 ml/min/1.73m² vs 124±138 ml/min/1.73m²; P=0.182) and when evaluating exclusively those patients with pre-existing CKD (N=6) (37±13 ml/min/1.73m² vs 56±28 ml/min/1.73m²; P=0.077), although there was a trend towards improved renal function associated with bortezomib-based regimens.

Conclusions: Our results suggest that in patients with MM bortezomib-based regimens are not harmful to kidney. On contrary, such regimens can improve renal function mainly in patients with pre-existing CKD.

Nephroprotection: PBI-4050, a Novel Orally Active Anti-Inflammatory/ Anti-Fibrotic Agent, Reduces Doxorubicin-Induced Nephrotoxicity in Mice

Background: The clinical use of doxorubicin (Dox), a potent anticancer agent, is associated with marked nephrotoxicity characterized by tubulo-interstitial lesions. The aim of this study was to examine the protective effect of PBI-4050 from day -3 to day 10.

Methods: BALB/c mice were randomized in three groups; Control, Dox (10 mg/kg) and Dox (10 mg/kg) + PBI-4050 (200 mg/kg). Mice were treated with oral administration of PBI-4050 from day -3 to day 10. On day 0, mice were immunosuppressed with intravenous administration of Dox.

Results: In LPS-induced air pouch model, PBI-4050 (200 mg/kg) inhibits PGE2 production to the same extent as indomethacin (50 mg/kg). However, in contrast to indomethacin, PBI-4050 has no effect on cyclooxygenase and lipoperoxidase pathways. Treatment with PBI-4050 significantly reduced Dox-induced nephrotoxicity as demonstrated by a reduction of serum albumin loss induced by doxorubicin. Histological lesions were also significantly reduced (p<0.05) in PBI-4050-treated mice (Lesion scores determined by HPE staining at Day 7: Dox: 1.03; Dox + PBI-4050: 0.18; at Day 11: Dox; 2.0; Dox + PBI-4050: 0.72). The Dox treated group showed an increase in TGFβ and CTGF mRNA expression in the kidney. Treatment with PBI-4050 gave a significant reduction (p<0.03) of the expression of TGFβ (26%) and CTGF (33%) in the kidney.

Conclusions: These results suggest that PBI-4050 exerts anti-inflammatory and anti-fibrotic effects in the acute phase of doxorubicin-induced toxicity. The nephroprotective effect of PBI-4050 relies, in part, on the anti-fibrotic activity as suggested by a decrease of TGFβ and CTGF expression in the kidney.

Funding: Pharmaceutical Company Support

Eliminating the Role of Notch Signaling in Acute Kidney Injury

Background: Notch signaling plays a crucial role in the regulation of cell proliferation, stem cell maintenance and differentiation during embryonic and adult development. Recent studies have reported that the activation of Notch signaling in tubule epithelial cells augments interstitial fibrosis. However, the role of Notch signaling in tubule cell maintenance and regeneration remains unclear.

Here we analyzed the role of Notch signaling in the regeneration of proximal tubules during AKI, utilizing RBP-β conditional knockout mice and proximal tubule-specific inducible Cre mice that we recently generated, in which Cre is activated only after the administration of tamoxifen.

Methods: We bred RBP-β conditional knockout mice to the proximal tubule-specific tubulin-αII antibody promoter (TUBAII:CreERT2) and control (RBP-β flox/+ :TUBAII:CreERT2) littermates.

Results: The expression of RBP-β mRNA in the whole kidneys of knockdown mice was reduced to about 30% of control kidneys, indicating the effective deletion of RBP-β in the proximal tubules. Unexpectedly, however, the proliferation of tubular epithelial cells and severity of tubular injury was indistinguishable between both genotypes. The expression of Cyclin D1, HSP47, and osteopontin in the knockdown kidneys tended to be lower compared to that of control kidneys, but the difference was not statistically significant.

Conclusions: Reduction of Notch signaling in proximal tubules during AKI did not cause significant change in the proliferation of tubular epithelial cells and severity of tubular injury. Considering the role of tubular Notch signaling in the progression of interstitial fibrosis, it is likely that epiblial Notch signaling affects the fibrogenic fibroblasts, but not neighboring epithelial cells.

Funding: Pharmaceutical Company Support

Six2-GDNF Pathway Is Activated during Experimental Acute Kidney Injury and Plays a Crucial Role in Renal Tubular Regeneration

Background: The transcriptional regulator Six2 emerged as a key factor in kidney development and maintenance of nephron progenitor cells. GDNF (glial cell-derived neurotrophic growth factor) is also reported to play an important role for nephrogenesis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
However, the roles of Six2-GDNF pathway in tubular regeneration and proliferation are poorly understood in acute kidney injury (AKI).

**Methods:** The aim of this study is to understand the functional roles of Six2 and GDNF in tubular damages in AKI in vivo and in vitro.

**Results:** To clarify the significance of Six2-GDNF pathway in AKI, we used a rat AKI model, in vivo in normal renal and tubular cells (NRK-52E cells) as an in vivo model. After clamping left renal artery for 1 h, kidney homogenates from 3 to 72 h after reperfusion were extracted. In Western blot analysis, Six2 and GDNF expressions were increased at 3-12 h and 6-24 h, respectively. In immunohistochemical examinations, Six2 positive cells and GDNF positive cells were observed in proximal tubules after AKI. In in vitro experiments, hypoxia stimulated mRNA expression and protein expression of Six2. To understand the downstream signaling of Six2, we transfected Six2 expression vector to NRK-52E cells. Overexpression of Six2 caused increased mRNA of GDNF promoter activity and increased immunofluorescence signals of GDNF in Six2 overexpressed cells (Fig 1B, immunofluorescence uptake). Furthermore, we used 3D gels to examine the role of Six2 for tubular formation. Overexpression of Six2 promoted tubular formation, while tubular formation was inhibited by transfection of Six2 siRNA.

**Conclusions:** Six2 and GDNF were upregulated in AKI at proximal tubular cells in vivo, and Six2 could regulate cell proliferation and tubular regeneration by regulating GDNF expression. The current study therefore unveils pathophysiological significance of Six2-GDNF pathway in AKI in vivo and in vitro.

**SA-PO2143**

Protective Effect of Human Umbilical Cord Blood-Derived Mesenchymal Stem Cells on Experimental Kidney Warm Ischemia-Reperfusion Injury in Mice

Yyeon Jang,1 Ji Hyeon Park,1 Seung Tae Han,1 Jae Young Yoon,1 Soo Jin Choi,2 Wonil Oh,2 Yoon Sun Yang,3 Jung Eun Lee,4 Wooseong Kim,4 Dae Joong Kim,1 Ha Young Oh,4 Yoon-Goo Kim;1 Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea; 2Biomedical Research Institute, MEDIPOST Co., Ltd., Seoul, Korea.

**Background:** Kidney ischemia-reperfusion injury (IRI), the leading cause of acute kidney injury (AKI), is characterized with robust inflammatory response. Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have been reported to show hypo-immunogenic property and exert anti-inflammatory and regenerative effects in a cerebral IRI model. We investigated the effects of hUCB-MSCs on renal injury following warm IRI in mice.

**Methods:** C57BL/6 mice were randomly allocated into 2 groups: control group (IRI without medication, n=13) and hUCB-MSCs group (n=15). A total of 1×10⁵ hUCB-MSCs were injected into the peritoneal cavity twice: 24 h prior to IRI and right before reperfusion. Serum creatinine was measured for 48 h. The trafficking of PKH-26 labeled hUCB-MSCs was assessed with immunofluorescence staining.

**Results:** Renal functional impairment was attenuated in the hUCB-MSCs group compared with the control group (serum creatinine mean ± SE, Day 0: 0.49±0.044 in the control group, 0.50±0.014 in the hUCB-MSCs group, Day 1: 2.16±0.037 in the control group, 1.75±0.097 in the hUCB-MSCs group, Day 2: 2.68±0.115 in the control group, 1.89±0.184 in the hUCB-MSCs group). PKH-26 labeled hUCB-MSCs were found in the post-ischemic kidneys of the hUCB-MSCs group on both day 1 and day 2 after IRI. hUCB-MSCs were trafficked into the post-ischemic kidneys and attenuated renal injury following IRI.

**Conclusions:** Human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) have been reported to show hypo-immunogenic property and exert anti-inflammatory and regenerative effects in a cerebral IRI model. We investigated the effects of hUCB-MSCs on renal injury following warm IRI in mice.

**SA-PO2144**

Improving Ischemia-Reperfusion-Induced Acute Kidney Injury by Treating with Induced Pluripotent Stem Cells

Pei-Ying Lee1,2, Der-Cheng Tang1,2

1Institute of Physiology, National Yang-Ming University, Taipei, Taiwan; 2Division of Nephrology, Department of Medicine, Taipei Veterans General Hospital, Taipei, Taiwan.

**Background:** Induced pluripotent stem (iPS) cells are novel stem cell populations induced from somatic cells, but the effects of iPS cells on acute kidney injury (AKI) are not currently known.

**Methods:** To investigate effects of IPS cells on AKI, iPS cells were injected via the renal artery into rats with ischemia-reperfusion (IR) AKI and compared with PBS-treated AKI rats. Renal function, histopathological findings, blood reperfusion ratio, and the levels of ROS-related factors and inflammatory cytokines were measured and analyzed.

**Results:** Our study underpins that iPS cells injected into kidney of AKI rats reduced the impairment of renal function and tissue injury compared to PBS-treated rats, and that this maximal improvement was observed at a cell dose of IPS 5 × 10⁶. However, transplantation of large number (5~10⁶) of iPS cells may decrease blood flow recovery, which was measured by laser Doppler imaging. At 48 hours after I/R, the iPS cells had mobilized to peritubular area and significantly diminished the histopathological changes associated with AKI, such as macrophages infiltration and the presence of apoptotic cells; in addition there was an enhancement of cell proliferation. Notably, iPS cell transplantation reduced the response to I/R injury in relation to ROS-related factors and inflammatory cytokines.

**Conclusions:** Injection of IPS cells into AKI rats provided a significantly beneficial effect by diminishing the impairment of renal functions, reducing tissue injury and protecting rats from the lethal effects of AKI. The effect to rescue AKI may be mediated by a reduction in ROS production and inflammation.

**Funding:** Government Support - Non-U.S.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.
Adipose-Derived Stem Cells Transfected with Hypoxia Inducible Factor-Lα Could Lessen the Injury of HK-2 Cells Induced by Cisplatin

Weini Wang, Jinnyuan Zhang.
Division of Nephrology, Jinlin Hospital, Shangai, China.

Background: Adipose-derived mesenchymal stem cells (ADSCs) may be an alternative cell source in cell therapy for some advantages. We hypothesize that ADSCs transfected by HIF-1α gene will have a positive effect on the cisplatin-induced HK-2 cells.

Methods: The hADSCs were transfected by lentiviral vector containing EGFP-HIF-1α genes. Cells expressing EGFP was sorted by flow cytometer. The expression of HIF-1α was examined by immunocytochemical method. The following groups were made: I: Cisplatin-induced group: Cisplatin (3μg/ml)-induced HK-2 cells; II: Transfected group: HIF-1α-transfected hADSCs and cisplatin-induced HK-2 cells were incubated; III: Normal group: hADSCs and cisplatin-induced HK-2 cells were incubated; IV: Control group: HK-2 cells. The ultrastructural changes of HK-2 cells was evaluated by electron microscopy. Apoptosis index of HK-2 cells was tested by TUNEL. Western blot was performed to examine the expression of caspase-3 and Bel-2 in HK-2 cells and also the expression of EPO, HO-1 and VEGF in hADSCs cells. The concentration of NGAL, KIM-1, EPO, HO-1, VEGF and HIF-1α in media were tested by ELISA.

Results: The apoptosis in co-incubation groups were decreased compared with the cisplatin group. The expression of caspase-3 and Bel-2 in co-incubation groups was lower than that in cisplatin group and the expression of Bel-2 in HK-2 cells in co-incubation groups was increased. The injury of HK-2 cells co-incubated with HADSCs transfected with HIF-1α were lesser. The concentration of KIM-1 was increased markedly in cisplatin-induced HK-2 group and that was decreased after co-incubated with hADSCs. The protein expression of EPO, HO-1 and VEGF in HK-2 cells transfected with HIF-1α were increased compared with the vector transfected group. The concentration of HO-1 and VEGF in media of gene-transfected group were higher than in vector transfected group and the variation of HIF-1α and EPO were not obvious.

Conclusions: The hADSCs could lessen the apoptosis of HK-2 cells induced by cisplatin, especially after transfected with HIF-1α and the protection may be related to the increase of higher expression of growth factors. Specific mechanism needs further experimental study.

Funding: Government Support - Non-U.S.

SA-PO2148

The Alteration of Macrophage Polarisation and Phenotype Via Mesenchymal Stem Cells In Vitro and In Vivo

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Background: Monocyte-derived macrophages comprise a heterogeneous population of cells that have the capacity to polarise in different ways depending on the nature of the stimulus. These different polarisation states (M1 and M2) may have different functions. For example, M1 macrophages are pro-inflammatory and have shown to exacerbate acute kidney injury, while M2 macrophages are anti-inflammatory and have been shown to attenuate acute kidney injury.

Methods: Monocyte-derived macrophages were isolated from human peripheral blood and plated in vitro. The macrophages were treated with lipopolysaccharide (LPS) to polarise them towards an M1 phenotype or with anti-CD203c antibodies to polarise them towards an M2 phenotype. The cytokine production of the macrophages was measured using a cytometric bead array. The mRNA expression of the macrophages was measured using real-time PCR.

Results: The macrophages treated with LPS showed an increase in the production of cytokines associated with an M1 phenotype, such as TNF-α and IL-1β. The macrophages treated with anti-CD203c antibodies showed an increase in the production of cytokines associated with an M2 phenotype, such as IL-10 and TGF-β.

Conclusions: The macrophages are able to polarise in different ways depending on the stimulus. This has implications for the treatment of acute kidney injury, as different therapies may be required to modulate the different polarisation states.

Funding: This work was supported by the National Health and Medical Research Council of Australia.

SA-PO2150

Mesenchymal Stem Cells and Endothelial Progenitor Cells Improve Renal Function in Experimental Swine Renal Artery Stenosis through Different Mechanisms

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Background: Both endothelial progenitor cells and mesenchymal stem cells may augment tissue repair. Whether cell phenotype affects the efficacy of cell therapy in the stenotic kidney is unclear.

Methods: Peripheral blood EPC and adipose derived MSC were expanded and characterized by cell surface markers (e.g. CD34 and KDR, or CD44 and CD90 respectively). Single kidney hemodynamics and function were assessed using multi detector CT in pigs after 10 weeks of renal artery stenosis treated 4 weeks earlier with an intra-arterial infusion of vehicle EPC (10x10^6, RSA+EPC, n=6) or MSC (10x10^6, RSA+MSC, n=6), and normal controls. Kidney microvascular structure, growth factors, apoptosis, oxidative stress and inflammatory pathways were evaluated in vitro.

Results: The degree of stenosis and hypertension were similar in RSA+EPC and RSA+MSC. Renal blood flow and glomerular filtration rate in RSA were lower than normal controls, but similarly improved in RSA+EPC and RSA+MSC. EPC mainly improved renal growth factor expression and oxidative stress, whereas MSC significantly attenuated inflammatory cytokines, endothelial reticulum stress related protein expression, and apoptosis.

Conclusions: Intra-renal delivery of EPC and MSC similarly improved renal function, but through different pathways. These results support development of selective cell based approaches for management of kidney disease.

Funding: Other NIH Support - DK73608, DK77013, HL71311, and HL085307

SA-PO2151

Human Umbilical Cord Blood CD133+ Cells Exacerbate Acute Kidney Injury in NOD-SCID Mice

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Background: Acute kidney injury (AKI) occurs in 5% of hospitalized patients, with a 50% mortality. An increase in circulating vascular progenitor cells occurs in humans with AKI, and certain blood-derived progenitor cells facilitate recovery in animal models of ischemia-reperfusion injury, including AKI. CD133+ cells isolated from human umbilical cord blood are associated with improvement following acute kidney injury in several settings. We examined the effects of CD133+ progenitor cells in a mouse model of AKI, induced by ischemia-reperfusion injury (I/R).

Methods: In non-obese diabetic severe combined immunodeficient (NOD-SCID) mice, I/R was induced by bilateral clamping of the renal artery and vein for 60 min, followed by clamp release. Human cord blood CD133+ cells or a mixed population of CD133+ cells (105/mouse) were injected via the jugular vein at reperfusion.

Results: Fluorescently-labeled CD133+ cells were detected in blood at 2 min following i.v. injection (35% bioavailability) but numbers decreased rapidly (2% at 1 hr). Homing to the kidney, liver, spleen, brain, lung, bone marrow, or heart was not reliably detected. Administration of CD133+ cells did not alter plasma urea or creatinine in sham-operated mice. In contrast, in mice subjected to I/R, CD133+ cells significantly increased plasma urea at 24 hrs (CD133+: 63 vs control: 34 mM, p<0.01, n=11-12), and serum creatinine at 6 and 24 hrs (respectively, CD133+: 47 and 95 vs control: 18 and 43 μM, p<0.05). Administration of CD133+ cells exacerbated histologic tubular necrosis. No change in blood pressure occurred over 6 hrs in mice treated with CD133+ cells. CD133 cells did not alter blood urea, serum creatinine or histologic indices of injury compared with I/R alone (p>0.05). Administration of CD133+ cells to immunocompetent mice (FVB/N) with I/R also increased renal injury.

Conclusions: Intra-renal delivery of EPC and MSC similarly improved renal function, but through different pathways. These results support development of selective cell based approaches for management of kidney disease.

Funding: Other NIH Support - DK73608, DK77013, HL71311, and HL085307

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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Conclusions: These data indicate that human cord-blood-derived CD133+ cells express the murine AKI renal injury, possibly via release of soluble factors. Our study highlights the importance of organ-specific responses and caution in any cell-based therapies for AKI.

Funding: Clinical Revenue Support

SA-PO2152

Adult Renal Progenitor Cells Revert Acute Renal Tubular Cells Injury by Means of TLR2-Driven Specific Paracrine Factors Fabio Sallustio,1 Vincenzo Costantino,1 Sharon N. Cox,2 Antonia Loverre,2 Marco Rizzi,2 Francesco Paolo Schena,2,3 Department of Emergency and Organ Transplantation, University of Bari, Bari, Italy; 2C.A.R.S.O. Consortium, Valenzano, Bari, Italy; 3Department of Human Anatomy and Histology, University of Bari, Bari, Italy.

Background: Acute Kidney Injury (AKI) is emerging as a public health problem worldwide, characterized by acute tubular apoptosis and necrosis. Tubular damage may recover, although a critical number of surviving cells is required to reconstitute structural integrity. Recently, many researchers focused their attention on the possibility of using adult renal progenitor cells (ARPCs) to improve kidney function. In this study, we investigated the influence of ARPCs in the regenerative process of cisplatin-injured renal proximal tubular epithelial cells (RPTECs) and injured Human Kidney 2 (HK2) cells.

Methods: We set up an in vitro model of cisplatin-induced toxicity, in which RPTECs were co-cultured with ARPCs, and performed proliferation and apoptosis assays to study regenerative effect of ARPCs on RPTECs. Moreover, we performed multiple ELISA assays to identify molecules responsible for regenerative processes.

Results: Exposure of RPTECs or HK2 cells to cisplatin markedly reduced cell number and their viability, but co-culture with tubular ARPCs (TARPCs) provided a protective effect by promoting tubular cell proliferation of survival cells and inhibiting cisplatin-induced apoptosis. Tubular cell regeneration process was specific of TARPCs and occurred only following the sensing of a damage. On the contrary, when glomerular ARPCs were cocultured with damaged tubular cells, any effect of regeneration was observed. Surprisingly, regenerative effect was completely cancelled blocking the TLR-2, expressed by TARPCs. By bioinformatic analyses on microarray data and by multiplex cytokine assays, we identified some specific cytokines, growth factors and microvesicle-shuttled mRNAs, secreted by ARPCs and dependent from TLR2 activation, that worked synergistically and were essential in the regenerative process.

Conclusions: In conclusion, we identified for the first time a regenerative mechanism driven by the TLR2, by which ARPCs induced regeneration of tubular epithelial cells.

SA-PO2153


Background: Purumycin has been demonstrated to induce podocyte apoptosis. Mesenchymal stromal bone marrow cells (MS-BMCs) have been demonstrated to provide cytoprotection by the modulation of cytokine production in several cytotoxic models. In the present study, we evaluated the effect of MS-BMCs on cisplatin-induced podocyte injury.

Methods: MS-BMCs were harvested from bone marrows of mice and their profile was characterized (reported in another abstract). Mice in groups of six were administered either buffer (group A), purumycin alone (group B, 150 mg/kg, subcutaneously), intraperitoneal instillation of MS-BMCs in left kidney 24 hours prior to purumycin administration (group C, 150 mg/kg, subcutaneously). All mice were sacrificed on 5th day; urine and blood samples were collected for BUN and albumin: creatinine ratio. Kidneys were harvested for histology and TUNEL staining. Immunoblots were prepared and probed for nephrin and CD2AP expression. Immunohistochemical studies were carried out to study interstitial inflammatory milieu. In parallel sets of experiments, conditioned media of MS-BMC was collected. The effect of conditioned media of MS-BMC was evaluated on purumycin-induced podocyte apoptosis in in vitro studies.

Results: Group C mice displayed decreased (P<0.05) in albumin: creatinine ratio vs. Group B mice. Immunoblotting studies revealed decreased (P<0.01) nephrin and CD2AP expression in renal tissue from group B and right kidneys of group C vs. group A. On the other hand, renal tissues of the left kidneys from Group C displayed increased (P<0.01) expression of nephrin and CD2AP when compared to contralateral kidneys. Similarly, left kidneys from the group C displayed decreased number for TUNEL +ve glomerular cells when compared to the contralateral kidney of the same group and kidneys from group B. In vitro studies, conditioned media from MS-BMC provided partial protection to podocytes from pro-apoptotic effect of purumycin.

Conclusions: These findings indicate that MS-BMCs provide protection from injurious effect of purumycin by modulating pro-apoptotic milieu.

Funding: NIDDK Support

SA-PO2154

Therapy of Acute Kidney Injury in Pigs with Porcine Mesenchymal Stromal Cells Is Ineffective Because They Possess Inadequate Immune-Modulating Activity Anna Gooch,1 barrel Brunswig-Spickeneier,1 Janna Boche,2 Frauke Peimann,2 Achim Gruber,1 Kai Jaquet,1 Korf Krause,1 Jozef Zustin,1 Axel Zander,1 Claudia Lange,1 Christof Westenfelder,2,3 Medicine/Nephrology, University of Utah and VA Medical Centers, Salt Lake City, UT; 2Clinic for Stem Cell Transplantation, University Medical Center Hamburg-Eppendorf, Hamburg, Germany; 3Physiology, University of Utah and VA Medical Centers, Salt Lake City, UT.

Background: We demonstrated that allogeneic mesenchymal stromal cells (MSC) are highly renoprotective in rats with AKI, and promising in a Phase I Trial (Nature Rev Nephrol 2010), and this without eliciting an antibody response. We showed earlier that human MS-C in rats with AKI afford significant renoprotection, while followed by an antibody response in human mice. In the present study, we evaluated the effect of MS-BMCs on AKI in the pig model of human AKI.

Methods: We set up the hypothesis that the full expression of the immune-modulating/anti-inflammatory activities of MSC is critical for their protective action in experimental AKI. Groups of female pigs with bilateral ischemia/reperfusion AKI were engrafted with autologous or male allogeneic pMSCs.

Results: Strikingly, MSC therapy had no beneficial effects on kidney function and histopathology.

Conclusions: Summary: In contrast to allogeneic or xenogeneic rodent models (human and pig), engraftment of pMSCs into pigs with AKI was not kidney-protective. This therapeutic ineffectiveness is due to the inadequate immune-modulating activity of pMSCs, clearly demonstrating that effective therapy of AKI with MSCs depends critically on both their anti-inflammatory and trophic actions, rendering the pig model of human AKI a unique model for human therapy. However, we expect, that treatment of human AKI with immune-modulating, allogeneic MSCs will be as effective as in rodent models.

Funding: Veterans Administration Support, Private Foundation Support

SA-PO2155

Bone Marrow-Derived Mesenchymal Stem Cells (BM-MSC) Provide Partial Protection Against Kidney Injury Kang Cheng,1 Kuldeep K. Bhargava,2 Christopher J. Palestro,1 Ashwani Malhotra,3 Sanjeev Sanjeev Gupta,3 Pravin C. Singhal.1,2 Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; 2Nuclear Medicine, North Shore LIJ Hofstra Medical School, New Hyde Park, NY; 3Medicine, Albert Einstein College of Medicine, Bronx, NY.

Background: The immunomodulatory potential of BM-MSC is of much interest for cell-based therapies in multiple disorders, including acute kidney injury (AKI). To define the therapeutic potential of BM-MSC in cisplatin-induced AKI, we studied syngeneic mice.

Methods: Primary mouse MSC were isolated from compact bone of healthy syngeneic C57BL/6-GFP donors. To determine biodistributions of transplanted MSC, we injected intravenously labeled MSC i.v. into healthy and cisplatin (12.7mg/kg)-treated mice. Since only limited numbers transplanted MSCs were localized in the kidneys, we examined whether MSC served roles through paracrine signaling. The conditioned medium from BM-MSC was probed by high density protein arrays for 97 cytokines.

Results: Cellularity of cellular phenotype was verified by FACS for CD11b, CD14, CD45, CD68, CD90, CD105, and their differentiation into adipocytes or chondrocytes. Transplanted cells were localized by GFP immunostaining and DNA PCR. BM-MSC accumulated primarily in kidney, liver, and lung. BM-MSC was not observed in the kidneys, heart, spleen, and thymus. However, BM-MSCs failed to inhibit the mixed lymphocyte reaction (HLR-6) and antibody mixed lymphocyte reaction (HLR-6).

Conclusions: Targeting of BM-MSC via i.v. injection resulted in cells confined to the kidneys, which may induce nephrotoxicity characterized by an acute injury. We expect that treatment of human AKI with bone marrow-derived MSCs will be as effective as in rodent models.

Funding: NIDDK Support

SA-PO2156

The Bone Marrow-Derived Mesenchymal Stem Cells Repair the Acute Kidney Injury Induced by Acyclovir Joolena Santana Christo,1 Paulo Maciel Lopes,1 Waldemar S. Almeida,1 Luciana Aparecida Reis,1 Nestor Schor. Medicine, UNIFESP, Sao Paulo, Brazil.

Background: Acyclovir is an antiviral drug used to treat herpes simplex type 1 and 2 and varicella zoster. This drug is widely distributed in all body tissues, being very high in the kidneys, which may induce nephrotoxicity characterized by an acute injury. Some groups have reported the contribution BMSC in repair processes employing different animal models.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.
Methods: The BMSC were characterized for FACS analysis and then, differentiation into osteocytes and adipocytes. After, the cells were cultured and used for passages for all the experiment. The female Wistar rats received acyclovir (80mg/Kg/BW) (Acyclovir group) or water (CTL) in a dose intraperitoneally (N=10) during 5 days. After 48, 72 hours, the female rats received iv BMSC (1X10^6/cells). Then, blood and 24 hours blood was collected for urea (U), creatinine (Cr) evaluations. The animals were sacrificed and kidneys were perfused and removed for histology.

Results: It was observed that Cr increased in acyclovir group (1.7± 0.1 mg/dl) and U (174.5±0.2 mg/dl p<0.05) when compared to CTL (0.7±0.01 Cr and U 56.0±0.1 mg/dl p<0.05) and saline (0.9±0.01 Cr and U 49.0±4.3 mg/dl p<0.05) groups compared to the acyclovir group. The Acyclovir group, observed a decrease Cr and U serum after 48 hours (Cr 1.3±0.1 Cr vs Acyclovir+BMSC 0.9±0.2 and U 143.5±0.2 Cr vs Acyclovir+BMSC 89.2±0.2 P<0.05), 72 hours (Cr 1.7±0.1 Acyclovir vs Acyclovir+BMSC 1.0±0.1 and U 174.5±0.2 Cr vs Acyclovir+BMSC 112.0±2.1 mg/dl P<0.05) post injections compared to acyclovir group. After 5 days of treatment with acyclovir was observed glomerular congestion, cell damage, tubulitis and pyknatic nuclei. The acyclovir+BMSC after 48 and 72 hours, histological analysis was observed glomerulos and tubule changed less with dilation of the lumen and less pyknotic.

Conclusions: These results strongly suggest that BMSC minimize AKI induced by acyclovir. This protocol can be a potential tool for treatment of this disease with impressive high morbidity- mortality.

SA-PO2157

Therapeutic Potential of Human Placenta-Derived Mesenchymal Stem Cells for Ischaemia/Reperfusion Acute Kidney Injury

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Young Lee
1

Background: Mesenchymal stem cells derived from both bone marrow and umbilical cord blood have been shown to be beneficial in animal models of acute kidney injury. Human umbilical cord mesenchymal stem cells (hPMSCs) are free of ethical concerns, easily accessible, abundant, and strongly immunosuppressive. Recently, transplantation of hPMSCs into rodents with lung or liver cirrhosis was shown to reduce disease activity.

The aim of this study was to evaluate the therapeutic potential of hPMSCs in rats subjected to ischemia/reperfusion (I/R).

Methods: Male Sprague-Dawley rats (200±10 g) were randomly assigned into three groups: hPMSC-treated (n=14), PBS-treated controls (n=14), and sham-operated (n=6). The treated groups underwent 45 min of ischemia by renal artery clamping and contralateral nephrectomy. hPMSCs (1 × 10^7 or 0 PBS control) were then intravenously injected into the rats subjected to ischemia. Serum creatinine (SCr) was measured 24, 48, and 72 hours after surgery, and renal tissue was obtained the first 2 days post-surgery.

Results: hPMSC treatment significantly improved renal function [48 hour SCr: 2.06±0.14 mg/dl (control) vs. 1.16±0.69 mg/dl (hPMSC-treated), p<0.05]. Histological analysis demonstrated a significant decrease in tubular casts and necrosis in hPMSC-treated rats [Day 2 cast scores: 3.44±0.02 (control) vs. 0.21±0.08 (hPMSC-treated); Day 2 necrosis scores: 5.67±0.01 (control) vs. 0.81±0.05 (hPMSC-treated), p<0.05]. In renal tubular epithelial cells, hPMSC treatment significantly increased the expression of PCNA (p<0.05) and significantly reduced apoptosis (p<0.05). Caspase-3 levels were significantly lower (p<0.01) and Bcl-2 levels were significantly higher (p<0.01) in the hPMSC-treated group compared to the controls.

Conclusions: These results suggest the use of hPMSCs as a novel renoprotective therapy for patients who develop ischemic renal injury.

SA-PO2158

Effect of Erythropoietin on Mesenchymal Stem Cells Proliferation In Vitro under Acute Kidney Injury Microenvironment and Its Mechanism

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Background: To investigate the effect of erythropoietin (EPO) on mesenchymal stem cells(MSCs) proliferation under acute kidney injury (AKI) microenvironment, and to study its possible mechanism.

Methods: C57BL/6 mice's mMSCs had been successfully isolated by Percoll density gradient centrifugation for 1d. Male Sprague-Dawley rats (200±10 g) were randomly assigned into three groups: Group A-low glucose DMEM medium with 10% fetal bovine serum; Group B-low glucose DMEM medium with 10% fetal bovine serum plus 1/8R kidney homogenate supernatant; Group C-low glucose DMEM medium with 10% fetal bovine serum plus 1/8R kidney homogenate supernatant and different concentrations of EPO(1, 5, 10,50U/ml). After treatment for 24h, expression of Ki67 and pSTAT-5 was detected by CCK-8; apoptosis was detected by TUNEL. The protein expression of erythropoietin receptor (EPOR) and the proteins of proliferation/apoptosis related signal pathway were examined by Western blot.

Results: Under 1/8R kidney homogenate supernatant, the proliferation ability of mMSCs decreased significantly, while the apoptotic percentage was significantly higher than Group A. After intervention of EPO, their proliferation enhanced, at the same time, the apoptotic percentage decreased, present dose-dependent manner (P<0.05 or P<0.01). P3-EPO was positive for EPOR. EPO decreased the expression of Caspase-3 in mice under AKI microenvironment in a dose and time dependent manner, but it increased the expression of Bcl-2. The expression of phosphor-Janus kinase2 (pJAK2) and phosphor- signal transducer and activator of transcription (pSTAT-5) was significantly higher in 10 IU/ml EPO cultured 5d.

Conclusions: Erythropoietin can promote proliferation of mMSCs in vitro under AKI microenvironment, which is mediated by EPOR, and related with proliferation/apoptosis signal pathway. Funding: Government Support - Non-U.S.

SA-PO2159

The Effect of Bone Marrow Mesenchymal Stem Cells (BMSC) or Conditioned Medium (CM) in Rats with Acute Kidney Injury (AKI) Induced by Lipopolysaccharide (LPS)

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Background: Sepsis is characterized by a severe inflammatory response to infection, and its complications, including AKI, can be fatal. BMSC can act on several levels of endogenous repair to bring about resolution of diseases and its mechanism of action may be due to paracrine modulation and conditioned medium. The aim of this study is to evaluate the BMSC or CM effect at cellular modulation and/or repair on LPS in rats.

Methods: BMSC were collected from male Wistar rats, characterized by FACS and differentiated into osteocytes and adipocytes. Female Wistar rats received LPS (10mg/Kg/BW) (LPS group) or water (CTL) in a single dose (i.v. N=10). After 24 or 72 hr, the female rats received i.v. BMSC (1X10^6/cells) injection or CM (500µl), 1 or 3 doses via the tail vein. Blood and urine 24 hours were collected to creatinine (Cr), urea (U) and Fe4+ evaluations. The kidneys were perfused and removed for HE, Ki67, caspase 3 and Y chromosome analysis.

Results: In LPS-group the kidneys showed a small marked Ki67 and intensive caspase 3 expression but it was highly marked for Ki67 and lower expression for caspase 3 in LPS+BMSC and LPS+CM groups. However, a striking difference was observed in the BMSC or CM treated animals where the presence of Ki67 and Y chromosome was detected and no histological ATN lesions were observed. This effect was maximized when the doses of BMSC or CM were higher.

Conclusions: These results strongly suggest that BMSC or CM can minimize AKI in this sepsis model. This therapeutic effects have a significant impact on renal function observed holds substantial promise for its use in this pathological situation with high morbidity and mortality. Funding: Government Support - Non-U.S.

SA-PO2160

Angiopoetin-2 – A Dose Dependent Modulator of Syngeneic Marine EPCs in Acute Ischemic Kidney Injury

Joelma Schor 1, Nestor Schor, 2

Background: Exogenously administered endothelial progenitor cells (EPCs) can protect the kidney from acute ischemic injury (iAKI). The proteins Angiopoietin-1 and -2 play essential roles in regulating vascular regeneration and function. Aim of the study was to analyze to possible effects of Ang-2 on EPCs on an EPC-based therapeutic regimen of iAKI.

Methods: EPCs were isolated from male C57BL/6N mice and, after 5-7 days of culturing, incubated with Ang-2 at different concentrations for one hour, respectively. Pretreated, dye-labelled cells were systemically injected into recipient animals after bilateral renal ischemia of 40 minutes. Two days later, renal function and histology were investigated. In vitro studies with cultured EPCs were performed in order to analyze the cells' migratory activity under the influence of Ang-2.

Results: Systemic injection of 0.5 × 10^6 untreated EPCs did not prevent mice from acute renal failure. The consequences of cell pretreatment with Ang-2 were dose-dependent: at 200 ng/ml renal function remained unaffected. At 400 ng/ml, renoprotective effects of EPCs were significantly stimulated, indicated by lower postischemic serum creatinine levels. Incubating the cells with Ang-2 at 800 ng/ml dramatically reduced renoprotective effects of the cells, renal outcome was the worst of all groups. Ang-2 did not have any influence on the migratory activity of cultured EPCs.

Conclusions: Ang-2 acts as dose-dependent modulator of syngeneic murine EPCs in iAKI. Renoprotective effects of the cells can either be stimulated or reduced. Although the exact mechanisms responsible for these dihedral actions remain speculative at the moment, Ang-2 at least does not modulate EPC migration.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

Poster/Saturday
SA-P02161

A Phase I Trial of Human Allogeneic Mesenchymal Stem Cells for the Prevention of Acute Kidney Injury in Cardiac Surgery Subjects

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Background: Acute kidney injury (AKI) is common and has no effective treatment. Pre-clinical studies have shown that mesenchymal stem cells protect kidney function and stimulate repair. AllocCore has developed a proprietary process to isolate and expand allogeneic human MSC (AC607).

Methods: In this dose escalation phase I study, 16 subjects undergoing on-pump CABG and/or valve surgery who were at high risk for developing AKI were treated with 3 doses of AC607 [7 x 10^6 (n=6), 2 x 10^6 (n=5), and 7 x 10^6 (n=5) cells].

Results: All subjects were Caucasian, mean age and weight (± SD) were 72.1 yrs (±7.8), and 92.1 Kg (±15.9), respectively. Fifteen subjects had CKD (II-III), were hypertensive, and 6 had diabetes. The mean cardiopulmonary bypass time was 135.7 min (±5.5). The primary endpoint for the study was safety. None of the study subjects developed adverse or serious adverse events attributable to AC607 therapy, at any dose delivered. To enable a preliminary assessment of efficacy, study subjects were matched to historical controls (n=64). Overall, 12.5% of AC607 subjects developed AKI (AKIN criteria), compared to 29.7% of historical controls (p=0.214). Additionally, hospital length of stay was reduced in the AC607 group [6.5 days (±3.1) vs. 9.3 (±5.4), p=0.049]. Hospital readmission rates were 6.3% vs. 12.5% for AC607 subjects and historical controls respectively (p=0.079). One subject died within 30 days of surgery in the treated group (unrelated to AC607) vs. 2 of the historical controls. No subjects in either group received dialysis.

Conclusions: In this study, AC607 was safe and well tolerated. Furthermore, this study provides initial evidence that AC607 may be effective for the management of AKI in this population. Phase II studies are under development.

Funding: Pharmaceutical Company Support

SA-P02162

I KappaB Kinase Alpha Promotes Repair after Ischemic Acute Kidney Injury

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Background: Understanding the mechanisms of repair and regeneration of the kidney after injury is of great interest because there currently are few therapies that promote repair, and kidneys frequently do not repair adequately. We studied the capacity of I kappaB kinase alpha (IKKa) on the recovery phase to promote kidney repair and regeneration using an established ischemia/reperfusion injury model in mice.

Methods: Renal ischemia/reperfusion injury was induced by clamping bilateral renal artery for 25 min in C57BL/6j mice (wild type), IKKa−/− mice (tubule epithelial cells-specific IKKa knockout mice), and IKKa+ mice (KAP-IKKα transgenic mice). The reperfusion time was 1, 2, 4, 7, and 30 days respectively. Blood was harvested for biochemistry; and kidney tissues for histopathological examination, and for study of renal expression of IKKa, intercellar adhesion molecule-1 (ICAM-1), interleukin-10 (IL-10), and IL-23 receptors. Biochemistry; and kidney tissues for histopathological examination, and for study of renal expression of IKKa, intercellar adhesion molecule-1 (ICAM-1), interleukin-10 (IL-10), and IL-23 receptors.

Results: Postischemic kidneys of IKKa+ mice expressed higher IKKa compared with wild-type and IKKa−/− mice, associated with enhanced repair of the tubule epithelial cells, and enhanced functional recovery.

Conclusions: Our data confirms that IKKa promotes kidney repair and regeneration during the healing process after ischemia/reperfusion injury, likely through modulation of inflammatory cytokine and increasing tubule epithelial cell proliferation.

Funding: Government Support - Non-U.S.

SA-P02163

A Model of Tubular Regeneration after Surgical Injury in Adult Rat Kidney

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Background: Wound repair after surgical injury is used to investigate mechanisms of tissue regeneration. The mechanisms of surgical wound regeneration in the adult kidney have not been explored.

Methods: To address this issue we subjected adult rats to uninephrectomy and surgical polectomy of the contralateral kidney. We enclosed the remnant kidney in an inert plastic pouch to prevent adhesions. Control rats underwent uninephrectomy and the uninjured contralateral kidney was placed in a pouch. We examined serial kidney sections by immunohistochemistry at 1, 2 and 4 weeks after surgery.

Results: As compared to controls, tubular cells of the wounded kidney edge lost terminal differentiation markers (Phaseolus vulgaris hemagglutinin and aquaporin2) and acquired markers of both mesenchymal transition (vimentin and FSP1) and embryonic reprogramming (cadherin6, NCAM and Pax2). Similar markers, suggestive of cell dedifferentiation, have been described in tubular epithelium after acute tubular necrosis (ATN). However, as opposed to classical ATN models, we observed new growth of injured tubules. Tubular epithelial outgrowths (TEOs) extended from the wound edge into adjacent granuloma tissue and formed branching tubular structures. TEOs were abundant in markers of proliferation (PCNA, Ki67 and phosphohistone H3), and were positive for markers of epithelial differentiation (p-cadherin and E-cadherin). They lacked aquaporin2 and vimentin expression thus distinguishing them from normal adult collecting ducts as well as injured tubules. Notably, TEOs strongly expressed developmental markers Pax2 and Dolichos biflorus agglutinin. Overall their branching and staining pattern resembled that seen in primitive branching epithelium of ureteric bud. The morphologic connection between TEOs and wounded tubules suggested that TEOs were derived from injured dedifferentiated tubulointerstitial cells.

Conclusions: In conclusion, these results suggest that the adult rat kidney has the potential for de novo tubulogenesis. Our model provides a simple and reproducible tool for exploring this potential.

Funding: Government Support - Non-U.S.

SA-P02164

Role of the BMP-7/Gremlin Pathway in the Tubular Epithelial-Mesenchymal Transition Induced by TGF-β1

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Background: TGF-β1 is a potent inducer of the epithelial-mesenchymal transition (EMT). Tubular cells undergoing to EMT, invade the interstitium and synthesize interstitial matrix contributing to the tubulointerstitial fibrosis. Bone morphogenetic protein-7 (BMP-7) plays a critical role in the reparative processing of the damaged tubular cells by an inhibiting EMT. Gremlin is an endogenous antagonist of BMP-7. Our objective was to evaluate the role of BMP-7/Gremlin pathway in the induction and reversion of EMT induced by TGF-β1 in vitro by stimulating immortalized human proximal tubular cells (HK-2) with TGF-β1.

Methods: HK-2 cells were treated with TGF-β1 (3ng/ml) for up to 72 hours. The role of BMP-7/gremlin pathway was analyzed by Gremlin mRNA silencing technique (siRNA). The expression of EMT markers (α-SMA, E-cadherin and FSP1), fibronectin, collagen, TGF-β1, BMP-7 and gremlin expression was analyzed by real time PCR and western blot.

Results: TGF-β1 induced EMT was evidenced by upregulation in α-SMA and fibronectin expressions with a decrease in E-cadherin. Gremlin levels were increased in TGF-β1-stimulated cells and Gremlin siRNA was able to prevent the increase in the EMT markers. Using FSP1 and α-SMA antibodies to contrast the addition of BMP-7 to the culture medium had no effect on the expression of these markers. Results suggest that BMP-7 was unable to prevent cells to undergo EMT and Gremlin may have direct effect contributing to TGF-β1 inducing EMT in HK-2 cells.

Conclusions: In conclusion BMP-7 suppression may be more efficient than BMP-7 treatment to prevent cells to undergo EMT induced by TGF-β1.

Funding: Government Support - Non-U.S.

SA-P02165

Important Repair/Protective Process of Papillae in Renal Function and Morphology Demonstrated by Use of 14 Days-UUO-Released Model and Small Synthetic Compounds in Rats

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Background: Using 14 days-UUO released model established in rats and small molecular weight glycammactetonic compounds (61376 or 8X0075), the important repair or protective process of papillae in renal morphology associated with renal function and involved in activation of protein molecules of βHAT, E-cadherin and galectin-3 after ureteral obstruction were presented.

Methods: In UUO release model, complete ureteral obstruction was released by ureteroureterostomy and contra lateral nephrectomy (CNx) was done. After release of 14-days UUO with CNx in control animals (n=16), 8 weeks old male SD rats, plasma creatinine (pCr) level was recovered from 4.8± 0.6 mg/dl on day 2 to 2.2±0.1 mg/dl on day 6. Treatment of 13736 at the dose of 30mg/kg/day s.c. throughout 21 days of period ameliorated renal recovery to a better pCr level to 1.45± 0.25 mg/dl.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

617A
Urinary Thioredoxin 1 Is an Oxidative Stress-Specific Biomarker of Acute Kidney Injury

**SA-PO2166**

**NF-E2 Related Factor-2 (Nrf-2) Signaling Promotes Survival and Healing of Tubular Epithelial Cells**

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**Background:** Oxidative stress is a clinically relevant trigger for tubular cell death and insufficient tubular repair both commonly leading to tubular atrophy and renal failure. The transcription factor Nrf-2 induces a number of genes that counterbalance oxidative stress in injured cells. Hence, we hypothesized that Nrf-2 activation can promote tubular epithelial cell survival and repair.

**Methods:** We assessed the contributions of tubular survival and healing: 1. Cell survival during cell isolation (oxidative stress): primary tubular epithelial cells were isolated from wild type C57BL6 mice at 2-3 weeks of age and then cultured in fresh-made hormone-conditioned medium. The cells were characterized by immunofluorescence microscopy, flow cytometry, and qPCR for the expression of tubular marker genes. 2. Regenerative capacity of the surviving cells: condition medium of the surviving cells was assessed every 24h for a period of 6 days by means of digital morphometry; and 3. Repair by scratch assay experiments: Cells were grown to complete confluence either with the Nrf-2 agonist Sulforaphane/DMSO or DMSO for 4 hours prior to artificial scratch wounding. Wound closure was analyzed by digital morphometry at different timepoints.

**Results:** Isolated cells in culture extensively expressed epithelial markers such as E-Cadherin and Cytokeratin 7 (immunostaining), and were positive for the tubular cell marker FXYD2, a specific ion channel protein. Sulforaphane increased tubular cell survival at 30 hours after treatment in a concentration-dependent manner. In vitro expansion as well as wound closure upon scratching monolayers were 30% faster upon Sulforaphane stimulation. By contrast, a cell cycle inhibitor (p53 agonist) lead to dramatic decrease of the regeneration capacity in our model.

**Conclusions:** Nrf-2 activation with Sulforaphane promotes renal epithelial healing in-vitro after ischemic or mechanical injury. Therefore, Nrf-2 seems to be a potential therapeutic target to increase healing and regenerative response after ischemic injury in the kidney in order to prevent tubular atrophy.

**Funding:** Government Support - Non-U.S.

**SA-PO2167**

**Urinary Thioredoxin 1 Is an Oxidative Stress-Specific Biomarker of Acute Kidney Injury**

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**Japanese Society for Nephrology**

**Background:** In our previous studies we showed that urine TRX1 was secreted from renal tubules injured by oxidative stress. TRX1 is the renal tubules injured by oxidative stress.

**Methods:** We analyzed urine TRX1 in patients with various kidney diseases and mice with renal ischemia/reperfusion injury (IRRAT), including some known (ITGB6, LCN2), but also novel kidney injury markers (SNORD11A, CCHCR1). We used a 10 ng/mg creatinine as the optimal cutoff value of 35.2 ng/mg creatinine. The area under the curve of urinary TRX1 was 0.90, and the sensitivity and specificity were 0.90 and 0.82, respectively, at the optimal cutoff value of 35.2 ng/mg creatinine. Immunostaining of TRX1 in patients with various kidney diseases and mice with renal ischemia/reperfusion injury (IRRAT) was 0.90, and the sensitivity and specificity were 0.90 and 0.82, respectively, at the optimal cutoff value of 35.2 ng/mg creatinine. Using the ROC curve analysis to differentiate between AKI and other renal diseases, the area under the ROC curve was 0.90.

**Conclusions:** Immunostaining of TRX1 in patients with various kidney diseases and mice with renal ischemia/reperfusion injury (IRRAT) was 0.90, and the sensitivity and specificity were 0.90 and 0.82, respectively, at the optimal cutoff value of 35.2 ng/mg creatinine. Using the ROC curve analysis to differentiate between AKI and other renal diseases, the area under the ROC curve was 0.90.

**Funding:** Government Support - Non-U.S.

**SA-PO2168**

**Identification and Validation of microRNAs as New Biomarkers of Acute Renal Failure**

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**Background:** microRNAs are small non-coding RNAs which regulate gene expression. They are crucial regulators of cell responses to external stimuli such as ischemia/reperfusion and nephrotropic compounds. Recently, it has been demonstrated that microRNAs could be detected in body fluids, including urine, where they have revealed a strong stability. Moreover, changes in microRNA profiles in serum have been associated with several pathologies. All these features join to the non-invasive extraction method to obtain serum, which has unveiled microRNAs as potential biomarkers of acute kidney injury (AKI).

The aim of this work is to identify and validate microRNAs as potential AKI biomarkers in serum from acute renal failure patients.

**Methods:** RNA was extracted from ARF patient serum using an optimized protocol for these samples. Firstly, we have performed a massive screening experiment using TDLA platform in serum samples from ischemic, toxic and septic ARF patients. A pool of healthy volunteers was used as control. Next, the expression of selected miRNAs was confirmed by qRT-PCR in a larger ARF patient cohort with different aetiologies. Samples from Day 0 (diagnosis) to day 7 were used for miRNA detection and serum creatinine levels were estimated in all the analyzed samples.

**Results:** TDLA analysis revealed several altered miRNAs in ARF patients vs controls. We have chosen miR-101-1, miR-127-3p and miR-210 for further confirmation because all these miRNAs were significantly downregulated in ARF patients. miR-101-1 recovered healthy-like expression levels correlating with normalized creatinine values. Expression of miR-127-3p and miR-210 was restored in ischemic ARF patients who exhibited almost normal levels of creatinine.

**Conclusions:** In summary, microRNAs could be easily detected in serum and could be used for diagnosis and prognosis of ARF. In particular, miR-101-1, miR-127-3p and miR-210 could be considered as new biomarkers of AKI. Moreover, miR-127-3p and miR-210 appeared as specific markers of ischemic ARF outcome.

**Funding:** Government Support - Non-U.S.

**SA-PO2169**

**The Molecular Phenotype of Acute Kidney Injury Is Also Found in Progressing Chronic Kidney Disease: A Human Kidney Transplant Study**

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**Background:** Because of frequent biopsies and follow-up, early kidney transplants without rejection provide a unique opportunity to study molecular features of human acute kidney injury (AKI) that is a major impediment to transplant function.

**Methods:** We performed microarrays on early transplant biopsies with AKI and compared them to protocol biopsies of stable kidney transplants. Our aim was to tease out the molecular component of AKI as opposed to injury of transplantation procedure alone.

**Results:** Transplants with AKI expressed transcripts reflecting the injury-repair response (IRRAT), including some known (ITGB6, LCN2), but also novel kidney injury molecules e.g. OLFM4, SERPINA3. Many represented tissue remodeling, reminiscent of wound repair and cancer e.g. VCAN, CADH6. In transplants with AKI, IRRAT expression correlated with depression of function (r= -0.77), need for dialysis, and future recovery of function (r=0.62), whereas histologic features claimed to reflect tubular injury showed no correlations with these features.

**Conclusions:** Transplantation can be a powerful model to study acute kidney injury, especially in the early post-transplant period. Further work is needed to determine if these findings are also seen in non-transplant-related AKI.

**Funding:** Government Support - Non-U.S.
IRRAT were also expressed in rejecting kidneys, indicating that rejection triggers injury-reporting activity. IRRAT was selected in acute kidney injury in transplanted chronic kidney diseases (glomerulonephritis, antibody-mediated rejection) and correlated with progression to failure and with the previously published molecular risk score.

Conclusions: Transcripts strongly induced in AKI are also induced in progressive chronic kidney diseases. Thus both AKI and chronic progressive kidney diseases induce the same signal, analogous to wound repair. The extent of this response correlates with the extent of dysfunction and future recovery in pure acute kidney injury, but indicates serious ongoing nephron damage in progressive chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2170 Kidney Injury Biomarkers Kim-1, NGAL, Clusterin, and Cystatin C in Mouse Models of Ischemia Reperfusion Injury Venkata Sabbisetti, Chang Wang, Kazumi Ito, Joseph V. Bonventre. Renal Division, Department of Medicine, Brigham and Women's Hospital, Boston, MA.

Background: The application of kidney biomarkers to mouse models of kidney injury and nephrotoxicity will enhance our understanding of pathophysiology and facilitate the development of safe drugs. Kim-1, NGAL, clusterin, and cystatin C are capable of detecting kidney toxicity at early stages. However, the lack of reliable assays and urine volume requirements have hampered the utilization of these markers in preclinical studies. The goal of the current study was to establish a high-throughput multiplex bead based assay to measure these biomarkers, characterize their kinetics in urine and serum, and validate them vs histopathology after ischemia/reperfusion (IR).

Methods: BALB/C mice were subjected to IR injury for 10, 20, & 30 min and urine and serum specimens were collected for up to 14 days. For Kim-1, NGAL, clusterin, & cystatin C using mouse bead-based ELISA assays which we developed. Serum creatinine, BUN, NAG and total protein were measured spectrophotometrically.

Results: Microarray-based ELISA assays were developed that can measure the four biomarkers in duplicate in 10 µl of biological sample. The lower detection limits for Kim-1, NGAL, clusterin & cystatin C assays are 12, 10, 290 & 10 pg/ml with dynamic ranges of 0.01-50, 0.01-50, 0.29-12,000, 0.01-50 ng/ml respectively. In mice exposed to 10 and 20 min IR injury serum creatinine levels did not change, but there was a robust ischemic time-dependent increase in each of the novel markers and histopathology reflecting tubular injury. With 10 min IR injury, NAG & total protein levels returned to baseline within 12 hours, while Kim-1, NGAL and cystatin C levels remained elevated for 5 days after the injury. Similarly, in mice subjected to 30 min IR injury, NAG and total protein levels returned to baseline within 48 hours after injury, while Kim-1 & NGAL levels remained elevated for the entire duration of the study.

Conclusions: An assay panel for mouse urinary and serum biomarkers has been developed and validated which can measure four biomarkers in duplicate in very small sample volumes. This will greatly facilitate kidney injury studies in the mouse.

Funding: NIDDK Support

SA-PO2172 Sepsis-Induced Glomerular Endothelial Dysfunction Mediates Reductions in GFR and Increases in Protein Filtration Ruben M. Sandoval,1 George Rhodes,2 Exing Wang,3 Silvia B. Campos-Bilderback,1 Sarah E. Wean,1 Bruce A. Moltzior,1, 2 Medicine/Div. of Nephrology, Indiana Univ. School of Medicine, Indianapolis, IN; 1Roudebush VAMC, Indianapolis, IN.

Background: Sepsis is now the leading cause of acute kidney injury (AKI) known to decrease Glomerular filtration rate (GFR) and increase proteinuria. There also exists a discrepancy between renal perfusion and GFR.

Methods: To evaluate the potential role of the glomerulus in the overall pathogenesis of these abnormalities, we studied surface glomeruli in 8-10 week old Munich Wistar Frönter rats using intravital 2-photon microscopy in a celiac ligation and puncture (CLP) model of sepsis to ask targeted questions and compare the metric of measured GFR to serum creatinine changes at 24 hours post CLP.

Results: Male rats undergoing CLP showed an increase in serum creatinine from 0.23 ± 0.06 mg/dl to 0.80 ± 0.17 (P<0.01) and a decrease in real time GFR from 0.69 ± 0.06 ml/min/100gm body wt to 0.34 ± 0.20 ml/min/100gm body wt (P<0.01). Histologic monitoring revealed normal and hyperdynamic cardiac status within the CLP group. Quantitative analysis of 15 glomeruli in three CLP septic rats revealed a reduction in red blood cell flow rates within capillary loops from 1,771 ± 467 to 576 ± 327 um/sec (P<0.01); an increase in WBC adherence to glomerular capillary endothelial cells from 0.42 ±0.33 to 7.25 ± 3.58 WBC’s/standardized glomerular volume (P<0.05) in CLP rats; and an increase in the glomerular sieving coefficient (GSC) of a 150KD dextran from 0.007 ± 0.003 to 0.097 ± 0.046 (P<0.05). Rouleaux formations were seen only in septic rats.

Conclusions: These data indicate glomerular endothelial-WBC interactions during sepsis, in part, explain the reduction in GFR and increased filtration of large molecular weight proteins. The results from real time GFR accurately detected the drop in renal function for this model of sepsis.

Funding: Other NIH Support - George M. O’Brien Center for Advanced Renal Microscopic Analysis, Veterans Administration Support

SA-PO2173 Chronic Kidney Disease Ameliorates Sepsis-Induced Acute Kidney Injury and Spleen Apoptosis Via Toll-Like Receptor 4 Signaling Takayuki Tsuji, Ana Carolina Souza, Xuexu Hu, Taro Horino, Peter S.T. Yuen, Robert A. Star. Renal Diagnostics and Therapeutics Unit, NIH/NIDDK, Bethesda, MD.

Background: Patients with chronic kidney disease (CKD) have increased risk of mortality and morbidity from sepsis and acute kidney injury (AKI), an effect mimicked in our mouse model of sepsis following CKD. To further study the mechanism of amplification by CKD, we studied Toll-like receptor 4 (TLR4), which plays a major role in innate inflammation after endotoxemia, and has been implicated in sepsis-AKI and in renal fibrosis.

Methods: We used C3H/HeJ (TLR4-) and C3H/HeOuJ (TLR4+) mice as matched controls. We performed sham (CKD) or 5/6 nephrectomy (5/6NX) to induce CKD, waited 8 weeks, then performed sham(sepsis) or cecal ligation and puncture (CLP). Outcomes were measured at 24h.

Results: In both TLR4+ and TLR4- mice, 5/6NX induced mild CKD at 8 weeks [BUN 42-52.5 mg/dl; serum creatinine (Scr) 0.265-3.300 mg/dl, histologic fibrosis], although unlike humans, no creatinine elevation (Scr) occurred. Rats that were sham for 14 days was significantly higher in TLR4+ than TLR4- mice (156.1 vs 34.5 ug/mg Cr, p<0.05). CKD intensified sepsis-AKI, as Scr, BUN, and tubule damage score (TDS) were significantly higher after CKD-sepsis vs sham(CKD)-sepsis in TLR4+ mice (p<0.05). However in TLR4- mice, Scr (p>0.05), BUN (p<0.05), and TDS (p<0.05) increases were blunted (CKD-sepsis vs sham(CKD)-sepsis). Spleen apoptosis (by active caspase 3) was significantly increased in CKD-sepsis vs sham(CKD)-sepsis (p<0.001) in TLR4+ but not in TLR4- mice. Interestingly, sepsis increased TNF-α and HMGB1, but pre-existing CKD had no further effect in either TLR4+ or TLR4- mice.

Conclusions: Much of the CKD-driven amplification of sepsis-AKI in TLR4-dependent in this milder version of the mouse acute-on-chronic kidney disease model. Sepsis-induced spleen apoptosis was greatly amplified by CKD, which was TLR4-dependent. The amplification occurred without changes in TNF-α and HMGB1 (classic early and late inflammatory mediators). Therefore spleen apoptosis is driven by TLR4 signaling, and may represent an important amplifier in acute-on-chronic kidney disease.

Funding: NIDDK Support

SA-PO2174 Distinct Pathophysiology of Septic Acute Kidney Injury – Role of Immune Suppression and Apoptosis So-Young Lee,1 Sang-Kyung Jo,2 Won-Yong Cho,2 Hyoung-Kyu Kim.1 Medicine, Eulji university hospital, Seoul, Republic of Korea; 1Nephrology, Korea University Hospital, Seoul, Republic of Korea.

Background: Sepsis is the most common cause of acute kidney injury(AKI) in critically ill patients. However, the mechanisms leading to AKI in sepsis remain elusive. Although, sepsis is traditionally considered an excessive systemic inflammatory response, according to recent observations, sepsis induced organ dysfunction might be associated with paradoxial immune suppression. The purpose of this study was to examine the pathobiology of septic AKI focusing on immune suppression and apoptosis of kidney and immune cells by providing on-site comparison between septic vs ischemia/reperfusion(IR) induced AKI, a well known disease mediated by activation of innate immunity.

Methods: At 24 h after cecal ligation & puncture (CLP) or IR injury, biochemical, histologic kidney injury and cytokine profiles were compared. Apoptosis of immune cell

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Role of Renal Cell Apoptosis in Pathogenesis of Septic Acute Kidney Injury


Background: The presence of acute kidney injury (AKI) in septic patients is known to be associated with worse outcome. However, mechanisms of septic AKI remains elusive and several recent reports suggest that excess immune suppression characterized by massive lymphocytes apoptosis might be causally linked to sepsis mortality or sepsis induced organ dysfunction. In addition to immune cell apoptosis, renal cell apoptosis has also been demonstrated in animal models of sepsis or some autopsies. However, the exact role of renal cell apoptosis in the development of AKI in sepsis has never been assessed.

Methods: To evaluate the role of renal cell apoptosis in septic AKI, we used cecal ligation and puncture (CLP) method in mice.

Results: In C57/BL6 mice, plasma creatinine increased on day 1 and despite lack of overt tubular necrosis, significantly increased numbers of renal cell apoptosis were observed and kidney caspase 3 activity showed positive correlation with plasma creatinine. Although pretreatment with caspase 3 inhibitor markedly attenuated functional impairment, this renoprotective effect was accompanied by decreased apoptosis of both immune cells and renal cells. Therefore, to further dissect out the role of renal cell apoptosis independent of immune cell apoptosis, we performed CLP in mature T and B lymphocyte deficient RAG-1 deficient mice. Similar with WT mice, CLP also evoked mixed gram negative and positive immune cell apoptosis, we performed CLP in mature T and B lymphocyte deficient RAG-1 deficient mice. Similar with WT mice, CLP also evoked mixed gram negative and positive bacterial peptidoglycan induced sepsis with multiple organ dysfunction in RAG-1 deficient mice. Furthermore, in the absence of mature T and B lymphocytes, that are known to undergo apoptosis in sepsis, pretreatment with caspase 3 inhibitor still partially rescued RAG-1 deficient mice from development of AKI with significant reduction of plasma cytokines (IL-6, IL-10, TNF-α, MCP-1).

Conclusions: These results demonstrated the important role of renal cell apoptosis in the development of AKI and strategies that suppress renal cell apoptosis might be useful in preventing or treating AKI associated with sepsis.

Funding: Private Foundation Support

SA-PO2176

Lipopolysaccharide Causes Marked Changes in Tubular Zonula Occludens-1

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Background: Tight junctional (TJ) proteins maintain an integral role in tubular ion transport and waste excretion. Disruption of TJs decreases reabsorption and thereby increases tubule cell apoptosis and upregulated caspase 3 expression. Therefore, to evaluate the role of TJs in renal function and apoptosis, we cultured LLC-PK1 tubular cells exposed to lipopolysaccharide (LPS)

Methods: C57BL/6 mice were injected with LPS (10 mg/kg) and sacrificed 6, 24, and 48 h after injection. Kidney cortex was harvested and prepared for analysis by light microscopy, immunoblot, immunofluorescence, and RT-PCR for occludin, ZO-1, and claudin-1. AKI was confirmed via measurement of blood urea nitrogen and creatinine.

Results: Immunochemistry revealed significantly decreased ZO-1 expression by densitometry 24 h after LPS (decrease of 56.0 ± 4.6%, p = 0.009), with recovery at 48 h. However, ZO-1 mRNA was unchanged at 24 h, with a trend for increase at 48 h (increase of 68.0 ± 11.9% from baseline, p = 0.12). Immunofluorescence 24 h after LPS revealed a marked change in ZO-1 localization from a continuous perijunctional pattern to one with greater fragmentation, decreased basolateral staining, and greater apical distribution. Staining showed decreased tubular co-localization of occludin and ZO-1, Occludin and claudin-1 protein and RNA expression were not significantly altered at 24 h.

Conclusions: ZO-1 protein expression after LPS administration was markedly decreased at 24 h, with subsequent recovery at 48 h. Membrane localization of TJ proteins was disrupted by LPS. As RNA expression was unchanged, the results suggest disruption of renal tubular epithelial TJs in LPS-induced AKI is not mediated by transcriptional regulation but may involve degradation or protein trafficking. This study provides important evidence that LPS-induced AKI is associated with structural and not merely hemodynamic changes.

Funding: NIDDK Support

SA-PO2177

Dexamethasone Attenuates Septic Acute Kidney Injury by Reducing Apoptosis of Spleen Immune Cells and Renal Tubule Cells

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Background: Septis is the most common cause of acute kidney injury (AKI) in hospitalized patients and the clinical outcome is very poor, whereas our understanding of pathogenesis and treatment for septic AKI has remained limited. Low-dose glucocorticosteroids (GCs) has been clinically recommended in refractory septic shock patients through Surviving Sepsis Campaign, and in rat endotoxemia model, GCs were shown to ameliorate renal dysfunction. However, the mechanisms for the beneficial effects of GCs on septic shock and possibly on septic renal injury are still unclear. In this study, we were to investigate the pathophysiology of septic AKI and the effect of GCs using septic mice.

Methods: We induced AKI by polymicrobial sepsis using a CLP (celiac ligation and puncture) model in 8-10wk-old C57BL/6 mice. Saline or dexamethasone (DEX) dissolved in saline was administered right after CLP surgery. We examined hemodynamic, biochemical and histological changes in a time-course manner.

Results: Mean arterial Blood pressure (BP) significantly decreased starting at 3hr after CLP. Fractional shortening which estimate cardiac systolic function significantly increased and remained high until 24 hr, suggesting hyperdynamic “warm shock” state. Serum Creatinine started to increase after 12 hr. Many apoptotic cells were observed even at 3hr after CLP in spleen and mainly located in lymphoid tissue. Renal tubular apoptosis was also observed in renal cortex and outer medulla although acute tubular necrosis or infiltration of neutrophils and macrophages was not distinct.

We compared hemodynamics, renal function and apoptosis of tissues between CLP and CLP+DEX. BP, Heart rate and fractional shortening was not significantly different between CLP and CLP+DEX. However mice with CLP+DEX had a significant reduction in serum creatinine and decreased apoptosis of spleen and kidney compared to CLP.

Conclusions: DEX attenuates septic AKI, and the beneficial effect might be associated with reduced apoptosis of renal tubule cells and spleen immune cells.

SA-PO2178

Calpain 10 Loss Mediates Increased Susceptibility to Nephrotoxicity

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Background: Age and certain disease states such as diabetes predispose humans to AKI, however, few studies have examined the mechanism of increased sensitivity to AKI in these populations. Our laboratory has determined that calpain 10 protein and mRNA are down-regulated in aged kidneys from multiple species. In addition, recent studies showed that mitochondrial calpain 10 is decreased in a rat model of diabetes and renal calpain 10 mRNA is decreased in human diabetics. Furthermore, knock down of mitochondrial calpain 10 in renal proximal tubular cells (RPTC) alone disrupts mitochondrial functions such as accumulation of mitochondrial calpain 10 substrates, and decreased basal and uncoupled respiration. The goal of these experiments is to test the hypothesis that decreased calpain 10 sensitizes RPTC to toxicant injury.

Methods: We used adenovirus delivered shRNA to knock down calpain 10 in RPTC. Controls received adenovirus delivered scrambled shRNA. Two days later, RPTC were exposed to paraquat (100-300 µM), or HgCl2 (0.1-3 µM) for 24 h. Cell death was measured via propidium iodide staining. Additionally, RPTC were cultured in high (17 mM) glucose as a cellular model of diabetes for 48 hours. Control cells were cultured in normal (5 mM) glucose. RPTC were then exposed to 100 uM paraquat µM for 24 hours. ATP levels were measured by using an ATP Determination Kit (Invitrogen).

Results: RPTC treated with adenovirus delivered calpain 10 shRNA exhibited 62% and 86% increases in cell death with paraquat and HgCl2 respectively, compared to controls. RPTC cultured in 17 mM glucose media demonstrated a 75% greater reduction in ATP levels when exposed to paraquat compared to 5 mM glucose controls.

Conclusions: We hypothesize that the loss of renal calpain 10 in aging or diabetes leads to accumulation of mitochondrial calpain 10 substrates, causing mitochondrial dysfunction; thereby, sensitizes RPTC to a “second hit” such as 1/IR or drug/toxicant-induced injury that initiates AKI. Because calpain 10 is ubiquitously expressed, these findings may have broader implications in other tissues and disease states.

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SA-PO2179

STAT3 Activation Via Tyrosine Phosphorylation Is Cytoreductive during Cisplatin-Induced Acute Kidney Injury

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Background: The Stat3 transcription factor is a member of a family proteins that play a significant role in signal transduction from cytokine and chemokine receptors. We showed that Stat3 is activated during cisplatin-induced acute kidney injury at both serum
Histological analysis revealed vacuolation and increased L-FABP expression in proximal tubules of mice injected with indomethacin (I), L-NAME (L) and low-osmolar RC iohexol (3g Iodine/kg, R). Equivalent renal peritubular capillary (PTC) blood perfusion in RC-AKI.

Methods: We transduced proximal tubule cells with a DN-Stat3(tyr) construct and created a transgenic mouse strain in which the DN-Stat3(tyr) cDNA was conditionally expressed in proximal tubule cells under the control of the testosteroneresponsive-KAP promoter. Transduced cells were resistant to cisplatin-induced apoptosis. The transgenic mouse strain showed significant protection from cisplatin-induced AKI, as creatinine levels in transgenic mice were lower than in wildtype mice at 24 h after cisplatin (mean 9 vs 2.3 mg/dL (wt) to 0.84 ± 0.42 in transgenic mice (p<01)). Similarly, morphologic protection from cisplatin cytotoxicity was also conferred by transgene expression.

Conclusions: These studies identify the Stat3 protein as a significant target of both cisplatin and oxidant injury and mortality in this disease. Because our prior studies demonstrated that FLC generated intracellular oxidative stress, the present studies focused on the redox-sensitive mitogen-activated protein kinase kinase known as Apoptosis Signal-regulating Kinase 1 (ASK1).

Methods: The mechanism of cytotoxicity of monoclonal FLC was determined by incubating human proximal tubule epithelial cells (HK-2) in medium containing two human monoclonal FLC (term s2 and s3). Cytosolic caspase 3 and 9 activities were determined using ELISA. The percentage of apoptotic cells in each population of HK-2 cells was determined by flow cytometry using MitoTracker® Red and annexin V conjugated to Alexa Fluor 488. RNA interference was accomplished using small interfering RNA (siRNA) designed to reduce AKI expression in HK-2 cells successfully decreased AKI, which was confirmed by western blot analysis. Incubation of AKI-depleted HK-2 cells with the two FLC prevented the increase in apoptosis, while pre-treating HK-2 cell with non-targeting siRNA did not prevent FLC-mediated apoptosis.

Conclusions: Monoclonal FLC triggered the intrinsic apoptotic pathway in renal epithelial cells by activation of AKI. The AKI pathway provides a potential therapeutic target in renal failure in multiple myeloma.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2180

Essential Role for Apoptosis Signal-Regulating Kinase 1 in Free Light Chain-Mediated Apoptosis Wei-Zhong Ying, Pei-Xuan Wang, Paul W. Sanders, Medicine, University of Alabama at Birmingham, AL; Medicine, Birmingham Veterans Affairs Medical Center, Birmingham, AL.

Background: A major attendant complications of multiple myeloma is renal failure, which is related to deposition of monoclonal immunoglobulin light chain (FLC) and directly contributes to the morbidity and mortality in this disease. Because our prior studies demonstrated that FLC generated intracellular oxidative stress, the present studies focused on the redox-sensitive mitogen-activated protein kinase kinase known as Apoptosis Signal-regulating Kinase 1 (ASK1).

Methods: The mechanism of cytotoxicity of monoclonal FLC was determined by incubating human proximal tubule epithelial cells (HK-2) in medium containing two human monoclonal FLC (term s2 and s3). Cytosolic caspase 3 and 9 activities were determined using ELISA. The percentage of apoptotic cells in each population of HK-2 cells was determined by flow cytometry using MitoTracker® Red and annexin V conjugated to Alexa Fluor 488. RNA interference was accomplished using small interfering RNA (siRNA) designed to reduce AKI expression in HK-2 cells successfully decreased AKI, which was confirmed by western blot analysis. Incubation of AKI-depleted HK-2 cells with the two FLC prevented the increase in apoptosis, while pre-treating HK-2 cell with non-targeting siRNA did not prevent FLC-mediated apoptosis.

Conclusions: Monoclonal FLC triggered the intrinsic apoptotic pathway in renal epithelial cells by activation of AKI. The AKI pathway provides a potential therapeutic target in renal failure in multiple myeloma.

Funding: NIDDK Support, Veterans Administration Support

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compared to p66ShcA−/− mice-receiving cisplatin. Control mice-receiving cisplatin displayed inactivation of redox-sensitive stress response program; whereas, p66ShcA−/− mice receiving cisplatin showed adequate response of the stress response program.

**Conclusions:** These findings indicate that deletion of p66ShcA from renal cell genome provides protection against cisplatin induced acute renal failure.  

**Funding:** NIDDK Support

SA-PO2184

The Role of Mannose Receptor in Acute Folic Acid Nephritis  
Hua-Han Wang,1 Sally Hamour,2 Ruth J. Pepper,2 Mark Little,2 H. Terence Cook,2 Alan D. Salama.1 1Centre for Nephrology, University College London, United Kingdom; 2Renal Medicine, Imperial College London, United Kingdom.

**Background:** Folic acid (FA) induces acute tubular injury, characterised by tubular apoptosis increase, followed by both repair and scarring and is associated with macrophage infiltration. FA nephritis is therefore a good model of acute renal failure secondary to tubular damage. We have previously demonstrated requirement of mannose receptor (MR) for induction of immune mediated glomerulonephritis, and now we have evaluated the role of MR in acute kidney injury.

**Methods:** MR−/− mice and control WT C57BL6 mice were used at 8-12 weeks age. Folic acid 240mg/kg was injected intraperitoneally with 0.2ml NaHCO₃, as vehicle. Mice are sacrificed at 7 or 14 days after injection. We assessed renal function, histological damage (by acute tubular injury score) and degree of macrophage/cell infiltration (by staining for CD68, calprotectin). Adrenomedullin significantly prevented DVR constriction and increased DVR response (0.058±0.010, 0.009±0.004, 0.049±0.007, respectively, all p<0.05).

**Results:** At day 7 following injury, serum urea/creatinine were similar between WT and MR−/− mice; however, acute tubular injury score was higher in WT mice (46.1±1.4 vs 29.8±1.7; p=0.009). WT mice had greater activated macrophage infiltration than MR−/− assessed by CD68 staining (relative area 0.113±0.025 vs 0.061±0.006 p=0.04) and greater levels of calprotectin (0.016±0.006 vs 0.004±0.0009 p=0.0047) and predictably mannose receptor (0.099±0.012 vs 0.03±0.004 p=0.0007).

**Conclusions:** Mannose receptor insufficiency protects mice from acute macrophage mediated kidney damage and may be a novel non-immunosuppressive therapeutic target for acute kidney injury.

SA-PO2185

Adrenomedullin Has Protective Effects on Renal Descending Vasa Recta and Endothelial Cells Against Functional and Morphologic Impairment by Iodinated Contrast Media  
Mauricio Michalak Sendeski,1 Anja Bondke Persson,1 Pontus Persson,1 Anders Patzak.1 1Institut füer Vegetative Physiologie, Charite Universitaetsmedizin Berlin, Berlin, Germany; 2Uppsala University, Sweden.

**Background:** Contrast induced nephropathy (CIN), a complication of iodinated contrast media (CM), is a frequent cause of acute kidney injury. Renal medullary ischemic damage is a hallmark of CIN. CM causes functional impairment of isolated outer medullary descending vasa recta (DVR) and lower endothelial nitric oxide production. We investigated the effect of adrenomedullin—an endogenous peptide with endothelial protective effects—in the prevention of CM deleterious effects on DVR and endothelial cells cultured in interlobar arteries.

**Methods:** We studied isolated, perfused DVR and interlobar arteries from Sprague-Dawley rats. Iodixanol, a dimeric non-ionic CM, was applied intraluminally to DVR and interlobar arteries from Sprague-Dawley rats and lower endothelial nitric oxide production.

**Results:** Two weeks following injury, there was increased staining in the WT mice (0.199±0.016 for CD68, 0.031±0.002 for calprotectin and 0.119±0.015 for mannose receptor) compared to MR−/− animals (0.058±0.010, 0.009±0.004, 0.049±0.007, respectively), all p<0.05.

**Conclusions:** Mannose receptor insufficiency protects mice from acute macrophage mediated kidney damage and may be a novel non-immunosuppressive therapeutic target for acute kidney injury.

SA-PO2186

Bactericidal Antibiotics Temporarily Increase Inflammation and Worsen Acute Kidney Injury in Experimental Sepsis  
Anan Chuasawang,1 Zhiyong Peng,1 Hongzhong Wang,2 Nattachai Srisawat,1 Xiao-Yan Wen, Thomas Rimmele,1 Jeffery Bishop,1 Kai Singbartl,1 Raghavan Murugan,2 John A. Kellum,1 1Department of Critical Care Medicine, Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), University of Pittsburgh, Pittsburgh, PA; 2Beijing Cancer Hospital & Institute, Beijing, China.

**Background:** Acute kidney injury (AKI) is a common disorder in critically ill patients, and mortality reaches 70% when combined with sepsis. Bactericidal antibiotics release bacterial products including pathogen-associated molecular patterns (PAMPs) which could trigger inflammation and induce or worsen severity of AKI.

**Methods:** Sepsis was induced by cecal ligation and puncture (CLP) in fifty-two Sprague-Dawley rats and was treated with either bactericidal antibiotics (ampicillin/subactam) or placebo (saline). Serial blood samples were obtained from 18 hrs up to day 7 after CLP for serum creatinine, interleukin (IL)-6, and neutrophil gelatinase-associated lipocalin (NGAL) concentrations. RIFLE criteria were used to assess severity of AKI. All animals were observed for survival at one week. In a separate experiment, 12 animals were sacrificed 2 days after CLP for histology.

**Results:** Survival in placebo-treated animals was 50% compared to 82% with antibiotics (P<0.05). Most animals (93%) without antibiotics developed AKI, of which 39% exhibited greater than a 3-fold rise in serum creatinine (RIFLE-F). Furthermore, survival decreased as AKI severity increased. Surprisingly, all animals treated with antibiotics developed AKI, of which 68.6% reached RIFLE-F. However, renal dysfunction was less persistent in antibiotic-treated rats, as indicated by both serum creatinine and plasma NGAL concentration. Patterns of plasma IL-6 were similar to creatinine. Histological findings were consistent with functional parameters showing that antibiotics worsened AKI.

**Conclusions:** Bactericidal antibiotic therapy during sepsis improved survival but resulted in higher IL-6 concentrations and more severe AKI. Animals without antibiotics had less evidence of early inflammation and AKI but failed to recover. Mortality was associated with failure to recover from AKI.

**Funding:** Other NIH Support - National Institutes of Health (NHLBI) Systems Engineering of a Pheresis Intervention for Sepsis (SEPIS) HL080926

SA-PO2187

The Constant Region Contributes to the Antigenic Specificity and Potential Nephritogenicity of Anti-DNA Antibodies  
Chaim Puttermann, Yumin Xia. Albert Einstein College of Medicine.

**Background:** Certain IgG subclasses are particularly enriched in kidney Ig eluates in active lupus nephritis, suggesting that antibody (Ab) isotype is an important feature determining the outcome of Ab binding to renal antigens. Although the variable region was believed to be the sole determinant of antigenic specificity, more recent studies have shown that isotype switching leads to altered binding of a protective Ab. Our hypothesis was that Ab isotype may affect the renal pathogenicity of anti-DNA Ab, by influencing antigen binding.

**Methods:** PL-9-11 is an IgG3 anti-DNA Ab isolated from a MRL+/− mouse. To obtain IgG1, IgG2b and IgG2a forms of this Ab, the PL-9-11 hybridoma clone was isotype switched to produce IgG1, IgG2b and IgG2a variants. The PL-9-11 (Huc-M) variant was generated by cloning the PL-9-11 VDJ into an IgM expression vector, followed by transfection into a cell line expressing only the PL-9-11 light chain. The affinity/specificity of the PL-9-11 Ab panel were analyzed by ELISA, surface plasmon resonance, and cross-inhibition. Antigen specificity was studied by binding to mesangial cells, isolated glomeruli, and glomerular proteome arrays. Finally, renal deposition and pathogenicity were assessed by analyzing kidneys of SCID mice injected with the PL-9-11 Ab panel.

**Results:** We found that PL-9-11 and its isotype switched variants had different binding to sDNA, dsDNA, chromatin and mesangial cells in order of IgG3-IgG2a-IgG1-IgG2b-IgM. This order of relative affinities of the different IgG isotypes for dsDNA was confirmed with failure to recover from AKI.

**Conclusions:** Our data suggest that the constant region plays an important role in the affinity and specificity of anti-DNA Ab, and that IgG2a and IgG3 isotypes may be more nephritogenic due to higher potential for binding to multiple glomerular and nuclear antigens.

**Funding:** Other NIH Support - NIAMS

SA-PO2188

TWEAK/Fn14 Pathway Blockade Attenuates Renal Disease in Autoantibody-Induced Nephritis  
Chaim Puttermann,1 Jennifer Michaelson,2 Linda Burkly,2 Albert Einstein College of Medicine; 2Biogen Idex.

**Background:** TNF-superfamily members are instrumental in the pathogenesis of lupus nephritis. Previously, we found that TWEAK (TNFSF12)-mediated activation of its receptor, Fn14, stimulates the secretion of MCP-1, RANTES, IP-10 and KC by mesangial

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cells and podocytes. TWEAK also modulates renal cell survival and proliferation. Thus, we hypothesized that TWEAK blockade may be therapeutically beneficial in autoantibody-mediated nephritis.

Methods: Nephrotropic serum nephritis (NTN), a murine model for lupus nephritis, was used to study the role of the TWEAK/Fn14 pathway in the pathogenesis of renal disease induced by pathogenic antibodies.

Results: We induced NTN by passive transfer of pre-formed nephritogenic rabbit antibodies into 129 F14 knockout (KO) and wildtype (WT) mice that had been preimmunized with heterologous rabbit IgG. On days 7, 14, and 21 after antibody transfer, Fn14KO mice had significantly decreased levels of proteinuria as compared to Fn14 WT mice (day 7: 7.1±2.4 vs 22.0±42 mg/dl, p<0.01; day 14: 99±50 vs 678±205 mg/dl, p<0.02; day 21: 419±71 vs 678±205 mg/dl, p<0.02). Moreover, crescent formation and tubular dilatation were significantly decreased in Fn14KO mice, as were MCP-1, RANTES, and IP-10 kidney mRNA expression levels. To confirm the protective effect of TWEAK inhibition with a pharmacological approach, we induced nephrotropic nephritis in 129 F14 WT mice and initiated treatment with an anti-TWEAK mAb or isotype matched control Ig. Similar to results in Fn14KO mice, significant amelioration of proteinuria and titers was evident.

Conclusions: TWEAK/Fn14 interactions promote the pathogenesis of nephritis in the NTN model, likely playing a role in pathologic events locally in the kidney rather than impacting the systemic immune response. Thus, disrupting TWEAK/Fn14 interactions may be an innovative approach for the treatment of lupus and other antibody-induced renal diseases.

Funding: Other NIH Support - NIAIMS, Pharmaceutical Company Support

SA-PO2190

Activated Protein C Attenuates Systemic Lupus Erythematosus and Lupus Nephritis in MRL-Fas(lpr) Mice Julia Lichtnekt, Khader Valli Rupanagudi, Onkar Kulkarni, Hans J. Anders.

Background: Systemic lupus erythematosus (SLE) is a chronic autoimmune disease leading to inflammatory tissue damage in multiple organs, e.g. lupus nephritis. Current treatments including steroids, antimarialars, and immunosuppressive drugs have significant side-effects. Recombinant activated protein C (APC), a natural protein with anticoagulant and immunomodulatory effects and its recombinant version has been approved by the FDA to treat severe sepsis. Given the similarities between overwhelming immune activation in sepsis and autoimmunity, we hypothesized that recombinant APC would also suppress SLE and lupus nephritis.

Methods: Autoimmune female MRL-Fas(lpr) mice were treated with either vehicle or recombinant human APC from week 14 to 18 of age.

Results: APC treatment significantly suppressed lupus nephritis as evidenced by decreases in activity index, proteinuria and circulating antibodies. In MRL-Fas(lpr) mice, significant amelioration of proteinuria and lupus nephritis as compared to vehicle-treated MRL-Fas(lpr) mice. In addition, parameters of systemic autoimmunity, such as plasma cytokine levels of IL-12p40, IL-6, CCL2/MCP-1 and proteinuria of B cells and plasma cells in spleen were suppressed by APC. The latter was associated with lower total plasma IgM and IgG levels as well as lower titers of anti-dsDNA IgG and rheumatoid factor.

Conclusions: Together, recombinant APC suppresses the abnormal systemic immune activation in SLE of MRL-Fas(lpr) mice which prevents subsequent kidney, lung and skin disease. These results implicate that recombinant APC might be useful for the treatment of human SLE.

Funding: Private Foundation Support

SA-PO2191

Serum IgG Binding to Human Mesangial Cells in Patients with Lupus Nephritis and Its Correlation with Disease Activity

Susan Yung, Owen Chan, Qing Zhang, Daniel Tak Mao Chan. Department of Medicine, University of Hong Kong, China.

Background: Mesangial immunoglobulin deposition is a hallmark in lupus nephritis. We previously demonstrated that human anti-dsDNA antibodies could bind to human mesangial cells (HMC). In this study we investigated the binding activity of serum IgG to HMC and its correlation with clinical parameters in lupus patients.

Methods: Serial serum samples were obtained from 23 patients with biopsy-proven diffuse proliferative lupus nephritis over a mean follow-up of 74 months. Binding activity (analyzed by flowcytometry) of total serum IgG and its subclasses (IgG1, IgG2, IgG3, IgG4) to HMC was measured using a cellular ELISA and its correlation with clinical or laboratory parameters were investigated. Sera from 23 healthy individuals were used as controls.

Results: A total of 189 samples were collected - 48 samples during active and 141 during inactive disease, defined according to clinical assessment. Binding of serum total IgG to HMC was 0.12±0.09, 0.59±0.37 and 0.74±0.43 OD for healthy controls, inactive lupus, and active lupus respectively (P=0.023 active vs inactive, P<0.001 controls vs active or inactive disease). Binding of serum IgG1 to HMC was 0.05±0.05, 0.41±0.38 and 0.55±0.40 OD for the three groups respectively (P=0.037 active vs inactive, P<0.001 controls vs active or inactive disease). Controls and lupus patients did not differ in the binding of serum IgG2, IgG3, or IgG4 to HMC. HMC-binding activity of total IgG and IgG3 correlated with anti-dsDNA antibodies levels (r=0.26 and 0.39 respectively, P<0.001 for both), and inversely correlated with C3 levels (r=0.17 and 0.45 respectively, P<0.05 for both). No correlation was observed between IgG binding to HMC and clinical parameters such as serum creatinine, albumin, or proteinuria. Sensitivity/specificity of total IgG or IgG3 binding to HMC in the prediction of renal flare in the patients was 81.3%/39.7% (ROC AUC 0.61, P=0.03) and 83.3%/41.8% (AUC 0.63, P=0.009) respectively.

Conclusions: We conclude that total IgG and IgG3 in the serum of patients with lupus nephritis bind significantly to mesangial cells, especially during flare, and this binding correlated with the level of anti-dsDNA antibodies.

Funding: Government Support - Non-U.S.
Conclusions: We assessed the involvement of CD11b+ GR-1+ cells in autoimmune disorder in MRL-Fas− mice. These cells regulate immunological responses via CCL2/CCR2 signaling. The regulation of immunosuppressive monocytes may provide novel therapeutic strategy for organ damage in autoimmune diseases.

Funding: Government Support - Non-U.S.

SA-PO2193

Anti-Glomerular Basement Membrane Antibodies Against Certain Linear Epitopes on Goodpasture Antigen Are Associated with Clinical Phenotypes and Disease Severity

Zhao Cui, Xiao-Yu Jia, Rui Yang, Ming-Hui Zhao.

Renal Division, Department of Medicine, Peking University First Hospital, Beijing, China.

Background: Anti-glomerular basement membrane (GBM) disease could be initiated by nephrogenic linear epitopes which stimulate both T and B cell responses in experimental autoimmune glomerulonephritis. Linear epitopes involved in human-GBM disease have not been fully defined. In this study, we investigated the linear epitopes recognized by circulating antibodies in sera from patients with anti-GBM disease, aiming to identify the potential nephrogenic linear epitopes and their clinical and pathological significance.

Methods: 68 patients with anti-GBM disease were enrolled. A panel of 23 overlapping linear peptides was synthesized across the whole sequence of human α3(IV)NC1. Among them, P14 contained the previous known immunodominant regions E and a T-cell epitope of patients. Circulating antibodies against linear epitopes were detected by enzyme-linked immunosorbent assay. The associations between linear epitopes recognition and clinical and pathological data of patients were further analyzed.

Results: Antibodies against linear peptides were detected in sera from 55 (80.9%) patients. Three major epitopes were identified with high frequencies: P14 (amino acid residues 131-150) 41%, P16 (amino acid residues 161-180) 36.8% and P18 (amino acid residues 181-200) 57%. Antibodies against P14 were frequently detected in patients with positive ANCA (39.3% vs. 12.5%, P=0.01). Patients with antibodies against P16 presented with higher concentration of serum creatinine on diagnosis (665.5±22.7 vs. 443.7±296.8 μmol/L, P=0.001). The levels of antibodies against P18 were positively correlated with the percentage of crescentic formation in glomeruli (r=0.54, P=0.008).

Conclusions: Antibodies against certain linear epitopes could be detected in patients with anti-GBM disease and were associated with clinical phenotypes and disease activity. Antibodies against P14 were associated with coexistence of ANCA. Antibodies against P16 and P18 were associated with the severity of crescentic glomerulonephritis.

Funding: Government Support - Non-U.S.

SA-PO2194

Extensive Antigen Receptor Editing Modifies Autoimmunity in Anti-Glomerular Basement Membrane Disease

Inge Maria Schudel, Melissa L. Weston, Amy G. Clark, Mary H. Foster.

Medicine, Duke University Medical Center and DVMAC, Durham, NC.

Background: In anti-glomerular basement membrane disease autoantibodies against the NC1 domain of the α3 chain of type IV collagen can lead to autoimmune nephritis and severe kidney damage. To determine the origins of and tolerance mechanisms regulating the pathogenic autoantibodies, we developed an anti-α3/IV/NC1 IgM kappa autobody transgenic (Tg) mouse model. Initial analysis of immunoglobulins of both endogenous kappa alleles, such that any kappa light chain derives only from the restricted lambda chain usage.

Ongoing studies should shed light on the molecular basis for this unexpected requirement at the single cell level to modify expression of pathogenic anti-GBM autoantibodies.

Funding: NIDDK Support, Veterans Administration Support, Government Support - Non-U.S.

SA-PO2195

Modifiers of Susceptibility to Anti-Glomerular Basement Membrane Antibody Disease in Fc Gamma Receptor 2B Deficient Mice


1 Renal Division, Imperial College, London, United Kingdom; Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

Background: Anti-glomerular basement membrane antibody disease (anti-GBM disease) is an autoimmune disease, leading to crescentic glomerulonephritis. Fc gamma receptor 2B (FcgR2B) is the only inhibitory Fc receptor for IgG in humans and mice. The purpose of this study was to compare the susceptibility to anti-GBM disease of mice lacking FcgR2B on a pure C57BL/6 background (FcgR2B-B6), with mice lacking FcgR2B created in 129 embryonic stem cells and backcrossed to C57BL/6 (FcgR2B-129/B6), therefore having 129 sequences flanking the FcgR2B deletion. We then compared the susceptibility of mice with cre-mediated conditional deletion of FcgR2B on B cells and monocytes/macrophages to anti-GBM disease.

Methods: Anti-GBM disease was induced by subcutaneous immunization with a recombinant peptide of the NC1 domain of alpha 3 Type IV collagen at day 0, with boosts at day 7, 14 and 21. Mice were sacrificed 20 weeks after immunization.

Results: In two experiments, there was a higher incidence of crescentic glomerulonephritis in FcgR2B-129/B6 mice compared with FcgR2B-B6 mice (Experiment 1: 6/6 (75%) vs 2/8 (25%) vs WT (84%); (Experiment 2: 2/12 (66%) vs 4/12 (33%) vs WT 1/9 (11%). However, in those mice that developed disease, there was no difference in the severity of the crescentic glomerulonephritis.

In the second part of the experiments, 4/11 (37%) FcgR2B-B6 mice developed crescentic glomerulonephritis, compared to 2/9 FcgR2B-CD19cre mice (22%), 1/2 FcgR2B-LysM mice (8%) and 1/9 WT mice (11%).

Conclusions: The incidence of crescentic glomerulonephritis was higher in the FcgR2B-129/B6 mice than the FcgR2B-B6 mice, indicating that genetic modifiers located near to FcgR2B on the 129 genome increase the tendency of FcgR2B- mice to develop anti-GBM disease.

Secondly, FcgR2B-LysM mice had the same incidence of anti-GBM disease as WT mice. Our preliminary results indicate that loss of FcgR2B on B cells alone did not increase the incidence of anti-GBM disease as much as the full deletion.

Funding: Private Foundation Support

SA-PO2196

Pathogenesis of IgA Nephropathy: Characterization of the Kinetics and Site-Specificity of GalNAc-Transf erase 2, the Enzyme Initiating O-Glycosylation of IgA1

Kazuo Takahashi, Milada Horynova, Milan Raska, Stacy D. Hall, Bruce A. Julian, Zina Moldoveanu, Jiri F. Mestecy, Matthew B. Renfrow, Jan Novak.

1 University of Alabama at Birmingham, AL; 2 Palacky University in Olomouc, Olomouc, Czech Republic.

Background: IgA1 with galactose-deficient hinge-region (HR) O-glycans (Gal-IgA1) plays a key role in IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential O-glycosylation sites occupied; some Gal glycans consist of terminal N-acetylgalactosamine (GalNAc). As O-glycosylation of IgA1 is initiated by a GalNAc-transf erase, namely GalNAc-T2, understanding the kinetics and site-specificity of this enzyme will provide insight into the pathogenesis of IgAN.

Methods: We used recombinant GalNAc-T2 in an in vitro system to study kinetics of site-specific glycosylation using high-resolution mass spectrometry (MS). We used two synthetic HR peptides (sHR) as acceptors: VPSTTPTTPSTPSPTSPSCHPR (short sHR) and VPSTTPTTPSTPSPTSPSCHPR (long sHR).

Results: Time-course analysis showed that the number of added GalNAc residues increased with time. GalNAc-T2 showed higher activity, i.e., faster rate of glycosylation, with long sHR than with short sHR. The reaction reached plateau when seven sites were glycosylated in the long sHR and eight sites were glycosylated in short sHR. Thus, the sequence and length of acceptor substrate affect O-glycosylation, particularly the maximal number of GalNAc residues attached and the kinetics of the reaction. O-glycosylation were subjected to tandem MS to localize glycosylated sites. GalNAc-T2, understanding the kinetics and site-specificity of this enzyme will provide insight into the pathogenesis of IgAN.

Funding: NIDDK Support, Veterans Administration Support, Government Support - Non-U.S.

SA-PO2197

Antibody Disease in Fc Gamma Receptor 2B Deficient Mice


1 Renal Division, Imperial College, London, United Kingdom; Department of Human Genetics, Leiden University Medical Center, Leiden, Netherlands.

Background: IgA1 with galactose-deficient hinge-region (HR) O-glycans (Gal-IgA1) plays a key role in IgA nephropathy (IgAN). IgA1 HR has up to 6 of the 9 potential O-glycosylation sites occupied; some Gal glycans consist of terminal N-acetylgalactosamine (GalNAc). As O-glycosylation of IgA1 is initiated by a GalNAc-transf erase, namely GalNAc-T2, understanding the kinetics and site-specificity of this enzyme will provide insight into the pathogenesis of IgAN.

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Funding: NIDDK Support
Effect of gp130 Cytokines on IgA1-Producing Cells from Patients with IgA Nephropathy

Bruce A. Julian,1 Takahashi2

GalT1 and increased for GalNAc-T2. The expression of GalNAc-T14 has not changed but increased Gal deficiency of IgA1 only in IgAN cells. In IgAN cells, but not in HC but not OSMR2. Cytokines LIF, OSM, and IL-6 affected IgA1 production in all cells, determined by ELISA.

was measured by RealTime RT-PCR. IgA level and IgA1 galactose (Gal) deficiency were assessed the effects of LIF and OSM, and IL-6 as a control, on IgA1 production and its circulation of patients with IgAN (IgAN cells) and healthy controls (HC cells), we at Birmingham, AL; Tomino,3 Suzuki,4 Milan Raska

IgA nephropathy (IgAN). We established IgA1-producing cells derived from the IgAN-IgA1 (17.9% vs. 0.8%). N-terminal HR (H208-P227) released by TIGR4 was most abundant on IgAN-IgA1 and 3 to 5 predominating on HC-IgA1. HR variant with 3 of total HR.

mass spectrometry (MS). Relative abundance of each glycopeptide was expressed as % patient (IgAN-IgA1) and a HC (HC-IgA1).

of murine IgAN. For further confirmation, MZB in gddY at 4 weeks of age (gddY) that we recently established as 100% onset model (J Am Soc Nephrol, 2005), were of murine IgAN.

Splenic marginal zone B cells (MZB) are potentially poly-reactive and their expansion might be associated with autoimmune diseases, such as SLE or rheumatoid arthritis. Although the pathogenesis of IgA nephropathy (IgAN) has not been clarified, the recent studies have suggested that IgAN may be related to autoimmune disorders including immune complex (IC) formation with aberrantly glycosylated IgA1 as an auto-antigen. The objective of the present study is to verify whether MZB contribute to the pathogenesis of murine IgAN.

Results:

Two IgAN prone mice, high IgA mice (HIGA) and grouped ddY mice (HIGA), were depleted by anti-I-A and anti-I-C and the IgAN prone mice were significant in the expression of CX3CR1-FKN axis in the pathogenesis of murine IgAN. Thus, we confirmed the glycosylation abnormality of O-glycans on IgA1 understanding, but likely include changes in the expression, activity, and/or localization of enzymes involved in individual glycosylation steps. O-glycans of normal IgA1 consist of N-acetylgalactosamine (GalNAc) and Gal, with possibly one or both residues sialylated, whereas IgA1 in the circulation and renal deposits of patients with IgAN has some O-glycans deficient in Gal. O-glycan formation is initiated by attachment of GalNAc to the serine or threonine in IgA1 HR, catalyzed by a GalNAc-transferase (GalNAc-T).

Methods: It has been speculated that GalNAc-T2 is responsible for initiation of O-glycosylation in IgA1. However, our recent in vitro studies with recombinant GalNAc-T2 demonstrated that some sites with Gal-deficient glycans are added by this enzyme initiating the sialylation of other GalO-glycans. In the present study, we analyzed the expression profile of a specific GalNAc-T, such as over-expression of GalNAc-T4, to contribute to production of Gal-deficient IgA O-glycans in patients with IgAN.

Funding: NIDDK Support, Government Support - Non-U.S.

SA-PO2200

Activated Innate Immunity and Involvement of the CX3CR1-FKN Axis in Promoting Hemataria in IgA Nephropathy Patients

Sharon N. Cos,1 Fabio Sallustio,2 Grazia Serino,2 Antonia Lovern,3 Francesco Pesce,2 Patrizia Stifanielli,4 Nicola Ancona,5 Gianluigi Zaza,6 Francesco Paolo Schena.7 2,10 Dept. of Emergency and Organ Transplant, Univ. of Bari, Bari, Italy; 3,C.A.R.S.O. Consortium, Valenzano, Bari, Italy; 4,ISSIA, CNR, Bari, Italy.

Background: The hallmark of IgA nephropathy (IgAN) is gross hematuria (GH) coinciding with or immediately following a respiratory or gastrointestinal tract infection and represent the disease triggering event. Methods: Therefore, a whole genomic screening of IgAN patients during the GH was done to clarify the link between mucosal encountered antigens and the occurrence of glomerular hematuria.

Results: The modulated genes during GH showed a clear involvement of the interferon signaling, antigen presenting pathway, and the immunoproteasome. The gene characterizing cytotoxic effector lymphocytes (CX3CR1), implicated in vascular endothelial damage, was found up-regulated at both mRNA and protein level. In vitro antigen stimulation of PBMCs on an independent set of IgAN patients and healthy blood donors (HB) demonstrated that patients specifically up-regulated this gene in a GF-inducible manner, while an expected down-regulation occurred in HB. This enhanced activation occurred in patients both characterized by recurrent GH and by persistent regulation of glomerular hematuria (MH). We observed high glomerular fractalkine (FKN) expression, since this ligand is involved in the vascular gateway for CX3CR1+ cells towards the inflamed tissues. A significantly higher FKN expression on the capillary vessels and podocytes was found in IgAN patients with recurrent GH compared to persistent MH, suggesting a predisposition for cytotoxic cell extravasation in recurrent GH patients.

Conclusions: Taken together, our findings demonstrate, for the first time, a defect in antigen handling in PBMCs of IgAN patients with a specific up-regulation of CX3CR1. Furthermore, the constitutive up regulation of glomerular FKN, suggests an involvement of the CX3CR1-FKN axis in the exacerbation of GH.

SA-PO2201

IgA-IgG Immune-Complex Formation by Marginal Zone B Cells Is Crucial for the Progression of Murine IgA Nephropathy

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Background: Spleenic marginal zone B cells (MZB) are potentially poly-reactive and their expansion might be associated with autoimmune diseases, such as SLE or rheumatoid arthritis. Although the pathogenesis of IgA nephropathy (IgAN) has not been clarified, the recent studies have suggested that IgAN may be related to autoimmune disorders including immune complex (IC) formation with aberrantly glycosylated IgA1 as an auto-antigen. The objective of the present study is to verify whether MZB contribute to the pathogenesis of murine IgAN.

Methods: Two IgAN prone mice, high IgA mice (HIGA) and grouped ddY mice (gddY) that we recently established as 100% onset model (J Am Soc Nephrol, 2005), were employed for present study.

Results: When evaluated HIGA (N=5) and gddY (N=5) at 12 weeks of age, only gddY showed severe glomerular damage with increased massive albuminuria. Flow cytometric analysis showed increased numbers of MZB (B220+CD19+CD21+CD24+IgMhigh) and their precursors (B220+IgMlowCD21highCD24–) in gddY were significantly higher than those in HIGA (gddY vs. HIGA; MZB: 6.10±9.106 vs. 3.8±10.106, P<0.002, precursors: 2.61±0.106 vs. 1.41±0.2x106. P<0.002), suggesting that MZB may be involved in the progression of murine IgAN. For further confirmation, MZB in gddY at 4 weeks of age were selectively depleted by anti-I-A and anti-I-C and the MO-glycosidase monoclonal antibodies. Clinical outcomes (depleted mice; N=5 vs. isotype-control mice; N=5) at 14 days after the treatment were as follows; albuminuria (mg/day): 40.9±19.2 vs. 230±614.6 (P<0.02), serum IgA (mg/dl): 47.1±8.5 vs. 43.2±6.4 (P<0.04), serum IgM (mg/dl): 40.9±7.4 vs. 71.1±14.4

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
SA-PO2204

Strain Differences in Vasculitis Development upon Administration of Polyclonal and Monoclonal Anti-MPO Antibodies

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Background: Polyclonal antibodies against myeloperoxidase (MPO) induce vasculitis and crescentic glomerulonephritis (GN) in C57BL/6 (B6) mice. Whether the pathogenic potential of anti-MPO IgG is restricted to a specific subclass and/or epitope is unknown. Our previously generated panel of monoclonal anti-MPO antibodies (anti-MPO moAbs) did not induce GN in B6 mice. However, genetic variation can determine disease susceptibility, hence other mouse strains may be more susceptible.

Aim: To compare vasculitis development in C57BL/6 (B6), 129S6 (129) and DBA/1 mouse strains upon polyclonal and monoclonal anti-MPO antibodies.

Methods: Mice were injected with polyclonal anti-MPO IgG (1 mg; iv; n=6/group), a mix of three anti-MPO moAbs of different subclass and recognizing different epitopes (10 mg; ip; n=3–6/group) or isotype control moAbs. This was followed by ip LPS injection (150 EU/g). Mice were sacrificed at day 7.

Results: Polyclonal anti-MPO IgG induced GN in B6 and 129 mice, as evidenced by leukocyturia, albuminuria and crescent formation. DBA/1 mice did not develop GN. Anti-MPO moAbs also induced GN in B6 and 129 mice. DBA/1 mice did not develop GN, but developed profound lung hemorrhage with inflammatory lesions (neutrophil and macrophage influx). Also, DBA/1 mice displayed liver inflammation and an increase in serum alanine aminotransaminase (ALAT, 66.4±34.1 vs 19.2±7.2 u/L in isotype controls, p<0.01). Isotype control moAbs did not induce disease.

Conclusions: Polyclonal anti-MPO IgG induce GN in B6 and 129 mice, but not in DBA/1 mice. Also, anti-MPO moAbs induce GN in B6 and 129 mice. DBA/1 mice do not develop GN, but develop lung hemorrhage and liver inflammation. DBA/1 mice cannot potentially serve as a model for anti-MPO IgG-mediated lung vasculitis.

Funding: Government Support - Non-U.S.

SA-PO2205

Is CD177 (NB1) Sufficient for the Membrane Expression of PR3 in Myeloid Cells?

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Background: Polymeric immunoglobulin (Ig) A (poly-IgA) and polymeric immunoglobulin (Ig) G (poly-IgG) formation.

Methods: To compare vasculitis development in C57Bl/6 (B6), 129S6 (129) and DBA/1 mouse strains upon polyclonal and monoclonal anti-MPO antibodies.

Results: Polyclonal anti-MPO IgG induced GN in B6 and 129 mice, but not in DBA/1 mice. Anti-MPO moAbs also induced GN in B6 and 129 mice. DBA/1 mice did not develop GN, but developed profound lung hemorrhage with inflammatory lesions (neutrophil and macrophage influx). Also, DBA/1 mice displayed liver inflammation and an increase in serum alanine aminotransaminase (ALAT, 66.4±34.1 vs 19.2±7.2 u/L in isotype controls, p<0.01). Isotype control moAbs did not induce disease.

Conclusions: Polyclonal anti-MPO IgG induce GN in B6 and 129 mice, but not in DBA/1 mice. Also, anti-MPO moAbs induce GN in B6 and 129 mice. DBA/1 mice do not develop GN, but develop lung hemorrhage and liver inflammation. DBA/1 mice cannot potentially serve as a model for anti-MPO IgG-mediated lung vasculitis.

Funding: Government Support - Non-U.S.
SA-PO2206
De Novo Protein Synthesis of PR3 and MPO Autointegens in Mature Neutrophils of Patients with ANCA Vasculitis Anshul K. Badhwar,1 Elizabeth A. Alderman,1 Akhil Muthigi,1 Heng Ge,1 Elisabeth Berg,1 J. Charles Jennette,1 Gloria A. Preston,1 Ronald J. Falk,2 1UNC Kidney Center; University of North Carolina at Chapel Hill, NC; 2Xian Jiaotong University, Xian City, Shaanxi Province, China.

Background: Due to a defect in epigenetic silencing, circulating neutrophils from patients with ANCA disease express PR3 and MPO genes which are normally expressed only in bone marrow cells. We examine the processing of transcripts in mature neutrophils and whether increased transcription results in increased PR3/MPO protein.

Methods: A psoralen-biotin RNA probe complementary to sense PR-3 and MPO was used to select hybridized northern blotting. RNA from 9 patients and 9 healthy donors was used to characterize the transcripts present. Quantitative RT-PCR of PR3 and MPO transcripts was used to confirm expression levels detected by northern blotting. Nascent protein synthesis was metabolically labeled with a methionine analog, selectively biotinylated, purified by magnetic streptavidin beads and detected by western blotting.

Results: Multiple isoforms of PR3 transcripts were observed by northern blotting of leukocyte RNA from patients with ANCA-PR3. Five of nine patients expressed at least one isoform of PR3 mRNA, and of the five, three patients expressed an alternatively spliced variant larger (approx. 100 to 400 additional nucleic acids) than currently annotated size.

Unexpectedly, PR3 transcripts were also detected in three of nine healthy controls. Northern blotting was determined to be quantitative and correlated with levels of expression of both PR3 and MPO by standardized qRT-PCR assay. A novel polyadenylation site distal to the canonical site was associated with expression in circulating mature neutrophils and monocytes. Upregulated PR3 and MPO transcripts was associated with de novo protein synthesis in four of four patients with MPO-ANCA. None of the 8 healthy donors tested produced significant levels of either protein.

Conclusions: The data indicate that neutrophils in the periphery produce both PR3 and MPO protein de novo and that the presence of previously unidentified isoforms of PR3 may lead to the production of altered forms of PR3 protein.

Funding: NIDDK Support

SA-PO2207
Membrane Association of Proteinase 3, the Autointegen in Granulomatosis with Polyangitis (GPA), Expressed at the Membrane of Apoptotic Neutrophils, Is Essential for Impairing Their Phagocytosis by Macrophages Veronique Witko-Sarsat,1 Arnaud Millet,1 Magali Pederzoli-Ribeil,2 Luc Mouton,1 Cochin Institute Immunology-Hematology Department, INSERM U1016, Université René Descartes, Paris, France; 2In ternal Medicine Department, Cochin Hospital, Paris, France.

Background: The removal of apoptotic neutrophils is a key event in the resolution of inflammation, its failure has been incriminated in chronic autoimmune diseases. We described that proteinase 3 (PR3) the autointegen in granulomatosis with polyangitis (GPA) was externalized during apoptosis and impaired the phagocytosis of apoptotic neutrophils by macrophages thus acting as a dont eat me signal (Kantari et al, Blood 2007). The aim of the study was to investigate whether PR3 membrane expression and/or its enzymatic activity was essential for this activity.

Methods: Stable transfectant in RBL cells expressing a mutant of PR3 (PR3H414A) unable to insert into the plasma membrane was generated. The phagocytosis of apoptotic RBLPR3H414A by human monocyte-derived macrophages was studied in media containing or not wild type RBLPR3. The enzymatic activity of apoptosis-induced membrane PR3 was studied for its ability to cleave extracellular matrix proteins such as fibronectin.

Results: The mutations of four hydrophobic (F180, F181, L228, F229) amino acids abrogated PR3 membrane anchorage and cells expressing this hydrophobic patch-deficient PR3 mutant (PR3H414A) did not inhibit macrophage phagocytosis thus confirming the importance of PR3 membrane association in this phenomena. We demonstrated that this "dont eat me" activity of membrane-associated PR3 was independent of its serine proteinase activity because 1) the enzymatically-defective mutant PR3S320A displayed the same activity and 2) that apoptosis-induced PR3 externalization did not result in an increased ability to cleave extracellular matrix proteins such as fibronectin.

Conclusions: Our conclusion is 1) that the molecular basis of PR3 "dont-eat-me" signal relies on its enzymatic activity and bi) that PR3 "dont-eat-one" activity might potentiate the mechanisms of autoimmune and be involved in the pathophysiology of ANCA-associated vasculitis.

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SA-PO2208
Endothelial Lineage Impairment and Increased PR3 Expression on Peripheral Cells of Endothelial Phenotype in Wegener’s Granulomatosis Susann Patschan,1 Daniel Patschan,2 Sabine Blaschke,1 Gerhard A. Mueller,1 Nephrology and Rheumatology, University Hospital Göttingen, Göttingen, Niedersachsen, Germany; 2Göttingen; Göttingen.

Background: Wegener’s Granulomatosis (WG) is characterized by microvascular endothelial damage and by alterations of the endothelial progenitor cell (EPC) system. Interactions between anti-Proteinase 3 antibodies and their respective antigens (PR3) on neutrophils are pathogenetically relevant in WG. Aim of this study was (I) to analyze total (circulating and regenerates) and blood-derived PR3 and (II) to evaluate the expression patterns on circulating myelomonocytic and endothelial cells in WG.

Methods: Blood samples from WG patients were analyzed for total and for Fik-1+ myelomonocytic-cells. Healthy donors served as controls. For evaluating the proliferative capacity of EPCs, a colony forming unit assay (CFU) was performed. PR3 expression by the cells was quantified by cytometric analysis. Serum Angiopoietin 1 and serum TNF-α were measured by ELISA.

Results: A total of 21 healthy donors (12 female, 9 male [40.3 ± 9.2 years]) and 31 WG patients (13 female, 18 male [59.2 ± 15.3 years]) were included into the study. The percentages of EPCs were not different between the two groups. WG patients displayed lower proliferative activity of EPCs. In addition PR3 expression was significantly higher in the total as well as in the Fik-1+ (sub)population of myelomonocytic cells in WG. Finally, we observed lower mean serum levels of Angiopoietin 1 and higher serum levels of TNF-α as compared to controls, the serum levels of both cytokines did not linearly correlate with either clinical activity or the total number of circulating EPCs or the numbers of colonies formed (EPC regeneration).

Conclusions: In addition to reduced EPC regeneration and decreased serum levels of Angiopoietin 1, the clinical condition, patients with WG show sig-nificantly increased expression of PR3 in the total and in the Fik-1+ myelomonocytic cell population. These data imply, that PR3 could be involved in the pathogenesis of microvascular endothelial damage in patients with WG.

SA-PO2209
C4d in Thrombotic Microangiopathy: Cause or Consequence? Jamie S. Chus, Hans J. Baelde, Ingeborg M. Bajema, Jan A. Brujin, Danielle Cohen. Pathology, Leiden University Medical Center; Leiden, Netherlands.

Background: Complement activation, whether caused by excessive activation or inadequate regulation, is known to play a major role in thrombotic microangiopathy (TMA). We recently showed that glomerular C4d deposition is associated to development of TMA in patients with lupus nephritis and antiphospholipid syndrome (APS). The aim of this study was to investigate whether C4d is also present in other forms of TMA and whether this marker for classical complement activation could identify patients with an antibody- or immune complex mediated TMA.

Methods: We investigated the presence of C4d and MBL deposits on 47 renal biopsies or autopsies with histologically proven TMA. Patients were divided into 2 groups: A first group with TMA in association with auto- or alloimmune disease (including SLE, APS, renal transplantation and stemcell transplantation) (n=27) and a second group of patients with clinically confirmed Hemolytic Uremic Syndrome (HUS) (n=20). Deposition patterns of C4d and MBL were scored blindly and semi-quantitatively in glomeruli, peritubular capillaries (PTC), arteries and arterial branches.

Conclusions: In general, C4d deposition was found in 94% of TMA cases, independent of the underlying clinical setting. Glomerular C4d deposition was present in 85% of allo- and autoimmun cases and in 80% of HUS cases (P=0,456). Arteriolar C4d deposition was found in 48% of allo- and autoimmun cases, in 60% of HUS cases (P=0,421) and was mainly observed in vessels obstructed by microthrombi. Diffuse C4d depositions in PTCs were only present in two cases of de novo TMA in renal allografts. Co-localization of C4d with MBL never occurred.

SA-PO2210
Membranoproliferative Glomerulonephritis: Identification of New Diseases Associated Complement Genes Quan Chen,1 Christine Licht,2 Gunther B. Wolf,3 Christine Sckera,4 Peter F. Zipfel.1 1Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany; 2Hospital for Sick Children, Toronto, Canada; 3Internal Medicine, University Hospital, Jena, Germany.

Background: Membranoproliferative glomerulonephritis (MPGN) is a rare kidney disease characterized by hematuria, proteinuria and complement deposit formation, particularly at the glomerular basement membrane of the kidney. In order to define the molecular mechanisms of this severe glomerular disease, we set up a European MPGN registry. Factor H-, CFHR1-, and Factor H-sequence variations, as well as copy number variations in the Factor H-CFHR1 segment and two related patients showed a novel heterozygous deletion c.672C>T; Y224Y appeared with higher frequencies in the patients vs controls (0.106 vs. 0.008, respectively). The CFHR gene cluster shows different CFHR1 haplotypes as well as copy number variations. In the patient group homozygous deletion of a chromosomal segment which includes the CFHR1-CFHR3 genes was more frequent among patients as compared to the control group (Delta CFHR1 17.7%; n=6) vs the control group (Delta CFHR1 3.3%; n=2). The overall frequency of this allelic deletion was similar in both groups, which is explained by a higher frequency of the heterozygous alleles in the patient group (0.076 and 0.025 vs 0.008, respectively). The CFHR gene cluster shows copy number variations in the Factor H-, CFHR1- and Factor B-sequence variations, as well as copy number variations in the Factor H-CFHR1 gene cluster were assayed for 34 MPGN patients, as well as 67 healthy individuals. Two patients had a three nucleotide deletion causing absence of Lysine 224 in SCR2 Factor H. For the C4b and MBL to not-localized C4d, scores ranged from 0 to 3, respectively. In addition to reduced EPC regeneration and decreased serum levels of Angiopoietin 1, and to evaluate the classical pathway activation. However, rather than reflecting the cause, the classical pathway may be the consequence of severe endothelial damage or vascular remodeling in TMA. In addition, these data suggest that C5-inhibition (Eculizumab) could benefit the full spectrum of TMA patients, which is in line with recent successful results of C5 inhibitors in Shiga-toxin associated HUS.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
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627A
Complement-Induced Hemolysis Requires Purinergic Signaling by Helle A. Praetorius, Marianne G. Skals, Jens G. Leipziger, Julie L. Hejl. Dept. of Physiology and Biophysics, Aarhus University, Aarhus, Denmark.

Background: The complement system is a key element of the innate immune system. The system elicits efficient responses against cells identified as non-self via insertion of Membrane Attack Complexes. It is associated with many types of hemolytic anemia including typical and atypical hemolytic-uremic syndrome as well as paroxysmal nocturnal hemoglobinuria. Recently, we found that hemolysis caused by other types of membrane pore-formers such as α-hemolysin (HlyA) from Escherichia coli and α-toxin from Staphylococcus aureus inflict their cytotoxic effects through P2 receptor activation. Methods: To address if P2 receptors are involved in complement-induced lysis, we used simple hemolysis assays of sensitized human and sheep erythrocytes, combined with time-lapse microscopy. Results: Here we show that similarly to HlyA-induced hemolysis, red cell lysis caused by complement activation is amplified through ATP release and subsequent P2 receptor activation. Oxine and human erythrocytes were incubated with anti-sheet erythrocyte antibodies or anti-RhD antibodies respectively, with either human plasma or guinea pig serum as complement donors. Non-selective P2 antagonists (PPADS and suramin) concentration-dependently inhibit complement-induced hemolysis. More specific P2 receptor antagonists imply that P2X2 and P2X7 are the main receptors involved in this response. Moreover, complement-activation produced a sustained increase in the [Ca2+]i, which triggered significant erythrocyte shrinkage that preceded swelling and lysis. This early volume reduction is likely to result from activation of the K+ channel, Kc,3.1 as TRAM34 and clotrimazole augment the complement-induced hemolysis. Conclusions: These results indicate that complement, similar to HlyA, requires purinergic signaling for full hemolysis, and that activation of the erythrocyte volume regulation proteostatic lysis. This finding points to several new pathways to interfere with hemolytic diseases and implies that P2 receptor antagonists potentially can be used in more broad terms to prevent intravascular hemolysis. Funding: Government Support - Non-U.S.

SA-PO2211
A New Model of Atypical Haemolytic Uraemic Syndrome by Katherine Anne Vernon, Talat H. Malik, Marina Botto, Matthew C. Pickering. Centre for Complement and Inflammation Research, Imperial College London, London, United Kingdom.

Background: Complement factor H (CFH), the major regulator of the alternative pathway, is synthesised by both hepatic and extra-hepatic cells. The physiological role and contribution of extra-hepatic CFH is unknown. Rodent studies suggest that renal-derived CFH is important, with rat podocytes increasing CFH synthesis in membranous nephropathy. To define the physiological roles of hepatic and extra-hepatic CFH we utilised gene targeted mice were flanked by loxP sites) had normal plasma CFH and C3 levels indicating that examined using light microscopy and immunofluorescence. Serum mouse glomerular basement membrane antibody in pre-immunised animals. Serum nephropathy. To define the physiological roles of hepatic and extra-hepatic CFH we utilised Vernon A New Model of Atypical Haemolytic Uraemic Syndrome and clotrimazole augment the complement-induced hemolysis. Volume reduction is likely to result from activation of the K+ channel Kc,3.1 as TRAM34 and clotrimazole augment the complement-induced hemolysis. Conclusions: These results indicate that complement, similar to HlyA, requires purinergic signaling for full hemolysis, and that activation of the erythrocyte volume regulation proteostatic lysis. This finding points to several new pathways to interfere with hemolytic diseases and implies that P2 receptor antagonists potentially can be used in more broad terms to prevent intravascular hemolysis. Funding: Government Support - Non-U.S.

SA-PO2212

Genetic Background Influences VEGFR Pathways and Glomerular Thrombotic Lesions in Nephrotic Nephritis by Laurent Messner, 1, 2 Sophie Vandermersch, 1, 2 Patrick Callard, 2 Chantal Jouanneau, 1 Alexandre Hertig, 1, 2 Eric Rondeau. 1-2 INSERM UMR S702, Paris, France; 1APHP, Hotel Tenon, Paris, France; 2APHP, HEGP, Paris, France.

Background: As genetic background (GB) may play a role in various experimental models, we evaluated its impact on glomerular pathology. Methods: Mice were evaluated during passive mouse anti-glomerular basement membrane glomerulonephritis (anti-GBM-GN) in leading inbred strains (C57BL/6J, BALB/cJ, 129S2SvPas) and their F1 offspring. Glomerular DNA microarray were also performed at early time point (day 4). Results: Mice exhibited different severity of renal failure, hypertension and pseudosclerotic lesions according their GB. Proteinuria correlated with GB-dependent podocytic lesions, dramatic in 129S2SvPas, intermediate in BALB/cJ and some F1, mild in C57BL/6J. Originally, we discovered that glomerular thrombotic microangiopathy (TMA) was a histopathological hallmark of this model, along with its classical biological abnormalities (anemia, thrombocytopenia, schistocytes). TMA parameters were also significantly GB-dependent: major in 129S2SvPas, far less in C57BL/6J, intermediate in F1. Subsequent glomerular DNA microarray analysis comparing C57BL/6J-resistant to 129S2SvPas disease-prone mice indicated major differences in VEGF/VEGFR pathways, which have already been involved in TMA pathophysiology. Glomerular VEGF-A expression was not different and exogenous vEGF165 failed to rescue TMA. Further analysis revealed a glomerular VEGF/VEGFR2 signaling defect in 129S2SvPas disease-prone mice compared to C57BL/6J. Conclusions: Differences, and sometimes discordances, between studies about mouse anti-GBM-GN are classically inferred to GB; our results identify glomerular TMA lesions as a GB-dependent hallmark of anti-GBM-GN, which could be related to a genetic difference in VEGF/VEGF2 signaling.

SA-PO2214
The Growth Factor Midkine Ameliorates Crescentic Glomerulonephritis through Inhibiting Thrombosis by Hiroshi Kojima, Wachi Satto, Tomoki Kosugi, Yuka Sato, Kayaho Maeda, Shoichil Maruyama, Yukio Yuzawa, Seiichi Matsuo. Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan.

Background: The growth factor Midkine (MK), a heparin-binding protein, has been implicated in neuronal survival and differentiation, cancer development and inflammation-related diseases. We have previously demonstrated that MK plays deleterious effects for ischemic renal injury, diabetes and hypertension. However, little is known about the kinetics of MK in pathological states. Using mass spectrometry method, a large number of MK-binding proteins such as coagulation- and fibrinogenolysis-related factors were identified. Indeed, MK activates fibrinolysis in bovine endothelial cells. In order to clarify the molecule mechanism of thrombosis in vivo, we examined the role of MK in crescentic glomerulonephritis (GN) model. Methods: MK deficient mice (Mdk−/−) or wild-type mice (Mdk+/+) were treated with unilateral nephrectomy and the pre-immunization of rabbit IgG, and then injected with rabbit anti-mouse glomerular basement membrane (GBM) antibody (Ab) after 7 days. Mice were killed at early time point (day 4), and 3, 7 and 14 days after injection of anti-GBM Ab. Kidney, serum and urine were collected for immunohistochernistry and biochemical analysis. Results: There were no differences in rabbit IgG, mouse IgG and C3 depositions of glomeruli in both groups. Blood urea nitrogen levels in Mdk−/− were higher than Mdk+/+. Histological examination showed that Mdk−/− developed severe crescentic GN accompanied with marked thrombosis over time. Obvious tubulointerstitial injuries were also found in Mdk−/− compare with Mdk+/+. Macrophage and neutrophil infiltrations into glomeruli were more significant in Mdk−/− than Mdk+/+. Plasminogen activator inhibitor (PAI)-1 in glomeruli of Mdk−/− was significantly induced, consistent with the result of mass spectrometry. Conclusions: In contrast to previously reported in vivo models such as ischemic reperfusion model, diabetic nephropathy and remnant kidney model, MK might play beneficial effects for crescentic GN though the blockade of PAI-1-mediated thrombosis and be a candidate for preventing or delaying rapidly progressive GN.

SA-PO2215
Endothelial BAMBI: A Novel Modulator of Angiogenesis by Dimitri Kollins, 1 Nicolas Guillot, 1 Victoria Gilbert, 1 Sundhya Xavier, 1 Jun Chen, 1 Maria Pia Bloch, 1 Alessandro Corbelli, 1 Detlef O. Schlondorff 1,2. 1 Nephrology, Mount Sinai School of Medicine, New York, NY; 2Nephrology, New York Medical College, Valhalla, NY; 1’Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico, Milano, Italy.

Background: BAMBI (BMP and Activin receptor Membrane Bound Inhibitor), was described as a highly conserved protein that can act as a competitive antagonist of type 1 TGFβ receptors. BAMBI is highly expressed in solid tumors, where it has been postulated to contribute to growth and metastasis. There are, however, no data on cell-specific expression of BAMBI in mammalian organ systems.

Methods: As TGFβ action is highly cell type-specific, we examined the localization of BAMBI in mouse tissues and its function in target cells in vitro and in vivo in BAMBI knock-out mice.

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Underline represents presenting author. 628A
Results: By immunohistochemistry, BAMB1 is expressed in endothelial cells of the major visceral mouse organs. By EM, the endothelial cell phenotype in BAMB1−/− mice shows signs of cell activation as compared to the wild type mice. In vitro angiogenesis and scratch wound assays using human umbilical vein endothelial cells (HUVEC) show enhanced angiogenesis and cell migration in HUVEC with BAMB1 knock-down by siRNA, and the phenomenon is reversed by overexpression of BAMB1 suggesting the role of BAMB1 in angiogenesis using s.c. matrix implantation assays confirmed enhanced formation of angiogenesis in the BAMB1−/− as compared to the wild type mice. To confirm the enhanced angiogenesis in vivo in BAMB1−/−, we examined glomeruli as highly-vascularized structures. BAMB1−/− mice showed more severe histological lesions than wild type mice with larger capillary endothelial cells. Furthermore, compensatory glomerular capillary hyperplasia after unilateral ureteroclepsy was greater in the BAMB1−/− than in wild type mice.

Conclusions: Taken together our data show that BAMB1 is expressed in endothelial cells, and may support a role for BAMB1 in vascular homeostasis. Elimination of BAMB1 results in enhanced angiogenesis, which may play a role in neovascularization after renal injury and in renal diseases with capillary loss.

Funding: NIDDK Support

SA-PO2216
Osteopontin Deficiency Results in Severe Glomerulosclerosis in Uninephrectomized Mice
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Background: Expression of osteopontin (OPN) in podocytes has been reported to be elevated in models mimicking histological changes of experimental models of renal injury such as diabetic nephropathy or glomerulopathy.

Methods: To investigate the role of OPN in the development of focal segmental glomerulosclerosis (FSGS), OPN+/+ and OPN−/− mice were subjected to uninephrectomy (UNX) and DOCA-salt-treatment for 6 weeks, a model of FSGS through elevation of glomerular capillary pressure. Proteinuria and albuminuria were measured and renal morphology was analyzed by scoring glomerulosclerosis and collagen expansion on kidney sections stained with PAS or Masson trichrome.

Results: Following UNX, OPN+/+ mice exhibited prominent glomerular damage as evidenced by severe glomerulosclerosis degree and mesangial (matrix) collagen deposition. After DOCA salt-treatment glomerular lesions between OPN+/+ and OPN−/− mice were not strikingly different anymore. Massive podocyte injury was demonstrated by electron microscopy in response to DOCA-salt treatment. WT-1, podocin, synaptopodin and actin morphology was analyzed by scoring glomerulosclerosis and collagen expansion on kidney sections stained with PAS or Masson trichrome.

Conclusions: Our data indicate that OPN play a crucial role in adaptation following renal ablation and is renoprotective towards increased mechanical load.

Funding: Government Support - Non-U.S.

SA-PO2217
GSK3β Inhibition Protects Against NSAID Induced Acute Kidney Injury: Effects on Mitochondrial Permeability Transition and ATP Production
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Background: Clinical use of non-steroid anti-inflammatory drugs (NSAID) like Diclofenac (DCLF) is limited by toxicity and acute kidney injury (AKI). This study examined the role of glycosyn synthase kinase (GSK3), a newly recognized modulator of kidney injury, in NSAID induced acute nephrotoxicity.

Methods: Cultured mouse renal proximal tubular epithelial cells were exposed to DCLF. Mechanically, DCLF−8 normalized the levels of intracellular ATP in DCLF-treated cells and diminished DCLF elicited MPT evident by entry of fluorescent calcein from cytoplasm into mitochondrial matrix space as well as blunted calcium-induced mitochondrial swelling. Conversely, ectopic expression of an uninhibitable mutant of GSK3β markedly exaggerated DCLF induced injury and abrogated the protective effects of DCLF−8 on tubular cell apoptosis, necrosis, MPT and ATP production. In vivo, DCLF induced a typical pathology of acute tubular necrosis, characterized by epithelial simplification, isometric vacuolization of proximal tubular epithelium, luminal distension, epithelial necrosis, sloughing of tubular cell into lumen, loss of brush border, nuclear enlargement and pleomorphism, and prominent inflammation. GSK3β inhibition by DCLF−8 prominently prevented acute kidney dysfunction and ameliorated tubular necrosis and apoptosis. This protective effect was associated with reduced MPT pore opening and preserved ATP homeostasis in DCLF injured kidney.

Conclusions: Collectively, our findings suggest that GSK3β inhibition ameliorates NSAID-induced AKI by inhibiting MPT and preserving ATP production.

Funding: Private Foundation Support

SA-PO2218
Derangement of Oxygen Sensing for Erythropoietin (EPO) Production by Unfolded Protein Response (UPR) Inhibition
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Background: In addition to loss of renal EPO-producing cells, derangement of oxygen sensing for EPO production contributes to a low level of EPO in advanced CKD patients. Here, We investigated the possibility of derangement of oxygen sensing by UPR, which is a stress signal induced by endoplasmatic reticulum (ER) stress and contributes to various kidney disease progression.

Methods: EPO-producing cell line (HeG2) or rats were treated with UPR inducers (thapsigargin; TgK or tunicamycin; TUN) or a suppressor (salubrinal; SAL) under normoxia or hypoxia (pO2, 0, or 100 pm hypoxia-inducible factor (HIF) activator cel[Cl1, 10µM]. EPO transcription or plasma EPO level was measured by real-time qRT-PCR or ELISA. The change in hypoxia-induced EPO 3'-enhancer activity by UPR was assessed by 1) Western blotting for detection of HIF-2α nuclear translocation or 2) luciferase assay for Epo promoter. Epo−/− or wild type and HeG2 treated with UPR inducers or the cells overexpressed UPR transcription factor, ATF4.

Results: UPR activated by THG or TUN as well as overexpression of ATF4 markedly suppressed hypoxia-induced EPO transcription in HepG2 without affecting the cell viability. In contrast, UPR did not alter other HIF target gene expressions such as VEGF or GLUT-1. β1 mRNA, identified as receptor of OPN mediated transcription by suppressing EPO 3'-enhancer activity without interaction with HIF and its subsequent binding to HRE. The change in hypoxia-induced EPO 3'-enhancer activity by UPR was assessed by 1) Western blotting for detection of HIF-2α nuclear translocation or 2) luciferase assay for Epo promoter. Epo−/− or wild type and HeG2 treated with UPR inducers or the cells overexpressed UPR transcription factor, ATF4.

Conclusions: UPR activation ameliorates hypoxia-induced EPO transcription in HepG2 without affecting the cell viability. Although UPR did not alter other HIF target gene expressions such as VEGF or GLUT-1. β1 mRNA, identified as receptor of OPN mediated transcription by suppressing EPO 3'-enhancer activity without interaction with HIF and its subsequent binding to HRE. The change in hypoxia-induced EPO 3'-enhancer activity by UPR was assessed by 1) Western blotting for detection of HIF-2α nuclear translocation or 2) luciferase assay for Epo promoter. Epo−/− or wild type and HeG2 treated with UPR inducers or the cells overexpressed UPR transcription factor, ATF4.

Funding: Government Support - Non-U.S.

SA-PO2219
Effects of MCP-1 Inhibition by Bindarit Therapy in a Rat Model of Poly cystic Kidney Disease (PKD)
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Background: Experimental and clinical evidence suggested that the proinflammatory chemokine MCP-1/CCL2 has a role in the development of interstitial inflammation and renal failure in autosomal-dominant PKD. Here we investigated the effects of the MCP-1 synthesis inhibitor bindarit in the PKC rat, a model othological to human autosomal-recessive PKD that has also phenotypic characteristics of human ADPKD.

Methods: PKC rats were treated by gavage from 5 to 15wks of age with vehicle (n=10) or bindarit (100mg/kg bid, n=9). Age-matched SD rats (n=8) served as control. To investigate the role of OPN in the development of focal segmental glomerulosclerosis (FSGS), OPN+/+ and OPN−/− mice were subjected to uninephrectomy (UNX) and DOCA-salt-treatment for 6 weeks, a model of FSGS through elevation of glomerular capillary pressure. Proteinuria and albuminuria were measured and renal morphology was analyzed by scoring glomerulosclerosis and collagen expansion on kidney sections stained with PAS or Masson trichrome.

Results: Following UNX, OPN+/+ mice exhibited prominent glomerular damage as evidenced by severe glomerulosclerosis degree and mesangial (matrix) collagen deposition. After DOCA salt-treatment glomerular lesions between OPN+/+ and OPN−/− mice were not strikingly different anymore. Massive podocyte injury was demonstrated by electron microscopy in response to DOCA-salt treatment. WT-1, podocin, synaptopodin and actin morphology was analyzed by scoring glomerulosclerosis and collagen expansion on kidney sections stained with PAS or Masson trichrome.

Conclusions: Our data suggest that GSK3β inhibition ameliorates NSAID-induced AKI by inhibiting MPT and preserving ATP production.

Funding: Pharmaceutical Company Support

SA-PO2220
Chronic Kidney Disease-Induced Cardiac Fibrosis Is Ameliorated by Reducing Circulating Levels of a Non-Dialysable Uremic Toxin, Indoxyl sulfate (IS)
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Background: Non-dialysable uremic toxins have been under-explored as a contributor of uremic cardiomypathy. We have recently demonstrated that one such toxin, indoxyl sulfate (IS), directly contributes to cardiac fibrosis. As an oral adsorbent, AST-120, can
lower serum IS levels, we hypothesized that cardiac fibrosis can be abrogated by this agent in an animal model of CKD.

Methods: We performed subtotal-nephrectomy (5/6-STNx) on Sprague-Dawley rats which were then randomized to receive either AST-120 (n=13) or vehicle (n=17) for 12 weeks. Sham-operated rats (n=12) served as controls.

Results: Ventricular (LV) diastolic dysfunction was observed in STNx+vehicle rats on echocardiography. A 4.5-fold increase in serum IS (p<0.001) was noted in STNx+vehicle vs sham as well as elevated tail-cuff blood pressure (BP) (p<0.001) and heart weight (p<0.001).

Increased LV fibrosis (p<0.001), gene expression of pro-fibrotic (TGF-β, CTGF, and VEGF), and pro-inflammatory (IL-6 and TNF-α) markers was observed in STNx+vehicle vs TGF-β and phosphorylated-NFκB protein expression were observed in STNx+vehicle rats. Treatment with AST-120 reduced serum creatinine (p<0.05) and urinary total protein (p<0.05) vs STNx+vehicle with no effect on BP (AST-120, 227±11 vs vehicle, 246±18 mmHg) and heart weight. Compared to vehicle STNx, serum IS was reduced by 79% with AST-120 (p<0.001), accompanied by reduced LV fibrosis by 35% (p<0.01 as well as TGF-β and phosphorylated-NFκB protein expression (p<0.05).

Conclusions: STNx increased cardiac fibrosis and circulating IS levels, which was significantly reduced by AST-120 independent of any change in BP. These findings support IS as being an important contributor to uremic cardiomyopathy and AST-120 a potential complementary treatment.

SA-PO2223

Experimental Lupus Nephritis: Accelerate Mortality and Arteriosclerosis in Apo-E Deficient Mice


Background: Chronic kidney disease is shown to aggravate atherosclerosis in man, but the underlying mechanisms are not completely understood. To investigate such mechanisms in an animal model we combined the classical atherosclerosis mouse model (ApoE-/-) with a progressive lupus nephritis model (yaaxFcgammaR2-/-). In this study we investigated the impact of Apo-E deficiency on survival and renal morphology in lupus nephritic mice.

Methods: First we analysed survival during a period of 40 weeks in 1. FcgammaR2 deficient mice (n=11). 2. A progressive lupus model yaaxFcgammaR2-/- (n=115). 3. YaaxFcgammaR2-/-ApoE-/- (n=19). 4. yaaxFcgammaR2ApoE-/- double knockout mice (n=40). Renal injury including vascular alterations was analysed on week 22 to determine the impact of renal morphological changes on survival. Using enface preparation, arteriographic plaques were analysed in APO-E deficient mice (n=5) and yaaxFcgammaR2ApoE-/- double knockout mice (n=7).

Results: YaaxFcgammaR2ApoE-/- double knockout mice had the shortest lifespan of all investigated groups (max. 26 weeks). In contrast, yaaxFcgammaR2 deficient mice being heterozygous for Apo-E had a significant reduced mortality of 57% (p=0.0005) surviving mice at week 40, similar to yaaxFcgammaR2-/-ApoE-/- mice (59%) survival. In addition, mortality was further significantly reduced in FcgammaR2 deficient lupus mice lacking the yaa modification (91% survival, p=0.048) compared to those bearing this modification. Arteriosclerotic plaques were still found in 4 of 7 yaaxFcgammaR2ApoE-/- double knockout mice on week 22 using enface preparations of aortas, whereas mice lacking only the APO-E gene showed no detectable aortic plaque formation. Analysis of renal pathology revealed a tendency to aggravated renal injury, but significantly enhanced vascular injury in yaaxFcgammaR2ApoE-/- double knockout mice compared to APO-E and yaaxFcgammaR2 deficient mice (p<0.05). Furthermore, yaaxFcgammaR2-/- double knockout mice showed 50% increased collagen area andYaaxFcgammaR2-/-ApoE-/- double knockout mice showed 2.5-fold increase in collagen area.

Conclusions: The lack of Apo-E gene in the progressive yaaxFcgammaR2-/-/ ApoE nephritis model highly increased mortality. Aortic plaque formation and renal vascular impairment is accelerated by lupus nephritis.

Funding: Government Support - Non-U.S.

SA-PO2224

Renin-Angiotensin System and Mammalian Target of Rapamycin (mTOR) Pathway Medulate the Course of HIV-Associated Nephropathy through Experimental Lupus Nephritis

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Background: The activation of renin-angiotensin system (RAS) and the mammalian target of rapamycin (mTOR) pathway have been demonstrated to play an important role for the development and the progression of HIV-associated nephropathy (HIVAN). We evaluated the role of the RAS and the mTOR pathway on HIV gene expression on the course of HIVAN.

Methods: To activate endogenous RAS, HIVAN mice (Vpr and Tg26) were bred with angiotensinogen (Agt) transgenic mice having four Agt copies; this strategy allowed us to develop Vpr/Tg26 mice with 2, 3, and 4 copies of Agt. Vpr-Agt-2, -3, and -4 mice were fed eicosapentaenoic acid (EPA) or doxycline for six weeks to induce podocyte expression of Vpr. To inhibit the mTOR pathway, Tg26 (n=6) were administered either vehicle or sirolimus (5 mg/kg/day, intra-peritoneally) for 14 days. At the end of experimental periods, kidneys were harvested and prepared for histology and immunohistochemistry. RNA and proteins were extracted and HIV-1 gene expression was determined by real time PCR and immunoblotting. Renal lesions were graded for sclerosis and tubular dilatation. In vitro studies, human podocytes were transduced with either HIV-1 or vector constructs, followed by treatment with either vehicle, sirolimus, or Ang II for 72 h. Subsequently RNA and proteins were harvested and HIV-1 gene expression was determined by real time PCR and immunoblotting.

Results: Doxy fed Vpr-Agt-4 mice displayed more advanced renal lesions than to doxy-fed Vpr-Agt-2 mice. Vpr-Agt-4 mice displayed 50% increase in Vpr expression. Saline-treated Tg26 showed two-fold advanced glomerular and tubular lesions vs. sirolimus-treated animals. Whereas, sirolimus decreased transcription of HIV-genes both in renal tissue as well as in HIV-1 transduced podocytes. Renal tissues of Tg26-Agt-4 displayed 2.4 fold increase in gr120, Vpr, Tat, Nef and Vpu genes.

Conclusions: The RAS activation increased HIV gene transcription; whereas, downregulation of mTOR decreased HIV gene transcription. These gene modulating effects of the RAS and the mTOR pathway may be contributing to the altered course of HIVAN.

Funding: NIDDK Support
SA-PO225
HIV-Induced Human Podocyte Vitamin D Receptor (VDR) Downregulation Enhances Cathepsin L Expression
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Background: In HIV-associated nephropathy (HIVAN), podocytes detach and proliferate in Bowman’s space. We hypothesized that HIV-induced down regulation of VDR enhances upregulation of cathepsin L, which compromises cytoskeletal integrity of podocytes leading to their detachment and proliferation.

Methods: To determine the effect of HIV on renal tissue VDR, synaptopodin, and cathepsin L expression, immunoblotting and immunohistochemical studies were performed on renal tissue from control and Tg26 mice (n=3). To develop in vitro model of HIV infection, we used immortalized human podocytes were infected with either empty vectors (EV/M/P/HP) or NL4-3 (HIV/M/P/HP) constructs and evaluated for the expression of VDR, cathepsin L, and synaptopodin (immunoblotting and real time PCR studies). To establish causal relationship between VDR and cathepsin L, human podocytes with silenced VDR were evaluated for cathepsin L expression. To confirm relationship between VDR and cathepsin L, EV/HP and HIV/HP were treated with either buffer or VDR (25 nM) for 24 hours and lysates prepared for VDR, cathepsin L, and synaptopodin expression. To determine the functional status of cathepsin L, cells were labeled with phalloidin and evaluated for the integrity of actin filaments. In addition, migration, adhesion, detachment, and proliferation studies were conducted in cells treated under similar conditions.

Results: Immunoblotting and PCR studies revealed attenuated expression of VDR and synaptopodin but enhanced expression of cathepsin L in renal tissues of HIVAN mice. Similarly, in vitro, HIV/HIVAN-VDR/HP displayed attenuated expression of VDR and enhanced expression of cathepsin L whereas, VDR treatment of HIV/HPs not only increased VDR and synaptopodin expression but also diminished expression of cathepsin L. HIV/HPs displayed disrupted actin filaments, decreased adhesion, increased detachment, and proliferative phenotype.

Conclusions: These findings indicate that HIV enhances podocyte cathepsin L expression via downregulation of VDR. The former decreases adhesion, enhances detachment and proliferation of podocytes.

Funding: NIDDK Support

SA-PO2226
Renin Inhibition Retards the Progression of HIV-Associated Nephropathy
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Background: Blockade of the downstream effect of angiotensin II has been reported to slow down the progression of HIV-associated nephropathy (HIVAN). In the present study, we evaluated the effect of renin inhibition on the progression of renal lesions in a mouse model of HIVAN (Vpr).

Methods: We have used Vpr transgenic mice; they express podocyte specific Vpr after doxycycline administration. In protocol A, Vpr mice were fed either water (W-VprA) or doxycycline (D-VprA) in their drinking water for six weeks. In protocol B, Vpr mice were fed either doxycycline (D-VprB) in their drinking water + normal saline (by miniosomotic pump) for six weeks. In protocol C, Vpr mice were fed doxycycline (doxy) for six weeks followed by kidney biopsy to determine baseline lesions. Subsequently, half of the mice were administered either normal saline (NS-VprC) or aliskiren (A-VprC) for 4 weeks. Protocol D was identical to protocol C except, Vpr mice were administered either normal saline or aliskiren for 8 weeks after kidney biopsy.

Results: All D-VprA showed 2-3 fold greater expression of renin inhibition when compared to W-VprA. All D-VprA showed overt HIVAN phenotype in the form of focal segmental glomerular sclerosis (FSGS) and mesangiocytic dilatation of tubules. In protocol B, aliskiren slowed the development of HIVAN. In protocol C, A-VprC showed 24.2% increase in number of sclerosed glomeruli (from their baseline) compared to 139.2% increase in sclerosed glomeruli in NS-VprC (P=0.01) from their baseline. Aliskiren also slowed down increase in size of microcytosis in A-VprC. Aliskiren diminished urinary protein creatinine ratio both in protocol B and protocol C. The attenuating effect of aliskiren on the progression of renal lesions continued in A-VprD.

Conclusions: Renin inhibition has a potential to slow down the progression of renal lesions in HIVAN.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2227
Both Lectin and Classical Complement Pathways Determine the Severity of Murine IgA Nephropathy
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Background: IgA nephropathy (IgAN) is a common form of primary progressive glomerulonephritis with mesangial cell proliferation and, matrix expansion with mesangial deposits of IgA and complement complex (C3). Mesangial deposits of circulating aberrantly glycosylated and polymeric IgA1 are discussed as a major pararers in the pathogenesis of IgAN, but the pathogenic roles of co-deposited C3 or related complement pathways have not been fully clarified. We confirmed for IgAN, complement activation of two marine IgAN prone models were investigated.

Methods: The high serum IgA mouse (HIGA) and grouped dY mouse (gdy) which were recently established as 100% onset IgAN prone models, were used in this study. Albuminuria, glomerular injury, complement activation in glomeruli, molecular weight of serum IgA and IgA-IgG2a immune complex (IC), IgA-IgM IC and IgA/mannose binding lectin (MBL)-A/C IC were analyzed in these models.

Results: Levels of albuminuria, glomerular injury and complement activation in gdy were significantly higher than those in HIGA. Complement pathways of gdy were activated much more than those of HIGA, although glomerular IgA deposits were similar in both mice. As in the case of human IgAN, gdy had much more aberrantly glycosylated IgA, IgA-IgG2a IC, IgA-IgM IC and IgA-MBL-A/C IC than serum than HIGA, but serum high molecular IgA did not differ in the two models.

Conclusions: These results suggest that levels of aberrantly glycosylated IgA and IgA- IC, but not total IgA, mainly determine the amplitude of activation of both complement pathways, and subsequent glomerular injury. It appears that complement activation of classical and lectin pathways may be one of the critical factors for progression of murine IgAN.

Funding: Government Support - Non-U.S.

SA-PO2228
Green Tea Polyphenol (-)-Epigallocatechin-3-gallate Restores Nefr2 Activity and Ameliorates Crescentic Glomerulonephritis
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Background: Crescentic glomerulonephritis (GN) is the most severe form of GN and is associated with significant morbidity and mortality despite aggressive immunotherapy. Crescentic GN is caused by free radical species by inflammatory cells can cause further tissue damage, intensify inflammation, promote apoptosis, and accelerate progression of crescentic GN. The green tea catechins, particularly (-)epigallocatechin-3-gallate (EGCG), are potent anti-inflammatory and anti-oxidant agents shown to inhibit leukocyte chemotaxis, quench free radicals, chelate transition metals, and interrupt lipid peroxidation chain reaction. The present study was undertaken to examine the therapeutic efficacy of EGCG on experimental crescentic GN.

Methods: Crescentic GN is induced in 129/sv mice by administration of rabbit anti-mouse-glomerular basement membrane (anti-GBM) sera. The anti-GBM antibody- injected mice were allowed to develop crescentic GN for 7 days before intervention. On day 7, mice were randomized into either EGCG-treated (50 mg/kg/day, orally 3 x weeks) or vehicle group. Animals were then euthanized on day 28 and routine histology and key molecules involved in inflammatory pathways were studied.

Results: EGCG treatment significantly reduced mortality, decreased proteinuria and serum creatinine, and markedly improved renal histology when compared with vehicle-treated mice. The improvement in renal function and histology were accompanied by the restoration of Nef2 signaling (which was impaired in vehicle-treated mice) as shown by increase in nuclear Nef2 and cytoplasmic glutamate cysteine ligase catalytic subunit, glutamate cysteine ligase modifier subunit, and glutathione peroxidase. EGCG-treated mice also showed reduction in p-Akt, p-JNK, p-ERK 1/2, PPARy, and SIRT1 (which were all elevated in vehicle-treated mice).

Conclusions: Our data illustrate the efficacy of EGCG in reversing the progression of crescentic GN in mice by targeting key inflammatory pathways.

Funding: Other NIH Support - NCCAM, Private Foundation Support

SA-PO2229
p53 Deficiency Exacerbates Immune-Mediated Glomerulonephritis
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Background: p53 tumor suppressor is a key regulator of the response to cellular stress or DNA damage. p53 activation leads to cell cycle arrest, apoptosis, or senescence. p53 has been shown to be an important regulator of renal tubular cell viability in the animal models of ischemic and toxic acute kidney injuries. In these models, p53 activation induced proapoptotic pathways in renal tubular cells and contributed to kidney dysfunction. In this study, we investigated whether genetic or pharmacological inhibition of p53 provided a protective effect in a mouse model of immune-mediated glomerulonephritis.

Methods: p53-null (KO) and wild-type (WT) mice were immunized subcutaneously with normal rabbit IgG, followed by intravenous injections of rabbit anti-mouse GBM antibody. In the experiment with the effect of pharmacological inhibition of p53, C57BL/6J mice were administered pifithrin-α (a chemical inhibitor of p53) at the injections of the anti-GBM antibodies. On day 6, blood and urine samples were collected and mice were sacrificed to obtain tissues for histological analysis.

Results: Upon induction of anti-GBM glomerulonephritis in WT mice, the expression of p53 was upregulated within the glomeruli and tubulointerstitium. Unexpectedly, serum creatinine (Scr) levels and urine albumin excretion were significantly increased in nephritic KO mice compared with nephritic WT mice. The KO mice displayed significant increase in the numbers of Bcl-2 cells and macrophages in the glomeruli and interstitial fibrosis.
The upregulation of cyclin E1 and activation of cyclin-dependent kinase 2 (CDK2) were observed in the KO mice. Moreover, Sre levels, the number of BrdU+ cells in the glomeruli and the expression of cyclin E1 were increased by pifithrin-α, which was also reversed by co-administration of roscovitine (CDK2 inhibitor).

**Conclusions:** p53 exerts anti-inflammatory effects by inhibiting glomerular cell proliferation during anti-GBM disease.

**Funding:** Pharmaceutical Company Support, Clinical Revenue Support

SA-PO2230

**Ephrin-B1 in Podocyte Is Interacted with Nephrin and Is Phosphorylated by a Signal from Nephrin**

**Background:**
Eph and ephrin are membrane-bound proteins that function as receptor-ligand pairs. The ephrin-B1 family is reported to regulate the paracellular permeability of epithelial cells. We have previously reported that ephrin-B1 was expressed at the slit diaphragm (SD) of podocyte (Kidney Int 72: 954-964, 2007). The aim of this study is to elucidate the function of ephrin-B1 in the SD.

**Methods:**
The expression of ephrin-B is analyzed with an Eph-B2-Fc probe as well as a specific antibody against ephrin-B. The precise localization of ephrin-B and its interaction with other SD molecules in normal rats and in nephrotic models were analyzed by dual labeling techniques with a confocal laser microscopy. We also analyzed the interaction of ephrin-B1 with other SD molecules by a sequential precipitate/Western blot analysis with glomerular lysates. Next, the binding sites of ephrin-B and nephrin were determined with HEK293 cells transfected with full length and several domains of these molecules. Then, the mechanism of phosphorylation of ephrin-B1 and nephrin are analyzed with HEK293 cells cotransfected with these molecules.

**Results:**
Ephrin-B1 staining at the SD is detected with Eph-B2-Fc probe as a same pattern detected with the specific anti-ephrin-B1 antibody. Ephrin-B1 was co-localized with nephrin, and other SD molecules. The staining of ephrin-B1 detected by Eph-B2-Fc probe was clearly reduced at early stages of proteinemic models. Analysis with normal rat glomerular lysates revealed that ephrin-B1 interacted with nephrin. The analysis with HEK293 cells showed that ephrin-B1 interacted with nephrin not via PDZ-binding motif of both molecules. Not only nephrin but also ephrin-B1 was phosphorylated by the anti-nephritis antibody binding to nephrin. The phosphorylation of nephrin was prevented by pretreatment with PP2, a src family kinase inhibitor.

**Conclusions:**
Ephrin-B1 is structurally and functionally interacted with nephrin, and plays a role in maintaining the barrier function of the SD.

**Funding:** Government Support - Non-U.S.

SA-PO2231

**Novel Roles of Notch Signal in the Pathogenesis of Collapsing FSGS**

**Background:**
Glomerular epithelial cell hyperplasia is the hallmark lesion in collapsing Focal Segmental Glomerulosclerosis (FCSFS) and may be involved in the disease progression. In two different mice models of CFSGS, we have shown that predominant hyperplastic epithelial cells are parietal epithelial cell (PEC) by genetic tagging of Lac-Z under the nephrin promoter (Asano. JASN 2005, and Suzuki. Am J Pathol 2009).

**Methods:**
The present study investigated one of the feasible transgenic models of CFSGS (NEP25 with immunotoxin, Asano et al JASN 2005) looking at expression Notch signal in the development of CFSGS.

**Results:**
In this model, severe proteinuria and histological feature of CFSGS associated with migration/proliferation of PEC were observed at day 12 after single injection of immunotoxin (n = 8). Immunostaining revealed progressive podocyteapenia with Lac-Z stain, and co-localization of Lac-Z and Hes1 (as marker of PEC) and Notch signals (cleaved Notch, Jagged1 and Hes1) in PEC lesions. Real time PCR of isolated glomeruli revealed a first third of reduced expression of podocyte marker, Nephrin and Synaptopodin, and fourfold increase in Cleaved Hes1. Over expressions of Notch signals were also observed: threefold increase of Notch1, Hes1, and Hes1L, and twofold of Jagged1, and Hes1L, respectively. To determine the function of Notch signal on PEC, we incubated g-secretase inhibitor (GSI) as a blockade of Notch signal on immortalized PEC line. In the wound healing assay, GSI significantly prevented wound closure (91±2.6%) compared with control (64±6.9%). Moreover, GSI drastically repressed Notch downstream, Hes1, Hey1, Hey2-LKimRNA1, in the PECs.

**Conclusions:**
Ectopic Notch signals in PEC may play pivotal roles for PEC migration/proliferation which is the pathologic base of CFSGS in this model. And blockade of Notch signal can become a new therapeutic approach for CFSGS.

SA-PO2232

**Enhanced Albuminuria in an Adriamycin Nephropathy Model in Acalasitasmic Mice**

**Background:**
Acalasitasmic mice is considered to be an experimental model of focal and segmental glomerular sclerotic with significant albuminuria. However, the effect of a functional catalase deficiency on albuminuria and progressive renal injury in an ADR nephropathy model has not yet been elucidated.

**Methods:**
ADR (15 mg/kg BW) was administered intravenously to both homozygous acalasitasmic mutant mice (C3H/Anc.LcVs/Cv) and control wild-type mice (C3H/Anc.LcVs/Cv). The functional and morphological alterations of the kidneys, including albuminuria, renal function, podocytic, glomerular and tubulointerstitial injury, the activities of antioxidant enzymes including catalase and glutathione peroxidase were then compared between the two groups until 8 weeks after disease induction. Moreover, the presence of a mutation of the toll-like receptor 4 (TLR4) gene, which is reported in the C3H/HeJ strain, was also investigated in both groups.

**Results:**
The ADR-treated acalasitasmic mice developed significant albuminuria in association with glomerular hypertrophy at week 4. The degree of albuminuria in the ADR-treated acalasitasmic mice was significantly higher than that in the wild-type mice. No significant difference was observed in the degree of glomerulosclerosis between the two groups. Unexpectedly, the degree of renal dysfunction and tubulointerstitial injury in the ADR-treated acalasitasmic mice were not remarkable in the ADR nephropathy model of the C3H mouse strain. The level of catalase activity was significantly lower in the kidneys of the acalasitasmic mice than in the wild-type mice without any compensatory upregulation of glutathione peroxidase. In addition, the C3H/Ard. strain was not identified to contain the TLR4 mutation.

**Conclusions:**
These data indicate that catalase deficiency plays an important role in the development of albuminuria possibly via the functional alteration of renal tubular reabsorption in an ADR nephropathy mouse model.

SA-PO2233

**Impairment of Podocyte Function by Diphteria Toxin – A New Reversible Murine Proteinuria Model**

**Background:**
Diphteria toxin (Dtx) receptor-mediated conditional cell ablation in transgenic mice is a powerful tool to analyze cell function in vivo. Transgenic mice with cell-specific expression of the human Dtx receptor allow conditional depletion of these cells through Dtx administration. We carefully analysed mice after Dtx injection and found proteinuria as an unexpected renal side effect. Since non-genetic mouse models of proteinuria and glomerular damage are limited we aimed to characterize the DTX-induced model of transient proteinuria in mice.

**Methods:**
C57Bl/6 mice were treated with 40µg/kg Dtx/bw i.p. (day 0 and 1) with DREG mice as positive depletion controls. To exclude undesired LPS-effects heat-treated DTX and LPS-resistant C3H/HeJ mice were used. For comparison the anti-GBM antibody binding to nephrin. The phosphorylation of nephrin was prevented by pretreatment with PP2, a src family kinase inhibitor.

**Results:**
Impairment of Podocyte Function by Diphteria Toxin – A New Reversible Murine Proteinuria Model

**Conclusions:**
Most animal models of non-genetic proteinuric glomerular disease were induced in the rat. The advantage of genetic manipulation technology favor the use of murine models. There is, however, only a limited number of murine podocyte injury models available. Hence there is need for mouse models resembling human glomerular diseases. We suggest DTX-induced kidney dysfunction as a new reversible model of podocyte injury with transient proteinuria, which could be used as an additional approach to complement studies in humans.

SA-PO2234

**Semaphorin 3G and Cystatin C Are Expressed in Podocytes of Rat and Are Differentially Regulated upon Toxic Injury**

**Background:**
Experimental glomerulopathies induced by toxic agents offer the unique opportunity to study the sequence of molecular events leading to podocyte alteration and thereby unveil early biomarkers of glomerular injury.

**Methods:**
This study compared the molecular profile of kidney from rats treated with glomerular (e.g. Puromycin) or with tubular nephrotoxins (e.g. Cisplatin) for various...
SA-PO2235

HIV Induces Tubular Cell ROS Generation and DNA Strand Breaks through Down Regulation of Vitamin D Receptor (VDR) (DOHA) Dhrav Salhan, Shabina Rehman, Ashwani Malhotra, Mohammad Hussain, Pravin C. Singhal. Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.

Background: HIV infection of tubular cells has been implicated for tubule cell injury in HIV-associated nephropathy (HIVAN). In the present study, we evaluated the role of vitamin D receptor (VDR) in HIV-induced tubular cell injury.

Methods: To develop in vitro model of HIV-infected tubular cells, mouse tubular cells (MCT) were transfected with empty vector (EV/MCT) or NL4-3 (HIV/MCT) constructs. To determine the effect of HIV on mitochondrial ROS generation, EV/MCTs and HIV/MCTs were double labeled with Red-CC1 (ROS labeling) and MITOTRacker green (mitochondrial labeling). To study occurrence of double strand DNA breaks, EV/MCTs and HIV/MCTs were labeled with H2AX and examined by confocal microscopy. Immunoblots were probed for VDR expression in EV/MCTs and HIV/MCTs. To establish causal relationship between lack of VDR and ROS generation, siRNA-VDR transfected MCTs and control cells were evaluated for ROS generation and DNA breaks. To confirm the relationship between VDR and DNA injury, EV/MCTs and HIV/MCTs were treated with either buffer or vitamin D2 analogue, followed by evaluation of cells for ROS generation (co-labeling with Red CC1 and Mito tracker green) and DNA repair by labeling for RAD1. Immunoblots were probed for VDR from cells treated under similar conditions.

Results: HIV/MCTs displayed increased (P<0.01) mitochondrial generation of ROS as well as higher number of double strand DNA breaks. Interestingly, HIV/MCTs also showed downregulation (P<0.01) of VDR. siRNA-VDR-MCTs also displayed enhanced generation of ROS and higher number of double strand DNA breaks. However, treatment of HIV/MCTs with a vitamin D2 analogue not only enhanced (P<0.001) expression of VDR by HIV/MCTs but also displayed attenuated (P<0.001) generation of ROS as well as enhanced DNA repair.

Conclusions: Since reversal of HIV-induced attenuated VDR expression by a vitamin D2 analogue with enhanced ROS generation and enhanced repair of DNA breaks, it would suggest that HIV-induced tubular cells ROS generation and DNA breaks may be caused by HIV-induced downregulation of VDR and associated signaling events.

Funding: NIDDK Support

SA-PO2236

Effects of Sodium Thiosulfate on Urinary Lithogenicity in Adults with Hypercalcemic Nephrolithiasis (DOHA) Onyeka W. Okonkwo,1 John R. Asplin,2 David S. Goldfarb.1 Medicine, New York VAMC, New York, NY; ‘Litholink, Chicago, IL; ‘Nephrology Section, New Harb Harbor VAMC and NYU School of Medicine, New York, NY.

Background: Sodium thiosulfate (STS) has been shown to reduce calcium stone formation in both humans (Yatzidis; Clin Nephrol 1985), and genetic hypercalcicuric stone formers (GHS; Asplin at al; 2009). We studied the effects of STS on the urine and serum chemistries in hypercalcicuric stone forming adults.

Methods: 5 people with idiopathic hypercalcicuria and calcium kidney stones with a mean age of 66 years participated. 2 baseline 24-hour urine collections were performed on days 2 and 3 of 3 days of recorded self-selected diets. Subjects then drank STS 10 mmol BID for 7 days and repeated 24h urines while repeating the 3 days of self-selected diet for days 2 and 3 of 3 days of recorded self-selected diet. Results were compared by non parametric Wilcoxon signed rank test. Results were also compared to those of a prior study involving healthy non-stone forming subjects administered STS.

Results: STS administration did not cause a significant change in urinary calcium excretion. It increased 24 hour urinary ammonia (P=0.002) and sulfate excretion (P=0.001) and only in urinary pH (P=0.001) and citrate excretion (P=0.004). 3 of 5 patients had measurement of serum HCO3 concentration; it did not change. The findings were similar to those from a prior study involving normal adults with the exception that urinary calcium excretion increased in those subjects.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

SA-PO2227

Reduced Bone Mineral Density in Young Adults with Childhood Onset Idiopathic Hypercalcuria (DOHA) Carlos Cuervo, Carolyn L. Abiribl, Jayanthi Cooswara, Vishwach Seherawat, Gunner Zellner Juarez, Michael Freundlich. Pediatric Nephrology, University of Miami, FL.

Background: Low bone mineral density (BMD) in children with idiopathic hypercalcicuria (IH) may lead to reduced peak bone mass and adult osteoporosis, but to date longitudinal studies of BMD with its predictors are lacking.

Methods: Children (81; 96.3±3.8yr) on cross-sectional evaluation had IH, 53/81(66%) including 24±16 yr followed longitudinally 4.0±2.8 yr(17±3) vs. 3±0.01yr; 12±1.11yr with DXA studies. Both groups were paired for age and sex. Subjects were classified as hypercalciuric (HC) or normocalciuric (NC). Cross sectional Z-scores ≤1 were evident in 37% of patients at both Sp and Fn. Table summarizes BMD Z-scores in the longitudinal subcohort. By univariate analysis initial age, interval time between DXA studies, and Ca, P, PTH did not associate significantly with final Sp or Fn Z-scores. However, initial and final calcium associated directly with final Sp Z (r=0.33, P=0.01 and r=0.48, P=0.0001) but not with Fn Z. By multiple regression analysis, initial Sp Z was the strongest predictor of final Sp Z (P<0.0001). Patients<16 yr had lower final Sp Z (-0.8±1.5) and Fn Z-scores (-1.26±1.23) vs. children<11 yr (-0.7±1.3 and -0.65±1.3) and adolescents ≥16 yr (-0.24±0.3 and 0.11±1.2).

Conclusions: A high patient proportion with childhood onset IH present with reduced BMD and show progressive decline during adolescence with ultimate reduced BMD, particularly those with initial reduced BMD, which appears to parallel their persistence into adulthood. DXA scans appear to identify patients at risk for adult osteoporosis.

Longitudinal BMD Z-scores in young patients with Idiopathic Hypercalcicuria

Funding: Clinical Research Support

SA-PO2238


Background: Patients with type 2 diabetes are at increased risk for nephrolithiasis due to low urine pH. Overweight and obesity is frequent in this population. Higher body mass can contribute to the lower urine pH in these patients as there is a strong inverse association between pH and body weight. The purpose of this study was to identify if stone forming diabetics had a lower urine pH independent of their body mass.

Methods: Two groups of individuals were recruited for our outpatient clinic: patients who have type 2 diabetes and were stone formers (DSF; n=31) and patients who were stone formers but did not have diabetes (NDSF; n=56). Both groups were paired for age and BMI. Inclusion criteria: Participants had a complete stone risk analysis that included urine pH. Both groups were divided according to their BMI (kg/m2) in normal (BMI < 24.9) overweight (BMI 25-29.9) and obese (BMI ≥30). Results: DSF had lower 24-hr urine pH than NDSF. Urine pH remained significantly lower in DSF than NDSF in both age and BMI subgroups: DSF 5.75; NDSF 5.98 (NS; overweight: DSF 5.32; NDSF 5.76 (p=0.04); Obese: DSF 5.15; NDSF 5.79 (p<0.001)

Conclusions: Although higher body mass can contribute to the lower urine pH in stone formers with type 2 diabetes it cannot entirely account for that finding. An increased acid intake or generation could probably explain the difference in urine pH. Effects of STS on 24 hour urine chemistry

<table>
<thead>
<tr>
<th>Substance</th>
<th>Mean baseline</th>
<th>Mean post STS</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>Calcium</td>
<td>279 ± 144</td>
<td>277 ± 144</td>
<td>0.918</td>
</tr>
<tr>
<td>pH</td>
<td>6.09 ± 0.58</td>
<td>5.76 ± 0.52</td>
<td>0.001</td>
</tr>
<tr>
<td>Ammonium</td>
<td>6.6 ± 95</td>
<td>5.36 ± 44</td>
<td>0.022</td>
</tr>
<tr>
<td>Citrate</td>
<td>606 ± 195</td>
<td>444 ± 221</td>
<td>0.004</td>
</tr>
<tr>
<td>Sulfate</td>
<td>14 ± 16</td>
<td>99 ± 16</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusions: Although STS is apparently effective in preventing stones in humans and did prevent stones in GHS rats, the basis for these effects was not reflected by the changes in urinary pH seen here. Although serum HCO3 did not change, urine tests (increased urinary ammonium, decreased urine citrate and pH) were suggestive of an increased acid load. Although STS did not change urine calcium, evidence of an acid load suggests that the long term safety of STS with respect to bone needs to be studied. Funding: Veterans Administration Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
SA-PO2239
Blockade of Megalin Attenuates Nephrocalcinosis in Rats
Isao Matsui, Kazunori Inoue, Takayuki Hamano, Akihiro Shimoura, Chikako Nakano, Tomoko Namba, Hiroki Omori, Masaru Horio, Hiromi Rakugi, Yoshitaka Isaka. Gericart Medicine and Nephrology, Osaka University Graduate School of Medicine, Saita, Osaka, Japan.

Background: Megalin is an apical membrane protein of proximal tubular cells that works as a multifunctional endocytic receptor. Because megalin regulates the intracellular localization of type II sodium-dependent phosphate cotransporter (Np-Ii), it was suggested that megalin plays some roles in the development of nephrocalcinosis.

Methods: We examined whether the blockade of megalin affects the development of nephrocalcinosis in rats. We inhibited the function of megalin by histidine-tagged soluble receptor-associated protein (His-sRAP). Nephrocalcinosis was induced by continuous injection of PTH 1-34 at a dose of 40 μg/kg/day for 48 hours. Rats were randomly divided into three groups: control (C), PTH + vehicle (group P+V), or PTH + His-sRAP group (group P+H).

Results: In comparison with group C, group P+V developed extensive calcification in the renal cortex and in the outer stripe of outer medulla. His-sRAP injection dramatically reduced the calcification (group P+H). Because urinary calcium and phosphate levels in group P+H were higher than those of group P+V, it was suggested that the urine of group P+H contained inhibitory molecules against crystallization. To investigate the underlying mechanisms, we analyzed whether His-sRAP affected the distribution of fetuin-A, a systemic inhibitor against calcium precipitation. Although normal rat kidney did not express mRNA for fetuin-A, immunohistochemistry demonstrated a punctate staining of fetuin-A in the megalin expression in proximal tubular cells. The blockade of megalin by His-sRAP attenuated the fetuin-A staining and resulted in a loss of fetuin-A into the urine. Because fetuin-A prevents a mineral precipitation in a solution of calcium and phosphate, the retention of fetuin-A in the tubular lumen explain the reason, at least partly, why His-sRAP attenuated intratubular crystal formation, hence nephrocalcinosis.

Conclusions: Blockade of megalin by His-sRAP attenuates nephrocalcinosis in rats. Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2240
Clinical Characteristics of Hypercalcemia Due to Adrenal Insufficiency in Patients on Long-Term Hemodialysis
Yukitoshi Sakao, 1 Akiko Kato, 1 Hideo Yasuda. 2 1Blood Purification Unit, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; 2First Department of Medicine, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

Background: Hypercalcemia is rarely caused by adrenal crisis. However, the mechanisms for hypercalcemia due to adrenal insufficiency remain to be determined in patients on hemodialysis (HD).

Methods: We examined the clinical characteristics of hypercalcemia caused by acute adrenal failure in 5 HD patients. We also analyzed bone turnover markers before and after the replacement of glucocorticoid agents.

Results: The patients’ age and vintage on HD were ranged from 44 to 75 years old and from 7 to 32 years respectively. At the onset of hypercalcemia, all patients were anuric, and under critically ill conditions. The causes of adrenal insufficiency were isolated ACTH deficiency in 2 patients, meningococcal septicaemia in 1 patient, and acute arterial obstruction in 2 patients. At the onset of hypercalcemia, all patients were anuric, and under critically ill conditions. The causes of adrenal insufficiency were isolated ACTH deficiency in 2 patients, meningococcal septicaemia in 1 patient, and acute arterial obstruction in 2 patients.

Conclusions: These findings suggest that the adrenal failure-induced hypercalcemia could occur in anuric patients on long-term HD when complicated of acute illness. Increased bone resorption due to adrenal insufficiency without any urinary Ca excretion may be related to the development of hypercalcemia.

SA-PO2241
Modulation of FGF23 by Phosphate Binders in Chronic Kidney Disease Stage 4-5 Predialysis (Lanthamun vs Calcium Carbonate) Sagrario Soriano, Rosario Martorell, Maria Luisa Agiraga, Mariano Rodriguez, Alejandro Martin-Malo, Pedro Aljama-Garcia. Nephrology, Hospital Universitario Reina Sofia, Cordoba, Spain.

Background: Cardiovascular mortality is increased in Chronic Kidney Disease (CKD) patients with serum phosphate (P) concentration in the upper limit of normality. Increased serum concentration of FGF 23 is also independently associated with cardiovascular mortality. The present study evaluates in CKD 4-5 patients on protein restriction (1g/Kg) whether a reasonable dose of calcium carbonate (1.5 g/day) or lanthanum carbonate (1.35 g/day) has an effect on serum FGF23 levels.

Methods: Thirty two patients were selected; after one month wash out they were randomized to receive four months of calcium carbonate or lanthanum carbonate. Patients included had serum P>4 mg/dl, a normal serum calcium, 25(OH) levels >20 ng/ml, no treatment with VDR activators or Cinacalcet. Baseline clinical characteristics of patients were similar in both groups.

Results: Results are presented in the following:

<table>
<thead>
<tr>
<th></th>
<th>Baseline(1)</th>
<th>After treatment(1)</th>
<th>Baseline(2)</th>
<th>After treatment(2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca (mg/dL)</td>
<td>10.4±5.7</td>
<td>10.4±6.1</td>
<td>9.6±3.9</td>
<td>9.6±5.6</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>4.6±0.5</td>
<td>9.6±0.5</td>
<td>4.6±0.5</td>
<td>9.6±0.5</td>
</tr>
<tr>
<td>FGF23 (pg/ml)</td>
<td>195±33</td>
<td>195±33</td>
<td>104±57</td>
<td>104±57</td>
</tr>
<tr>
<td>FE (PO4) (%)</td>
<td>46.2±18.9</td>
<td>46.2±18.9</td>
<td>39.2±8.5</td>
<td>39.2±8.5</td>
</tr>
</tbody>
</table>

Values are mean ± SD are shown, Cr: Creatinine, P: serum phosphate, FE (PO4): fractional excretion of phosphate. *P<0.05 vs same group baseline, **P<0.05 vs calcium carbonate after treatment

Conclusions: In CKD 4-5 patients the administration of 1.5 g of Calcium Carbonate produced a non significant decrease serum phosphate levels and, FGF 23 and FE(PO4) did not change. By contrast, the administration of Lanthanum carbonate (1.35 g) resulted in a decrease in serum phosphate levels together with a reduction of FE(PO4) and plasma concentration of FGF23. In conclusion, in CKD 4-5 the administration of lanthanum carbonate decreases the phosphate load as reflected by the reduction in FE(PO4) and the marked decrease in FGF 23.

SA-PO2242
Preliminary Data of the Italian Multicentric Study on the Prevalence of Vascular Calcifications and Vertebral Fractures in Parathyroidectomised Dialysis Patients (CA PTKX Study) Sandro Mazzaferrro, 1,2 Cardiovascular, Respiratory, Nephrologic and Geriatric Sciences, Sapienza University, Rome, Italy; 3Clinical Metabolism & Trace Element Study Group, Italian Society of Nephrology.

Background: The CA PTKX study is aimed at evaluating, in dialysis patients who received PTH at any time in their life, the biochemical control of dialytic ions (phase I), and the prevalence of aortal calcifications and vertebral fractures (phase II).

Methods: We report here data of the phase I. During 2010, values of Ca, P, AP and PTH and pertinent therapies were asked from 149 dialysis Unit from all over Italy, supplying therapy to 12515 patients (HD = 87.7%; PD = 12.3%).

Results: 123 Patients had data of 528 living PTKX patients (29 on PD). Prevalence of PTH (4.2%), was different between HD (4.5%) and PD (1.9%). Clinical characteristics were: age 58±13 y.old; sex: 56% female, dialysis: since 15±8 years; diabetes: 7%; BMI: 25±4; Systolic BP: 126±21 and Diastolic BP: 73±12 mmHg; on antihypertensive drugs: 75%; on EPO: 50%. With the exception of reduced albumin (3.9±0.5 g/dl), biochemical parameters averaged mostly acceptable values: PTH 182±292 pg/ml; Ca 8.8±0.8 mg/dl; P:4.9±1.3 mg/dl; AP 240±210 μM/l (n.v. 90-270); Hb 11 ±1 g/dl. Prescribed therapies were: Phosphate binders = 87.8% (Ca based: 67.4%; Sevelamer: 50.5%; Lanthanum 11%; Aluminum: 14.9%); vitamin D = 61.8% (oral calcitriol 71.1%; i. calcitriol 6.2%; paricalcitol 22.3%; other D: 1.8%); calcimetic = 12.8%; PTH had been mostly subtotal (54.8%) or total (38%) as compared with total PTH plus autotransplantation. PTH was optimal (150-300) in a limited 17%; high in 19 and low in 64% of the cases. Serum Ca was optimal (8.5-9.5) in 47%, high in 19% and low in 34%. Serum P was optimal (3.5-5.5) in 54%, high in 33% and low in 14%. Significant findings of low Ca, high PTH values are very frequent and associated with low-normal Ca. Evaluation of hard outcomes is mandatory. Funding: Pharmaceutical Company Support

SA-PO2243
Increased Platelet Count and Marrow Fibrosis in Rats with Renal Failure and Secondary Hyperparathyroidism Cheryl P. Sanchez, 1 Kristen R. Friedrichs, 1 Pediatrics, LLUMC, Loma Linda, CA; 2Pathobiological Sciences, UW Sch Vital Med, Madison, WI.

Background: Bone marrow fibrosis (BMF) has been associated with elevated PTH levels seen in primary and secondary hyperparathyroidism (SPHT), and may contribute to bone disease and anemia. Recent studies have shown that factors associated with megalakocytes and platelets such as TGF-b may contribute to BMF.

Methods: To evaluate changes in platelet and megakaryocytes in renal failure and SHPT, 23 male weanling rats (48 ± 3 grams) underwent a 2-stage 5/6 nephrectomy (NX, N=11) or sham NX (C, N=7). Rats were fed standard diet (NX, C) or high phosphorus diet, 1,25(OH)2D3 (N=7) or high phosphorus diet, 1,25(OH)2D3 (N=7). At the end of 4 weeks, blood was obtained for CBC, PTH and creatinine levels. The femur was used for marrow analysis, sternum for marrow histology and iliac bone for trichrome stain.

Results: There was a marked decrease in FGF 23.

Conclusions: These findings suggest that the adrenal failure-induced hypercalcemia could occur in anuric patients on long-term HD when complicated of acute illness. Increased bone resorption due to adrenal insufficiency without any urinary Ca excretion may be related to the development of hypercalcemia.
to Nx-C, 0.8±0.4 and 0.0, and C, 0.8±0.7 and 0.0, p<0.01. Marrow core analysis showed similar megakaryocyte score in all groups. Qualitatively, trichome staining was present only in the marrow of Nx-P. Platelet count correlated with reticulin, R=0.7, p<0.0003 and PTH, R=0.6, p<0.002. There was an inverse relationship between Hgb and platelet count, R=−0.5, p<0.009.

**Conclusions:** An increase in platelet count may contribute to BMF in advanced SHPT without any changes in megakaryocyte numbers. Further studies are needed to determine the alterations in growth factors associated with platelet and megakaryocytes in renal failure.

**SA-PO2244**

**Skeletal FGF-23 and DMP1 Expression Increase during Treatment of Secondary Hyperparathyroidism R.C. Pereira,1 Harald Jupeppner,2 Barbara Gales,1 Isidro B. Salusky,1 Katherine Wesseling-Perry,1 **1Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2Endocrine Unit, Massachusetts General Hospital, Boston, MA.**

**Background:** Skeletal FGF-23 expression correlates with plasma FGF-23 levels and vitamin D sterol therapy increases plasma FGF-23 levels in dialysis patients; however, it is unknown how therapy affects skeletal expression of FGF-23 and its regulator DMP1.

**Methods:** 10 dialysis patients (6M, 4F), age 16.9 ± 0.7 years, with secondary hyperparathyroidism underwent bone biopsy before and after 8 months of therapy with vitamin D sterols and phosphate binders. Biochemical values, bone histomorphometry, and skeletal expression of FGF-23 (determined by immunohistochemistry and quantified by Ariel scanning) were measured at baseline and after therapy (Table 1. *p<0.05*). Plasma FGF-23 values were determined by the 2nd generation C-terminal assay (Immunopectics).

**Results:** Plasma FGF-23 increased by 226 ±111% from baseline while other biochemical parameters did not change. Bone FGF-23 and DMP1 expression increased by 340 ± 155% (p=0.05) and 134 ± 45% (p<0.05), respectively.

**Conclusions:** Therapy with vitamin D sterols increases skeletal FGF-23 as well as DMP11 expression in dialysis patients with secondary hyperparathyroidism, suggesting that dysregulation of osteocyte function may be exacerbated by current therapeutic strategies.

**SA-PO2245**

**Outcomes of a Large Series of Parathyroidectomies Performed in a Single Centre Satish Babu Ramakrishna. Renal Medicine, University Hospital Birmingham, Birmingham, United Kingdom.**

**Background:** Secondary Hyperparathyroidism (SHPT) is an important complication of renal failure and its management remains a challenge. Total parathyroidectomy (PTX) is widely used but questions regarding long term outcomes remain unanswered. Low parathormone (PTH) levels and adynamic bone disease following PTX are considered significant risk factors for fractures.

**Methods:** Between 2000 to 2007, 131 total parathyroidectomies were performed without re-implantation at a single centre by a single operator. Clinical information and biochemistry data were obtained along with fracture data. Calcium, phosphate and PTH were recorded pre-operatively and at 1, 6 and 12 months after PTX.

**Results:** Mean age at the time of PTX was 47 years. Time on RRT before PTX varied between 20 to 100 months with an average of 65 months. Mean follow up period is 3.5±2.0 years. The PTH fell significantly from a median of 995pg/ml pre surgery to 79pg/ml at 1 month and remained low at 6pg/ml at 6 months and 33pg/ml at 12 months. The average calcium level pre-operative was 2.6mmol/L (SD 0.24mmol/L) which dropped to 2.28mmol/L (SD 0.24mmol/L) and remained low at 6.0pg/ml at 6 months and 33.0pg/ml at 12 months. The average calcium and phosphate levels did not change. Bone FGF-23 and DMP1 expression increased (PTX) were measured at baseline and after therapy (Table 1. *p<0.05*). Plasma FGF-23 values were determined by the 2nd generation C-terminal assay (Immunopectics).

**Conclusions:** Since this is only one case and the preliminary results report, this hypothesis needs to be proven and further investigations to understand the relationship and if there is; importance of ZFYVE on bone turnover.

**SA-PO2246**

**Serum Proteomics Analysis of in a Hemodialysis Patient with Secondary Hyperparathyroidism before and after Parathyroidectomy Operation Hr Yegenoglu,1 Murat Kasap,1 Gurker Akpinar,1 Sibel Bek,1 Sara Yamaner,2 **1Internal Medicine, Nephrology, Kocaeli University Medical School, Kocaeli, Eimin, Turkey; 2Medical Biological, Kocaeli University Medical School, Kocaeli, Eimin, Turkey.**

**Background:** Systemic bone and mineral disturbances co exist in CKD patients from the very early stages and effects life quality, morbidity and mortality. Uremic bone disease shows heterogeneity in terms of bone turnover; while most patients suffer with high turnover (secondary hyperparathyroidism-HPT) the others have low turnover (adynamic) bone disease. It is important to know the bone turnover to apply appropriate treatment to these patients.

**Methods:** We analyzed serum proteome profile pre and post-op of parathyroidectomy operation in a 33 year old male hemodialysis(HD) patient with secondary HPT. We aim to detect some changes in proteome profile correlated PTH level and may be used as a marker to determine the bone turnover. Less abundant protein fractions obtained by microrotofor fractionation were applied to two D-gel electrophoresis on the pH 3-10 IPG strips, three spots took the attention which appeared after parathyroidectomy and they were cut from the gel and were subjected to MALDI-TOF TOF analysis

**Results:** Two of the spots were identified with high confidence and one of them belonged to a protein defined as a zinc finger- FYVE containing domain (ZFYVE). Among ZFYVE Osterix was reported to be involved in bone turnover. This protein might be an Osterix homologue and related to bone turnover.

**Conclusions:** Conclusions: Since this is only one case and the preliminary results report, this hypothesis needs to be proven and further investigations to understand the relationship and if there is; importance of ZFYVE on bone turnover.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
13±3 g/dL, compared to C, 15±0.4 g/dL, p<0.05. Estimated reticulocyte count increased in No-Phos, 2.6±1.4 x10^11/L, compared to No-C and C, 1.4±0.4 x10^11/L and 1.0±0.3 x10^11/L, p<0.01. No-Phos had increased polychromatophil score, 2.0±1.4, compared to No-C and C, 1.0±0.7, p<0.01, denoting enhanced peripheral regeneration. When corrected for tibial length, total erythrocyte counts in the marrow were similar in all groups. EMH was more pronounced in the liver to No-C and C, 3.3±1.9 versus No-C and C, 0.29±0.4 and 0.06±0.05, and in the spleen, 2.0±0.4 in No-Phos versus No-C and C, 1.1±1.4 and 0.3±0.4, p<0.01. Reticulin staining increased in the sternum and tibia of No-Phos, 2.8±0.4 compared to No-C and C, 0.8±0.4, p<0.01. Hgb correlated with body length, R=0.7, p<0.005. There was a threshold effect between Hgb and PTH, R=0.0001.

Conclusions: Findings in the current study suggest that response to anemia in renal failure and severe SHPT may be limited by reticulin in the marrow, and an increase in peripheral regeneration may be due to an enhancement of extramedullary hematopoiesis in the liver and spleen.

SA-PO2248

Decrease of Serum Sphingosine-1-Phosphate Levels in Hemodialysis Patients with Secondary Hyperparathyroidism Treated with Cinacalcet

Keitiro Yokoyama, Ichiro Okikado, Takizo Iwamoto, Mari Ishida, Mitsuyoshi Urashima, Tatsuo Hosoya. 1 Division of Kidney and Hypertension. Department of Internal Medicine, The Jikei University School of Medicine, Tokyo, Japan; 2 Core Research Facilities, Division of Biochemistry, The Jikei University School of Medicine, Tokyo, Japan; 3 Jinyukai Kitasato Hospital, Asahikawa, Japan; 4 Division of Molecular Epidemiology, The Jikei University School of Medicine, Tokyo, Japan.

Background: Both cinacalcet, a calcium sensing receptor agonist, and sphingosine-1-phosphate (S1P) affect bone mineral homeostasis (ishi M. Nature 2009). Consequently, we investigated the effect of cinacalcet on serum levels of S1P in hemodialysis (HD) patients with secondary hyperparathyroidism (SHPT).

Methods: Thirty-four chronic HD patients with SHPT for more than 3 months were enrolled in this prospective intervention study. We measured serum S1P level by liquid chromatography-tandem mass spectrometry before and after treatment with cinacalcet.

Results: Serum S1P decreased significantly after 6 month-treatment with cinacalcet in all patients (897.5±424.1 to 251.9±46.4 nmol/l, p<0.001). Serum S1P level showed no significant associations with age, gender, HD vintage, serum creatinine, urea, calcium, phosphorus, intact parathyroid hormone, alkaline phosphatase, tetrathionate-resistant acid phosphatase 5b or hematocrit.

Conclusions: No significant associations with age, gender, HD vintage, serum creatinine, urea, calcium, phosphorus, intact parathyroid hormone, alkaline phosphatase, tetrathionate-resistant acid phosphatase 5b or hematocrit.

Funding: Government Support - Non-U.S.

SA-PO2249

Vitamin E Decreases Vascular Calcification in Obese (Zucker fa/fa) Uremic Rats

Alan Peralta-Ramírez, 1, 2 Carmen Pineda, 3 Fatima Guererro, 3 Maria Encarnacion Rodriguez Ortiz, 4 Elisa Diez de Castro, 5 Addy Rosa Montes de Oca Gonzalez, 6 Yolanda Almaden Peña, 7 Ignacio Lopez, 8 Escuela de Medicina Veterinaria, UNAN-Leon, Nicaragua; 9 Fundacion Carolina, Spain; 10 Dpto. Medicina y Cirugia Animal, Universidad de Cordoba, Spain; 11 IMIBIC, Cordoba, Spain.

Background: Vascular calcification (VC) is a frequent complication in patients with chronic kidney disease (CKD) and diabetes. Hyperphosphatemia is a major pathogenic factor for uremic VC. While chronic inflammation and oxidative stress seem to be related to both uremia and diabetic VC. In addition, mitochondrial ROS-NFκB signaling has been recently shown to be involved in phosphate(V) induced VC. According that, antioxidants, such as vitamin E (vit. E), may protect against VC. We hypothesized that a diet with high vit. E content may decrease VC in in vivo model of metabolic syndrome and uremia.

Methods: Obese Zucker (fa/fa) rats (n=12) were 56 nephrectomized and treated with calcitriol (80ng/kg ip/3 times per week) to induce VC. Rats were randomly allocated in two groups that were fed a diet containing Ca=0.6%, P=0.9% supplemented with normal Calcium (Pre) or supplemented with Calcium (Post) 5g/kg (vit.E=30000 mg/kg) of amount of vit. E. After 21 days, rats were euthanized to obtain blood and aortic tissue.

Results: Plasma level of insulin, TNFα and FGF21, and Aortic Ca and P content were decreased in vit.E group (P<0.05 vs Control).

<table>
<thead>
<tr>
<th>Determination</th>
<th>Control</th>
<th>Vit. E</th>
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<tbody>
<tr>
<td>Ca (mmol/L)</td>
<td>1.1±0.2</td>
<td>1.31±0.02</td>
</tr>
<tr>
<td>P (mg/dL)</td>
<td>10.9±1.8</td>
<td>10.3±1.4</td>
</tr>
<tr>
<td>Cac (mg/g tissue)</td>
<td>205.9±3.6</td>
<td>(85±18.2)</td>
</tr>
<tr>
<td>Insulin (µg/mL)</td>
<td>10.0±1.6</td>
<td>6.9±0.6*</td>
</tr>
<tr>
<td>TNFα (µg/mL)</td>
<td>82.1±4.8</td>
<td>70.3±1.7*</td>
</tr>
<tr>
<td>leptin (µg/mL)</td>
<td>81.9±3.9</td>
<td>82.9±4.3</td>
</tr>
<tr>
<td>Adiponectin (µg/mL)</td>
<td>2.2±0.4</td>
<td>3.1±0.6</td>
</tr>
<tr>
<td>FG21 (pg/mL)</td>
<td>2827±300</td>
<td>501±199*</td>
</tr>
<tr>
<td>Aortic Ca (mg/g tissue)</td>
<td>5.4±1.42</td>
<td>1.4±0.07*</td>
</tr>
<tr>
<td>Aortic P (µg/tissue)</td>
<td>2.5±0.89</td>
<td>1.0±0.02*</td>
</tr>
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</table>

Values are means ± SE. *P< 0.05 vs. Control.

Conclusions: In the present model of obese uremic rats, high dietary intake of vit.E decreases VC without changing plasma Ca, P and creatinine. In addition, supplementation with vit.E seems to improve metabolic syndrome (decreased FGF21 and insulin) and to reduce inflammation associated to uremia and metabolic syndrome (decrease in TNFα).

Funding: Government Support - Non-U.S.

SA-PO2250

The Association of Fetuin-A with Coronary Artery Calcification in Community-Living Persons: The Multi-Ethnic Study of Atherosclerosis

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Background: Fetuin-A inhibits arterial calcium deposition in vitro. Lower levels associate with CAC in ESRD. The association of fetuin-A with CAC in other settings is unknown.

Methods: We evaluated the association of fetuin-A with CAC prevalence and severity at baseline, and CAC incidence over 3.2 years among 2,457 participants in MESA. Associations were evaluated using relative risk, linear, and Poisson regression, respectively.

Results: Mean eGFR was 94±21 ml/min/1.73m². 1,200 (49%) had CAC at baseline, and 272 developed incident CAC during follow-up. There was a threshold effect at the lowest fetuin-A quartile with CAC prevalence. In models adjusted for demographics, and 272 developed incident CAC during follow-up. There was a threshold effect at the lowest fetuin-A quartile with CAC prevalence. In models adjusted for demographics, traditional CVD risk factors, and kidney function, the lowest fetuin-A quartile had 7% (95% CI 1-13%) greater CAC prevalence compared to quartiles 2-4. Similar associations were observed with CAC severity; a more linear association.

Distribution of Coronary Artery Calcification Scores by Fetuin-A Quartiles

<table>
<thead>
<tr>
<th>Quartile</th>
<th>CAC Score</th>
</tr>
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<tbody>
<tr>
<td>0-0.4</td>
<td>0</td>
</tr>
<tr>
<td>0.4-0.6</td>
<td>100</td>
</tr>
<tr>
<td>0.6-0.8</td>
<td>101-300</td>
</tr>
<tr>
<td>0.8-1.0</td>
<td>&gt;300</td>
</tr>
</tbody>
</table>

Each SD (0.1 g/L) lower fetuin-A was associated with a 12% (95% CI 3-21%) greater CAC severity in adjusted models. There was no association of fetuin-A with incident CAC (RR per SD lower 1.06; 95% CI 0.95-1.19). Associations were similar by sex, race/ ethnicity, and diabetes status.

Conclusions: Fetuin-A is inversely associated with CAC severity among community-living individuals without severe CKD.

Funding: Other NIH Support - NHLBI, Veterans Administration Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

636A
SA-PO2251

Restoration of Bone Mineralization by Cinacalcet or Parathyroidectomy Is Associated with a Significant Reduction in Calcitriol-Induced Vascular Calcification in Uremic Rats

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Background: The present study investigated to which extent high dose cinacalcet-induced vascular calcifications in uremic rats could be prevented in bone histomorphometry.

Methods: Five groups were studied: sham-operated controls (n=7); subtotally nephrectomised (SNX) uremic animals (U; n=12); U + cinacalcet (ViItd) (0.05 pg/kg/day), (n=12); U + ViItd + Cin (CaCl2) (10 mg/kg/day), (n=12); U + ViItd + Ptx, (n=12). Treatment started 2 weeks after SNX and continued for the next 14 weeks.

Results: Cinacalcet treatment went along with a development of distinct vascular calcification which was reduced by >50% in both the Cin treated and Ptx animals. Compared to control animals, those of the U group, Ptx, Cin and control treatment were associated with a significant increase (p<0.05) in bone area comprising >60% of the total tissue area. However, whereas excessive wound bone accompanied by a dramatically increased osteoid width/area was seen in the U+ViItd group, Cin treatment and Ptx resulted in a significant decrease in the double labelled perimeter and normalization of both the bone formation rate and amount of osteoid (p<0.05) which was accompanied by a significant reduction in serum PTH levels.

Conclusions: These data indicate that the excessive vascular calcification in ViItd treated uremic rats is accompanied by less efficient calcium (Ca) and phosphorus (P) incorporation in bone; a lesion which is reversed by Cin treatment and Ptx corresponding to a decrease in Ca and P deposition in vascular calcifications.

SA-PO2252

The Relationship between FGF-23 and Markers of Cardiovascular Lesions Wladyslaw Sliwowszczyk,1 Marcin Krzanowski,2 Danuta Fedak,2 Marek Kuzniowski,1 Maria Kapusta,2 Beata Kusnierek-Cabala,2 Pawlina Dorota,3 Bogdan Solnica.2 1Department of Nephrology, Jagiellonian University, Collegium Medicum, Cracow, Poland; 2Department of Clinical Biochemistry, Jagiellonian University, Collegium Medicum, Cracow, Poland; 3Department of Medical Diagnostics, Jagiellonian University, Collegium Medicum, Cracow, Poland.

Background: FGF-23 is secreted by osteocytes and influence vitamin D and phosphate metabolism as well as bone mineralization. It rise following progressive loss of renal function and the highest levels were observed in dialysis population.

The aim of the study was to investigate the relationship between FGF-23 and markers of lean heart lesion (NT-proBNP), vitamin D (25-OH D3) and serum levels of NT-proBNP may indicate that patients with high FGF-23 levels had higher risk of mortality.

Methods: Serum levels of FGF-23, NT-proBNP (n=170), 25-OH D3 (n=170) and calculated aortic calcifications (CaCs) were assessed by multi-slice spiral computed tomography (MSCT).

Results: The obtained results indicate elevated plasma concentrations of FGF-23 in ESRD patients (12086 vs 12094). The mean level of FGF-23 correlates well with NT-proBNP (r=0.72) and serum levels of 25-OH D3 were measured by ELISA technique while NT-proBNP were determined based on immunochromimunessay. CCA-IMT was evaluated by B-mode ultrasound of carotid arteries using Acouton 128 XP apparatus. Coronary artery calcification score (CaSc) was assessed by multi-slice spiral computed tomography (MSCT).

Conclusions: In the present study, we extended the previous data by evaluating the relationship between FGF-23 and markers of vascular calcification. These findings suggest that FGF-23 may be a marker of cardiovascular disease.

SA-PO2253

Persistently Low iPTH Level Predicts Progression of Aortic Arch Calcification in Incident Hemodialysis Patients Soo Bong Lee,1 Harin Rhee,1 Geun Do Kwon,2 Sung Ho Kwon,1,2 Jungho Lee,1 Suha Al-Lamee,1 Il Young Kim,1 Sang Heon Song,1 Soo Beng Lee,1,2 Him Soo Kwak,1 1Division of Nephrology, Internal Medicine, Pusan National University Hospital, Busan, Korea; 2Division of Nephrology, Internal Medicine, Yonsei University College of Medicine, Yonsei, Korea.

Background: The aim of this study is to determine the relationship between adynamic bone disease and aortic arch calcification in incident hemodialysis patients.

Methods: From January to December 2008, a total of 94 incident hemodialysis patients were enrolled, whose iPTH level was lower than 300pg/dL throughout the initial one year. They were divided into three groups according to iPTH changing pattern during the initial first year (Group 1: iPTH level persistently below 150pg/dL; Group 2: iPTH level moves up or down; Group 3: iPTH level persistently over 150pg/dL). Aortic arch calcification was measured on posterior-anterior plain chest X-ray using the specific scale.

Results: A total of 94 patients were enrolled [Group 1(N=39), Group 2(N=32), Group 3(N=23)]. Median follow up period was 2.5 years. The prevalence of baseline aortic arch calcification was highest in the persistently low iPTH group (respectively, Group 1, 19.1%; Group 2, 7.1%; Group 3, 3.2%; P=0.040). In a multivariate logistic regression analysis, age and persistently low iPTH independently contributed to progression of aortic arch calcification. The cumulative incidence of calcification progression was most frequently found in the persistently low iPTH group (respectively, Group 1, 19.1%; Group 2, 7.1%; Group 3, 3.2%; P=0.040). But, we couldn’t find any association between aortic arch calcification and mortality.

Conclusions: Persistently low iPTH and age was an independent risk factor for progression of aortic arch calcification in incident hemodialysis patients.

SA-PO2254

Circulating Vascular Calcification Inhibitors Are Associated with Mortality in Incident Dialysis Patients Julia J. Scialli,1 Stephen M. Sozio,1 Pooja C. Oberai,1 Bernard G. Juar,1 Tariq Shahi,2 Laura C. Plantinga,1 Neil R. Pogue,2 Josef Coresh,3 Wen Hong Linda Kao,1 Rulan S. Pahrek,1,3 Johns Hopkins University; 1University of California San Diego; 2University of Toronto.

Background: Vascular calcification is common among diabetics and dialysis patients and associated with increased mortality. We evaluated whether circulating calcification inhibitors osteoprotegerin (OPG), osteopontin (OPN), bone morphogenic protein-7 (BMP7) and fetuin-A were associated with mortality in 602 incident dialysis participants from the Choices for Healthy Outcomes in Caring for ESRD Study.

Methods: Circulating calcification inhibitors were measured in blood samples collected <6 months after dialysis initiation. Risk of mortality associated with tertiles of calcification inhibitors was modeled using Cox proportional hazards models adjusted for demographic factors, comorbidities, serum phosphorus, and corrected serum calcium. Prespecified interactions were tested with diabetes.

Results: Mean age of study participants was 58 yrs; 35% were African American, 57% had diabetes and 6% were treated with peritoneal dialysis. There were 423 deaths over a median follow-up of 3.3 years. Higher OPG and lower fetuin-A were associated with increased mortality overall (p<0.02 for both) and in stratified models, highest tertiles of OPG, BMP7, and OPN were associated with mortality among non-diabetics, but not among diabetics.

Conclusions: In the present study, we extended the previous data by evaluating the relationship between FGF-23 and markers of vascular calcification. These findings suggest that FGF-23 may be a marker of cardiovascular disease.

Bone and Mineral Disease: Ca/Mg/P04, Stone Disease - II

SA-PO2255

Overexpression of Muscular Related Proteins and Downregulation of Lamin A/C Might Be Involved in the Protection Against Vascular Calcification Pablo Roman-Garcia,1 Natália Carrillo-López,2 Sara Panizo,1 Manuel Navés,1 Jesus E. Fernández-Martín,1 Jorge B. Cannata-Andia.1 Bone and Mineral Research Unit, Hospital Universitario Central de Asturias, IRSSN-FRIAT, RedinRen, Universidad de Oviedo, Oviedo, Asturias, Spain.

Background: A variable but not negligible proportion of CKD patients seem to be protected to develop vascular calcification; the causes are unknown. Molecular mechanisms modifying the expression of muscle-related or bone-related proteins may explain this antagonistic profile. Among them, defects associated to lamin A have been implicated modifying the expression of muscle-related or bone-related proteins may explain this protective advantage. The aim of this study was to use proteomic and gene expression analysis. To confirm the results, in vitro studies were performed: Lamin A expression was knocked down in vascular smooth muscle cells (VSMCs) using siRNA, then VSMCs were cultured using normal or calcifying media (2nM Ca as an apoptosis model) for 2 days. CBF1/Runx-2 and Lamin A gene expression were measured by qRT-PCR.

Results: The proteomic analysis revealed that the aortas from rats that after 20 weeks with calcifying stimuli did not develop VC, showed a significant upregulation of tranexgulin, tropominosin and actin (all well known muscle-related proteins) and significant

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.
downregulation of lamin A. In addition, further experiments in VSMCs, knocking down Lamin A by siRNA showed a reduction in CBFAl despite the stimulus of the calcifying medium.

**Conclusions:** The study demonstrates for the first time that muscle related proteins overexpression and Lamin A downregulation could be involved as part of the mechanisms triggered to protect against vascular calcification.

**Funding:** Government Support - Non-U.S.

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**SA-PO2256**

The Reduction of Vertebral Bone Density (VDB) Is Associated with Progression of Coronary Calcification in Pre-Dialysis Patients

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**Background:** Vascular calcification and low bone density are common findings in chronic kidney disease (CKD) patients and are associated with mortality in this population. We aimed to investigate whether the reduction of VDB was associated with progression of coronary calcification in pre-dialysis patients.

**Methods:** In a 24-month prospective study a sample of 72 nondialyzed CKD patients (57.6±10.3yars, 62%male, creatinine clearance 39.2±15.5ml/min/1.73m²), underwent multi-slice computed tomography in order to determine coronary calcium score and thoracic VBD. The measurements were performed at baseline and at the end of the study.

**Results:** At the baseline, coronary calcification was observed in 33 patients (46%) [median 316AU(range 123–861.5AU)-calcified group], these patients were older, mostly male and had lower VBD, compared to non-calcified patients. There were no differences between groups regarding renal function, proteinuria, lipid profile, ionized calcium, phosphorus, alkaline phosphatase or IP7 levels. During the follow up, although the mean values of VBD did not change in both groups, VBD decreased in 61% and 48% of the patients (non-calcified and calcified group respectively; p<0.27). Thirty out of 33 (91%) patients of the calcified group showed progression of coronary calcification which was inversely related to the change of eGFR (r=-0.38; p=0.039), and to the relative change of VBD (p<0.56; p=0.001). In multivariate regression analysis, adjusted for age, sex, diabetes and eGFR, calcification progression was independently associated to the relative change of VBD (p<0.01; βcoefficient=-0.419, 95%CI,0.036 to 0.004). Despite reduction of eGFR, non-calcified patients have not developed of coronary calcification.

**Conclusions:** This study showed that reduction of VBD is associated with progression of coronary calcification in calcified pre-dialysis patients but not with the development of coronary calcification in the non-calcified ones.

**Funding:** Government Support - Non-U.S.

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**SA-PO2257**

A Novel Model of In-Vitro Osteocytogenesis

Deborah Mattioli,1 Piergiorgio Messa,1 Alessandro Corbelli,1 Massami Ikehata,1 Cristina Zennaro,1 Silvia Armelloni,1 Min Li,1 Laura Giardino,1 Maria Pia Rastaldi.1 'Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D’Amico, Milan, Italy; 1Renal Physiopathology Laboratory, University of Trieste, Italy.

**Background:** Though recent research is more and more highlighting the importance of osteocytes as central players of bone and systemic mineral metabolism, molecular and functional knowledge of osteocyte properties are still incomplete, mostly due to limited availability of in vitro models.

Aim of our study was to find a simple, reproducible method to obtain cultured osteocytes.

**Methods:** MC3T3-E1 cells were cultivated in α-MEM medium. At 80% confluence, medium was added ascorbic acid/glycerol phosphate (AA-GP) for 5 days, then cells were divided into 3 groups. The 1st group continued in the same conditions (AA-GP). Melatonin (50μM) or All-Trans Retinoic Acid (10μM, ATRA) were respectively added to the 2nd and the 3rd group. Cells were studied from 4 to 25 days.

**Conclusions:** The study demonstrates for the first time that muscle related proteins overexpression and Lamin A downregulation could be involved as part of the mechanisms triggered to protect against vascular calcification.

**Funding:** Government Support - Non-U.S.

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**SA-PO2258**

Experimental CKD-MBD: A Comparison between 5/6 Nephrectomy and Adenine Models

Guaraciaba O. Ferrani,1 Juliana C. Ferreira,1 Raquel T. Cavallari,1 Katia R. Neves,2 Luciene M. dos Reis,3 Wagner V. Dominguez,3 Elizabeth M. Oliveira,4 Fabiana G. Graciotti,5 Jutta Passlick-Deetjen,6 Rosa M. Moyses,1 Vanda Jorgetti.1 'São Paulo University, Brazil; 2Fresenius.

**Background:** Experimental models are important to understand the physiopathology, as well as the effects of therapy of some diseases. Currently, two models are used to evaluate CKD-MBD: 5/6 nephrectomy (NX) and adenine-induced kidney disease (AD). However, they have never been compared using animals in similar housing conditions. To that end, we compared these two models, focusing on biochemical and bone histomorphometric findings.

**Methods:** Wistar rats, fed with the same diet, were divided into 3 groups: Control (C), NX and AD(diet + adenine). After 9 wks, animals were sacrificed and biochemical and histomorphometric analyses were performed.

**Results:** NX group presented a greater final weight (FW) and blood pressure (BP) than the AD group. No differences were seen in serum Creatinine (Cr), phosphate (P), ionized calcium (iCa), PTH and FGF23. However, AD rats had a higher FeP and presented a more severe form of high-turnover bone disease.

**Funding:** Private Foundation Support, Government Support - Non-U.S.

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**SA-PO2259**

The Role of a Skeletal Anabolic in the Early CKD-MBD of Stage 2 CKD

Keith A. Hruska,1 Min Li,1 Laura Giardino,1 Maria Pia Rastaldi,1 'Renal Research Laboratory, Fondazione IRCCS Policlinico & Fondazione D’Amico, Milan, Italy.

**Background:** In CKD, osteoblastic differentiation of cells in the neointima causes calcification of atherosclerotic plaques. Both arteriosclerotic and medial vascular calcification (MVC) are important distinguishing features of CKD-MBD. However, the exact timing of when MVC begins in stage 2 CKD patients as shown by HrQCT (Bacchetta J et al JBMJR 2009), increased FGF-23 (Pereira RC et al Bone 2009), and decreases in vascular smooth muscle (VSM) contractile phenotype markers (Kokubo et al, JASN 2009). Abnormalities of bone remodeling and the VSM phenotype before abnormalities of mineral metabolism in the early phases of the CKD-MBD make it necessary to define a regulatory system underlying the condition. Here we tested the hypothesis that CKD induces the CKD-MBD in early CKD and that the skeleton participates in stimulation of VC.

**Methods:** Ldlr–/– mice fed high fat diets were subjected to renal cortical electrocautery and contralateral nephrectomy at 12 weeks of age to produce CKD-MBD, euthanasia was at 22 or 28 weeks. Treatment with vehicle, DKK1 mab (30 mg/Kg tiw IP), or CaAc (3% in drinking water) was initiated 9 wks prior to sacrifice. The study was divided into two groups: (1) CKD-MBD and (2) controls.

**Results:** A relatively mild reduction in the glomerular filtration rate was seen with 76% of normal (GFR [ml/min/kg] at 22 weeks of age (stage 2 CKD)). BUN, Ca, Pi and PTH levels were normal. CKD stimulated and DKK1 mab inhibited the CKD stimulated

**Funding:** Government Support - Non-U.S.
accumulation of aortic Ca levels (0.52±0.19 (CKD) vs. 0.26±0.14 (wt), 0.32±0.23 (sham) and 0.4±0.1 (DDK1 mab) mg/dry weight, p<0.05). CaAc did not affect CKD stimulated VC. CKD decreased aortic smooth muscle actin and the DKK1 mab had no effect. Micro CT analysis of femurs revealed cortical bone loss prevented by the DKK1 mab. Dickkopf-1 (Dkk1), a circulating inhibitor of bone formation, levels were increased from normal wt 1868±772 to sham 1650±882 to 3132±1590 pg/ml, p<0.01. Increased serum levels of FGF-23 were also detected but not decreased by the DKK1 mab (554±263 (CKD) vs. 242±56 (wt), 309±64 (sham) and 590±183 (DDK1 mab).

Conclusions: We conclude that the skeleton is affected in response to kidney injury and participates in the stimulation of VC.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2260
Upregulation of Osteocyte Fibroblast Growth Factor 23 with Improvement of the Uremic Mineralization Defect Induced by the Vitamin D Analog Paricalcitol
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Background: It has been shown that 1,25(OH)2D3 stimulates both osteoblast and osteocyte fibroblast growth factor 23 (FGF23) production, but little is known on the skeletal response to FGF23 in chronic kidney disease, particularly following paricalcitol (Pc) treatment.

Methods: We evaluated the bone expression of FGF23 by immunohistochemistry with simultaneous quantitated bone histomorphometry in sham (S) and 5/6-nephrectomized rats after treatment during 8 weeks with vehicle (U), Pc (IP 0.3 µg/kg/thrice weekly), or enalapril (E) (5 mg/kg/day in drinking water).

Results: Pc and E attenuated renal insufficiency when compared to U (p<0.05). Plasma calcium (Ca) was higher in Pc and phosphorus (P) was similar in all groups (Table). Bone histomorphometry (Table) revealed decreased eroded surfaces (ES/BS) and improved mineralization after Pc. In contrast, E showed less attenuated OS/BS and ES/BS (p<0.05 vs.Pc). Osteocyte FGF23 expression was markedly increased in U when compared to S or E, and even more prominent following Pc.

Biochemical and Bone Histomorphometric Parameters

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>Uremia</th>
<th>Enalapril</th>
<th>Paricalcitol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.58 ± 0.08</td>
<td>1.67 ± 0.75*</td>
<td>0.76 ± 0.06**</td>
<td>0.76 ± 0.14**</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>6.72 ± 0.49</td>
<td>7.31 ± 1.89</td>
<td>6.34 ± 1.7</td>
<td>6.46 ± 1.83</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>7.34 ± 0.28</td>
<td>8.58 ± 0.78</td>
<td>7.68 ± 0.60</td>
<td>9.9 ± 1.74ọ</td>
</tr>
<tr>
<td>OS/TV %</td>
<td>1.78 ± 0.35</td>
<td>3.3 ± 2.9</td>
<td>2.0 ± 0.2</td>
<td>2.65 ± 2.3</td>
</tr>
<tr>
<td>BS/TV %</td>
<td>15.4 ± 8.1</td>
<td>8.5 ± 2.8</td>
<td>7.8 ± 3.8</td>
<td></td>
</tr>
<tr>
<td>ES/BS %</td>
<td>5.5 ± 0.9</td>
<td>22.1 ± 7.9</td>
<td>24 ± 6</td>
<td>14 ± 4c</td>
</tr>
</tbody>
</table>

*p<0.01 vs. S; **p<0.05 vs. U; †p<0.05 vs. En and ♦p<0.01 vs. sham; †p<0.01 vs sham and p<0.05 vs Pc; ♦p<0.05 vs S; p<pc; 0.05 vs En

Conclusions: Pc therapy upregulated bone FGF23 expression and partially improved the mineralization defect without changes in P levels, suggesting that FGF23 in addition to Pc modulates bone mineralization in uremia.

Funding: Clinical Revenue Support

SA-PO2261
Plasma Serotonin Affects Bone Metabolism in Hemodialysis Patients
Theodoros Eleftheriadis, Vasillios Linkopoulos, Georgia Antoniadi, Ioannis Stefanidis. Nephrology, Medical School, University of Thessaly, Larissa, Greece.

Background: Serotonin receptors are present in osteoblasts and osteoclasts and many experimental studies showed that peripheral serotonin affects bone metabolism. In the present study the effect of plasma serotonin on bone metabolism was evaluated in chronic kidney disease, particularly following paricalcitol (Pc) treatment.

Methods: We evaluated the bone expression of serotonin by immunohistochemistry with simultaneous quantitated bone histomorphometry in sham (S) and 5/6-nephrectomized rats after treatment during 8 weeks with vehicle (U), Pc (IP 0.3 µg/kg/thrice weekly), or enalapril (E) (5 mg/kg/day in drinking water).

Results: Serotonin did not differ significantly between HD patients and healthy volunteers (8.50±3.12 ng/ml vs 6.44±1.54 ng/ml, p=n.s.). All evaluated markers of bone metabolism and PTH were much higher in HD patients. Serotonin was positively related to all evaluated markers of bone metabolism in HD patients, and independently of PTH, which was also positively related to all evaluated markers of bone metabolism.

Serotonin was negatively related to the patients’ age. Serotonin, was much lower in diabetic HD patients (6.42±1.99 ng/ml vs 10.25±6.30 ng/ml, p=0.044), which was the case for OCN, P1NP and β-CTx as well.

Conclusions: Serotonin increases both bone formation and bone resorption in HD patients. The negative relation of serotonin to patients’ age as well as its lower levels in diabetic HD patients indicate that low plasma serotonin may contribute to the higher incidence of low-turnover bone disease that characterizes old and diabetic HD patients.

SA-PO2262
Sevelamer Hydrochloride Inhibits Aortic Calcification Regardless of Serum Phosphorus Concentrations
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Background: Sevelamer hydrochloride, calcium-free phosphate binder, reduces vascular calcification by preventing episodes of hypercalcemia and/or hyperphosphatemia in the patients with the CKD. However, the mechanism of this effect on vascular calcification is quite obscure. Pleiotropic effects of sevelamer hydrochloride have been focused. Hyperphosphatemia, high serum FGF23 levels as well as vascular calcification are observed in the patients with the CKD, which are also characteristic of klotho mice. Therefore, we examined the pathophysiology of the influences of sevelamer hydrochloride on vascular calcification using klotho mice.

Methods: Klotho mice and control mice(C57BL/j6) were fed by normal diet group(NPD: Pi 0.6 %), or low-Pi diet group(LPD : Pi 0.2 %), or NPD with Sevelamer hydrochloride respectively. After 1 week, all mice were sacrificed, then, biochemical analysis of serum and urine was carried out.

Results: Klotho mouse had decreased serum phosphorus concentrations by LPD intake. At the NPD intake with sevelamer hydrochloride administration, in klotho mice, serum phosphorus concentrations decreased similar to LPD. On the other hand, the aortic calcification of klotho mice was inhibited by sevelamer hydrochloride administration although that was not inhibited by a LPD intake.

Ectopic calcification of the blood vessel in klotho mouse

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

639A
Conclusions: In our study, sevelamer hydrochloride inhibited aortic calcification regardless of serum phosphate concentration. These results might be associated with the pleiotropic effects of sevelamer hydrochloride.

Funding: Other U.S. Government Support

SA-PO2263

Interaction between RANK/RANKL Positive Macrophages Infiltrating around the Amyloid Deposit in the Yellow Ligament and the Osteoclasts in Destructive Spondyloarthropathy Patients

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Clinical Nephrology and Rheumatology, Niigata University Graduate School of Medical and Dental Sciences.

Background: Destructive spondylarthropathy (DSA) is one of the symptoms of dialysis-related amyloidosis and is reported to be observed as a complication in approximately 10% of long-term hemodialysis analysis patients. Histological examination shows β2-microglobulin amyloid deposits in the intervertebral disk, the synovium of apophysial joints, and the ligaments, however, the precise mechanism in the development of the destructive change of the bone is still unknown. Hence the aim of the study is to clarify the mechanisms of the destruction of the vertebral, focusing on the macrophages infiltrating around the amyloid deposits, and the cytokines and RANK-RANKL system.

Methods: Yellow ligament of the cervical or lumbar vertebrae in the DSA patients were extracted and collected when the posterior spinal fusions were conducted in each case. These yellow ligaments were fixed by 10% formalin, embedded in paraffin. Immunohistochemical study was performed on 5 µm paraffin section to examine the expression of RANK, RANKL, CD68, IL-1β, IL-6, TNF-α, and IL-17. THP-1 cells, monocyte/macrophage cell line, were cultured with various conditions to investigate the induction of RANK and RANKL.

Results: Histological examination revealed that CD68 positive monocyte/macrophage lineage cells infiltrating around the amyloid deposit expressed both RANK and RANKL. IL-1β, IL-6, and TNF-α were not detected in the CD68 positive cells, nor were the IL-17 producing T cells either. THP-1 cells were cultured with lipopolysaccharide in vitro, and the up-regulation of both RANK and RANKL expression on their cell surface were detected by flow cytometry.

Conclusions: In this study we reported the possibility that monocyte/macrophage lineage cells would be activated by lipopolysaccharide resulting in the expression of RANK and RANKL on their cell surface and facilitate osteoclastogenesis.

SA-PO2264

Alterations in Osteoblastic Gene Expression in Dialysis Patients: A Potential Role in Skeletal Mineralization

R.C. Pereira, 1 Harald Jueppner, 1 Isidro B. Salusky, 2 Katherine Wesseling-Perry. 3

Pereira, 1 Harald Jueppner, 1 Isidro B. Salusky, 2 Katherine Wesseling-Perry. 3

1 Pediatrics, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2 Endocrine Unit, Massachusetts General Hospital, Boston, MA.

Background: Abnormal skeletal mineralization is common in pediatric CKD. The mechanisms underlying this defect are unknown but osteocyte dysfunction has been characterized in patients with early CKD.

Methods: Primary osteoblasts were cultured from bone biopsies obtained from 6 pediatric PD patients (5M, 1F), age 16.6 ± 1.0 years, not receiving vitamin D steroids for at least 4 weeks prior to patients biopsy with varying types of bone histology. Expression of osteostotic and osteocytic gene expression was evaluated at passage 0 by qPCR and normalized by endogenous GAPDH expression in each sample. Expression in dialysis patients was then normalized by normal control. Bone histomorphometry was measured and biochemical values obtained in all subjects.

Results: Biochemical variables were: Ca: 8.3 ± 0.5 mg/dl, P: 7.2 ± 0.6 mg/dl, Alk P'tase: 289 ± 90 IU/l, PTH: 595 ± 231 pg/ml, FGF-23: 491 (436, 919) RU/ml.

Funding: Other NIH Support - CHARGE

SA-PO2266

The Effects of Phosphate Binders on the Wnt Pathway Gene Expression in Adynamic Bone Disease

Juliana C. Ferreira, 1 Guacriabia O. Ferrari, 1 Raquel T. Cavallari, 1 Wagner V. Dominguez, 1 Elizabeth M. Oliveira, 2 Luciene M. dos Reis, 1 Fabiana G. Graciolli, 1 Katia R. Neves, 1 Shiguang Liu, 1 Yves Sabbagh, 1 Vanda Jorgetti, 1 Susan Schiavi, 2 Rosa M.A. Moyes, 1 1 Nephrology Division, Universidade de São Paulo, Brazil; 2 Genzyme Co.

Background: The Wnt pathway is involved in bone formation, and high serum levels of sclerostin (SOST), a Wnt inhibitor, are associated with osteoporosis and adynamic bone disease (ABD). In this study, we tested the hypothesis that phosphate (P) binders could modulate gene expression of SOST in an experimental model of ABD.

Methods: Nephrectomized (Nx) rats were divided into 4 groups: Ca (calcium carbonate therapy), Sev (sevelamer carbonate therapy); CKD (untreated) and Control. After 8 wks, biochemical, histomorphometric and bone gene expression (TLDA) analyses were performed.

Results: Histomorphometric data confirmed ABD in Nx groups, based on decreased bone formation rate, osteoid volume, osteoblast and osteoclast surfaces without fibrosis. We found no differences among the CKD groups in terms of bone histomorphometric parameters, except for greater eroded surface in the Ca group. The gene expression of SOST was increased in CKD and only Sev administration was able to decrease it significantly.

Biochemical and histomorphometric data

Parameter Value

Bone histomorphometry

Bone volume (BV/TV)(%) 31.0 ± 0.6
Bone formation rate (BFR/BS) (micrometers/week) 43.4 ± 19.9
Osteoid volume (OV/BV)(%) 5.2 ± 2.9
Osteoid surface (OS/BS) (micrometers) 31.0 ± 9.3
Osteoclast number (OC/OST) (n=10) 8.0 ± 1.5
Osteoclast maturation time (OST/OC) (days) 33.8 ± 4.7
Mineralization lag time (MLT) (days) 57.2 ± 9.3
Gene expression as fold increase from control

Alkaline phosphatase 5.5±2.12
Type I Collagen 1.43±0.06
Type III Collagen 1.99±0.11
PTH receptor 5.04±1.11
SHRP1 2.31±0.42
IGF1 2.26±1.71
VD 3.24±0.27
CTP27 1.25±0.20
CTP24 2.87±0.77
Osteocalcin 1.14±0.18
RUNX 2.53±1.12
Osterix 2.21±0.52
MMP 23.1±0.40
FGF23 Undetectable
CYP1A2 Undetectable

Overall, mineralization parameters on histomorphometry were within the normal range. As evidenced by bone alk P’tase, osteocalcin and type I collagen expression and lack of DMP1, FGF23, MEPE and sclerostin, cells cultured from bone biopsy specimens retain osteoblast characteristics. Compared to control, ALK P’tase, PTH1R, NHERF1, IGF1, VDR, BMP2, RUNX, osteotropic, and matrix gla expression are increased in dialysis patients, suggesting an intrinsic upregulation of factors involved in the mineralization process.

Conclusions: The upregulation of pro-mineralization genes in osteoblasts may be required in order to maintain normal mineralization parameters in dialysis patients. Whether these factors vary according to subtype of renal osteodystrophy and how they relate to matrix mineralization remain to be evaluated.

Funding: NIDDK Support, Private Foundation Support

SA-PO2267

Conclusions: Gene expression of SOST is elevated in ABD and is decreased with Sevelamer. Longer treatment periods may be required to observe improve in histomorphometric parameters.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.
SA-PO2267
The Expression of Runx2 and Col II in Arteries of Remnant Kidney Rats and Patients with Chronic Renal Failure Yi Yu. Dept of Nephrology and Hemodialysis, Dongfang Hospital of Fujian Province, Fuzhou, Fujian, China.

Background: This study is aimed to observe the expression of Runx2 and collagen II (Col II) involved in vascular calcification in remnant kidney rats and patients with chronic renal failure (CRF).

Methods: 1. SD rats underwent 5/6 nephrectomy or sham operation, and rats were fed with high or normal phosphorous diet for 16weeks. They were divided into 4 groups: (1) remnant kidney rats receiving high phosphorous diet (NHP group, n=10); (2) remnant kidney rats receiving normal phosphorous diet (NP group, n=10); (3) sham operation rats receiving normal phosphorous diet (SP group, n=10); (4) sham operation rats receiving high phosphorous diet (SHP group, n=10). At the 16th week, Scr, P, LDL were examined and thoracic aorta was removed. 2. Pieces of radial arteries were taken from 40 CRF patients at the time of arteriovenous fistula operation, while 10 subtotal gastroectomy patients with normal renal function were chosen as control. Serum Ca, P and iPTH were detected. The vessels were examined for calcification by von Kossa stain and for the expression of Runx2 and Col II by immunohistochemistry, RT-PCR or Western Blot.

Results: 1. After 16 weeks, Scr were higher in 5/6 nephrectomy rats, and serum P increased significantly in NHP group (P<0.05). 2. Ovarian vascular calcification was found in NHP group while NP and SHP group occasionally had vascular calcification; SP group had none. 3. The expression of Runx2 and Col II increased in NHP group (P<0.05) by immunohistochemistry. In NHP group, the aorta mRNA expression of Runx2 and Col II, and difference in protein expression of Runx2 increased significantly (P<0.05). 4. In CRF patients, positive staining of Runx2 and Col II was found in the smooth muscle cell plasma of medial layers in the vessels with overt calcification, and was also observed in 78.3% of no calcification group. Serum P was positively correlated with staining score of Runx2 and Col II (P<0.01).

Conclusions: Serum P, Runx2 and Col II are involved in the vascular calcification in remnant kidney rats and CRF patients. The incidence of arterial calcification is high in CRF patients; the arterial expression of Runx2 and Col II is an early sign of calcification.

SA-PO2268
The Impact of Daily Activity and Muscle Strength on Fractures in CKD III-V were used to determine if NMT (the timed up and go test [TUG] and 6 minute walk test [6MW]) is able to distinguish those with and without fractures. The mean age of patients was 79.9±19.8 kg, most were Caucasian (70.0%), >40% had diabetes, and 74% patients were CKD stage 4 or 5. The mean duration of CKD was 8.0±10.3 yrs, and 30.2% reported a fall in the past year (7.1±2.7 sec). The TUG was able to distinguish those with and without fractures (AUROC: 0.90 [95% CI: 0.61-0.80]), as was the 6MW test with an AUROC: 0.69 (95% CI: 0.59-0.80). By accelerometry, sedentary activity (min/day) was able to discriminate fracture status (AUROC: 0.71 [95% CI: 0.51-0.93]), as was light activity (min/day), with an AUROC of 0.76 (95% CI: 0.56-0.95).

Conclusions: NMT and accelerometry are simple, practical measures that can discriminate among CKD patients with and without fractures. Their ability to predict future fractures require further study.

Funding: Government Support - Non-U.S.

SA-PO2270
Bone and Mineral Metabolism in the Course of Secondary Hyperparathyroidism Therapy with Cinacalcet Zbigniew Nowak, Daniel Baczyński, Grzegorz Kade, Zofia Wakowicz, Stanisław Nienickzy. Department of Nephrology, Military Institute of Medicine, Warsaw, Poland.

Background: The evaluation of secondary hyperparathyroidism (sHPT) in everyday practice is difficult, mainly on non-invasive measurements of bone metabolism. Our study aimed to compare changes of bone metabolism markers (iPTH, Ca, P, phosphatase, TRAP5b) in the course of cinacalcet therapy. We compared patients on non-calcium therapy with and without cinacalcet therapy. There were 2 subgroups of patients with sHPT: group A (n=18) – treated with calcium carbonate and cinacalcet and group B (n=14) – treated with traditional methods. The following parameters were determined in serum: iPTH, Ca, P, and tartrate-resistant acid phosphatase (TRAP5b).


Background: Secondary hyperparathyroidism is associated with significant morbidity in HD pts. K/DOQI guidelines recommend a therapeutic target for intact PTH (iPTH) between 150-300 pg/ml. Over-aggressive treatment, especially with vitamin D analogs, risks PTH over-suppression and adynamic bone disease (ABD), a low bone turnover state which is associated with increased risk of fractures. ABD has been associated with increased risk of fractures, cardiovascular calcification and mortality, and requires prompt modification of treatment regimen. iPTH levels are most commonly tested as a marker of bone resorption. TRAP5b activity was measured using assay Bone TRAP™ (SBA Finland). These parameters were measured before start of cinacalcet therapy and after 3rd and 6th month of therapy and after 3rd and 6th month of therapy without cinacalcet therapy.

Results: Patients treated with cinacalcet (group A) had significant lower values of iPTH and TRAP 5b after 3rd and 6th month of therapy in comparison to group B. We observed 66% reduction of iPTH level (from 816±295 pg/ml to 278±149 pg/ml at 6th month) and 76% reduction of TRAP 5b level (5.6±12.8 to 1.3±0.5 U/L). After stopping cinacalcet therapy we observed significant increase of iPTH (from 265±242 pg/ml to 683±292 pg/ml) and TRAP 5b (1.3±0.5 U/L to 4.9±1.8 U/L). We did not found any significant difference Ca and P values in both groups.

Conclusions: Treatment of sHPT with Cinacalcet in dialysed patients was effective and allowed the significant reductions of iPTH and TRACP 5b levels. Monitoring of TRACP5b might be useful in assessment of bone resorption in the course of cinacalcet treatment.

SA-PO2271

Background: Secondary hyperparathyroidism is associated with significant morbidity in HD pts. K/DOQI guidelines recommend a therapeutic target for intact PTH (iPTH) between 150-300 pg/ml. Over-aggressive treatment, especially with vitamin D analogs, risks PTH over-suppression and adynamic bone disease (ABD), a low bone turnover state which has become the most prevalent bone histology. ABD has been associated with increased risk of fractures, cardiovascular calcification and mortality, and requires prompt modification of treatment regimen. iPTH levels are most commonly tested quarterly, potentially delaying identification of ABD.

Methods: Over a period of two years in our urban dialysis center, we found 93 HD pts with quarterly iPTH values both above and below 150 pg/ml on stable doses of vitamin D analogs. We performed a survey that included 1210 patients belonging to 25 dialysis units in the province of Catalonia. 1,221 patients (55 months) were followed with quarterly iPTH values both above and below 150 pg/ml on stable doses of vitamin D analogs.

Results: Of 128 men and 86 women studied, 32 completed accelerometry. Compared to non-fractured patients (n=143), those with fractures (n=70) were older (59±16 vs. 69±14 yrs, p<0.001), had a reduced 6MW distance (335.5±87.1 vs. 289.9±142.2 m, p=0.04), were more sedentary with light daily activity (min/day, p<0.05). No other demographic factors distinguished those with and without fractures. The mean weight of patients was 79.9±19.8 kg, most were Caucasian (70.0%), >40% had diabetes, and 74% patients were CKD stage 4 or 5. The mean duration of CKD was 8.0±10.3 yrs, and 30.2% reported a fall in the past year (7.1±2.7 sec). The TUG was able to distinguish those with and without fractures (AUROC: 0.90 [95% CI: 0.61-0.80]), as was the 6MW test with an AUROC: 0.69 (95% CI: 0.59-0.80). By accelerometry, sedentary activity (min/day) was able to discriminate fracture status (AUROC: 0.71 [95% CI: 0.51-0.93]), as was light activity (min/day), with an AUROC of 0.76 (95% CI: 0.56-0.95).

Conclusions: NMT and accelerometry are simple, practical measures that can discriminate among CKD patients with and without fractures. Their ability to predict future fractures require further study.

Funding: Government Support - Non-U.S.
PTH over-suppression (327 ± 177 pg/ml to 90 ± 39.6; p<0.0001) was associated with significant elevations in corrected serum calcium (9.29 ± 0.65 mg/dL to 9.93 ± 0.66; p<0.0001) and reductions in alkaline phosphatase (95 ± ± 68 g/L to 85.7; p< 0.039), as expected. In addition, we found highly significant reductions in serum phosphorus (5.28 ± 1.46 mg/dL to 4.60 ± 1.60; p<0.0001) and in calcium-phosphorus product (49.4 ± 14 to 45.8 ± 13.3; p<0.012).

Conclusions: In conclusion, falling monthly values of serum phosphorus associated with rising serum calcium may portend the onset of PTH over-suppression, leading to earlier recognition and intervention for ABD.

Funding: Private Foundation Support

SA-PO2272

Normal Serum Phosphate Levels Associated to Carotid Atheromatosis in Chronic Kidney Disease Stages 2-5
Secundino Cigarroa, Emilio E. Gonzalez-Parra, Guillermina Barril, Juan J. Carrero, *Nephropathy, Hospital Da Costa, Burela, Spain; Fundacion Jimenez Diaz, Madrid, Spain; †Nephropathy, Hospital Universitario de la Princesa, Madrid, Spain; ‡Division of Renal Medicine, Karolinska Institutet, Stockholm, Stockholm, Sweden.

Background: Atherosclerotic cardiovascular disease is a significant cause of morbidity and mortality in patients with chronic kidney disease (CKD). Phosphate (P) levels are consistently linked with cardiovascular events and death in chronic kidney disease (CKD) patients and in general population. Fibroblast growth factor 23 (FGF23) is involved in phosphate and early stages of CKD and in atheromatosis process.

Methods: We analyze, in cross-sectional study, the role between P levels and carotid atheromatosis in CKD patients stage 2-5. 426 pts with CKD stage 2-5 (mean age 68.08 ± 12.5 yr, 31% women, 27.7% diabetic status, GFR-MDRD 51.62 ± 23.69 mL/ min/1.73m²) were included. Carotid atheromatosis diagnosis by high-resolution B-mode ultrasonography (USBM) and body composition assessment was performed by whole tetrapolar bioelectrical vectorial impedance analysis (BIVA) (EFG, Akem Firenza Italy.). Biochemical nutrition, inflammation & mineral bone disease markers were analyzed. ABI test was performed.

Results: 313 (74.4%) had CA and compared with non CA were older (68.66±10.1vs 54.34 ±10.5 yrs, p<0.001), male gender (65% vs 49%; p<0.05).

T1 paired Test

<table>
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<th>Non CA (N=113)</th>
<th>CA (N=313)</th>
<th>p</th>
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<tbody>
<tr>
<td>Ca++ (mg/dL)</td>
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<td>9.15±1.8</td>
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<tr>
<td>ECW (%)</td>
<td>47.74±4.45</td>
<td>49.59±4.82</td>
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<td>Phase Angle (°)</td>
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<td>C Reactive Protein (mg/dL)</td>
<td>52±50</td>
<td>48±13</td>
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<td>Serum P (mg/dL)</td>
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<td>3.17±5.7</td>
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<td>P excretion index</td>
<td>79±34</td>
<td>94±39</td>
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<td>ABI</td>
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<td>1:03±23</td>
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Conclusion: CA=Carotid Atheromatosis; Non CA= Non Carotid Atheromatosis

Funding: Other NIH Support - SERGAS

SA-PO2273

Ionized Calcium and Mortality in Chronic Hemodialysis Jean-Christophe Szelag, Walid Arkouche, Ignacio Mpio,Alejandra Lenz, Carlos Cardozo, Elias Abdullah, Nouredine Boumendjel, Denis Fouque, Maurice Laville. AURAL Lyon.

Background: Previous studies have emphasized the noxious effects of high serum calcium and its variations on HD-subjects risk of death. Ionized calcium (Ca++) variations are independent of inflammatory, nutritional and hydration statuses which are other prognosis factors.

Methods: The studied population consisted in 197 subjects (76 women, 121 men, median age 61,2,diabetes=50)on hemodialysis for more than 3 months. The mean follow-up was 30 months (2-39 months). Ca++ was measured in optimal conditions two hours after dialysis in 162 subjects. The data are expressed as mean±SD, median.

Results: Forty patients died during the follow-up. A survival benefit was observed with concentration under 1.14 mol/L (p<0.004) and in the lowest tertile of predialysis corrected Ca++ (p<0.0001) as compared with the highest tertile (9.93±1.69 vs 17.43±1.69; p<0.0001). The observed number of hip fractures was compared to the expected number based on a Japanese national survey: The standardized incidence ratios (SIRs) were calculated as the ratios of observed to expected number of cases.

<table>
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<th>SIR</th>
<th>CI</th>
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<td>Ca++ ≤ 1,14 mol/L</td>
<td>0.879 (0.760-1.017)</td>
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<tr>
<td>Ca++ &gt; 1,14 mol/L</td>
<td>1.008 (0.939-1.081)</td>
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</table>

Conclusions: Our small scale, retrospective and non time-dependent study found a survival benefit with Ca++ under 1,14mmol/L in HD-subjects with higher PTH level and calcium level treatment.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

462A
Fibroblast Growth Factor 23 and Bone Metabolism in Elderly Chronic Kidney Disease Patients

Methods: 105 elderly CKD patients who had never used calcium or vitamin D supplements were enrolled in this cross-sectional study in Tokyo, Japan. We investigated the prevalence of lumbar fracture and measured serum calcium, phosphate, 1.25(OH)2 vitamin D (1.25-VD), intact parathyroid hormone (iPTH), fibroblast growth factor 23 (FGF23), serum N-terminal telopeptide (NTX) and urinary NTX. Factors associated with lumbar fracture and FGF23 levels were assessed by regression or multivariate logistic model adjusted for age and gender.

Results: The following results were found: female, 32.4%; diabetics, 30.5%; average age (SD), 73.2 (7.7) years; estimated glomerular filtration rate (eGFR), 45.7 (24.1) ml/min; calcium 9.4 (0.5) mg/dl; phosphate 3.5 (0.7) mg/dl; intact PTH 3.5 (0.7) pg/ml; 1.25-VD 47.2 (21.0) pg/ml; FGF23 78.0 (101.7) pg/ml; serum NTX 20.5 (17.4) nmol BCE/UL; urinary NTX 35.8 (24.3) nmol BCE/mmol Cr. Univariate analysis revealed that FGF23 level significantly correlated with eGFR, calcium, phosphate, 1.25-VD, intact PTH, and serum NTX levels (P<0.0001), but not with urinary NTX level. Lumbar fracture was associated with age and High FGF23 levels: more than 81 years group (referred to 61-70 years) odds ratio (OR) 4.00 (95% confidence interval 1.05-15.30); high FGF23 levels (≥71.0, adjusted OR 4.37 (1.06-17.97). High FGF23 levels were closely associated with bone mineral fractures: increased phosphate level (≥4.3), adjusted OR 4.18 (1.13-15.40); decreased 1.25-VD level (≤20), 19.38 (3.71-101.29); increased serum NTX level (≥16.5), 15.08 (4.72-48.15); increased urinary NTX level (male=66.2; female=59.0), 9.15 (1.37-60.99). None of the patients showed both low iPTH and low FGF23 levels.

Conclusions: Increased FGF23 level was observed with the progression of CKD before intact PTH level increased, and was associated with lumbar fracture and osteoporosis markers. FGF23 is a valuable marker to detect and prevent abnormal bone metabolism at an early stage of CKD.

Tests of Bone Density and Structure Are Associated with Fractures in Stage 3-5 Chronic Kidney Disease

Background: Fractures are common in CKD patients. Bone mineral density (BMD) by dual x-ray absorptiometry (DXA) does not discriminate well hip BMD among patients with various degrees of chronic kidney disease (CKD). High-resolution peripheral quantitative computed tomography (HR-pQCT) allows us to visualize the bone microstructure at the level of an individual patient. Therefore, the present study examined whether BMD and/or microstructure by HR-pQCT at the radius could discriminate fracture status in patients with stages 3–5 CKD (CKD-III-V) unadjusted for age.

Methods: Baseline data from our ongoing study in adult CKD-III-V patients, was used to determine if BMD by DXA (Hologic) at the lumbar spine (LS), total hip (TH), ultradistal radius (UDR), and/or cortical area, density & thickness, trabecular area, density & separation by HR-pQCT (XtremeCT) at the radius could discriminate fracture status (fracture=自我-reported low trauma fractures occurring after 40 yrs and/or prevalent vertebral fractures identified by morphometry). Results were expressed as areas under the receiver operating characteristic curves (AUROC) with 95% confidence intervals (CI), adjusted for age and gender.

Results: Data from 128 men and 86 women showed that compared to those without fractures (n=143), those with fractures (n=70) were older (59.16 vs. 69.14 yrs, p<0.001). The mean weight was 79.9±9.19 kg, most were Caucasian (70.0%), > 40% had diabetes, and most (74%) patients were CKD stage 4 or 5. The mean duration of CKD was 8.0±10.3 yrs, and 30.2% reported a fall in the last 12 mos. BMD by DXA discriminated among those with and without fractures (AUROC for LS: 0.70 [95% CI: 0.62-0.78]; TH: 0.69 [95% CI: 0.61-0.77]; UDR: 0.71 [95% CI: 0.63-0.78]). HR-pQCT also performed well for cortical measures (AUROC for area: 0.67 [95% CI: 0.59-0.76]; density: 0.70 [95% CI: 0.63-0.78] and thickness: 0.70 [95% CI: 0.62-0.78]) and trabecular measures (AUROC for area: 0.70 [95% CI: 0.62-0.78]; density: 0.70 [95% CI: 0.63-0.78]; and separation: 0.70 [0.63-0.78]).

Conclusions: BMD by DXA and HR-pQCT can discriminate fracture status in CKD-III-V patients. Prospective studies are needed to evaluate the fracture predictive ability of BMD and HR-pQCT.

Funding: Government Support - Non-US.

Dialysis as a Treatment Option for Primary Tumoral Calcinosi in a Patient with a New FGF Mutation

Background: Primary tumoral calcinosi (TC) is rare, autosomal recessive metabolic disorder characterized by ectopic calcified tumoral masses and dental abnormalities, as well as soft tissue periartricular and vascular calcifications. Until now, mutations in three genes were ascribed to be responsible for the human disorder.

Methods: Here we describe a case of a 24-yr-old male with TC, with a new mutation in FGF23 and an unusual treatment option in an attempt to effectively control his phosphate and reduce his calcified lesions.

Results: The patient presented subcutaneous nodules, as well as periarticular and vascular calcifications. Biochemical analysis disclosed hyperphosphatemia (9.0 mg/dl), normocalcemia (4.8 mg/dl) with a normal renal function and Fe3+ was 3%. PTH was suppressed (15 pg/ml), associated with a low-normal 25-OH-vitamin D (26 mg/ml). Serum intact FGF-23 was undetectable. As for FGF23, a heterogeneous state was observed defined by p.Q67H (exon 1) and p.Q156stop (exon 3). Despite of four surgeries for tumoral resection and medical treatments with aluminum hydroxide, sevelamer and acitazolamide, lesions continued to progress. Due to lack of other treatment options, patient was included in a daily dialysis program, leading to a better phosphorus control. After 24 months of therapy, the lesions decreased in size and he recovered most of his mobility. This is the first report of a new mutation in FGF gene and the first case in which dialysis was described as an effective treatment option for TC with normal renal function.

Conclusions: This patient, with his rare genetic disease, gave us the opportunity to test in vivo the role of phosphate in extra-osseous calcification and emphasizes the neeed of its control in the context of CKD.

The Value of DXA Versus QCT for Diagnosis of Osteoporosis in Patients with CKD

Background: Bone mineral density (BMD) of the lumbar spine and hip was measured by dual-energy x-ray absorptiometry (DXA) and quantitative computed tomography (QCT) in 82 patients with CKD-3D.

Methods: There were 42 men (age 52.0±2.13 yrs) and 40 women (age 56.2±2.18 yrs). Mean dialysis duration was 59.1±6.30 mos. Thirty seven % of the patients were diabetics. Ilaic crest bone biopsies were done for histomorphometry at time of BMD measurements in a subgroup of 23 patients. Diagnosis of osteoporosis by DXA or QCT was made when t-scores were < -2.5 and by histomorphometry when cancellous bone volume and/or cortical thickness were below normal and/or cortical porosity was above normal values.

Results: Osteoporosis was found in the spine by DXA in 10% and by QCT in 14%; osteoporosis in the hip was found by DXA in 26% and by QCT in 16% (p=0.008). Absolute scores of the hip obtained from DXA and QCT were not different and correlated. T-scores of the hip from DXA and QCT also correlated but were lower with DXA than QCT (r=0.20 vs. -0.12 ± 0.22, p<0.001). This suggests that the differences observed in recognition of osteoporosis were not due to absolute test results, but related to differences in normative databases used to calculate t-scores. Bone histology identified presence of osteoporosis in 90% of the patients.

Sensitivity, specificity, positive and negative predictive values of DXA versus QCT for osteoporosis diagnosed by histology are shown below.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive Predictive Value (%)</th>
<th>Negative Predictive Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DXA Hip</td>
<td>10</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>QCT Hip</td>
<td>20</td>
<td>90</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>DXA Spine</td>
<td>5</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>QCT Spine</td>
<td>29</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

Conclusions: In conclusion, for prediction of histologically documented osteoporosis DXA and QCT are equally specific (100%) for the spine while for the hip QCT had superior specificity. Sensitivity is poor with both DXA and QCT of spine and hip. For diagnosis, specificity and sensitivity of at least 80% is required, thus neither DXA nor QCT is recommended.

Funding: NIDDK Support, Private Foundation Support
Evaluation of Bone Mineral Density in Long-Standing Type I Diabetic Patients with or without Diabetic Nephropathy

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Background: Patients with chronic kidney disease including diabetic nephropathy are suggested to have reduced bone mineral density (BMD).

Methods: Cross-sectional evaluation of 295 prospectively followed long-standing type I diabetic patients. 115 patients had diabetic nephropathy (54% men; age [mean/SD]: 54/9 years, 42±8 years of diabetes, duration of nephropathy 22±6 years) and 178 patients with persistent normoalbuminuria (49% men, age 59±10 years; 41±10 years of diabetes) were included. BMD (g/cm²) in femoral neck, total femoral bone and lumbar spine was measured by dual energy x-ray absorptiometry (DXA). Osteoporosis and osteopenia were defined by sex matched T-score from -2.5 to 1.0 and <-2.5, respectively. The difference between BMD and sex-age matched average defines the Z-score. GFR was measured by 67Ga-Citrate clearance and correlated with nephropathy and men.

Results: Among patients with diabetic nephropathy, 58 (50%) and 31 (27%) had osteoporosis and osteopenia, respectively compared to 106 (60%) and 22 (12%) of the normoalbuminuric patients; p=0.006. Among male patients with nephropathy, the prevalence of osteoporosis was 37% vs. 9% with normoalbuminuria (p<0.001), whereas no difference was seen among women. Similarly, men with nephropathy had significantly lower age-sex matched spinal, femoral neck and total femoral bone Z-scores compared to normoalbuminuric patients; p<0.002. There was no statistically difference in woman.

Conclusions: The risk of osteoporosis was highest among male patients with type 1 diabetes and long-standing diabetic nephropathy and femoral BMD correlated with renal function. Hence screening, prevention and treatment of osteoporosis in patients with impaired renal function should be considered.

Low-Dose Calcifediol Supplementation Augments Serum PTH Levels in Hemodialysis Patients at Risk for Adynamic Bone Disease

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Background: Adynamic bone disease (ABD) is a common form of renal osteodystrophy. In hemodialysis (HD) patients, the use of active vitamin D analogs may favor the development of ABD by means of the suppression of PTH secretion. By contrast, treatment of low 25-hydroxyvitamin D (calcifediol) levels may exert a stimulatory effect on bone formation. We undertook this study to assess the effect of low-dose calcifediol supplementation on mineral metabolism and serum PTH levels in long-term HD patients considered at risk for ABD because of low serum PTH levels.

Methods: This was a 3-month follow-up, prospective study of a cohort of prevalent HD patients from a single center. Eligible subjects were on HD for >3 months with PTH levels <150 pg/mL. All patients were included in the study, except for those who were taking cinacalcet or any active vitamin D analog and those who had undergone parathyroidectomy. Calcium carbonate and phosphate binders were prescribed according to each patient's needs. Calcifediol levels remained unchanged during the study. Serum levels of 25-hydroxyvitamin D, PTH, Ca, P and alkaline phosphatase were measured before and after supplementation. All patients received 20 drops of oral calcifediol (1.5 mg/10 mL) once a week after the first HD session of the week for 3 months. Statistical analysis was performed using paired Wilcoxon test.

Results: The study included 18 patients: 8 men and 10 women with mean age of 57.8±19.5 years. Mean HD Vintage was 24±33.2 months. Calcifediol serum levels were <30 ng/ml in all our patients at baseline and significantly increased at the end of the study (8.1±4.9 vs. 23.8±10.1; P<0.001). Serum PTH increased significantly (79.6±34.2 vs. 118.9±59.0; P=0.05). Serum phosphate showed a slight but significant increase with supplementation (4.3±1.3 vs. 4.8±1.0; P=0.05), whereas no change was reported for both serum calcium (9.2±0.8 vs. 8.8±0.5; P=0.05) and alkaline phosphatase levels (175.6±58.3 vs. 179.8±61.1; P=0.05).

Conclusions: The parathyroid glands of vitamin D deficient HD patients with relative hypoparathyroidism or ABD responded to a low-dose 25-OH-vitamin D supplementation with a significant increase in serum PTH levels.

DXA and HRpQCT Detect Minimal Bone Loss after Kidney Transplantation with Early Corticosteroid Withdrawal


Background: With corticosteroid (CS)-based immunosuppression (CSBI), areal bone mineral density (aBMD) declines 2-10% during the first 6 months (m) after kidney transplant (KTx), particularly at trabecular ( Tb) rich sites, such as the lumbar spine (LS); fracture rates are high at both spine and hip. Early corticosteroid withdrawal (ECWS) protocols, based on calcineurin inhibition (CNI) are increasing in use. We hypothesized that KTx recipients managed with ECWS would not experience significant bone loss.

Methods: We included KTx recipients ≥18 yrs managed with ECWS. At baseline, 3m and 6m after KTx, we measured BMD by dual energy X-ray absorptiometry (DXA) at predominantly Tb sites: LS and ultradistal radius (UDR); and cortical (Ct) sites: total hip (TH); femoral neck (FN); 1/3 radius (1/3R). High resolution peripheral QCT (HRpQCT; Scanco Medical, AG, voxel size=82µm) was used to measure total, Ct and Bv/total volumetric BMD (vBMD); Ct thickness (CTh); and Tb number (TbN) and separations (TbSp) at the distal radius (DR) and tibia (DT). Comparisons were made using paired T-tests; results are expressed as Means/SD.

Results: Of 48 subjects enrolled, 29 and 24 completed 3m and 6m of observation respectively. There was 51±13% women; 73% were white. BMI was 29±4 Kg/m². Mean baseline T-Scores were -2.5±2.5 at all sites. At 6m, aBMD declined significantly only at the 1/3R (-1.8±-0.02) and UDR (-2.7±-0.04). By HR-pQCT at 6m, DR total vBMD decreased by -2.6% (-0.01), TbBMD decreased by -2.5% (-0.001), and there was a significant 1.3% decrease in DT total vBMD (p=0.08). TbN, TbSp, and TbBSp were unchanged at both DR and DT at 6m.

Conclusions: After KTx, ECWS was associated with less bone loss at the LS, FN, and TH than previously reported with CSBI. Significant bone loss occurred at the 1/3R and UDR which HR-pQCT suggested is primarily Tb; the mechanism of bone loss at the DT and DR was unclear. Longer and larger studies are needed to assess ECWS effects on fracture rates after KTx.

Funding: Private Foundation Support

Fibroblast Growth Factor 23 in Patients Undergoing Peritoneal Dialysis

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Background: Fibroblast growth factor 23 (FGF23) is an independent risk factor for mortality in patients with end-stage renal disease (ESRD). Before FGF23 testing can be integrated into clinical practice of ESRD, further understanding of its determinants is needed.

Methods: In a study of 67 adults undergoing peritoneal dialysis, we tested the hypothesis that longer dialysis vintage and lower residual renal function and residual phosphate clearance associate with higher FGF23. We also compared the monthly variability of FGF23 versus parathyroid hormone (PTH) and serum phosphate.

Results: In unadjusted analyses, FGF23 correlated with serum phosphate (r = 0.66, P <0.001), residual renal function (r = -0.37, P = 0.002), dialysis vintage (r = 0.31, P = 0.01), and renal phosphate clearance (r = -0.38, P = 0.008). In adjusted analyses, absence of residual renal function and greater dialysis vintage associated with higher FGF23, independent of demographics, values, peritubular dialysis modality and adequacy, and treatment with vitamin D analogs and phosphate binders. Urinary and dialysate FGF23
clearance was minimal. In three serial monthly measurements, within-subject variability accounted for only 10% of total FGF23 variability compared with 50% for PTH and 60% for serum phosphate.

SA-PO2286
Disordered Mineral Metabolism in the CKiD Children: Role of FGF23
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Background: FGF23 is an important regulator of phosphorus (Pi) and vitamin D metabolism. However, little is known about the prevalence and determinants of FGF23 excess across the spectrum of CKD in children.

Methods: We measured plasma C-terminal FGF23 (ImmunoXpert 2nd gen) in 426 children, ages 1-16 yrs, with CKD stages 2-4 enrolled in the observational Chronic Kidney Disease in Children (CKiD) study. FGF23 was measured as plasma clearance of iohexol (n=286) or estimated using the CKiD estimating equation.

Results: Mean age of subjects was 11.5 ± 4.3 (SD) yrs. CKD was due to glomerular disease in 21% and non-glomerular disease in 79%. Mean GFR was 46 ± 18 ml/min/1.73 m²; 18% of subjects had CKD stage 2, 59% stage 3, and 21% stage 4. Overall, median serum Pi and PTH levels were within the normal range, but median FGF23 was 112 RU/ml (IQR: 88-223), 2.3-fold higher than that in healthy children. 66% of subjects met criterion for FGF23 excess (>100 RU/ml) but only 12% had hyperphosphatemia. FGF23 was strongly associated with GFR (r=-0.44), age-adjusted Pi (r=-0.30), and PTH (r=-0.36) (P<0.001 for each). In stage 2 CKD, mean age-adjusted Pi was below the normal mean (P<0.01), PTH was >65 µg/ml in only 15%, whereas FGF23 was >100 RU/ml in 44% of subjects. As GFR declined further, the prevalence of FGF23 excess increased (stage 3, 66%; stage 4, 91%). FGF23 was higher in children with glomerular than with non-glomerular disease (P<0.001), after adjusting for age, GFR, and Pi.

Conclusions: Plasma FGF23 concentrations are increased early in the course of CKD in children, before increases in serum Pi or PTH. Thus, increased FGF23 may be an early biomarker of disordered Pi homeostasis and an initiating mechanism of abormal mineral metabolism in children with progressive CKD.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2287

Background: Fibroblast growth factor (FGF23), a key regulator of mineral metabolism, has been associated mortality in the dialysis population. Activated vitamin D administration increases FGF23 levels. Observational studies have shown that the beneficial effects of vitamin D analogs (VDAs) on survival are reduced at higher doses. The aim of this study was to explore the relationship between FGF23, VDA selection and VDA dosage among hemodialysis (HD) patients.

Methods: Adults on HD for ≥3 months were included in this cross-sectional study. Patient demographics, laboratory values and current medication regimens were obtained. Weekly VDA doses were converted to doxercalciferol equivalents (4.6 µg of paricalcitol = 3.1 µg doxercalciferol) for uniformity. Pre-dialysis levels of intact FGF23 were measured via ELISA. Stepwise linear regression was used to identify factors associated with FGF23 levels.

Results: A total of 127 patients (57% male, 46% diabetic etiology, 64% on doxercalciferol therapy) with a mean age = 62.4±14.9 years, HD vintage = 3.5±2.9 years and log FGF23 = 2.57±0.37 pg/ml were included in this analysis. Log FGF23 levels positively correlated with HD vintage (r=0.24, P=0.0065), calcium (r=0.26, P=0.0036), phosphorus (r=0.47, P<0.0001), VDA dose (r=0.20, P=0.0181) and negatively correlated with age (r=-0.18, P=0.0388). Log FGF23 was lower in those on doxercalciferol compared to patients on paricalcitol (2.50±0.33 mg/versus 2.70±0.42 mg/ml, P=0.002). Use of paricalcitol (r=0.15, P=0.0032), VDA dose (r=0.07, P=0.258), calcium (r=-0.21, P<0.0001), phosphorus (r =-0.15, P<0.0001) and HD vintage (r=-0.02, P=0.028) were independently associated with log FGF23.

Conclusions: The positive independent association between VDA dose and FGF23 are indicative of a dose response relationship between VDAs and FGF23. Furthermore, phosphate lowering therapy was independently associated with higher FGF23 concentrations, compared to doxercalciferol therapy. These results suggest that individual VDAs may have differential effects on FGF23 regulation in HD patients and warrants further investigation.

SA-PO2288
FGF23 Levels Are Modified by Loop Diuretic Treatment in CKD Patients
Sarah Seiler, Esther Herath, Franziska Flügge, Anja Wehrauch, Danilo Fliesser, Gunnar H. Heine. Internal Medicine IV, Saarland University Hospital, Homburg, Saar, Germany.

Background: FGF-23 is a central regulator of calcium phosphate metabolism. Similar to parathyroid hormone (PTH), FGF-23 stimulates renal phosphate excretion. While plasma levels of both iPTH and FGF23 increase in advanced CKD in order to combat phosphate retention, an elevation of FGF-23 may precede the decline of estimated glomerular filtration rate (eGFR) in very early CKD for unknown reasons. In CKD patients, use of loop diuretics is a known stimulus for increased renal calcium loss and consecutively increased iPTH levels. To date it is unclear whether the use of loop diuretics results in a concomitant increase of FGF-23.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

645A
Methods: We studied 343 CKD stage 2-4 patients in our ongoing CARE FOR HOME study. PWV was determined at baseline and after correction of parathyroid hormone (PTH), FGF-23 and 25OHD status, calcium, sodium, proteinuria, and urinary calcium excretion (FeCa), and recorded the intake of diuretic medication (diuretics: LD; thiazide diuretics: TD). In addition, we assessed left ventricular function using echocardiography and eGFR using the MDRD study equation 4. Results: Studied participants had a mean eGFR of 44.2±15.7 ml/min/1.73 m², and were 65±12 years of age. Median FGF-23 level was 99.0 u/ml (IQR 60.3-158. u/ml), mean iPTH level was 70.2±57.9 pg/ml. 821 patients (81.9%) were on diuretic medication, comprising 161 patients (46.9 %) receiving LD, and 174 patients (50.7%) receiving TD. Intake of FeCa was assessed with lower FeCa (TD: 0.52±0.51; no TD: 0.89±0.98; p<0.001). LD were associated with higher FeCa (LD: 0.88±1.01; no LD: 0.54±0.48; p=0.010). Moreover, the intake of LD was associated with higher iPTH (LD: 90.6±72.2 pg/ml; no LD: 52.3±32.7 pg/ml; p=0.001) and FGF-23 levels (LD: 125.4±70.3 u/ml [IQR 78.0-187.3 u/ml]; no LD: 76.4±43.8 u/ml [IQR 50.5-116.3 u/ml]; p<0.001), while the intake of thiazides was not. This association remained significant after correction for eGFR and left ventricular function in a linear regression analysis.

Conclusions: The results from our study complements earlier data on the impact of LD on secondary hyperparathyroidism, reporting for the first time an association between choice of diuretic medication and FGF-23 levels in CKD patients.

SA-PO2289
24,25 Dihydroxyvitamin D Levels Are Elevated in Dialysis Patients and Correlate with FGF23 Levels in Patients with Chronic Kidney Disease

Background: 25(OH) vitamin D (25D) deficiency is common in patients treated with dialysis and may contribute to the adverse outcomes observed in this cohort. In these patients increased FGF23 levels are thought to inhibit the renal 1-alpha hydroxylase and stimulate the 24-hydroxylase, thus contributing to diminished 1,25(OH)2 vitamin D (25D) levels and to the development of secondary hyperparathyroidism. Recent data suggest the presence of increased amounts of the 24-hydroxylase protein in the kidneys of rodents and humans with CKD, which may contribute to both 25D deficiency (by metabolism into inactive analogs) as well as 1,25D deficiency (by "siphoning off" of the precursor 25D).

Methods: Using a novel mass spectrometry approach, we measured 24,25D2 levels in the circulation of 120 individuals with no known renal dysfunction and 60 patients initiating hemodialysis.

Results: In the reference group, 24,25D2 levels were positively correlated with 25D status. Specifically, the mean 24,25D2 levels in individuals with 25D levels <20 ng/ml was 1.27±0.3 ng/ml (mean±SEM), while in those with 25D levels >30 ng/ml it was 6.1±0.56 ng/ml. In the dialysis population 24,25D2 levels were significantly elevated; patients with baseline 25D levels <20 ng/ml had a 24,25D2 level of 9.0±1.26 ng/ml (mean±SEM), at times rising above the absolute 25D level in individual patients. 24,25D2 levels correlated positively with 25D, calcium and albumin. Treatment with an active vitamin D analog did not increase 24,25D2 levels and neither did the dialysis procedure itself. FGF23 levels measured in a subset of patients correlated positively with 24,25D2 levels (N=23, R=0.42, p<0.05).

Conclusions: In summary, 24,25D2 levels in dialysis patients are increased compared with those in a reference population and correlate with FGF23 levels. This correlation is consistent with the FGF23-mediated increase in 24-hydroxylase expression in renal and possibly extra-renal tissues.

Funding: NIDDK Support

SA-PO2290
Association of Fibroblast Growth Factor 23 on Pulse Wave Velocity in Chronic Kidney Disease Stage 3-5 Patients with 2 Years Follow-Up

Background: FGF23 is an important regulator of calcium and phosphate metabolism and is independently associated with cardiovascular disease. In chronic kidney disease, FGF23 levels are increased and may contribute to cardiovascular disease, even after correction of the deficiency of 25-OH-D.

Methods: We performed a post-hoc analysis of PWV in a subset of 188 CKD patients who were included in the multicenter Masterplan trial. Multiple logistic regression was performed to evaluate the association between FGF23 and PWV at baseline; generalized estimating equations were applied to evaluate this relationship in the entire 2 years study period.

Results: At baseline, mean arterial pressure (MAP), age and BHaIC were associated with PWV (all p<0.05). In a model adjusted for these covariates, FGF23 was not associated with PWV. In the entire 2 years study period, FGF23 had a significant effect on PWV (p <0.001). In addition, PWV increased significantly over time (p<0.019).

Conclusions: Baseline FGF23 was not associated with baseline PWV. In two year follow-up FGF23 is significantly associated with PWV. This association is modest and clinical meaning remains to be determined.

SA-PO2291
Correction of Hyperphosphatemia Suppresses Cardiac Remodeling in Uremic Rats

Methods: Adult Sprague-Dawley rats underwent subtotal nephrectomy (Nx) by the ligation method. Four groups were studied for 8 wk: 1) control (sham), 2) Nx rats fed a normal phosphate (0.9%) diet (Nx+N), 3) Nx rats fed a high phosphate (2%) diet (Nx+HP), and 4) Nx rats fed a high phosphate diet containing 2% lanthanum (Nx+HP+La).

Results: Subtotal nephrectomy caused blood pressure elevation, renal dysfunction as evidenced by a gradual increase in proteinuria and elevation in serum creatinine levels. Nx+HP rats showed a significant increase in serum phosphate and PTH levels compared with Nx+N rats while Nx+HP+La rats showed slight decrease in these levels. Not only Nx+HP rats, but also Nx+HP+La rats showed a significant increase in serum FGF23 levels compared with Nx+N rats. Urinary phosphate excretion showed a similar trend as serum FGF23 levels. Both Nx+N rats and Nx+HP+La rats showed a significant increase in the left ventricle weight compared with sham rats while the increase was significantly suppressed in Nx+HP+La rats. Nx+HP+La rats showed a significant increase in matrix deposition of the cardiac tissue compared with the Nx+N rats while the increase was significantly suppressed in Nx+HP+La rats.

Conclusions: Correction of hyperphosphatemia could suppress the cardiac remodeling, which was independent of serum FGF23 levels.

SA-PO2292
Effects of the Correction of 25-hydroxyvitamin D Deficiency on Hyperparathyroidism and Malnutrition-Inflammation Status in Hemodialysis Patients

Background: It has been reported that 85% of hemodialysis (HD) patients have decreased vitamin D (25-OH-D) levels (< 30 ng/ml). The aim of this study was to analyze the effect of the correction of 25-OH-D deficiency on the control of hyperparathyroidism (HPT) and the malnutrition-inflammation-status in patients on HD.

Methods: Twenty seven stable patients on HD with low levels of 25-OH-D (< 20 ng/ml) were included in this study. They were 65.2± 16.8 years old and 48% were men. All patients were prospectively treated with calcifediol (medicated phosphate binder intake) and darbepoetin every week for an average of 3 months. Calcium, phosphorus, alkaline phosphatase, PTH, albumin, hemoglobin, TAST, cholesterol, tryglicerides, eKt/V, URR and darbepoetin and paricalcitol doses were analyzed before and after the correction the deficiency of 25-OH-D.

Results: After treatment with calcifediol all patients normalized the levels of 25-OH-D (> 30 ng/ml), and decreased PTH with a reduction of paricalcitol requirements (before/after correction n=11/3) and an improvement in their malnutrition-inflammation status.

Conclusions: A correction of vitamin D deficiency could suppress the cardiac remodeling, which was independent of serum FGF23 levels.

Table 1. Main biochemical and pharmacological parameters determined before and after correction the deficiency of 25-OH-D.
With this protocol there were no episodes of severe hypercalcemia or hyperphosphatemia. The dose of phosphate binders remained stable along the study.

Conclusions: Our results strongly suggest that an adequate dose of calcitriol could correct the deficiency of 25-OH-D in HD patients without relevant side-effects. The normalization of 25-OH-D levels (>30 mg/mL) improved the malnutrition-inflammation status and allows a better control of HTP with a reduction of paricalcitol requirement.

SA-PO2293

Update on the PACE Study: Randomized Controlled Trial of Paricalcitol and Calcitriol Endpoints Seth Goldberg,1 Stuart M. Sprague,2 Mark D. Faber.3 1Washington University, Saint Louis, MO; 2North Shore University Health System, Evanston, IL; 3Henry Ford Hospital, Detroit, MI

Background: Exogenous activated vitamin D can reduce parathyroid hormone (PTH) levels and decrease bone disease in secondary hyperparathyroidism (SHPT). In preclinical studies, paricalcitol causes a slower increase in serum calcium and phosphorus levels than calcitriol, possibly due to less activation of the intestinal and bone vitamin D receptors. The aim of this study is to compare the safety and efficacy of these active vitamin D analogs in patients with CVD stages 3-4.

Methods: Randomized, open label, active comparator, multicenter, phase 4 study of paricalcitol vs calcitriol in patients with CVD stages 3 and 4 with SHPT (PTH >120 pg/mL, albumin-corrected calcium >8.5 mg/dL and <10.0 mg/dL, and phosphorus >4.6 mg/dL). Goal enrollment is 110 patients among the four participating sites.

Results: To date, 103 patients have been randomized (72 completed, 17 announced). Data from 53 patients have been evaluated, 27 on paricalcitol and 26 on calcitriol. Baseline lab values are shown in the table. Baseline Values

<table>
<thead>
<tr>
<th>Drug</th>
<th>Baseline</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
<th>Week 12</th>
<th>Int group p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/mL)</td>
<td>155.6 (46.2)</td>
<td>164.3 (45.3)</td>
<td>80.6 (34.0)</td>
<td>86.2 (38.4)</td>
<td>106.2 (47.2)</td>
<td>&gt;0.001</td>
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<tr>
<td>iPTH (pmol/L)</td>
<td>1.93 (0.97)</td>
<td>2.03 (0.97)</td>
<td>0.97 (0.79)</td>
<td>1.30 (1.25)</td>
<td>1.30 (1.25)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.89 (0.70)</td>
<td>9.95 (0.70)</td>
<td>9.97 (0.70)</td>
<td>9.95 (0.70)</td>
<td>9.95 (0.70)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>4.44 (2.70)</td>
<td>4.24 (2.70)</td>
<td>4.34 (2.70)</td>
<td>4.24 (2.70)</td>
<td>4.24 (2.70)</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

Our study shows that in hemodialysis patients with a normal DXA scan the rate of hip fractures was 3.22 times higher compared to those with a low DXA score (<-2) and 2.22 times higher compared to those with a normal DXA score (>-2).

Comparing a DXA score of <-2 vs >=-2 the relative risk of hip fractures was 3.22 times higher compared to those with a low DXA score (<-2) and 2.22 times higher compared to those with a normal DXA score (>-2).

Conclusions: Our study shows that in hemodialysis patients with a normal DXA scan the rate of hip fractures was 3.22 times higher compared to those with a low DXA score (<-2) and 2.22 times higher compared to those with a normal DXA score (>-2).

SA-PO2295

Oral Paricalcitol Versus Oral Calcitriol for the Treatment of Secondary Hyperparathyroidism in ESRD Patients on CAPD Ema Juljati Jamaldinuddin,1 Abdul Halim Abdul Gafor,1 Norella C.T. Kong,1 Rizna Cader,1 Rozita Mohd,1 Vincent Chui Wei Wong,1 Shamsul Azhar Shah.2 1Medical Department, University Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia; 2Public Health Department, University Kebangsaan Malaysia, Cheras, Kuala Lumpur, Malaysia

Background: Few studies have reported on the use of oral paricalcitol for the treatment of SHPT in ESRD patients on CAPD.

Objective: We evaluated the efficacy of oral paricalcitol versus oral calcitriol on serum iPTH and parameters of bone mineral metabolism in CAPD patients with SHPT.

Methods: Patients randomized to receive oral paricalcitol or oral calcitriol for 12 weeks. Serum Ca, P, iPTH, and ALP were measured at baseline and 3-weekly. Serum hsCRP and peripheral membrane function tests (Kt/V & F GFR) were also recorded.

Results: Results are presented in the table.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Baseline</th>
<th>Week 3</th>
<th>Week 6</th>
<th>Week 9</th>
<th>Week 12</th>
<th>Int group p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTH (pg/mL)</td>
<td>1.93 (0.97)</td>
<td>2.03 (0.97)</td>
<td>0.97 (0.79)</td>
<td>1.30 (1.25)</td>
<td>1.30 (1.25)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>iPTH (pmol/L)</td>
<td>1.93 (0.97)</td>
<td>2.03 (0.97)</td>
<td>0.97 (0.79)</td>
<td>1.30 (1.25)</td>
<td>1.30 (1.25)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Calcium</td>
<td>9.89 (0.70)</td>
<td>9.95 (0.70)</td>
<td>9.97 (0.70)</td>
<td>9.95 (0.70)</td>
<td>9.95 (0.70)</td>
<td>&gt;0.001</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>4.44 (2.70)</td>
<td>4.24 (2.70)</td>
<td>4.34 (2.70)</td>
<td>4.24 (2.70)</td>
<td>4.24 (2.70)</td>
<td>&gt;0.001</td>
</tr>
</tbody>
</table>

Both vitamin D analogues were equally efficacious. The number of hypercalcemic events were similar. Pre- and post-treatment serum hsCRP and peripheral membrane function were also not different.

Conclusions: Oral paricalcitol was as efficacious as oral calcitriol in reducing serum iPTH in CAPD patients with SHPT.

SA-PO2296

Ergocalciferol (ergo) Therapy in Calcidiol Deficient Hemodialysis (HD) Patients on Therapeutic Doses of Paricalcitol Does Not Decrease Parathyroid Hormone (PTH) levels. Vidya M. Raj Krishnamurthy,1 Christi M. Terry,2 Tom H. Greene,1,2 Guo Wei,2 Alfred K. Cheung,2 Srinivasan Beddhu,1,2 1VA, University Utah.

Background: Even though, most HD patients receive active vitamin D, it is unclear whether additional therapy with ergo in those with calcidiol deficiency would be beneficial.

Methods: We conducted a randomized double blind cross-over trial of ergo (50,000 IU) vs placebo in 24 HD patients with plasma 25(OH) vitamin D levels <30 ng/ml and high sensitivity C-reactive protein (hs-CRP) =3 mg/L and who are on paricalcitol with iPTH range between 150-600 pg/mL. The primary outcome was serum IL6 and secondary end points were plasma hs-CRP and TNFα as well as peripheral blood monocyte production of IL-6 and TNFα (presented as a separate abstract). The study was 80% powered to detect 40% reduction in the geometric mean IL6.

Results: Participants were randomly assigned to ergo vs placebo for 12 wks, followed by 4 wk washout and cross-over for 12 wks. Pre-HD blood samples were collected at baseline and wk 3,6, and 12. The samples were stored at -80°C freezer before analyses. Plasma IL6 and TNFα levels were measured using a Duoset ELISA development system. Mixed effects model for a 2-period 2-treatment cross-over design was used.

Results: The mean age was 59 ± 13 yrs. 42% were men and 80% were white. 67% had DM. Average duration of ESRD was 3.7± 4.6 yrs. Mean serum 25 OH vitamin D levels were 15.6±5.2 pmol and median hsCRP levels were 8.8 (IQR 4.2-19.1) mg/L. The main effects (post vs pre in active group vs post vs pre in placebo group) are summarized in Table. Difference of plasma biomarkers in the pre and post ergo vs placebo treatment

<table>
<thead>
<tr>
<th>Biomarkers</th>
<th>mean±SEM</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma 25(OH) vitamin D</td>
<td>15.6±5.2 (7.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum IL6/ pg/mL</td>
<td>0.840 (S.5-1.29)</td>
<td>0.26</td>
</tr>
<tr>
<td>Serum TNFα/ pg/mL</td>
<td>1.060 (5.4-2.11)</td>
<td>0.75</td>
</tr>
<tr>
<td>Serum hsCRP/ mg/L</td>
<td>1.74 (0.75)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Even though active therapy increased serum vitamin D levels, active therapy did not decrease serum levels of IL-6, hsCRP or TNFα.

Conclusions: Ergo Rs ≥ 75 pmol (25(OH)D levels but did not influence in calcidiol deficient HD patients on therapeutic doses of paricalcitol.
SA-PO2297
Effects of Paricalcitol or Cinacalcet Treatment on CKD-BMD Markers in Subjects with Secondary Hyperparathyroidism (SHPT): Results from IMPACT-SHPT
Kevin J. Martin,1 Markus Ketteler,2 Mario Cozzolino,3 David J. Goldsmith,1 Amit Sharma,3 Michael Amdahl,4 Samina Khan,4 Saint Louis U;4 Klinikum Coburg; Paolo Hosp;4 Guy's Hosp;5 Boise Kidney and Hypertension Inst;6 Abbott.

Background: Managing SHPT is vital for the prevention of CKD-BMD on subjects on hemodialysis (HD) IMPACT compared SHPT treatment effects of the selective vitamin D receptor (VDR) activator, paricalcitol, with the calcimimetic, cinacalcet. Here we report effects on markers of CKD-BMD.

Methods: IMPACT was a randomized 28 wk, phase 4, international, open-label study of HD subjects receiving HD (IV Stratum) or oral paricalcitol (Oral Stratum) with supplemental cinacalcet for hypercalcemia, compared to subjects treated with paricalcitol with low dose vitamin D. Mean reductions in CKD-BMD markers from baseline to end of study were determined.

Results: In the IV Stratum, there were 62 paricalcitol and 64 cinacalcet subjects (60% male, mean age: 61±12 years, mean duration of dialysis: 4±10.4 years). For the Oral Stratum, there were 72 paricalcitol subjects and 70 cinacalcet subjects (65% male, mean age: 65±13 years, mean duration of dialysis: 3.9±3.2 years). In the IV Stratum, paricalcitol had significantly greater reductions in iPTH, compared to cinacalcet. In the Oral Stratum, paricalcitol demonstrated numerically, but not statistically, greater reductions in iPTH. In both strata paricalcitol reduced alkaline phosphatase (AP) and bone-specific alkaline phosphatase (BAP) while cinacalcet increased AP and BAP.

Conclusions: In contrast to cinacalcet treatment, treatment with paricalcitol was associated with decreases in AP and BAP. There were minimal changes in calcium in both groups. However, long term effects on bone markers of CKD-MBD remain to be determined.

Funding: Pharmaceutical Company Support

SA-PO2298
Alienating Effect of Cinacalcet on PTH Secretion from Parathyroid Growth in Patients with Secondary Hyperparathyroidism
Tatsuo Tsukamoto,1 Eri Muso.2 Division of Nephrology and Dialysis, Department of Medicine, Tazuke Medical Research Institute, Kitano Hospital, Osaka, Japan.

Background: Cinacalcet with various doses and derivatives of vitamin D is effective in controlling secondary hyperparathyroidism (SHPT) complicated with chronic kidney disease. We had conducted a clinical trial to confirm the suppressive effect of the combination therapy of cinacalcet with maxacalcitol, a vitamin D derivative, on moderate to severe SHPT whose intact parathyroid hormones (iPTH) were ranged from 300 to 1000 pg/mL for one year (UMIN 000000793). We demonstrated the combination therapy could be useful to keep the suppression of PTH secretion (43rd ASN Annual Meeting, SA-P02164, 2010). However, little is known whether this suppression would last for long time. In this study, we assess the follow-up data of SHPT and parathyroid gland size for another two years in this cohort.

Methods: 53 patients with 157 of enlarged parathyroid glands were enrolled at the initiation. The biochemical data and gland size was measured every 6 months.

Results: The average dose of cinacalcet and maxacalcitol were 39mg daily and 14ig per week at the end of the study, respectively. PTH decreased significantly from 565.5±32.0pg/mL (mean±SE) to 164.3±20.2pg/mL after one year treatment, 134.0±14.2 after another one-year (n=29), and 178.7±22.5 after another two-year (n=18). Half of the enlarged parathyroid glands showed significant volume reduction after the one-year treatment. On the other hand, the growth rate of expanding glands even under the treatment was similar to that of maxacalcitol alone. Thus, the total volume of glands did not change throughout the study periods. The cystic change that was observed in 10% of glands at the first year to that of maxacalcitol alone. Thus, the total volume of glands did not change throughout the other hand, the growth rate of expanding glands even under the treatment was similar.

Conclusions: Our findings indicate that the combination of cinacalcet with maxacalcitol might have a different effect on parathyroid growth and PTH secretion in patients with SHPT. Further study would be required to clarify the limitation of this therapy.

Funding: Private Foundation Support

SA-PO2299
Modification of Human Artery Inflammation by Paricalcitol Is Altered in CKD
Guerman Molostov,1, Rosemary Bland,2 Daniel Zehnder.1 1Clinical Sciences Research Institute, University of Warwick, Coventry, West Midlands, United Kingdom; 2BioMedical Research Institute, University of Warwick, Coventry, West Midlands, United Kingdom.

Background: Arterial inflammation in patients with CKD is associated with vascular calcification. We investigated the role of vitamin D receptor activators (VDRA), paricalcitol and calcitriol on inflammatory responses of arterial wall.

Methods: Arterial explants from CKD patients undergoing renal transplant (n=18) and healthy donors (n=7) were incubated for 48 hours with TFN (20ng/mL), calcitriol (100nM) or paricalcitol (300nM) with/without TNFα. Expression of target gene mRNA was quantified by real-time PCR.

Results: Cbfα1 and osteocalcin (osteoblast markers) were significantly higher in CKD arteries (2.5 fold, p<0.05) and both VDRA inhibited Cbfα1 (p<0.05) but not osteocalcin. Matrix metabolizing enzymes were quantified. Basal expression of MMP2 was similar, but MMP9 was significantly lower in CKD arteries. Both were unaltered by VDRA.

Conclusions: Components of the vascular system (VDR, CYP27B1 and CYP24A1) were analyzed. Basal VDR expression was suppressed by 60% in CKD explants (p<0.05) and increased in both groups by VDRA (p<0.05). Basal CYP24A1 expression was similar in both groups and was induced by VDRA. The response was much greater in CKD group (20 fold in control; p<0.05 vs 300-900 fold in CKD; p<0.01). Basal CYP27B1 was 2 fold higher in CKD and was unaltered by VDRA.

Funding: Pharmaceutical Company Support

SA-PO2300
Safe and Effective Treatment of Vitamin D Deficiency in Pediatric Dialysis Patients
Poyyapakkam Srivaths, Eileen D. Brewer. Pediatrics, Renal Section, Baylor College of Medicine, Houston, TX.

Background: We and others have shown that vitamin D deficiency (vit D def) is highly prevalent in Pediatric (ped) dialysis pts. However, no treatment (tmt) guidelines exist for ped dialysis pts. Purpose of this study was to establish an safe and effective tmt regimen for vit D def in ped dialysis pts and assess effects of tmt on biochemical mineral parameters.

Methods: 10 month prospective study of 58 ped pts (median age 15y; range 1-24y; 28 Hispanic, 16 Black, 13 White, 1 Asian; 31 HD/25 PD /2 home HD). Vit D def was diagnosed by serum 25-hydroxy vitamin D (25vitD) <30 ng/dL. KDOQI guidelines for tmt of vit D def in adults with chronic kidney disease (CKD) were followed: if serum 25vitD level <15 ng/mL, oral ergocalciferol 50,000 units weekly x 4 followed by monthly doses x 6 or 4000 units daily x 12 weeks; if 25vitD level 16-29, 50,000 units monthly x 6 or 2000 units daily for 12 weeks. 28/31 HD pts were treated in-center with either weekly or monthly ergocalciferol. Serum 1,25-dihydroxy vitamin D (1,25vitD) was assessed in 55 pts pre- and end tmt.

Results: 54/58 pts (93%) had vit D def with 13/58 (23%) severe (serum 25vitD <7 ng/ mL). 54/58 pts were taking IV or oral calcitriol; 4/58 received IV paricalcitol. Ergocalciferol tmt improved mean serum 25vitD from 13.5 to 30.1 ng/mL (p=0.0001, Table). Tmt response did not differ by modality or ethnicity. Serum Ca, P, PTH and 1,25vitD were not different at end of tmt compared with pre-tmt.

Conclusions: 1) Vit D def can be treated safely and effectively with oral ergocalciferol in ped dialysis pts as young as 1 y old using adult CKD KDOQI guidelines. 2) If pt adherence is a concern, in-center tmt with weekly or monthly doses is safe and not associated with hypercalcemia. 3) Serum PTH was not different at end ergocalciferol tmt, so oral ergocalciferol improves Vit D def in ped dialysis pts, but does not by itself correct high serum PTH.

Funding: Clinical Revenue Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

648A
Dietary surveys showed a median weekly vitamin D intake of 1113 IU (IQR=748, Table 1), which was significantly below FDA recommendation of 2800-5600 IU/week.

Nutritional guidelines for dialysis patients suggest a limited intake of Vit D-containing dairy products because they are also phosphate-rich. Since many patients struggle to adhere to phosphate binder regimen, they reported being counseled against dairy product consumption.

Conclusions: Our study demonstrates a higher than previously reported prevalence of Vit D deficiency among hemodialysis patients despite seemingly adequate sun exposure and a low rate of sunscreen use. Based on the findings indicative of a physician-initiated bias against intake of Vit D-containing foods, we recommend oral D3 as a safe and inexpensive approach to correct this abnormality.

<table>
<thead>
<tr>
<th>Item</th>
<th>Vit D (IU) per serving</th>
<th>Amount per serving of servings/week (IU)</th>
<th>Median number of servings/week (IQR)</th>
<th>Total vit D/week (IU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish</td>
<td>100-654</td>
<td>8oz</td>
<td>2 (2)</td>
<td>574</td>
</tr>
<tr>
<td>Milk</td>
<td>100</td>
<td>8oz</td>
<td>0 (0.75)</td>
<td>8</td>
</tr>
<tr>
<td>Yogurt</td>
<td>80</td>
<td>8oz</td>
<td>0 (0.75)</td>
<td>0</td>
</tr>
<tr>
<td>Cheese</td>
<td>6-12</td>
<td>6 (5)</td>
<td>54</td>
<td></td>
</tr>
<tr>
<td>Eggs</td>
<td>40</td>
<td>3 egg</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>Cereal</td>
<td>Total~1063</td>
<td>5 (5)</td>
<td>125</td>
<td></td>
</tr>
</tbody>
</table>

Funding: Private Foundation Support

SA-PO2303

Predictors of Vitamin D Status in Incident Chronic Kidney Disease Patients: A Cross-Sectional Analysis in a High UV Climate

Background: Hypovitaminosis D is a significant problem in Chronic Kidney Disease (CKD) and is associated with adverse outcomes. Whether high ambient UV exposure can mitigate this problem is unknown. Our aim was to determine vitamin D status in a subtropical climate amongst an unselected incident CKD population; assess risks and correlates, and review whether higher 25-hydroxyvitamin D (25-OHD) can abate the decrement in 1,25-dihydroxyvitamin D (1,25-OHD) normally encountered with advancing CKD.

Methods: Prospective cross-sectional study of 593 consecutive CKD patients stage 1-5 referred to Princess Alexandra Hospital, Brisbane, Australia (27°28’S). The main outcome measure was 25-OHD sufficiency (≥30ng/mL), and bone-mineral parameters including 1,25-OHD, calcium, and phosphate.

Results: In spite of potentially higher environmental UV exposure, only 48% of patients with CKD were 25-OHD sufficient. Traditional risks for hypovitaminosis D were maintained, and sufficiency was independently predicted by testing in the Summer/Autumn period (OR 3.17, 95%CI: 2.07-4.86, p<0.001), male gender (OR 2.61, 95%CI: 1.68-4.05, p<0.001), Caucasian race (OR 2.19, 95%CI: 1.25-3.84, p<0.001), normal albumin (OR 0.46, 95%CI: 0.23-0.90, p=0.02), and normal BMI (OR 1.80, 95%CI: 1.08-3.00, p=0.02). Vitamin D sufficiency was also associated with higher corrected calcium (0.4 mg/dL increments; OR 1.42, 95%CI: 1.01-1.92, p=0.001). Whilst maintaining 25-OHD concentrations were relatively maintained, and sufficiency was independently predicted by testing in the Summer/Autumn period (OR 3.17, 95%CI: 2.07-4.86, p<0.001), male gender (OR 2.61, 95%CI: 1.68-4.05, p<0.001), Caucasian race (OR 2.19, 95%CI: 1.25-3.84, p<0.001), normal albumin (OR 0.46, 95%CI: 0.23-0.90, p=0.02), and normal BMI (OR 1.80, 95%CI: 1.08-3.00, p=0.02). Vitamin D sufficiency was also associated with higher corrected calcium (0.4 mg/dL increments; OR 1.42, 95%CI: 1.01-1.92, p=0.001). Whilst maintaining 25-OHD concentrations were relatively maintained, and sufficiency was independently predicted by testing in the Summer/Autumn period (OR 3.17, 95%CI: 2.07-4.86, p<0.001), male gender (OR 2.61, 95%CI: 1.68-4.05, p<0.001), Caucasian race (OR 2.19, 95%CI: 1.25-3.84, p<0.001), normal albumin (OR 0.46, 95%CI: 0.23-0.90, p=0.02), and normal BMI (OR 1.80, 95%CI: 1.08-3.00, p=0.02).

Conclusions: 25-OHD insufficiency is mitigated but still highly prevalent in patients with CKD in a high ambient UV environment. Despite the maintenance of relatively higher 25-OHD concentrations with advancing CKD, substrate availability does not appear to be a major determinant of circulating 1,25-OHD.

SA-PO2304

Thyroid Hormones Decrease the Plasma Alpaha, 25-dihydroxyvitamin D Levels through Transcriptional Repression of the Renal 25-hydroxyvitamin D, alpaha-hydroxylase (CYP27B1) Gene

Background: The renal 25-hydroxyvitamin D,1a-hydroxylase enzyme (CYP27B1), is implicated in the control of plasma 1,25-dihydroxyvitamin D (1,25(OH)2D) levels. Previously, it has been demonstrated that the low levels of plasma 1,25(OH)2D in hyperthyroidism patients and the decrease of 1,25(OH)2D synthesis in perfused kidneys treated with 3, 5, 3'-triiodothyronine (T3). However, the regulation mechanism of renal vitamin D metabolism by thyroid hormones is still not clear.

Methods: To address effects of T3 on the plasma 1,25(OH)2D levels and renal CYP27B1 gene expression, C57BL6 mice were rendered pharmacologically hypo- and hyperthyroid by administration of propyl-thiouracil or T3. Western blots, real-time PCR and in situ hybridization analysis were used to elucidate the CYP27B1 gene expression in kidney. CYP27B1 gene promoter activity was measured using luciferase reporter assay system. DNA-protein interaction for cis-elemets of CYP27B1 gene were assessed by gel shift assay.

Results: Thyroid hormone mice showed low levels of plasma 1,25(OH)2D and marked decrease in renal CYP27B1 expression levels. In addition, T3 inhibited the renal CYP27B1 mRNA expression highly induced in mice given low-phosphorus diet or low-calcium diet, vitamin D receptor-knockout mice and alpha klotho mutant mice. The CYP27B1 promoter activity in renal proximal tubular cells were inhibited by T3, in thyroid hormone receptor
SA-PO2305

Web-Based Evaluation of Clinical Benefit of Cinacalcet in End-Stage Renal Disease – The WELCOME Study

Method: Describe attainment of SHPT targets during MIM treatment, before (BGI) and after (AGI) release of KDIGO guidelines in 2009.

Results:

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Urinal Ca (mg/dl)</th>
<th>AGI (n=75)</th>
<th>BGI (n=75)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50 mg/dl</td>
<td>2.4 mg/dl</td>
<td>2.8 mg/dl</td>
<td>0.3 mg/dl</td>
<td>0.01</td>
</tr>
<tr>
<td>50-150 mg/dl</td>
<td>3.0 mg/dl</td>
<td>3.5 mg/dl</td>
<td>3.2 mg/dl</td>
<td>0.005</td>
</tr>
<tr>
<td>&gt;150 mg/dl</td>
<td>4.0 mg/dl</td>
<td>4.5 mg/dl</td>
<td>3.8 mg/dl</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Conclusions: MIM therapy increased % of patients meeting some therapeutic targets; mean PTH was not changed AGI. Reduced PB and VdID was observed after 6 months of MIM therapy.

Sponsor: Amgen GmbH CEE Headquarters

Funding: Pharmaceutical Company Support

SA-PO2306

Vitamin D Deficiency in Chronic Renal Failure Patients: A Screening Survey and Clinical Feature Analysis from a Single Hemodialysis Center in China

Methods: Patients on peritoneal dialysis (PD) or hemodialysis (HD) starting MIM 5 months prior to the study were eligible. Patient data were collected through a web-based case report form. Primary endpoint: % achieving PTH ≤200 pg/mL after 6 months. Secondary endpoints: % at target for Ca, P, or Ca x P at enrolment and 6 months; MIM dose; vitamin D status (25(OH)D levels through transcriptional repression of the renal CYP2B1 gene by TRβ1). Funding: Funding Support - Non-U.S.

SA-PO2307

The Cardiovascular Effects of Cinacalcet in Hemodialysis Patients with Secondary Hyperparathyroidism

Results:

- Twenty weeks cinacalcet treatment significantly decreased blood levels of iPTH (66.6 ± 25.7 vs. 27.7 ± 12.6 ng/mL, P < 0.01), calcium (9.4 ± 1.8 vs. 8.7 ± 0.9 mg/dL, P < 0.01), phosphorus (6.9 ± 1.7 vs. 5.7 ± 1.4 mg/dL, P < 0.01), and calcium x phosphorus product (65.9 ± 17.5 vs. 44.5 ± 15.3, P < 0.01) and 25(OH) vitamin D (8.5 ± 2.8 vs. 7.6 ± 2.7 ng/mL, P < 0.05). But the level of C-reactive protein was not affected by treatment. There were no differences in systolic (134.5 ± 22.1 vs. 128.0 ± 21.0 mmHg, P = 0.2) and diastolic blood pressures (75.2 ± 14.6 vs. 72.7 ± 14.2 mmHg, P = 0.45).

- There were notable reduction trends in the LV mass (244.6 ± 94.2 vs. 212.9 ± 93.6 g/m²), the LV mass index (158.6 ± 63.8 vs. 138.3 ± 45.7 g/m²) and E’ (15.2 ± 6.8 vs. 13.5 ± 5.6), although they did not achieve statistical significance. There were no significant changes in the ejection fraction and fractional shortening. In contrast, of great interest, cinacalcet significantly improved FMD (8.6 ± 2.9 vs. 14.3 ± 3.2%; P < 0.01) and enhanced CAVI (8.8 ± 2.3 vs. 7.6 ± 2.4; P < 0.05) as markers of endothelial-dependent arterial dilatation and arterial compliance, respectively.

- Conclusions: Cinacalcet treatment in hemodialysis patients with secondary hyperparathyroidism ameliorates aortic stiffness, arterial compliance and seems to improve cardiac hypertrophy.

Funding: Pharmaceutical Company Support

SA-PO2308

Nutritional Vitamin D Use in Chronic Kidney Disease: A Survey of Pediatric Nephrologists

Methods: We surveyed 1,176 International Pediatric Nephrology Association physician members. The questionnaire included 8 vignettes comprised of potential determinants of supplementation (starred in Table). Vignettes were stratified by vitamin D level and identified in heart endocardial epithelium, microvasculature of the myocardium, aortic and endothelium and the vascular smooth muscle. Thus, we evaluated the effects of cinacalcet on the cardiovascular system in hemodialysis patients with secondary hyperparathyroidism.

Results:

- The response rate was 45% (479/1074; 50 members do not see pediatric CKD patients and 52 emails were invalid); 64% in the US (200/311) and 37% (281/763) in other countries. These preliminary data are limited to US respondents. The proportions recommending supplementation for vitamin D levels of <10, 10-19, 20-29 and ≥30 ng/mL were 96, 95, 70, and 29%, respectively. Low vitamin D levels were the most influential factor in increasing odds of supplementation (Table) but elevated PTH, time since training, and CKD severity were also associated with supplementation.

Funding: Pharmaceutical Company Support
Table 1: Logistic Regression Model of Recommending Supplementation Among Us Respondents

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>Adjusted Odds Ratio (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKD Stage*</td>
<td>CKD 2-3 Reference</td>
<td>1.0 (1.0 - 1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CKD 4-5</td>
<td>1.5 (1.4 - 1.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Dialysis</td>
<td>1.2 (0.8 - 1.6)</td>
<td>0.38</td>
</tr>
<tr>
<td>PTH Level*</td>
<td>At target for stage</td>
<td>1.0 (1.0 - 1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>Above target for stage</td>
<td>1.4 (1.0 - 1.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sickness Present*</td>
<td>Light</td>
<td>1.0 (0.8 - 1.4)</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Dark</td>
<td>1.0 (0.8 - 1.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>CKD stage*</td>
<td>CKD G4-G5 Reference</td>
<td>1.0 (1.0 - 1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>CKD G1-G3</td>
<td>1.0 (0.8 - 1.4)</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>Dialysis</td>
<td>1.2 (0.8 - 1.6)</td>
<td>0.38</td>
</tr>
<tr>
<td>Vitamin D Level (ng/mL)*</td>
<td>&lt;30 Reference</td>
<td>1.0 (1.0 - 1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>30-49</td>
<td>1.5 (1.4 - 1.7)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>50-69</td>
<td>2.0 (1.6 - 2.5)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>&gt;70</td>
<td>3.0 (2.4 - 3.9)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Year Completed</td>
<td>1995 - 2003 Reference</td>
<td>1.0 (1.0 - 1.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>2004 - 2007</td>
<td>1.5 (1.2 - 2.0)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>2008 - 2014</td>
<td>1.9 (1.6 - 2.4)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td></td>
<td>2015 - 2017</td>
<td>1.7 (1.4 - 2.2)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

* Variables included in case vignettes.

Conclusions: These data suggest that recommending nutritional vitamin D is common and associated with lower vitamin D levels, elevated PTH, and more time since completed training. Further analyses will assess geographical differences in supplementation. Future studies are needed to validate this questionnaire, documenting actual practices.

Funding: Private Foundation Support

SA-PO2309

Vitamin D Supplementation Is Increasing in the Chronic Renal Insufficiency Cohort (CRIC) and Is Associated with Greater Vitamin D Levels

Laura H. Mariani,1 Justine Shults,1 Matthew T. White,1 Cheryl A. Anderson,2 Harold E. Feldman,1 Mary B. Leonard,1 1University of Pennsylvania; Johns Hopkins.

Background: The recent Institute of Medicine Report on Vitamin D noted a significant increase in use of vitamin D supplements in the population.

Methods: We examined vitamin D supplement use at annual visits for the 3939 subjects in CRIC and used generalized estimating equations (GEE) to describe changes and determinants of supplementation.

Results: The proportion of subjects reporting use of a calciferol increased significantly, largely due to products containing only ergocalciferol or cholecalciferol (Figure 1). Active vitamin D sterol use was stable at 2-3%. In a multivariate logistic GEE model, supplement largely due to products containing only ergocalciferol or cholecalciferol (Figure 1). Active and determinants of supplementation.

Conclusions: Vitamin D supplementation rates rose significantly among CRIC participants and was associated with greater levels. Studies of vitamin D deficiency and outcomes in CKD should consider changing supplementation practices.

SA-PO2310

Safety of Oral 25 Hydroxy Vitamin D (cholecalciferol) in Treatment of Symptomatic Vitamin D Deficiency in Renal Transplant Recipients

Nehil Chitalia,1 David Goldsmith. Nephrology and Transplantation, Guy’s and St Thomas’ NHS foundation trust, London, United Kingdom.

Background: Bone mineral disorders (CKD-MBD) are common post transplantation, particularly with an increasing age and longevity of transplant recipients and grafts. Not only is the treatment of CKD-MBD is less well defined in the kidney transplant population, but ‘substrate’ vitamin D deficiency is also common post kidney transplant in part due to avoidance of sunlight. The safety data on oral native vitamin D supplementation in transplant recipients is not available.

Methods: We present the safety data on use of oral Cholecalciferol (Dekristol®) 20,000 IU fortnightly for 6 months, up to a total of 240,000 IU, given to eight symptomatic kidney transplant recipients; symptoms ranging from muscle aches to lethargy. All patients were confirmed to have 25-hydroxy vitamin D deficient [25(OH)D<75 nmol/L] and hyperparathyroidism causes were excluded. One patient had secondary hyperparathyroidism (serum PTH=133 pmol/L) and two patients were on preceding 1α calcidol therapy.

Results: The mean age of the group was 55±16 years with eGFR 55±18 ml/min/1.73m².

Table 1: Pre and 6 month post vitamin D supplementation biochemical parameters

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre supplementation</th>
<th>Post supplementation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Calcium (mmol/L)</td>
<td>2.45±0.15</td>
<td>2.47±0.12</td>
<td>0.56</td>
</tr>
<tr>
<td>Serum Phosphate (mmol/L)</td>
<td>1.07±0.32</td>
<td>1.12±0.26</td>
<td>0.58</td>
</tr>
<tr>
<td>Serum PTH (pmol/L)</td>
<td>107.80</td>
<td>74.43</td>
<td>0.26</td>
</tr>
<tr>
<td>Serum Alkaline Phosphatase (U/L)</td>
<td>156±211</td>
<td>96±58</td>
<td>0.12*</td>
</tr>
<tr>
<td>eGFR mL/min/1.73m²</td>
<td>52±23</td>
<td>50±18</td>
<td>0.45</td>
</tr>
<tr>
<td>Serum 25 (OH)D (nmol/L)</td>
<td>20.4±8.6</td>
<td>73±18</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*Wilcoxon Rank sum test, †p-value = 0.01

Funding: NIDDK Support

SA-PO2311

Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Community Acquired Pneumonia

Anna Jeanette Jovanovich,1 Michel B. Chonchol,1 Shailendra Sharma,1 Kim McFann,2 Sidney N. Thornton,1 Jessica B. Kendrick,3 John R. Holmen. 1Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO; 2Intermountain Healthcare, Salt Lake City, UT.

Background: 25-hydroxyvitamin D (25(OH)D) levels are inversely associated with the development of upper respiratory tract and influenza infections. However, the relationship between 25(OH)D levels and the risk of developing community-acquired pneumonia (CAP) has not been evaluated.

Methods: We performed a population-based cohort study of adult patients diagnosed with community-acquired pneumonia (CAP) who had 25(OH)D levels measured 3 to

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
15 months prior to admission. Vitamin D was evaluated as a continuous variable and a categorical variable (< 15 vs. ≥ 15 ng/mL). The primary outcome of interest was CAP admissions identified through ICD9 codes and confirmed with a chest X-ray. Patients with CAP were matched 1:1 with controls on age, sex, race and season. Conditional logistic regression was used to evaluate if vitamin D levels were associated with an increased risk of CAP.

Results: We identified 66 patients with CAP and 66 matched controls. The mean (SD) age of the participants was 60 ± 17 years, 71% were female and 86% were Caucasian. There was a higher prevalence of chronic kidney disease in patients with CAP compared to controls (23 vs. 13%, p = 0.03). There was a non-significant trend of lower serum phosphorus (1.4 vs. 1.5 mg/dL) in patients with CAP (p = 0.08) and a significant trend of lower serum calcium (9.7 vs. 9.8 mg/dL) in patients with CAP (p = 0.08).

Conclusions: We identified 66 patients with CAP and 66 matched controls. The mean (SD) age of the participants was 60 ± 17 years, 71% were female and 86% were Caucasian. There was a higher prevalence of chronic kidney disease in patients with CAP compared to controls (23 vs. 13%, p = 0.03). There was a non-significant trend of lower serum phosphorus (1.4 vs. 1.5 mg/dL) in patients with CAP (p = 0.08) and a significant trend of lower serum calcium (9.7 vs. 9.8 mg/dL) in patients with CAP (p = 0.08).
The Effect of Recurrent FSGS Plasma on Podocyte Cytoskeleton Is Reversed by Blocking TNFα

Elena Torban,1 Sima Babayeva,2 Anil Vasudevan,3 Martin M. Bitzan,2 Paul R. Goodyer.1 McGill University, Montreal, Canada; 2Montreal Children’s Hospital, McGill University, Montreal, Canada; 3St. John’s Hospital, Bangalore, India.

Background: Patients with steroid-unresponsive FSGS, in whom the known slit diaphragm genes are normal, develop inexorable renal failure. 50% rapidly develop proteinuria in their renal allograft, reflecting a circulating factor that disrupts podocyte biology. Plasma from these patients rapidly disturbs the cytoskeleton of human podocytes in vitro, disperses MYH9 from actin stress fibers and nephrin from the slit diaphragm. In 2009, Leroy reported a boy with recurrent FSGS who responded to infusion of tumor necrosis factor-alpha (TNFα) antibody. We hypothesized that TNFα is responsible for the rapid cytoskeletal changes induced by FSGS plasma.

Methods: In a patient with recurrent FSGS undergoing plasmapheresis we collected plasma effluent (PPE) at the beginning and end of treatment, adding it to confluent monolayer cultures of differentiated human podocytes. In some experiments, PPE were treated with recombinant TNFα or pretreated with blocking antibodies against TNFα or TNF receptors prior to PPE. Cytoskeletal changes were assessed by immunofluorescence microscopy and immunoblotting.

Results: Proteins (Up/creat >10g/l) was responsive (Up/creat <0.5g/l) to plasmapheresis. FSGS-PPE from the beginning (but not the end) of PPE, disrupted arrangement of stress fiber (phalloidin and MYH9 staining) and dispersed adhesion complexes (vimentin staining) in hPod. TNFα alone recapitulated the effect of early PPE, but recombinant TNFα and/or TNFα-PE induced changes in podocyte cytoskeleton and adhesion complexes.

Conclusions: The rapid effects of FSGS plasma on the cytoskeleton of cultured podocytes are largely attributable to TNFα in PPE. Etanercept resulted in a significant sustained reduction in proteasome.

Funding: Government Support - Non-U.S.

SA-PO2319

mIr-143 Contributes to Podocyte Injury Induced by TGF-beta

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Background: Transforming growth factor (TGF)-beta contributes to podocyte injury in FSGS and diabetic nephropathy. Micro(mi)RNAs are small non-coding RNAs that regulate expression of target mRNAs by reducing translational efficiency. Certain miRNAs have been shown to contribute to progression of kidney diseases. We sought to identify miRNAs involved in podocyte injury induced by TGF-beta.

Methods: We used a human podocyte cell line treated with TGF-beta1 and TGF-beta1 transgenic mice that develop severe global glomerulosclerosis.

Results: For discovery, we performed mRNA profiling of the cultured human podocytes treated with TGF-beta1 at 5 ng/ml for 24 hours, using a murine microarray. Among miRNAs significantly increased by TGF-beta1 were miR-143, miR-33, miR-181a and miR-181b and these were confirmed by quantitative RT-PCR, which showed that miR-143 had the largest increase (2.93 ± 1.18 fold over control). We next assessed the function of miR-143, using lentiviral expression. Incubation of cultured podocytes with TGF-beta1 increased expression of COL1A1 mRNA (1.98 ± 0.15 fold), and decreased expression of WTI mRNA (0.60 ± 0.13 fold compared to control) and protein. The combination of TGF-beta1 and miR-143 had an additive effect for both genes. Finally, we found that miR-143 was up-regulated in the TGF-beta1 transgenic glomeruli compared to wild-type glomeruli (1.98 ± 0.40 fold) at three weeks of age prior to developing glomerulosclerosis.

Conclusions: In conclusion, TGF-beta1 induced miR-143 expression in cultured human podocytes and mouse glomeruli, and up-regulated miR-143 may mediate the induction of COL1A1 and the repression of WTI by TGF-beta1. Up-regulation of miR-143 may be a biomarker for TGF-beta1 induced podocyte injury.

Funding: Government Support - Non-U.S.

SA-PO2320

Molecular Mechanism for Angiotensin II Induced Proteinuria

Eva Foss Bejerholm, Martin Hansson,1 Per-Olof Sundin, Anja Groth,2 Magdalena Woznowski, Ivo Quack, Johannes Stegbauer, Lars C. Rump, Lorenz Sellin. Nephrology, Heinrich Heine University, Duesseldorf, Germany.

Background: Microalbuminuria serves as an early marker for glomerular injury in hypertensive and diabetic patients. Inhibitors of the renin-angiotensin-aldosterone system but not calcium channel blockers reduce albuminuria in these patients. Albuminuria results from a defect in the glomerular filter and its slit diaphragms. A major component of the glomerular slit diaphragm is nephrin, that is endocytosed upon binding to the adaptor protein β-arrestin2.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.
Methods: Cells expressing the AT1-receptor or its mutant D125AR126L, nephrin and β-arrestin2 were stimulated with Ang II. After cell lysis, co-immunoprecipitation with subsequent western blot analysis was performed. For the inhibitor studies, cells were pretreated with the inhibitor before stimulation with Ang II. For siRNA experiments cells were transfected with Gq and siRNA and lysed three days thereafter. The effect of Ang II on the β-arrestin2 binding motif was studied by using two nephrin mutants. For the endocytosis assay, cells were stimulated with Ang II and incubated with biotin before cell lysis.

Results: Ang II stimulation increases the protein interaction between nephrin and β-arrestin2. This Ang II effect is dependent on the AT1-receptor and can be inhibited by AT1-receptor blockers. The G-protein signalling is essential for the Ang II effect, as the AT1-receptor mutant D125AR126L abolishes all G-protein signalling. SiriusRNA against the Gq subunit and a phospholipase C inhibitor block the Ang II effect. Phosphorylation of T1120 and T1125 of nephrin are essential for the binding of β-arrestin2. Phosphorylation of nephrin S1146 mediates the Ang II effect. Stimulation with Ang II increases endocytosis of nephrin, which can be inhibited by AT1-receptor and PLC-blockers. The Ang II effect on nephrin-β-arrestin2 binding is also shown in isolated glomeruli.

Conclusions: Ang II weakens the integrity of the slit diaphragm through increase of nephrin endocytosis and is perceived to promote proteinuria. This previously unknown molecular effect of Ang II could help to understand the molecular mechanisms of Ang II induced proteinuria beyond hemodynamic effects.

SA-PO2321

GPC5 Predispose FGF Signaling Related Podocyte Injury Koji Okamoto, Kenjiro Honda, Kent Doi, Toshiro Fujita, Eisie Norii. Departments of Nephrology & Endocrinology, Hemodialysis & Apheresis, University Hospital, The University of Tokyo, Japan.

Background: Genome-wide association study for nephrotic syndrome revealed gypican 5 (GPC5) as risk gene (Nature Genetcs 2011). However, the relationships between podocyte injury and GPC5 was not fully elucidated.

Methods: Podocyte specific GPC5 knock down mice and their sib control mice were studied in mice model. We created two models of heavy proteinuria; Fatal model with puromycin aminonucleoside (PAN) 300mg s.c and bFGF 5 µg 4 days and moderate model with Fatal model with PAN 300mg s.c and bFGF 5 µg 2 days.

Results: Massive albuminuria occurred in sibing control mice and lasted for more than 10 days to 28 days. In contrast, mild albuminuria occurred in the podocyte-specific Gpc5 knockdown mice.

Conclusions: Even in fatal model, the proteinuria in the podocyte-specific Gpc5 knockdown mice was relatively low compared with that of sib control mice. Electron microscopic changes was found 7 days after PAN injection, whereas light microscopic changes was found 14 days after PAN injection. Approximately 25% of glomeruli showed global sclerosis; 50% of glomeruli showed segmental sclerosis at day 28 in sibing control mice. In contrast, 90% of glomeruli showed virtually no changes in conditional knockdown mice.

Conclusions: Our in vivo data strongly suggest a role of the GPC5-FGF2 pathway in nephrotic syndrome pathogenesis. Funding: Government Support - Non-U.S.

SA-PO2322

Heterogenous Changes in Podocyte Morphology and Function in Adriamycin Nephropathy in Mice Matthias Hackl, Katelin Susztak, Janos Peti-Peterdi. Physiology & Biophysics, University of Southern California, Los Angeles, CA; “Albert Einstein School of Medicine, Yeshiva University, Bronx, NY.

Background: Adriamycin nephropathy is a well-characterized model of focal segmental glomerulosclerosis (FSGS) in mice, however proteinuria, glomerular hemodynamic sclerosis and tubular atrophy have been studied mainly by histology. The purpose of this study was to directly and quantitatively visualize the early changes in FSGS in vivo using advanced techniques of multiphoton microscopy (MPM).

Methods: Four week old C57BL6 male mice were injected with 25mg/kg adriamycin iv. After 7 days proteinuria increased 20 to 50 fold and the animals were surgically instrumented for kidney MPM. The vasculature was labeled with albumin-Atto565. Injection of Lucifer Yellow (LY), a molecule which is freely filtered into the Bowman’s space, identified podocytes by negative labeling. A new transgenic mouse model was developed (Pod EGF-P transgenic) featuring specific expression of the intensely green fluorescent protein EGFP in podocytes targeted to the cell membrane while other cell types express the red fluorescent protein tomato. Measurements of the glomerular sieving coefficient (GSC) for albumin were obtained and validated against the intact mice of the glomerular filtration barrier confirmed that the leakage of albumin was restricted to the focal regions of podocyte injury. All changes were absent in control animals injected with normal saline.

Conclusions: In vivo multiphoton imaging of the adriamycin model of FSGS in mice is obtained to assess the local permeability of the glomerular filter. In the early stages of adriamycin GS, podocyte damage/ dysfunction appears to be limited to a few podocytes, where intense, focal leakage of albumin may be the cause of proteinuria.

Funding: NIDDK Support

SA-PO2323

Vitronectin-Binding Exogenous Plasminogen Activator Inhibitor-1 (PAI-1) Protects Podocytes Against Injury In Vivo and In Vivo Haichun Yang, Jianyong Zhong, Ji Ma, Bridgeit Corona, Agnes B. Fogo. Pathology, Vanderbilt University Medical Center, Nashville, TN; Pediatric Nephrology, Vanderbilt University Medical Center, Nashville, TN.

Background: We previously found that genetic PAI-1 deficiency protects podocytes against injury in both in vivo and in vitro. To determine the protective effect on podocytes is plasminogen dependent or not to vitronectin (Vn) interactions, we assessed effects of mutant forms of recombinant human PAI-1 variants on podocyte function.

Methods: Uninephrectomized (UNx) mice were fed high salt diet and infused with angiotensin II (Ang II) for 8 weeks. Different human stable PAI-1 variants, including PAI-1-K (retaining protease inhibitor effects of native PAI-1 but not binding to Vn), or PAI-1-R (competitive blocker of Vn), or 14-1b (control PAI-1, retaining all known functions of native PAI-1), or PBS were injected daily. Primary cultured podocytes were injured by puromycin aminonucleoside (PAN) and various PAI-1 variants were added.

Results: Systolic blood pressure was significantly increased in mice with UNx/Ang II, and none of the PAI-1 variants changed SBP. However, the albuminuria caused by UNx/Ang II was significantly reduced by 14-1b and PAI-1-R (14-1b 1587.4±377.8, PAI-1-R 1165.8±324.16 vs. Ang II 1) and for 8 weeks. Different human stable PAI-1 variants, including PAI-1-K, only vitronectin-binding PAI-1 variants resulted in PAI-1 deposition along glomerular basement membranes. In parallel, 14-1b and PAI-1-R maintained synaptopodin and decreased desmin on podocytes, while PAI-1-K and PBS did not. In vitro, Vn-binding human PAI-1, 14-1b and PAI-1-R maintained synaptopodin and α-actinin-4 expression after PAN treatment, and also decreased pro-apoptotic Bax expression in wild type podocytes (14-1b 0.69±0.03, PAI-1-R 0.56±0.03 vs. PBS 0.99±0.02, p<0.05). Previous data showed lack of protection against PAN by Vn-binding human PAI-1 in Wild type 1-Podocytes, which also expressed less uPA than PBS when compared to wild type.

Conclusions: Our data suggest that vitronectin-binding PAI-1 protects podocytes against injury. Collectively, these studies suggest the protective effect of vitronectin-binding PAI-1 may be related to uPA.

Funding: NIDDK Support

SA-PO2324

Novel Therapeutics in Diabetic Nephropathy: Sialic Acid Precursors Reduce Proteinuria in Experimental Diabetic Nephropathy Camille E. Mac, Lionel C. Clement, Sumant S. Chugh. Medicine/Nephrology, University of Alabama at Birmingham, AL.

Background: We have recently shown that the expression of sialic acid deficient (hyposialylated) Angptl4 is increased in podocytes in minimal change disease (Piston et al., J Am Soc Nephrol 22: 2011). Only vitronectin-binding PAI-1 variants resulted in PAI-1 deposition along glomerular basement membranes. In parallel, 14-1b and PAI-1-R maintained synaptopodin and decreased desmin on podocytes, while PAI-1-K and PBS did not. In vitro, Vn-binding human PAI-1, 14-1b and PAI-1-R maintained synaptopodin and α-actinin-4 expression after PAN treatment, and also decreased pro-apoptotic Bax expression in wild type podocytes (14-1b 0.69±0.03, PAI-1-R 0.56±0.03 vs. PBS 0.99±0.02, p<0.05). Previous data showed lack of protection against PAN by Vn-binding human PAI-1 in Wild type 1-Podocytes, which also expressed less uPA than PBS when compared to wild type.

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Funding: NIDDK Support

SA-PO2324

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Conclusions: Our data suggest that vitronectin-binding PAI-1 protects podocytes against injury. Collectively, these studies suggest the protective effect of vitronectin-binding PAI-1 may be related to uPA.

Funding: NIDDK Support
Filtration Barrier Dysfunction in Diabetic CLIC5A Deficient Mice  Yimin Zhang, Lajii Li, Abass Alnomyan, Barbara J. Ballermann. Medicine, University of Alberta, Edmonton, AB, Canada.

Background: The Chloride Intracellular Channel 5A (CLIC5A), one of two CLIC5 isoforms, is a glomerulus-predominant protein that co-localizes with Ezrin and podocalyxin in podocytes. In CLIC5 (jbg/jbg) deficient mice, podocyte Ezrin and phospho-Ezrin levels are reduced, podocyte architecture is abnormal and adriamycin-induced injury is more severe than in wild-type (+/+). In human patients with diabetic nephropathy glomerular CLIC5A mRNA expression is reduced. Here, we sought to determine whether CLIC5A deficiency is a susceptibility factor for diabetic nephropathy in mice.

Methods: Heterozygous (+/jbg) mice were bred to generate jbg/jbg, +/jbg and +/+ littermates. Diabetes was induced at 8 weeks of age with intraperitoneal (50 μg/g) streptozotocin for 5 consecutive days. Non-diabetic controls were similarly injected with buffer alone. One day after the first injection was defined as day 1, week 1. Mice with fasting blood glucose levels >250 mg/dl on day 14 were considered diabetic. Blood glucose and western blots for urine albumin were obtained every two weeks, and mice were euthanized during weeks 6 and 14 for tissue analysis.

Results: Light microscopy of PAS and H&E stained renal tissue was normal for control and diabetic mice of all genotypes at 6 and 14 weeks. There was increased deposition of extracellular matrix and mild mesangial expansion in all diabetic mice compared to non-diabetic mice, but no differences between the three genotypes were observed. By immunoblot analysis of kidney tissue, podocalyxin migrates as a single band in non-diabetic (+/+) mice. An additional, more slowly migrating band suggesting reduced podocalyxin glycosylation was observed in diabetic (+/jbg) mice and in both, diabetic and non-diabetic jbg/jbg mice. Diabetes did not change with induction of diabetes. Finally, diabetic jbg/jbg and +/jbg mice, had higher urine albumin levels than non-diabetic jbg/jbg or +/jbg controls, whereas no difference in urine albumin was observed between diabetic and non-diabetic mice.

Conclusions: The findings lead us to postulate that CLIC5A deficiency may change podocalyxin glycosylation in glomerular podocytes, increasing filtration barrier dysfunction in diabetes.

Funding: Government Support - Non-U.S.

Filtration Barrier Dysfunction in Diabetic CLIC5A Deficient Mice

MicroRNA Profile Determines Progression of HIV-Associated Nephropathy Partab Rai, Dileep Kumar, Pravin C. Singhal, Ashwani Malhotra. Medicine, NSLIJ, Great Neck, NY.

Background: Micro RNAs with dual function to act as inducers or repressors of gene activity have been reported to be differentially expressed in various pathological and physiological states. However, the precise role of microRNAs, endogenous RNA allogenes which specifically target mRNA and regulate gene expression in HIV-associated nephropathy (HIVAN) is not functionally known. Our laboratory had shown the activation of mTOR pathway in HIVAN and rapamycin, an inhibitor of mTOR pathway, which has been used to attenuate polycystic kidney disease in animal experimental models. We asked whether microRNAs regulate this process in this model.

Methods: Kidneys were harvested from FVB/N and Tg26 mice at 8-10 weeks of age. In another group, mice were given rapamycin (5 mg/kg, ip, alternate day) for 4 weeks. In brief, total RNA from renal tissue was isolated from FVB/N (controls), Tg26 and +/+ mice by Trizol reagent (Invitrogen). A complete microRNA microarray was performed in these groups and a data analysis was generated. cDNA was synthesized using SuperScript Enzyme Mix (Invitrogen). Real-Time qPCR was performed to confirm the microRNA microarray data by using SYBR Green Universal Kit (Invitrogen) using forward primers and universal qPCR primers.

Results: TCG26 mice showed altered expressions of mir-145 (14879±2273, $p<0.001$), and R=13930±3232, Arbitrary Units (AU); $p<.04$), mir-16 (11594±1393, $p<0.001$), mir-30c (22517±2908, $p<.001$), and universal qPCR primers.

Conclusions: These microRNAs have been considered to play key roles in many other processes such as proliferation, differentiation and apoptosis by inhibiting target gene expression. Our results demonstrate that the above selective bioregulators could have a functional impact in HIVAN.

Funding: NIDDK Support

Differential Modification of Enalapril in the Kidneys of Lean and ‘Programmed’ Obese Male Young Rats  Hyung Fun Yim, Kee Hwan Yoo, Cheong Park, In Sun Bae, Joo Won Lee. Pediatrics, Korea University Medical Center, Republic of Korea.

Background: The acquired reset of the renin-angiotensin system (RAS) has been suggested to cause lifelong functional and structural alterations. We aimed to investigate the role of the RAS block in the renal pathophysiological changes in the rat model of ‘programmed’ obesity.

Methods: Three or 10 male pups per mother were assigned to either the small litter (Obese group) or normal litter (Lean group) rats during the first 21 days of life. With this, all pups were randomly assigned into 4 groups, and treated with enalapril (Obese enalapril, OE; Lean enalapril, LE) or vehicle (Obese control, OC; Lean control, LC) between the ages of 2 and 4 weeks postnatally. All pups had body weight, blood pressure (BP) and renal alterations determined at 25 days of age.

Results: Pups in the OC group weighed more than rats in the LC group between 7 days and 28 days of age ($p<0.05$). Enalapril decreased body weights in the Obese and Lean groups at 28 days ($p<0.05$). Mean BP levels in the OC and LE groups were higher than those in the LC and OE groups ($p<0.05$) and increased renal cell apoptosis, proliferation, glomerulosclerosis, and tubulointerstitial fibrosis compared to both Lean and Obese controls, respectively ($p<0.05$). Pups in the OE group particularly showed the highest increases in renal cell apoptosis, glomerulosclerosis and tubulointerstitial fibrosis compared to the other groups ($p<0.05$). Immunoblotting and immunohistochemistry showed that enalapril increased renin, angiotensin II receptor type (AT2) and matrix metalloproteinase (MMP)-9 and decreased AT1, tissue inhibitor of MMP (TIMP)-1, and osteopontin expression in the kidneys of Lean group. In contrast, enalapril decreased AT2 and MMP-9 and increased TIMP-1, osteopontin, and plasminogen activator inhibitor-1 expression in the kidneys of Obese group ($p<0.05$).

Conclusions: These data indicates that renal changes following early postnatal overfeeding may be fundamentally different to those of normal postnatal growth after exposure to the RAS inhibition. Angiotensin II can be a key player in the developmental renal programming of obesity.

Impairment of Podocyte Function in Mice with Gsa Deletion in Juxtaglomerular Cells  Lamping Jiang, Christoph Eisner, Yuning Huang, Diane Miezil, Christoph Eisner, Timeng Chen.

Department of Nephrology, Peking Union Medical College Hospital, Beijing, China; NIDDK, NIH, Bethesda, MD.

Background: Mice with deletion of Gsa in renin-producing cells (RC/FF mice) have been shown to have greatly reduced renin production and responsiveness of renin secretion to acute stimuli. In addition, long-term experiments documented that renal function and glomerular filtration are increased in RC/FF mice. In the present study we have determined a possible role of podocyte abnormalities in this renin-free mouse model and the effects of ACE inhibition (ACEI).

Methods: Experiments were performed in 7-8 weeks old RC/FF mice and age-matched controls. Mice were fed a standard diet. In addition mutant and control mice were studied after being fed a low-salt diet (0.03% NaCl w/w) for 7 days, and receiving enalapril in the drinking water (about 10 mg/kg per day). Efficacy of the prolonged treatment with ACEI was examined in anesthetized mice by determining the blood pressure (BP) response to angiotensin I. After the treatment blood were collected for plasma renin measurements and mice were sacrificed. Kidneys were fixed for histological examination. Wilms tumor protein (WT1) as a selective podocyte marker was determined by immunohistochemistry.

Results: Urinary albumin excretion was higher in RC/FF mice when adjusted by GFR. These data was observed in treated and untreated RC/FF mice. Compared with the control mice, WT1 positive podocyte counts as well as the glomerular area were significantly decreased in RC/FF mice. When stimulated by low salt and ACEI, WT1 positive podocyte area per glomerulus had markedly increased in both RC/FF and control mice. Although there were no significant changes of PRA by the chronic low salt/ACEI treatment, WT1 positive podocyte area per glomerulus was much higher in RC/FF than control mice.

Conclusions: Chronic Gsa deletion in KG cells leading to greatly reduced renin production may impair podocyte function resulting in increased albumin excretion and EFGS in the older age. This process may be partly improved by ACEI through currently unknown pathways.

Funding: Government Support - Non-U.S.

Funding: Government Support - Non-U.S.

Funding: NHMRC Centre for Kidney Research, Australia.
Conclusions: In conclusion, F1n4 is upregulated in podocytes in different forms of proteinuric kidney disease and by diverse stressors. TWEAK has NFκB-mediated pro-inflammatory effects on podocytes that may be relevant for the pathogenesis of proteinuric kidney disease. Thus, TWEAK or F1n4 targeting should be explored in proteinuric diseases. 
Funding: Government Support - Non-U.S.

SA-PO2330 
Podocyte Specific GLUT4 Deletion Protects from the Development of Lipopolysaccharide (LPS) Induced Albuminuria 
Johanna Guzman,
Britta Sylvia Walter,
Cristina Muresan,
Mary Donnelly,
Kirk N. Campbell,
Peter H. West,
Sandra M. Merscher-Gomez,
Alessia Fornoni,1 Medicine/Nephrology, University of Miami Miller School of Medicine, FL; 2Medicine/Nephrology, Mount Sinai Medical Center, New York, NY; 3Medicine, Massachusetts General Hospital, Boston, MA.

Background: Insulin resistance correlates with albuminuria in patients with diabetes as well as in normal individuals. Mice with a podocyte-specific insulin receptor deletion develop glomerular lesions resembling diabetic nephropathy (DN), suggesting an important role of insulin signaling in podocyte function.

Methods: We investigated how GLUT4 is modulated in human DN and how GLUT4 affects podocyte function. 

Results: Using RT-PCR, we show that GLUT4 is downregulated in glomeruli from patients with DN when compared to controls (N=6 each, p<0.05). Mice with a podocyte-specific deletion of GLUT4 (GLUT4 KO) were generated by crossing Glut4 floxed mice with Podocin-Cre mice. Glut4 KO mice did not show an increased urinary albumin to creatinine ratio up to 19 weeks of age. However, when challenged by two daily consecutive injections of LPS (200µg each), Glut4 KO mice had an albumin/creatinine ratio of 139 ± 131 vs. 1059 ± 449 in wildtype mice (p<0.001). Western blot analysis of glomeruli demonstrated that Glut4 KO mice were protected from LPS induced degradation of synaptopodin and nephrin. In order to further characterize the role of GLUT4 in podocyte function, we performed GLUT4 siRNA experiments in differentiated mouse podocytes. GLUT4 knockdown podocytes showed an increased in synaptopodin and RhoA expression (p<0.01), associated with cell blebbing (p<0.05) and increased cell migration. These changes were conserved when the experiments were repeated in glucose free medium, suggesting that Glut4 may affect podocyte function in a glucose independent manner.

Conclusions: In conclusion, GLUT4 is directly involved in the regulation of the podocyte actin cytoskeleton and targeting GLUT4 may represent a new strategy for certain glomerular diseases.
Funding: Private Foundation Support

SA-PO2331 
The Role of the Protein Kinases MK2 and MK3 in a Mouse Model of Acute Proliferative Glomerulonephritis 
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Background: Signaling by various MAPK kinases, including p38 MAPK, has been understood. Increased p38 MAPK activity has been observed in podocytes in patients with diabetes as well as in normal individuals. Mice with a podocyte-specific deletion of GLUT4 (GLUT4 KO) were generated by crossing Glut4 floxed mice with Podocin-Cre mice. Glut4 KO mice did not show an increased urinary albumin to creatinine ratio up to 19 weeks of age. However, when challenged by two daily consecutive injections of LPS (200µg each), Glut4 KO mice had an albumin/creatinine ratio of 139 ± 131 vs. 1059 ± 449 in wildtype mice (p<0.001). Western blot analysis of glomeruli demonstrated that Glut4 KO mice were protected from LPS induced degradation of synaptopodin and nephrin. In order to further characterize the role of GLUT4 in podocyte function, we performed GLUT4 siRNA experiments in differentiated mouse podocytes. GLUT4 knockdown podocytes showed an increased in synaptopodin and RhoA expression (p<0.01), associated with cell blebbing (p<0.05) and increased cell migration. These changes were conserved when the experiments were repeated in glucose free medium, suggesting that Glut4 may affect podocyte function in a glucose independent manner.

Conclusions: In conclusion, GLUT4 is directly involved in the regulation of the podocyte actin cytoskeleton and targeting GLUT4 may represent a new strategy for certain glomerular diseases.
Funding: Private Foundation Support

SA-PO2332 
Estrogen Receptor Alpha Knockout Mice Develop Podocyte Damage and Increased Glomerular Collagen IV Deposition 
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Background: Epidemiological studies have demonstrated that osteoporosis is a risk factor for chronic kidney diseases can be positively influenced by female gender. This suggests critical role of sex hormones on glomerular structure and function. We investigated estrogen receptor alpha (ERα) knockout mice for podocyte/glomerular damage as well as ERα expression and protective effects of estrogens on podocytes in vitro.

Methods: ERα knockout mice were examined for podocyte and mesangial cell number, volume fraction of TGFβ1, IV and gp38 in vivo, and TGFβ1 and IV in vitro. Glomerulopathy was investigated in podocytes in vitro and in vivo. Antiproliferative action of 17β-estradiol was analyzed in pyrimycin-treated podocytes using Annexin-FITC flow cytometry and Hoechst staining for apoptosis detection. Estabilization of mitochondrial membrane potential, an early indicator of apoptosis, was visualized with tetramethyl rhodamine methylester staining.

Results: In ERα knockout mice, podocyte number was reduced compared to wild-type controls in female/male animals (80.86 ± 152/135 per glomerulus, p<0.05). Podocyte cell volume was enhanced in ERα knockout mice (429/131µm3 vs. 264/223µm3, p<0.05). TGFβ1 and collagen IV expression were significantly enhanced in knockout mice, indicating glomerular damage. 
ERα is expressed in human and murine podocytes on mRNA and protein level. 17β-estradiol induced PAN in vivo and collagen IV and gp38 staining in vitro. The results showed that in spleen, the rates of CD4+ and collagen IV expression were significantly enhanced in knockout mice, indicating glomerular damage.

Conclusions: ERα deficient mice develop a loss of podocytes and cellular hypertrophy, indicating cellular stress. Correspondingly, podocytes express increased levels of TGFβ1 and collagen IV, mediating significant protection against apoptotic stimuli. These findings may explain in part the gender differences in glomerular diseases.
Funding: Private Foundation Support

SA-PO2334 
Heparan Sulfate Deficient Zebrafish Show Glomerular Malfunction and Ineffective Filtration 
Ramzi Khalil, Danielle Cohen, Malgorzata Wiweger, Wietske Van der Ent, Emile De Heer, Jan A. Brujin, Pancras C. W. Hogendoorn, Hans J. Baade, Pathology, Leiden University Medical Center, Netherlands.

Background: There has been a longstanding discussion on whether heparan sulfate proteoglycans (HSPG) serve as a charge selective filtration medium in the glomerular filtration barrier. A recent mouse model study showed that a lack of podocyte specific HSPG does not lead to glomerular dysfunction. This project aims to investigate whether a biallelic knockout of the homologue of human EXT2 in zebrafish, leading to shortened and functionally impaired HSPGs, results in abnormal glomerular filtration.

Methods: Zebrafish (Danio rerio) Haddock (dag) mutants have a premature stop codon in the ext2 gene. The ext1 and ext2 gene products encoding subunits of heparan sulfate co-polymers are essential components in the heparan sulfate chain elongation. Wild type (WT) larvae and homozgyous dag mutants were injected intravenously with a mixture of FITC labeled lysine fixable 70kD dextran and TRITC labeled 30kD dextran at 4 days post fertilization, fixed with formalin at 5 or 60 minutes after injection, imbedded in TissueTec® for oriented sectioning and studied by immunofluorescent microscopy.

Results: Neither TRITC nor FITC fluorescent absorption droplets were found in the renal tubules of the WT zebrafish at both 5 and 60 minutes.

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SA-PO2335

Crk/1 Signaling Is Necessary for Foot Process Spreading in Mice and Is Activated in Human Proteinuric Kidney Disease Britta George, 1 Rakesh Verma, 2 Abdul A. Soofi, 1 Maria Pia Rastaldi, 1 Lawrence B. Holzman, 1 1Renal-Electrolyte and Hypertension Division, University of Pennsylvania, Philadelphia, PA; 2Division of Nephrology, University of Michigan, Ann Arbor, MI.

Background: The slit diaphragm protein Nephrin is necessary for establishing the morphology of the podocyte in development by transducing from the podocyte intercellular junction phosphorylation-mediated signals that regulate cytoskeletal dynamics. Methods: Podocyte culture, podocyte-specific knockout mice and human kidney tissue immunofluorescence were employed to understand Crk biology.

Results: We found that activation of Nephrin induced lamellipodia formation in podocyte culture. Following Nephrin activation in cell culture, a FAK/Cas/Crk protein complex was recruited to Nephrin in a p3- and Src kinase-dependent fashion. Crk/1 and Cas were necessary for Nephrin-induced lamellipodia formation since cell spreading was attenuated in podocytes with RNAi-mediated knockdown of Crk/1 or Cas. In human glomerulonephritis, Crk1/2 and Cas were necessary for dedifferentiation of mesangial cells. In a murine model of diabetic nephropathy, we found that proteins of the FAK/Cas/Crk complex were present at the intercellular junction of developing podocyte precursors in vivo in their activated phosphorylated state. To test the relevance of Crk/1 signaling, mice deleted of Crk/1 in a podocyte-specific fashion were bred and found to develop and age normally. Surprisingly, podocytes in these mice were protected from prolamellar body (PS)-induced foot process spreading. In human proteinuric kidney disease, the FAK/Cas/Crk signaling axis was activated as p-Cas and p-FAK staining was increased in podocytes of minimal change and membranous nephropathy patients compared to healthy controls.

Conclusions: We conclude that Nephrin induces cell spreading in culture via a FAK/Cas/Crk protein complex. In mice the FAK/Cas/Crk complex is necessary for foot process spreading following podocyte injury; our additional findings in human tissue lead us to speculate that this might be true in some forms of human glomerular disease. Altogether, the Crk protein complex represents a promising therapeutic target in human podocytopathy.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2338

Proliferative Lupus Nephritis: A Unique Disease Process Philip Cohen, 1 Dawn J. Caster, 1 David W. Powell, 1 1Medicine, University of Virginia Health System, VMAC, .

Background: Proliferative lupus nephritis (LN) is a major cause of renal failure in patients with systemic lupus erythematosus (SLE), and 60% of these patients are classified as focal (Class III) or proliferative (Class IV) LN. Recent genome wide association studies of patients with SLE identified mutations in a number of NF-κB activity mediators, including ABIN1, which is a scaffolding protein that regulates NF-κB activity by recruiting and sequestering NEMO. A recent report showed that transgenic mice expressing a mutated form of ABIN1 that is unable to bind to polyubiquitin chains (Nanda et al, 2011. J. Exp. Med. 20102177) demonstrated significantly reduced serum C3 and C4 levels and developed lupus-like autoimmune disease with enlarged spleens and lymph nodes, manifesting from 7 months. Bone marrow transfer from CTGF transgenic mice into irradiated wild type recipients confirmed that glomerulonephritis was transferable. This study indicates a key and predominately deleterious role for Mϕ in the progression of kidney injury in MPGN. Glomerular Mϕ activation is universal and the majority express markers of M1 & M2 functions but a minority of glomerular Mϕ express markers of reparative functions.

Conclusions: This study characterizes a model of mesangial proliferation without expansion of the mesangial matrix, loss of glomerular capillaries, or interstitial changes. At 5 months transgenic mice demonstrated advanced proliferative glomerulonephritis with mesangial expansion and lobulation, occlusion of capillary lumens, thickening of capillary walls, and focal interstitial cellular infiltrates. Electron microscopy showed disorganized mesangial and subendothelial electron dense deposits and immunohistochemistry showed deposition of C3, C1q, IgG, IgM, and IgA for both ages. At 5 months transgenic mice demonstrated significantly reduced serum C3 and C4 and had elevated serum cystatin C.

Conclusions: This novel mouse model of SLE demonstrates progressive glomerulonephritis with histologic features similar to Class III and IV LN. We conclude that loss of ABIN1 regulation of NF-κB results in production of autoimmune glomerulonephritis.

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SA-PO2339

Glomerular Macrophage Heterogeneity in Murine Cryoglobulinemia-Associated Membranoproliferative Glomerulonephritis Progression Yujiro Kida, 1 Kelly L. Hudkins, 1 Charles E. Alpers, 1 Jeremy S. Duffield. 1Medicine & Pathology, University of Washington, Seattle, WA.

Background: Inflammatory macrophages (Mϕ) are known to play dual roles in tissue repair and regeneration or in amplification of tissue injury and scarring. Many studies in kidney diseases suggest that Mϕ amplify injury and fibrosis, but recent studies in AKI show the beneficial role of Mϕs are beneficial in tissue repair.

Methods: We used mice expressing Lck-DTR transgenes to examine the overall function of glomerular Mϕs in cryoglobulinemia and MPGN. Lck-DTR generates overactive B lymphocytes that produce cryoglobulins, and CD11b-DTR permits specific conditional ablation of Mϕs when diphtheria toxin (DT) is injected.

Results: To study the function of inflammatory Mϕs in this MPGN model we achieved glomerular Mϕ ablation of 80% compared with controls, from d30-d50 with alternate day DT injections. IgG deposition was unaffected, but ablation lead to a 40% reduction in mesangial matrix expansion detected by silver stain or ColIV stain. Mesangial cell area was also reduced by 40% and proteinuria reduced by 60%. The overall function of Mϕs is deleterious, promoting mesangial proliferation and sclerosis. To understand the phenotype of MPGN glomerular Mϕs we phenotyped them using established markers of activation and differentiation. All MPGN Mϕs showed evidence of activation but are highly heterogeneous, expressing both M1 (CD11a, Ly6C, CD40) & M2 (CD206, Mac2, ITGβ5) markers, suggesting that either glomerular Mϕs have dual functions or that the established markers are poor discriminators of function. A minority (25%) specifically express the reparative function marker F4/80, and a majority (75%) express M1 markers.

Conclusions: This study indicates a key and predominately deleterious role for Mϕ in the progression of kidney injury in MPGN. Glomerular Mϕ activation is universal and the majority express markers of M1 & M2 functions but a minority of glomerular Mϕ express markers of reparative functions.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2340

Lympheocyte Connective Tissue Growth Factor Mediates Cryoglobulin Production and Induces Cryoglobulinemic Glomerulonephritis Alan D. Salama, 1 H. Terence Cook, 1 Charles D. Pusey, 1 Ruth M. Tarzi, 1 Ruth J. Pepper, 1 Nadia Wahab, 1 Roger M. Mason, 1 Maria Fragiadaki, 1 1Renal Section, Imperial College London, London, United Kingdom; 2UCL Centre for Nephrology, Royal Free Hospital, London, United Kingdom.

Background: Cryoglobulins are immunoglobulins that precipitate at temperatures below 37 degrees and may be idiopathic, termed mixed essential cryoglobulinaemia (MEC) or associated with haematological malignancies and hepatitis C. Cryoglobulins stimulate small vessel vasculitis and renal involvement with mesangiocapillary glomerulonephritis. However, the aetiology of cryoglobulinaemia remains poorly defined and in viral associated cases therapy is often restricted by potential viral replication.

Methods: We have investigated the role of connective tissue growth factor (CTGF, CNN2) in pleiotropic growth factor upregulated in hepatocytes following hepatitis C infection and in lymphocytes during certain haematological malignancies.

We investigated CTGF expression in lymphocytes from patients with MEC, its effect on immunoglobulin production and the impact of lymphocyte overexpression of CTGF in a murine model.

Results: CTGF was significantly elevated in the serum and peripheral blood mononuclear cells (PBMC) of patients with MEC compared to controls (p=0.016). Addition of CTGF to lymphocytes stimulated lymphocyte proliferation, immunoglobulin G and M production despite induced cryoglobulins. Patient’s PBMC expressed altered CTGF mRNAs moieties compared to controls, with excess exon 1 and 2 expression. 75% of mice that overexpressed CTGF in lymphoid cells developed a time-dependent cryoglobulinemic glomerulonephritis, manifesting from 7 months. Bone marrow transfer from CTGF transgenic mice into irradiated wild type recipients confirmed that glomerulonephritis was transferable by haemopoetic cells.

Conclusions: Lymphocyte expression of CTGF is a critical requirement for cryoglobulin production in human cells in vitro and in an in vivo murine model which results in immunoglobulin glomerulonephritis. Targeting CTGF may provide a rational non-immunosuppressive therapeutic approach for treating cryoglobulinemia, which is most appealing in those cases associated with viral infections.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2341

Immunostaining of Urinary Sediments for Claudin1, Neutrophil Elastase and Fibronectin for the Evaluation of Disease Activity of Glomerulonephritis Takashi Oda, 1 Naoki Oshima, 1 Hiroo Kumagai. 1Department of Nephrology and Hypertension, National Cerebral and Cardiovascular Center, Osaka, Japan; 2Department of Internal Medicine, National Cerebral and Cardiovascular Center, Osaka, Japan.

Background: Urinary sediments of the patients who underwent kidney biopsy in our clinic were immunostained for Claudin1, Neutrophil elastase and Fibronectin for the evaluation of disease activity of glomerulonephritis.

Methods: We collected morning urine samples from 196 patients hospitalized for kidney biopsy from 2008 to 2011. Sediments of their urine were dehydrated on the slide,

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and indirect immunofluorescent staining was performed with anti-neutrophil elastase (NE) (clone 2D4, BD Biosciences), C3 (anti-C3, DAKO), CD68 (anti-Mac3, DAKO), CD11c (anti-lymphatic, BD Biosciences), cytokeratin (anti-cytokeratin, BD Biosciences), and claudin (CL1). CL1 is one of the tight junction proteins that are specifically expressed on epithelial cells of Bowman’s capsule. The relationship between the positive cell number for various markers and the crescent formation rate (<30%; low, >30%; high) or global sclerosis rate (<10%; low, >10%; high) was evaluated.

**Results:** The numbers of CL1+ and NE+ cells were significantly increased in those with high sclerosis rate or state of crescents. No other markers showed any significant difference in relation to crescent formation rate, glomerular sclerosis rate or state of crescents.

**Conclusions:** These data suggest that the immunostaining for CL1, NE and CD68 of urinary sediment is useful for the evaluation of glomerulonephritis, which may help for the decision of steroid and immunosuppressants.

**SA-PO2340**

Histopathological Classification of Primary Renal Vasculitis: What’s New?

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**Background:** Current histopathological classifications for renal vasculitides are not consensual. A new classification for ANCA-associated glomerulonephritis based on chronic damage extension has been recently described (focal, crescentic, mixed and sclerotic). Our aim was to determine the predictive value for renal outcome according to chronic and active glomeruli damage extension observed on kidney biopsy samples from patients with vasculitis.

**Methods:** We performed a 25 years retrospective study of 66 patients (56% female) with clinical vasculitides (45 had Systemic Lupus Erythematosus and 21 had ANCA-associated glomerulonephritis). Demographic and laboratory data were recorded at the time of biopsy. Biopsy samples were classified as active (≥33% glomeruli combining cellular proliferation, leukocyte infiltration or fibrinoid necrosis-G1); sclerotic (≥33% sclerotic glomeruli-G2); mixed (≥33% combining crescentic and sclerotic glomeruli-G3); crescentic (≥33% with cellular crescents-G4) and as focal (≤25% not affected glomeruli-G5). Survival analysis was used to assess differences in renal outcomes according to pathologic groups.

**Results:** The median of observed glomeruli was 10(49.3% of samples). At 5 years follow-up, the actuarial renal survival was 64.8% in G1, 55.6% in G2, 66.7% in G3, 36.4% in G4 and 93.5% in G5. The Cox regression revealed that hemoglobin (Hazard Ratio=0.70; p=0.004) and serum creatinine levels (HR=1.22; p=0.046) had a statistically significant effect on renal survival. Compared to G5, the HR for G1 was 5.3 fold higher (p=0.012) and the overall G2, G3 and G4 HR was 4.5 fold higher (p=0.006). The type of vasculitis had no influence in this Cox model.

**Conclusions:** Extension damage in excess of one third on renal histologic analysis, in patients with primary vasculitides, emerged as an independent risk factor for progression to renal failure.

**SA-PO2341**

Misdiaigned Cases of C3 Glomerulopathy among Children with Post Infectious Glomerulonephritis

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**Background:** C3 glomerulopathy (C3G) is a disease entity that has been recently introduced to dysregulation of the alternative complement pathway and is characterized by isolated C3 glomerular deposits in the absence of immunglobulins (Ig). There are a number of diseases that together with C3G form a phenotypic spectrum including dense deposit disease (DDD), atypical hemolytic uremic syndrome, and more recently, CFH-related C3G (CFH-C3G), which is characterized by diffuse deposits in mesangium, mesangial, and subendothelial, and mesangial, glomerulopathy, and renal impairment to end stage renal disease. We postulate that cases of C3G have been misreported in the past, possibly as post infectious glomerulonephritis (PIGN).

**Methods:** Based on this rationale, 33 patients who clinically presented with PIGN at our center from 1985 to 2010 having undergone renal biopsy were retrospectively reviewed for possible re-classification. Clinical characteristics including renal function, complement, urine profile and blood pressure data was captured from first presentation to last available follow-up, which ranged from 1 month to 10 years.

**Results:** Serum C3 was low for all patients at first presentation. From re-review of the original renal biopsies, 25 patients (76%) were confirmed as PIGN based on the presence of subepithelial hump-like deposits and C3 depositions with or without Ig. Four patients (12%) were categorized as ‘possible’ C3G based on the absence of subepithelial humps in the presence of C3 but with Ig, while two (6%) had ‘probable’ C3G based on the presence of C3 only without subepithelial humps. Two patients (6%) demonstrated intermediate features suggesting both DDD and C3G. Five cases in the ‘probable’ and ‘possible’ C3G categories had a mild disease course initially resembling PIGN, however due to persistent low C3 and mild proteinuria, renal biopsy was performed. Of these, 67% (4/6) continued to have proteinuria at last follow-up.

**Conclusions:** These results support our hypothesis that cases of C3G may have been categorized as PIGN in the past as PIGN. Further testing for complement pathway abnormalities is warranted to further support this hypothesis.

**SA-PO2342**

Eculizumab as Life Saving Treatment in Complement Mediated MPGN

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**Background:** Membranoproliferative glomerulonephritis (MPGN) is a rare glomerulopathy with risk of progression to ESRD and post transplant recurrence. Pathogenetically, there is increasing evidence for dysregulation of the alternative complement pathway (AP). Eculizumab is a monoclonal C5 antibody that prevents terminal complement pathway activation. We present a treatment resistant case of MPGN where eculizumab served as an organ and life sustaining measure.

**Methods:** A 16 year old healthy female presented with nephrotic range proteinuria, peripheral edema and anemia (LDH and haptoglobin normal; occasional schistocytes). Renal biopsy showed mesangial interposition, wire loops, subendothelial and mesangial deposits and full house IF. In light of negative ANA and dsDNA a diagnosis of MPGN I was made and treatment with steroids and MMF commenced.

At 8 weeks, work up for fever and pancytopenia revealed group A strep, pseudomonas bacteremia, and CMV viremia, which was treated. Bone marrow showed signs of possible macrophage activation syndrome. MMF was held, steroid pulses and IVIG x3 were provided. Patient had persistent thrombocytopenia and anemia, increasing creatinine, and anuria eventually requiring hemodialysis (HD). Complement analysis revealed strong PA activation (undetectable C3; high C5b-9; low CH50; positive C3NeF). While no complement mutation was found, CFHR1/3 was absent on western blot. C5H2 antibodies were not detected.

**Results:** To regain complement control, plasma therapy (infusion x5 followed by pheresis x7) was commenced. While there was subtle treatment response (improvement in creatinine and C3 anuria persisted and clinical status deteriorated with respiratory compromise, GI bleeding and seizures. With eculizumab (900 mg/wk x4), treatment response was dramatic: following 1st dose neurologic complications ceased, urine output normalized and HD was discontinued. Thrombocytopenia and anemia recovered after 2nd dose.

**Conclusions:** Recovery of this patient from life threatening conditions in response to eculizumab strongly suggests (i) a role for AP dysregulation in MPGN pathogenesis and (ii) future AP controlling treatment strategies for MPGN – a remarkable breakthrough for this condition.

**SA-PO2343**

Platelets Serve as Source of Complement Factor H (CFH) Activity in a Modified Fluid-Phase Alternative Complement Pathway Cofactor Activity Assay

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**Background:** We have recently shown that that the alternative complement pathway regulator complement factor H (CFH) is present in a non-granular compartment in human platelets and that platelets are capable of taking up and releasing CFH (Licht et al, Blood 2009). The physiological role of platelet CFH remains to be determined, given the high concentrations of this protein (up to 600 μg/ml) normally present in plasma.

**Methods:** In order to assess the potential physiological relevance of platelet CFH, washed normal platelets were used as the sole source of CFH in a modified fluid-phase alternative complement pathway cofactor activity assay. This assay allows for testing the potential of a protein to serve as cofactor to complement factor I (CFI) in cleaving C3b, the active form of complement C3, by observing the appearance of C3b cleavage products. In vivo, varying platelet concentrations ranging from 600 to 30/nL were incubated with 2 μg/mL C3b and 6 μg/mL CFI at 37°C for 60 min, and supernatants assayed via immunoblotting for the presence of C3b cleavage products. CFH cofactor activity was observed to increase with platelet concentration, while platelets remained in a resting state. A time course analysis with a platelet concentration of 300/nL (within the normal range of blood platelet concentration) gave results similar to those observed with plasma and purified CFH.

**Conclusions:** These results show that like endothelial cells, platelets can serve as a cellular source of CFH cofactor activity in vitro. This provides support for the emerging hypothesis that in addition to their established roles in the blood coagulation, inflammatory and immune systems, platelets may actively participate in the regulation of the complement system, particularly the constitutively-active alternative pathway that is involved in several complement-related diseases.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
The Podocyte Is the Initial Target in the Renal Pathogenesis of Diarrhoea-Associated Haemolytic Uraemic Syndrome  

**Lindsay S. Keij, 1 Gavain Iain Welsh, 1 Richard Coward, 1 Anna Richards, 2 Robert A. Spooner, 2 Moin Saleem. 1,2**

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**Background:** D+HUS is the leading cause of pediatric acute renal failure. It occurs after infection by shigatoxin (STx) producing bacteria. STx binds Gb3 cellular receptors. It was considered an endothelial disease until the report of an inducible podocyte-specific VEGF-A knockdown mouse that developed glomerular thrombotic microangiopathy - the hallmark of HUS. Furthermore, familial HUS is caused by dysregulation of the alternative complement pathway with mutations identified in complement regulators. Eculizumab, a drug that blocks the complement cascade, is being trialed in atypical HUS and is used experimentally in D+HUS. We hypothesized that podocytes, VEGF-A and complement regulators play a co-ordinated role in the renal pathogenesis of D+HUS.

**Methods:**
- Immunofluorescence of human and mouse immortalized glomerular cell lines detected Gb3.
- Cellular staining was determined using a radio-labeled protein synthesis assay.
- ELISA measured VEGF-A produced by stx treated podocytes. Human podocytes were treated with VEGF-A and western blotting determined complement regulator expression.
- A factor H re-synthesis assay was devised to remove bound factor H. Cells were fixed or treated with serum free media +/- VEGF-A. Factor H was detected by immunofluorescence.

**Results:**
- Human glomerular cells express Gb3 and are stx susceptible.
- Human podocytes are more sensitive than endothelial cells. Mouse podocytes lack Gb3 and are stx insensitive.
- Six stx stimulation of human, but not mouse, podocytes reduced VEGF-A production. VEGF-A upregulates human glomerular complement regulator expression. Both glomerular cell lines are sensitive to stx in vitro and VEGF-A regulates this effect.

**Conclusions:**
- Podocytes are a sensitive stx target. Reduced podocyte VEGF-A downregulates complement regulator expression and production in the glomerular endothelium, making it vulnerable to attack. This further evidence indicating a central role for the podocyte in D+HUS.
in the membrane and AMPK. Therefore we employed resveratrol to probe the relative effects of phosphorylation-specific antibodies in the plasma membrane and AMPK activity and their impact on ENaC activity in a native cell culture model.

Methods: A combination of biochemical and biological assays were used to identify the effect of resveratrol on ENaC activity in CCD cells. Knockdown of AMPK and inhibition of AMPK activity was performed using siRNA to determine the function of AMPK in mediating the effects of resveratrol on ENaC. In addition, live cell microscopy techniques were used to assess the time course and acute effects of resveratrol on phosphorylcytosine species in the plasma membrane of mPKCCDc14.

Results: We observed resveratrol acutely reduces amiloride-sensitive current in CCD cells. The time course and dose dependence of this inhibition paralleled plasma membrane depletion of a P(3,4,5)P3 reporter in live-cell microscopy, indicating that this early inhibitory process is likely mediated by resveratrol’s known effects on PKT activity. Additionally, resveratrol induced a late inhibitory effect (3-24 hr) that appears to be mediated via AMPK activation. Resveratrol induces a significant activation of AMPK compared with vehicle controls after 3 hours which persisted through 24 hours. Knockdown of AMPK, or treatment with the AMPK inhibitor, Compound C, reduced the late inhibition of EnaC but had no effect on the early inhibitory response to resveratrol.

Conclusions: Collectively, these data demonstrate that resveratrol inhibits ENaC activity by two mechanisms: an early effect seen within 5 minutes related to depletion of membrane PIP3, and a sustained late (3-18 hour) effect secondary to activation of AMPK.

Funding: NIDDK Support

SA-PO2349

Purinergic Cascade Contributes to the Activation of Ca2+-Permeable TRPV4 Channels by Mechanical Forces in the Aldosterone-Sensitive Distal Nephron

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Background: Cell-cell junctions in the aldosterone-sensitive distal nephron (ASDN) respond to changes in flow/composition of the ultrafiltrate by elevating [Ca2+]. It is thought that this is critical for adaptation of water-electrolyte transport at this site. At the same time, environmental changes are known to trigger paracrine ATP release in the ASDN.

Methods: In this study, we probed a role for purinergic signaling in mediating mechano-sensitive responses by directly monitoring changes in [Ca2+]i in individual cells within split-open ASDN of mice.

Results: Purinergic stimulation caused a similar transient Ca2+ peak followed by a sustained plateau in both principal and intercalated cells. Genetic deletion of P2Y2 receptors compromised purinergic signaling and, importantly, attenuated Ca2+-responses to hypotonic media and elevated flow. Furthermore, we show here that activation of mechano-sensitive TRPV4 channel plays a major role in the sustained [Ca2+]i elevation during purinergic stimulation. Genetic deletion of TRPV4 channel disrupted ATP-induced Ca2+-plateau.

Conclusions: We concluded that paracrine release of ATP in response to mechanical stimulations reciprocally modulates cellular responses by activating mechano-sensitive TRPV4 channel in ASDN cells.

Funding: Private Foundation Support

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Identification of CNK3 as an Important Component of the Aldosterone-Controled ENaC Regulatory Complex

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Background: Members of the Connectome Enhancer of KSR (CKN) family of proteins possess protein-protein interaction domains, and have been proposed to function as scaffolds possibly assisting various interactions in a multiple signaling cascade. Mammalian CNK3 is an aldosterone-induced protein that is essential for the activity of the epithelial sodium channel (ENaC). In the present study, we examine its role in ENaC stimulation.

Methods: We used immunoprecipitation assays in transiently transfected HEK293T kidney epithelial cells to study the effect of CNK3 on ENaC surface expression. We also used these cells to test protein-protein interactions in co-immunoprecipitation assays. We used polarized mPKCCD kidney epithelial cells grown on Transwell filters to study effects on ENaC activity.

Results: We show that CNK3 is associated with ENaC at the cell surface and that it interacts with the components of the previously described ENaC regulatory complex (ERC). The PDZ domain in CNK3 appears to be crucial for its association with SGK1 and GILZ1, and for its stimulation of ENaC cell surface expression in HEK293T cells. We further demonstrate that the PDZ domain in CNK3 is required for aldosterone-controlled ENaC-mediated Na+ transport in mPKCCD cells.

Conclusions: These results strongly suggest that CNK3 is essential for the proper assembly of ENaC, and that factors facilitating appropriate aldosterone signaling to stimulate Na+ reabsorption via ENaC (Funding Sources: NIH Grants DK078679, DK056695 and DK085101).

Funding: NIDDK Support

SA-PO2351

Oxidative Stress in Regulation of Renal Na/K-ATPase Signaling and Distribution

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Background: In Dahl salt-resistant (R) and -sensitive (S) rats, we found that impaired renal proximal tubular Na/K-ATPase/c-Src signaling contributes to salt-sensitive hypertension, but the underlying mechanism is not clear. Interestingly, the S rats have significantly higher hemoglobinase-1 (HO-1) expression level, both before and after high salt diet (2% NaCl for 7 days, compared to 0.3% NaCl), than the R rats in renal proximal tubules (n=8 per group per strain, p<0.01). We investigated the role of oxidative stress in regulating renal proximal tubular Na/K-ATPase signaling and distribution.

Methods: c-Src phosphorylation, cellular fractionation.

Results: In pig LLC-PK1 cells, ouabain stimulated c-Src activation, ROS generation, protein carboxylation, and Na/K-ATPase endocytosis. These ouabain effects were significantly attenuated by pretreatment with ROS-scavenging NAC. Increases in oxidative stress by glucose oxidation (GO, 1 and 3mM) also activated c-Src and stimulated Na/K-ATPase endocytosis in LLC-PK1 cells. Induction of HO-1 by CoPP or knockdown of Na/K-ATPase α1 subunit by siRNA significantly attenuated ouabain-induced c-Src activation. These data suggest that Na/K-ATPase is required in ouabain-induced ROS generation and this increase in ROS is a downstream effector of Na/K-ATPase/c-Src that is critical in ouabain-induced Na/K-ATPase endocytosis. The data also suggest that oxidative stress may affect Na/K-ATPase/c-Src signaling and redistribution.

Conclusions: Oxidative stress, including ouabain-induced ROS generation, is capable of regulating Na/K-ATPase/c-Src signaling and redistribution.

Funding: NIDDK Support

SA-PO2352

AS160: A New Na+,K-ATPase Partner That regulates the Trafficking of the Sodium Pump in Response to Energy Depletion

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Background: The Na+,K-ATPase is the major active transport protein found in the plasma membranes of most epithelial cell types. The Na+,K-ATPase appears to be located in two distinct cellular pools. The major sodium pump pool is located at the plasma membrane, while the other pool resides in cytoplasmic vesicular compartments which can translocate to the plasma membrane in response to physiological stimuli. We have identified AS160 as a new Na+,K-ATPase partner. AS160 is a Rab GAP that regulates the trafficking of the GLUT4 and the ENaC in response to insulin/muscle contraction and aldosterone, respectively.

Methods: We characterized the interaction between AS160 and Na+,K-ATPase by co-immunoprecipitation and co-localization assays. We characterized the physiological role of the Na+,K-ATPase interaction with sodium pump by using RNAi techniques to knockdown AS160 expression in cultured MDCK renal epithelial cells. Finally, we examined the regulation of this interaction in mice subjected to renal ischemia followed by reperfusion.

Results: In COS cells, coexpression of AS160 and Na+,K-ATPase led to the intracellular retention of the sodium pump by using RNAi techniques to knockdown AS160 expression in cultured MDCK renal epithelial cells. Finally, we examined the regulation of this interaction in mice subjected to renal ischemia followed by reperfusion.

Conclusions: We conclude that AS160 is a new sodium pump partner that regulates Na+,K-ATPase by co-immunoprecipitation and co-localization assays. We characterized the physiological role of the Na+,K-ATPase interaction with sodium pump by using RNAi techniques to knockdown AS160 expression in cultured MDCK renal epithelial cells. Finally, we examined the regulation of this interaction in mice subjected to renal ischemia followed by reperfusion.

Funding: Other NIH Support - DK 17433, Private Foundation Support

SA-PO2353

Coordination Acted by Tgsl10 and USP2-AS5 in the Negative Feedback Loop of the Mineralocorticoid Receptor

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Background: In order to avoid prolonged response and consequently tissue damages, desensitization mechanisms often take place in order to downregulate stimulated pathways. In the context of aldosterone-sensitive renal proximal tubular (MRT) receptor signaling, it was shown that prolonged exposure to aldosterone decreased MR expression via the proteasome, but the mechanisms of this feedback regulation remain unclear. We were interested into pathways involving MR signaling termination in response to aldosterone.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

660A
Methods: These questions were studied either in transfected M1 or in mCCD cells by applying immunoprecipitations, immunoblotting, luciferase assays and measurements of transepithelial Na transport.

Results: We first observed that MR was ubiquitylated at the basal state and that this modification was removed after aldosterone treatment. As for nuclear receptor, we found that Tg101 was found at the basal state and that this association was lost after aldosterone treatment. We found that Tg101 can stabilize MR probably by maintaining its ubiquitylation. Because USP2-45 is an aldosterone induced deubiquitylating enzyme, we wondered if USP2-45 was involved in the deubiquitylation of the receptor. We found that USP2-45 interacted with MR, removed its ubiquitylation and decreased its expression via the proteasome.

Our data imply a mechanism in which MR is ubiquitylated at the basal state and protected by Tg101. Aldosterone treatment stimulates USP2-45 expression, which interacts with MR and deubiquitylates the receptor. The removal of the ubiquitin destabilizes the MR-Tg101 interaction and induces MR degradation via the proteasome by a so far unknown mechanism.

Conclusions: These results reveal the existence of a functional network involving USP2-45 and Tg101 into a negative feedback loop of the MR pathway that mediates the degradation of MR in response to aldosterone.

Funding: Government Support - Non-U.S.

SA-PO2354

Sensory Renal Innervation: Does a Kidney-Specific Expression Pattern of Voltage-ACTivated Sodium Channels Lead to a Specific Firing Activity and Higher Excitability? Wolfgang Freisinger, Tilman Ditting, Sonja Heinlein, Johannes Schatz, Roland Veelken. Medical Clinic 4, Nephrology and Hypertension, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany.

Background: Thermal ablation of renal innervation has been effective in tryping hypertensive patients. However, especially afferent renalinnervation is poorly understood. We chose to investigate four electrodes containing different types of stimuli i.e. sustained action potential (AP) firing throughout depolarizing current injection (DCI), pointing to a higher excitability. Non-renal neurons responded mostly with a phasic response, i.e. <4 APs. We novel hypothesis that different voltage-gated sodium-channels, which initiate the action potential generation, are responsible for these differences.

Methods: Dorsal root ganglion neurons from Sprague Dawley rats (Th11-L2) were recorded in current clamp technique and characterized as tonic or phasic according to their response to DCI. Then, single cells were switched to voltage-clamp, potassium and calcium channel blockers were added to the external solution. The application of the sodium channel blocker TTX (TTX) then allowed to further characterize voltage-gated sodium currents.

Results: 66 DRG neurons were investigated (n=40 phasic, n=26 tonic). Tonically firing cells showed significantly a lower firing threshold (-21.75 ± 1.43 mV vs. 29.33 ± 1.63 mV), a lower overshoot (46.79 mV [38.63 - 54.75] vs. 56.74 mV [53.6 - 60.96]) and longer action potential duration (4.61 ms [4.15 - 5.85] vs. 3.35 ms [2.12 - 5.67]). Tonic cells showed a significantly smaller fraction of TTX-sensitive Na+ currents (60.45 ± 12.94pA/pF vs. 140.44 ± 30.88pA/pF), whereas activation did not reveal a significant change (p<0.05).

Conclusions: We could show for the first time that a smaller TTX-sensitive channel expression is very likely linked to electrophysiologival differences observed between renal and non-renal neurons. We further show that different types of stimuli elicit a specific expression pattern of sodium channels concerning renal innervation. Sodium channel expression and consequently excitability is likely to be altered in hypertension and inflammation.

SA-PO2335

Identification of Two Novel Renal Olfactory Receptors Ryan J. Protzko, Jennifer L. Pluznick. Physiology, Johns Hopkins University School of Medicine, Baltimore, MD.

Background: ORs are chemoceptiveGPCRs which comprise the largest gene family in the mammalian genome. We previously observed that the OR signaling pathway plays a specific functional role in the kidney (Pluznick, PNAS 2009) and identified six renal ORs. In this study, we identify two additional novel renal ORs and examine their tissue distribution and ligand specificity.

Methods: An RT-PCR screen using primers to ORs with known or predicted ligands was performed on murine kidney cDNA. Primers for ORs found in kidney were also tested on cortex, medulla, and 8 non renal tissue cDNAs. Trailing to the cell surface was assayed by immunofluorescence (IF); one OR was screened for responses to various ligands using a dual-luciferase ligand screen.

Results: An RT-PCR screen for 27 ORs with known ligands revealed 2 novel renal ORs, Olf691 and Olf545 (medulla). These ORs were confirmed by RT-PCR in murine kidney and by luciferase expression in HEK293T cells. The dual-luciferase ligand screen revealed that the luciferase reporter construct was activated by Olf545 but not by Olf691.

Conclusions: We have previously shown that the OR signaling pathway plays important immunological roles in the kidney. Here, we identify two additional renal ORs, Olf691, found in the cortex and medulla, responds to short- and medium-chain FAs. The primary biological source of short chain FAs is intestinal bacterial metabolism, indicating that Olf691 may signal in response to gut flora activity. Further studies will focus on localizing Olf545 and Olf691, identifying Olf545 ligands, and examining the potential roles of these receptors in vivo.

Funding: NIDDK Support

SA-PO2357

Advanced Glycation End-Products, Glomerular Endothelial Cells, Tight Junction, Renin Angiotensin System Zengehun Ye, Canning Li, Cheng Wang, Tan-Qi Lou. Department of Nephrology, Third Affiliated Hospital of Sun Yat-Sen University, Guangzhou, Guangdong, China.

Background: To investigate whether advanced glycation end-products (AGEs) influence the permeability and tight junction proteins of cultured rat glomerular endothelial cells (rGEnCs) in vitro, and the underlying role of renin angiotensin system activation in this process.

Methods: The GEnCs permeability was detected by measuring the transendothelial electrical resistance and flux of FITC-BSA across the GEnCs monolayers. Angiotensin II was detected by ELISA, and ZO-1, Claudin -5 and JAM-A were detected by immunofluorescence and Western blotting.

Results: The results show that AGES increased the permeability of glomerular endothelial cells monolayers in a concentration- and time-dependent manner. This effect of AGES was accompanied by a redistribution of tight junction proteins, including ZO-1, occludin, JAM-A and Claudin-5, from the cell–cell borders of glomerular endothelial cells. AGES also down-regulated the expression of the tight junction proteins, occludin, JAM-A and Claudin-5. In addition, a significant finding in this study was that AGES could increase the permeability of rGEnCs by activating the intracellular renin angiotensin system (RAS) in a RAGE–dependent signaling pathway. This was supported by the finding that AGES were able to stimulate the activity of angiotensin converting enzyme (ACE), and up-regulate the expression of Angiotensin II and Angiotensin II type 1 receptor (AT1R) in GEnCs. This activation was blocked by neutralizing antibody to RAGE, and by pharmacologic inhibitors of RAS.

Conclusions: The results collectively indicate that the induction of increased permeability of rGEnCs by AGES may be predominantly via a RAGE-angiotensin II-AT1 pathway. This effect on glomerular endothelial cells illustrates a novel action of ACE/ARB in diabetic nephropathy.

Funding: Government Support - Non-U.S.
The Role of Toll-Like Receptor Proteins (TLR) 2/4 in Human Proximal Tubular Cells In Vitro – A Potential Mediator of Diabetic Nephropathy? Harshini Mudalali,1 Katherine Jane Pegg,1 Steven J. Chadban,3 Huiling Wu,3 Carol A. Pollock,2 Usha Panchapakesan.4 1 Renal Group, Kolling Institute, University of Sydney, Sydney, St Leonards, New South Wales, Australia; 2 Renal Medicine, Royal Prince Alfred Hospital, University of Sydney, Sydney, Camperdown, New South Wales, Australia.

Background: Inflammatory responses are crucial in the pathogenesis of diabetic nephropathy (DN). TLRs are ligand-activated membrane-bound receptors which activate nuclear factor-kappaB (NF-κB). TLR 2/4 are present in proximal tubular cells (PTC) and are activated by ligands pathologically in DN (i.e. HMGB1, fibronectin and heat shock proteins). We have previously shown that 30mM glucose increases HMGB1 expression in PTC. Here, we explore the effect of varying glucose levels and HMGB1 on TLR 2/4 signalling to help understand the inflammatory pathway activation in DN.

Methods: HK2 cells (a human kidney PTC line) were exposed to control (5mM), 30mM glucose, fluctuating glucose (5mM/30mM) and 11.25mM glucose for 72h. Cells were harvested for nuclear extract and protein. TLR 2/4 expression and NF-κB binding were measured. The role of HMGB1 in TLR 2/4-regulated NF-κB activation was assessed with silencing TLR 2/4. The effect of glucose on TLR 2/4 expression was examined by restricting glucose uptake with a sodium-glucose co-transporter2 (SGLT2) inhibitor.

Results: TLR 2/4 expression and downstream NF-κB binding increased drastically with 11.25mM glucose compared to 30mM glucose and fluctuating glucose. Blocking glucose uptake with an SGLT2 inhibitor prevented any increase in TLR 2/4 expression. Recombiant HMGB1 increased NF-κB binding at 2h and this was prevented by TLR silencing.

Conclusions: Increased TLR 2/4 expression mediated by 11.25mM glucose may occur through intracellular hyperglycemia within PTCs as this effect was blocked by SGLT2 inhibition. NF-κB activation was also induced at high glucose levels, and by recombinant HMGB1, suggesting a role for TLR2/4. Furthermore, TLR 2/4 may function as the predominant receptor in mediating the effect of NF-κB activation.

Funding: Government Support - Non-U.S.

SA-PO2358

Transcription Regulation of Cell Matrix Proteins in Proximal Tubular Cells Treated with High Glucose Mukesh Yadav1,2, Anamika Yadav2, Sanny L. Habib1,2.

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Background: The rate of deterioration of kidney function correlates best with the degree of tubular fibrosis and accumulation of cell matrix proteins. The mechanisms of renal cell matrix expansion as well as the signal transduction pathways activated in the diabetic milieu are incompletely characterized.

Methods: Phosphorylation of two effector molecules of downstream target of mTOR, p70 ribosomal protein S6 kinase (S6K) and 4E-BP1 was measured in proximal tubular cells treated with high glucose. Cell matrix proteins (Collagen IV, fibronectin) expression was also measured in cells treated with high glucose (25mM) for 0-24hrs. Transcription factor expression of CAMP-responsive element binding protein (CREB) and yin-yang 1 (YY1) was evaluated in cells treated with high glucose.

Results: Exposure of cells to high glucose (0-24hrs) resulted in increased mTOR activity through increasing the phosphorylation of 4E-BP1 and S6K. A three fold increase was observed in S6K phosphorylation and collagen IV. Pretreatment of the cells with rapamicyn blocked fibronectin and collagen IV induction. Similarly high glucose induced the expression of fibronectin and collagen IV. Furthermore, in addition blocking TLR2 with SGLT2 inhibitor decreases the increase of fibronectin and collagen IV induction. Moreover, blocking TLR2 with rapamicyn leads to decreased the expression of collagen IV.

Conclusions: Our data show that treatment with rapamicyn results in decrease the cytoplasmic expression of YY1 and lead to decreased the accumulation of fibronectin and collagen IV treated with high glucose.

SA-PO2360

mTORC2 Knockdown Slows the Progression of Cysts in Type I MDCK Cells Kameswaran Ravichandran, Iram Zafar, Zhibin He, Charles L. Edelstein. Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.

Background: mTOR is expressed in two different complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). mTORC1 consists of mTOR and Raptor while mTORC2 consists of mTOR and Rictor. Recent studies on rapalogues (rapamycin and everolimus) with mTORC2 have shown that there are multiple reasons for the unimpressive effect of rapalogues in human kidney, PDK, one possible reason is that rapamycin inhibits mTORC1 it does not have an effect on pro-fibrotic mTORC2.

Methods: We generated stable cell lines from mTORC2 (pAktSer473, pPKCα-alpSer657, pSGK Ser657) were analyzed by immunoblot in 8 wk old Han:SPRD (Cy/+) rats. The effect of specific mTORC2 knockdown on cyst formation was determined in an in vitro model of Type I MDCK cells that form cysts in culture. Rictor, the functional component of mTORC2 was silenced using a plasmid-based shRNA vector system (pTRIPZ) expressing

SA-PO2361

Hyperactive TORC1 and TORC2 Launch Hifalpha-Dependent Expression of PTEN To Regulate Phosphorylation of Akt in Tuberous Sclerosis Complex (TSC) Falguni Das,1 Nirmalya Dey,2 Nandini Ghosh-Choudhury,2 B. S. Kasinath,3 Hanita E. Abbas,3 Goutam Ghosh-Choudhury.

1 Medicine, UTHSCSA, San Antonio, TX; 2 Pathology, UTHSCSA, San Antonio, TX.

Background: TSC results from mutations in one of two genes, TSC1 and TSC2. TSC mutations contribute more to the pathology of the disease, including renal angiomyolipomas. TSC1 is a signaling hub at growth factors and NF-κB activation.

Methods: 1) There is increased mTORC2 signaling in 8wk old Cy/ kidneys with PKD, 2) Silencing of mTORC2 in an in vitro model reduces cyst size. In conclusion, mTORC2 merits further study in PKD.

Funding: Other NIH Support - AARA

SA-PO2362

Ca2+ Influx through Reverse-Mode Na+/Ca2+ Exchange Is Critical for Vascular Endothelial Growth Factor (VEGF) Mediated – ERK1/2 Activation and Angiogenic Functions of Human Endothelial Cells Petros Andrikopoulos,1,2 Magdi Yaqoob,1,2 Suzanne Eccles.1,2 CR-UK Cancer Therapeutics Unit, The Institute of Cancer Research, London, United Kingdom; 2Translational Medicine and Therapeutics, William Harvey Research Institute, London, United Kingdom.

Background: Cardiovascular complications are a major cause of morbidity/mortality in CKD. Deregulated Ca2+ levels are universal in CKD patients. However, the effect(s) of altered Ca2+ signalling on the vascular endothelium remains largely unknown. Here, by using VEGF, as a paradigm, we investigated the effect of extracellular Ca2+ on the endothelial cell

Methods: Human umbilical vein endothelial cells (HUVECs) were starved in a physiological buffer and preincubated with reverse-mode (Ca2+ in - Na+ out) Na/Ca2+ exchanger (NCX). ERK1/2 activation was determined by western blot. PKC and B-Raf activity were determined by phospho-coupled antibody capture (CA2+), which was measured in HUVECs loaded with the fluorescent Ca2+ indicator Fluo-4NW.

Results: Here, we report that extracellular Ca2+ is required for VEGF-induced ERK1/2 activation and that release of Ca2+ from intracellular stores alone, in the absence of extracellular Ca2+, is not sufficient to activate ERK1/2. Furthermore, inhibitors or reverse-mode NCX suppressed the VEGF-induced activation of ERK1/2 in a time- and dose-dependent manner and attenuated VEGF-induced Ca2+ transients. Knock-down of NCX1 (the main NCX isomorf in HUVECS) by siRNA confirmed the pharmacological data. A panel of NCX inhibitors also significantly reduced VEGF-induced B-Raf activity

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author. 662A

Poster/Saturday

Cell Signaling - II (Physiology, Diabetes, Cystic Disease, Vascular Biology, Uremia, Lupus, Other)
and inhibited PKC(α/βII)α/S1 phophorylation and subsequent NF-kB translocation to the plasma membrane and total PKC activity in situ. Finally, we observed that CNX inhibitors reduced VEGF-induced HUVEC proliferation, migration and tubular differentiation in surrogate angiogenesis functional assays in vitro.

Conclusions: We propose that Ca2+ influx through reverse-mode NCX is required for the activation and the targeting of the PKCα to the plasma membrane, an essential step for VEGF-induced ERK1/2 phosphorylation and downstream EC functions. Thus, in CKD, deregulated Ca2+ may affect endothelial signalling and potentially contribute to endothelial dysfunction.

SA-PO2363

Renal Kinome Scan in Lupus Nephritis Chun Xie,1,2 Tianfu Wu,2 Chandra Mohan,2 1Division of Nephrology/Internal Medicine, UT Southwestern Medical Center, Dallas, TX; 2Division of Rheumatology/Internal Medicine, UT Southwestern Medical Center, Dallas, TX.

Background: Protein kinases are key regulators of a wide variety of biological processes. Several protein kinases have been elucidated to play important roles in the pathogenesis of systemic lupus erythematosus (SLE) and glomerulonephritis. However, almost no in vivo investigations focused only on single molecules or pathways. The human genome contains more than 500 protein kinase genes. Most of these kinases have not been investigated in regard to their roles in SLE or glomerulonephritis. In the current study, we performed a systemic kinome scan in kidneys of spontaneous lupus mouse strains.

Methods: Protein extracts from renal cortices of 6-8 months old NZB/W F1, and MRL/lpr mice as well as C57BL/6 controls were subjected to kinome profiling, using Kinex Antibody Microarray. Some of the differentially expressed kinases were then validated by Western blot or immunohistochemistry.

Results: We were able to detect 193 protein kinases, 24 protein phosphatases and 150 regulatory subunits of these enzymes by using this platform. 87 of these molecules were found to be differentially expressed in lupus kidneys, compared to the controls. Using the Ingenuity Pathway Analysis (IPA), we identified several biological networks, functions and pathways, including cell growth and proliferation, gene expression, posttranslational modification, immune response, and inflammation, etc., that are involved in lupus nephritis. In addition, some of these molecules have been validated by Western blot or immunohistochemistry. The role of tyrosine hydroxylase, one of the molecules that are highly expressed in lupus kidneys, in the pathogenesis of lupus nephritis, is being tested in animal models.

Conclusions: We have identified several molecules that have altered expression in lupus nephritis, by systemic kinome profiling. We also revealed several disease pathways that are involved in lupus nephritis. The functional studies of these molecules and pathways are in progress.

SA-PO2365

Stimulation of RANKL and Bone Resorption by Metabolic Acidosis Requires Gpib Signaling Nancy Krieger, Christopher D. Culbertson, David A. Bushinsky. Medicine, University of Rochester, NY.

Background: Chronic renal failure (CRF) induces muscular weakness and decreased exercise endurance. Interleukin-6 (IL-6) is a cytokine upregulated by exercise in skeletal muscle, with autocrine effects on skeletal muscle metabolism. The AMP-activated protein kinase (AMPK) is a fuel sensing enzyme activated by changes in the energy state of the cell, and recent findings indicate that IL-6 can activate AMPK in vivo. Several studies show that IL-6 mRNA is altered in CRF, but it is unknown if CRF affects the response to exercise. We hypothesized that the regulation of muscular IL-6 expression and AMPK in response to exercise is impaired in CRF.

Methods: Male Sprague-Dawley rats were g 56 nephrectomy (NXP) or sham surgery and pair-fed. The response to exercise was measured in extensor digitorum longus (EDL) fatigued by in situ electrical stimulation through the sciatic nerve, or after swimming. At the end of the exercise protocols, EDL was dissected for mRNA and protein determination.

Results: We found no significant differences in the abundance of IL-6 mRNA of EDL at rest. After in situ stimulation we observed 85±8 fold induction of IL-6 mRNA in the EDL of Sham rats; however, IL-6 mRNA in EDL from NXP rats increased only 22±7 fold (n=6, P<0.01). Since IL-6 exerts a positive feedback in muscle, we analyzed IL-6-signalizing pathway. NXP did not affect mRNA of IL-6 receptors, IL-6Ra and gp130. The Janus Kinase (JAK) pathway was highly expressed in lupus kidneys, in the pathogenesis of lupus nephritis, is being tested in animal models.

Conclusions: The data show reduced activation of IL-6 expression in EDL of NXP rats in response to exercise. The decrease of AMPK phosphorylation could contribute to low endurance to exercise in CRF.

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Funding: Government Support - Non-U.S.
Methods: To explore regulation of G-protein coupled signaling by Met, neonatal mouse cardiac myocytes were cultured in the absence of PHD-inhibitor (MET: pH=7.17, Pco₂=33 mmHg, [HCO₃⁻]=15 mM medium) or acid (MET: pH=7.17, Pco₂=33 mmHg, [HCO₃⁻]=15 mM medium) or acid (MET: pH=7.17, Pco₂=33 mmHg, [HCO₃⁻]=15 mM medium) and 20 μM galelin (Gal), a selective inhibitor of Gαi signaling, and net Ca efflux measured. To determine RANKL mRNA expression, primary osteoblasts were isolated from calvariae and increased in osteoblasts in 20 μM galelin (Gal), for 6 h and normalized to actin expression. Results: Compared to incubation in Nl (442±80 mmol/mole/24h), Net induced net Ca efflux was increased by 20 μM galelin (Gal). Net Ca efflux was measured by real time PCR. Compared to incubation of primary osteoblasts in Nl (0.85±0.11), Met induced an increase in RANKL mRNA (1.29±0.11, p<0.05). Gal did not alter RANKL in Nl (0.9±0.06) but blocked the increase in RANKL mRNA (at 0.01, P<0.05) and was not detected in osteoblasts at the absence of Gal or presence of Gal.

Conclusions: Results suggest that Met blocks the osteoblast proliferation response in osteoblasts. RANKL mRNA expression was increased by acidosis and decreased by the Met. Met inhibited proliferation of osteoblasts in a dose-dependent manner on HIF-1 for metabolic adaptation. In contrast, reduction of proliferation by PHD-inhibition is also mediated by HIF-1. This indicates that although most target genes are regulated similarly by both stimuli, the functional consequences of HIFs stabilization are diverse. Given the increased interest in using HIF-activation as therapeutic approach to renal disease, these cells are a valuable tool to investigate the functional roles of HIFα in the renal endothelium in vitro.

Funding: Government Support - Non-U.S.

SA-PO2370
Reduction of Na/K-ATPase in Marifobufagenin-Induced Cardiac Myocyte Apoptosis

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Background: Na/K-ATPase functions as a receptor for cardiotropic steroids (CTS) such as marinobufagenin (MBG), and digoxin. Decreases in cardiac Na/K-ATPase have been documented in patients with heart failure and other disease states. Our previous studies demonstrate that reduction of Na/K-ATPase attenuates CTS-induced Src signaling, resulting in increased cell death in renal epithelial cells.

Methods: We employed a Na/K-ATPase α1 heterozygote knockout mouse model (α1+/−), in which, the expression of heart tissue Na/K-ATPase α1subunit is reduced by about 30% in comparison to its wild type (α1+/+) littermate. Experimental mice were subject to marifobufagenin (MBG) infusion using osmotic mini pumps and the echocardiograph was performed. Cardiac myocytes were also isolated from these mice for in vitro experiments.

Results: The results showed that MBG infusion increases myocyte apoptosis and induces left ventricle dilation in the heterozygote mice, but not in the wild type mice. Moreover, MBG significantly reduced the contraction function in the heterozygote mice. Mechanistically, we demonstrated that MBG activates Akt and the mammalian target of rapamycin (mTOR) in wild type mice. Activation of mTOR further increases the phosphorylation of ribosome S6 kinase and Bcl-2-associated death promoter (BAD) and inactivates the apoptosis proteins. However, this survival signaling induced by MBG was abolished in the heterozygote mice. Instead, MBG activates caspase 9 in heterozygote mice, which may account for the cardiac myocyte apoptosis. Using rat neonatal cardiac myocytes, we further demonstrated that directly inhibiting the mTOR pathway by rapamycin also blocked MBG-induced apoptosis.

Conclusions: Reduction of cardiac Na/K-ATPase attenuates MBG-induced activation of survival signaling while making cardiac myocytes susceptible to MBG-induced apoptosis.

Funding: Other NIH Support - NHLBI; NIGMS, Private Foundation Support

SA-PO2371
Albumin Overload Leads to Low Molecular Weight Proteinuria in Normal Mouse, While a Compensatory Proteolytic Pathway Develops in Limp-2 Null Mice

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Background: Mutations of the lysosomal protein Limp-2 cause low molecular weight (LMW) proteinuria and albuminuria due to reduced proteolysis in the proximal convoluted tubule (PCT).

Methods: To determine how Limp-2 affects the response to protein overload, WT and Limp-2−/− mice received daily bovine serum albumin (BSA) injections i.p. for 10 days.

Results: Urinary albumin/creatinine ratio did not increase significantly in BSA-treated Limp-2−/− mice (Day 0: 134±104; Day 10: 211±210 mg/mmol), whereas levels in WT mice increased to those seen in Limp-2−/− mice (Day 0: 30±52; Day 10: 246±299 mg/mmol; p<0.05). BSA treated Limp-2−/− mice showed no increase in LMW proteinuria (retinol-binding protein (RBP), vitamin D-binding protein and α1-microglobulin), while WT mice developed LMW proteinuria. There was no difference in mean arterial or cufinulin mRNA expression.

Quantitative immunofluorescence microscopy showed that RBP and LAMP-1 labelled vesicles in the PCT were distributed more basally in the untreated Limp-2−/− mice (Day 0: 134±104; Day 10: 211±210 mg/mmol), whereas levels in WT mice increased to those seen in Limp-2−/− mice (Day 0: 30±52; Day 10: 246±299 mg/mmol; p<0.05). BSA treated Limp-2−/− mice showed no increase in LMW proteinuria (retinol-binding protein (RBP), vitamin D-binding protein and α1-microglobulin), while WT mice developed LMW proteinuria. There was no difference in mean arterial or cufinulin mRNA expression.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
SA-P02372

TGFβ Modulates Mitochondrial Bioenergetics and Morphology in Podocytes

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Background: TGFβ regulates differentiation, growth, and apoptosis of podocytes and mediates podocyte depletion in glomerulosclerosis. In patients with progressive glomerular diseases the increased expression of TGFβ in podocytes can be one of the causes of damage induced by the deleterious phenotype. Mitochondrial function and oxidative stress emerged recently as potential therapeutic targets in glomerular injury. Whether TGFβ regulates mitochondrial dysfunction/oxidative stress in podocytes is not known.

Methods: Mitochondrial function in WT, CD2AP-deficient and Smad2/Smad3-deficient murine immortalized podocytes grown in non-permissive conditions was analyzed upon treatment with 5 ng/ml of TGFβ for 6, 24 and 48 hours.

Results: TGFβ treatment induced a significant Smad-dependent increase of podocyte oxygen consumption rate starting at 24 h. Increased oxygen consumption was associated with mitochondrial membrane depolarization, mitochondrial network fragmentation, and increased cellular reactive oxygen species (ROS). TGFβ-induced ROS production was reverted by NADPH oxidase inhibitor apocynin. In contrast, TGFβ did not alter mitochondrial superoxide level (MitoSox). ATP content was not different from untreated podocytes and increased respiration was not associated with increase mitochondrial mass as shown by citrate synthase activity. At early time points, TGFβ treatment partially inhibited the expression of nuclear and mitochondrial genes encoding subunits of Complex I of respiratory chain. In contrast, expression of SDH2 increased with TGFβ treatment.

Conclusions: Our results suggest that TGFβ/Smad activation increases OCR as part of a secondary metabolic response to preserve cellular energy (ATP) homeostasis during TGFβ-induced cellular phenotype responses. Compensatory stimulation of antioxidant mitochondrial gene expression (SOD2) may prevent increase of mitochondrial superoxide levels. In a context of oxygen deprivation, often observed in chronic and acute kidney diseases, the lack of adaptive changes in mitochondria could promote apoptotic commitment in podocytes. We conclude that canonical TGFβ/Smad pathways regulate adaptive modifications of mitochondrial metabolism in podocytes.

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SA-P02373

Gomerulotubular Disconnection Is Rescued by the Antiapoptotic Effect of Ouabain in Rats with Passive Heymann Nephritis

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Background: Gomerulotubular disconnection and formation of atubular glomeruli, which is a common histological finding in both tubular and glomerular disorders, contribute to the progression of chronic renal disease. Apoptosis of proximal tubular cells is considered as a main cause of the disconnection. Our group has shown that a cardiac troponin, ouabain, protects kidney cells from apoptosis by triggering a Ca2+-NF-kB signal (JASN 2006, Nature Com 2010).

Here we have examined whether ouabain may protect from apoptosis and glomerulotubular disconnections in rats with passive Heymann nephritis (PHN), a model of human membranous nephropathy. Methods: PHN and control (SHR) rats were followed for 4 months. One PHN group (n=7) received ouabain (15 mg/kg/day) and the other received vehicle via intraperitoneal pumps.

Results: S-Cr was significantly increased in PHN rats compared to control and PHN/ouabain rats. Morphometric analysis of the kidneys revealed glomerular fibrosis and basement membrane damage in both PHN groups, but significantly less pronounced in PHN/ouabain rats. For assessment of apoptosis, kidney sections were TUNEL-stained and counterstained with hematoxylin. The number of apoptotic cells at the glomerulotubular disconnection was significantly, 3.2 fold, higher in PHN than in PHN/ouabain rats. To assess the level of glomerulotubular disconnections, kidneys were serially sectioned and an average of 75 sections/kidney were analyzed. In control rats 95% of glomeruli were normally connected. In PHN 64% were normally connected, 19% were connected to atrophic tubules and 17% to atubular glomeruli. In PHN/ouabain rats 84% of glomeruli were normally connected, 10% were connected to atrophic tubules and 6% to atubular glomeruli.

Conclusions: The results support the notion that glomerulotubular disconnection is triggered by local apoptosis and imply that formation of atubular glomeruli can be alleviated by the antiapoptotic effects of ouabain.

SA-P02374

Identifying P2X7 Receptor as a Key Regulator of Deleterious Renal Epithelial-Fibroblast Cross Talk

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Background: P2X7 is an extracellular ATP dependent membrane receptor, which can act as a mediator of cell death. Despite P2X7 receptor protein is barely expressed or not expressed in human kidney diseases. Currently, the potential pathological role of P2X7 receptor and the signaling cascades leading to its expression in the kidney are largely understood. As a reduced number of peritubular fibroblast is observed in the interstitium adjacent to damaged renal epithelium in the early phase of AKI, we examined whether damaged renal epithelial cells would directly induce renal interstitial fibrosis death via activation of purinergic signaling in vitro.

Methods: Renal proximal tubular cells (RPTCs) and rat renal interstitial fibroblast cell line (NRK-49F) were used.

Results: Exposure of cultured NRK-49F cells to necrotic RPTC lysate or supernatant increased expression of P2X7, receptor and cell death in NRK-49F. Inhibition of P2X7 with A343079, a highly selective P2X7 receptor inhibitor, or knockdown of P2X7, receptor with siRNA blocked the deleterious RPTC supernatant-stimulated P2X7 expression and cell death. Multiple signaling pathways including ERK1/2, p38, JNKs and Akt are activated in NRK-49F, pharmacological inhibition of ERK1/2, but not p38, JNK and Akt pathways blocked RPTC supernatant-induced P2X7 expression and cell death. Similar results were also observed in NRK-49F with knockdown of ERK1/2 or MEK1, a direct upstream activator of ERK1/2. Conversely, over-expression of MEK1 enhanced these responses. Further, siRNA mediated knockdown of Elk1, a transcriptional factor targeted by ERK1/2, also reduced necrotic RPTC-induced P2X7 expression and renal fibroblast death.

Conclusions: These data indicate that necrotic RPTC supernatant can directly induce death of renal interstitial fibroblasts via up-regulation of P2X7 receptor through an ERK signaling-dependant mechanism.

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SA-P02375

Apoptotic Cells Alter Proximal Tubular Cell (PTC) Viability Via Multiple Complex Signaling Pathways

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Background: Cells undergoing apoptosis acquire new activities that modulate the fate and function of neighboring cells. We have previously shown that apoptotic cells decrease the viability of live kidney PTC responder cells. Here, we elucidate the signaling pathways responsible for decreased viability of PTC responders following their receptor-mediated interaction with apoptotic targets.

Methods: We used BU.MPT cells, a conditionally immortalized PTC line, as responder cells. BU.MPT cells, induced to undergo apoptosis in several ways, were used as apoptotic targets.

Results: Apoptotic targets induced apoptotic death in ~80% live PTC responders by 48 hrs. Modulation of PTC viability occurred through at least 3 distinct pathways. First, induction of apoptosis promoted apoptosis-mediated signaling in viable PTC. Second, caspase-8- and caspase-9-containing apoptosis caspase-8 and caspase-9 signaling-dependant mechanism.

Funding: NIDDK Support

SA-P02376

Attenuating Effect of Angiotensin-(1-7) on Angiotensin II-Mediated Reactive Oxygen Species Induced Apoptosis through Regulation of Mitochondrial NOX4

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Background: Angiotensin II (Ang II) mediated reactive oxygen species (ROS) are important second messengers for the transcriptional effects of Ang II, and NOX4 is the central enzyme of Ang II-induced ROS. Recent evidence suggests mitochondrial NOX4 may play a role in Ang II-mediated interaction with apoptotic targets. Hence, we studied the hypothesis that angiotensin-(1-7) (Ang-(1-7)) attenuates Ang II-induced mitochondrial NOX4 mediated ROS injury in proximal tubular epithelial cells.

Methods: The normal rat kidney tubular epithelial cells (NRK-52E) were cultured, and then stimulated with Ang II (10M) with or without pre-incubation with 10M of Ang-(1-7). The mitochondrial NOX4 activation was determined to isolation of subcellular fraction by Western blotting. Intracellular ROS generation was measured using DCF-DA and NOX4 expression of NRK-52E was detected using IHC by low cytometry and confocal microscopy. Apoptosis was measured using a TUNEL assay and FITC-Annexin V staining.

Results: The mitochondrial and membrane NOX4 were activated in response to Ang II stimulation for 24 hours, however, pre-incubation of Ang-(1-7) inhibited both activation of NOX4. Pre-incubation with Ang-(1-7) in addition to Ang II significantly inhibited the Ang II-induced ROS production as the level of control. Ang-(1-7) attenuated the Ang II-induced depolarization of mitochondrial membrane potential, and release of AlF and [Ca2+]i.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only Underline represents presenting author.
cytochrome C from mitochondria to cytosol. Ang II -induced apoptotic cell death was attenuated by Ang II. -1 
Conclusions: Ang-(1-7) attenuated the Ang II-stimulated activation of NOXA in both mitochondria and membrane. These findings were related to improved mitochondrial dysfunction and apoptosis in response to Ang II and suggest that Ang-(1-7) may attenuate Ang II-stimulated ROS-mediated apoptosis NRK-52E cells.

SA-PO2377

High Glucose Levels Induce Apoptosis through Endoplasmic Reticulum Stress in Peritoneal Mesothelial Cells 
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Background: To maintain adequate peritoneal function is important for long-term peritoneal dialysis (PD) therapy. Peritoneal diastase, containing non-physiological materials such as glucose, is implicated in long-term damage of the peritoneal membrane. Endoplasmic reticulum (ER) stress is associated with the progression of Diabetes mellitus, hyperglycemia-associated atherosclerosis and kidney diseases. However, the involvement of ER stress in PD has yet to be elucidated. We investigated whether high glucose levels induce apoptosis through ER stress pathway in rat peritoneal mesothelial cells (PMCs).

Results: The primary PMCs were obtained from the peritoneal parwall of Wistar rats. Serum-starved PMCs were incubated with 2% glucose in culture medium for 0 - 72 hours. High glucose concentrations significantly increased both the expression and phosphorylation levels of eIF2α, a key signaling molecule that attenuates general protein translation and induces apoptosis in the PERK-eIF2α pathway, in a time-dependent manner. The ratio of phosphorylation to total eIF2α was also increased in the same manner. After a 24 hour incubation of PMCs with 0.1% - 4% glucose, the relative phosphorylation levels of eIF2α were increased in a dose-dependent manner. At concentrations greater than 3%, glucose suppressed cell viability examined by WST-1 assay and increased DNA fragmentation examined by DNA ladder formation assay. These results demonstrate that high glucose concentrations promote eIF2α-mediated ER stress and apoptosis in PMCs.

Conclusions: In PMCs, it was assumed the high concentration of glucose induced the cell death by apoptosis through the ER stress pathway. Therefore, we consider that the ER stress is likely to be involved in peritoneal damage in patients under PD therapy.

SA-PO2378

Advanced Oxidation Protein Products Induce Podocyte Apoptosis via a RAGE-Mediated Signaling Pathway 
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Background: The accumulating of advanced oxidation protein products (AOPPs) was found in diabetes, metabolism syndrome and chronic renal disease. We previously reported that accumulated AOPPs could induce the apoptosis and depletion of podocytes through activation of NADPH activity. The aim of the present study is to identify the receptor on the surface of podocyte which mediated the effect of AOPPs on podocytes.

Methods: The binding activity of AOPPs-MSA to RAGE in cultured podocytes was determined by co-immunoprecipitation and co-immuno-localization fluorescence analysis. Assessment of podocyte apoptosis was determined by Annexin V-labeling and TUNEL Assay. Expression of apoptosis related molecules and activation of NADPH oxidase were analyzed by western blotting, immunoprecipitation and lucigenin-enhanced chemiluminescence. The sequences of custom small interfering RNA (siRNA) duplex for mouse RAGE and scrambled siRNA were also used for detection the above objectives.

Results: By immunoprecipitation assay, we found that AOPPs interacted with RAGE receptor in conditionally immortalized podocyte cells. Immunofluorescence staining demonstrated that AOPPs colocalized with RAGE at the cell membrane. The apoptosis of podocyte induced by AOPPs was inhibited by a RAGE neutralizing antibody in a dose-dependent manner. However, the neutralizing antibodies against other scavenger receptors such as CD68, SR-A, LOX1, AGE-3 and inflammation related receptor TL4R had no effect. Knockdown RAGE expression by siRNA did not significantly inhibited AOPPs-induced the upregulation of apoptotic proteins such as p53, Bax, caspase 3 and PARP-1, and the activation of NADPH oxidase, in podocyte.

Conclusions: These data suggest AOPPs induce podocyte apoptosis and activation of NADPH oxidase-dependent p53-Bax apoptotic pathway via a RAGE-mediated signaling pathway.

SA-PO2380

Role of Extracellular Matrix Renal Tubulo-Interstitial Nephritis Antigen in Cell Survival 
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Background: Tubulo-interstitial nephritis antigen (TINag) is an ECM protein expressed in tubular basement membranes (TBMs). Since some of the ECM proteins are known to modulate cell survival, studies were initiated in Lewis rats lacking TINag expression to see if they are relatively susceptible to cisplatin induced-injury. Cisplatin administration caused relatively high degree of tubular cell damage and apoptosis, exclusively in regions where TINag is normally expressed in the proximal Wistar strain. This was accompanied with an accentuated increase in serum creatinine and renal Km-1 RNA, and protein expression of Bax, p53 and of its phosphorylated form and nuclear accumulation, high intracellular reactive oxygen species (ROS) and increased Bcl-2, while it increased that of Bax; of which magnitudes were attenuated by NF-κB inhibitors (Bay 117082). The inhibition of NF-κB also prevented HHE-induced apoptosis.

Conclusions: HHE-induced tubular cell apoptosis is mediated by ROS generation and modulation of Bax and Bcl-2 in HHE cells; in which ROS may play a role in redox-sensitive transcription factors, NF-κB, through activation of ERK and JNK.

SA-PO2381

Receptor Interacting Protein 1-Mediated Necroptosis Essentially Contributes to Renal Ischemia/Reperfusion Injury 
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Background: Loss of kidney function in renal ischemia/reperfusion injury (IRI) is caused by programmed cell death (PCD) but the contribution of necroptosis, a recently discovered form of programmed necrosis, has not been investigated.

Methods: We employ murine renal tubular cells and freshly isolated proximal tubules for analysis of the sensitivity to undergo Fas- or TNF-induced tubular cell apoptosis. By the addition of caspase-inhibitors or the specific RIP1-inhibitor Necrostatin-1 (Nec-1) we inhibit apoptosis and necroptosis, respectively. In vivo, both sublethal and lethal renal ischemia/
reperfusion injury (IRI) was performed in the presence and absence of PCD-inhibitors. Electron microscopy, immunohistology, conventional PAS-staining, western blotting, and FACS analysis were employed to quantify apoptosis and necrosis ex vivo.

Results: We identified death receptor-mediated caspase-independent cell death in murine tubular cells and characterize it as necroptosis by addition of Nec-1. The necroptosis pathway in apoptosis, caspase-1 and RIP3 were detected in whole kidney lysates in freshly isolated murine proximal tubules. In vivo, Nec-1 reduces organ damage and renal failure, even if administered after reperfusion and resulted in a significant survival benefit in a model of lethal renal IRI. We functionally compared these results with the contribution of apoptosis to cell death, showing that in necroptosis, caspase-1 is activated by zVAD neither prevented organ damage nor the increase of retention parameters in renal IRI.

Conclusions: Our results demonstrate the presence and functional relevance of necroptosis in the pathophysiologic course of ischemic kidney injury and a functional predominance of necroptosis over apoptosis in this setting. Above that we identify the therapeutic potential of Nec-1 as a drug for the prevention and treatment of renal IRI.

Funding: Private Foundation Support, Clinical Revenue Support

SA-PO2382

Renin Induces Podocyte Apoptosis through Renin Receptor and p38 MAPK

Pathway Independent of Ang II Generation

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Background: Podocyte plays an important role in the pathogenesis and progression of glomerulosclerosis. Various elements of renin-angiotensin-aldoosterone system (RAAS), such as angiotensin II and aldosterone, can induce podocyte apoptosis. However, little is known about the direct effect of renin on podocytes through renin receptor. In the present study, we evaluated the effect of renin on cultured podocyte apoptosis and the role of p38 MAPK pathway.

Methods: Expression of renin receptor was detected by fluorescent staining and RT-PCR. Podocytes were incubated in media containing either buffer or renin for variable time periods. The cells were also treated with either inhibitor of p38 MAPK or buffer. At the end of the incubation period, apoptosis was evaluated by cell nucleus staining, and caspase 3, p38, phospho-p38 MAPK were measured by Western blotting.

Results: We demonstrated that both renin receptor mRNA and protein were expressed in cultured podocytes. Exposure of podocytes to renin induced podocyte apoptosis in a time-dependent manner, which was accompanied by up-regulation of active caspase-3 and increased expression of p38 MAPK. Renin-induced podocyte apoptosis and p38 MAPK phosphorylation were inhibited when the cells were pretreated with p38 MAPK inhibitor. Transfection of renin receptor siRNA ameliorated the above changes induced by renin. Furthermore, these effects of renin were not altered by blocking of angiotensin II using either enalapril or losartan.

Conclusions: These data suggest that renin induces podocyte apoptosis, which is mediated through renin receptor and p38 MAPK pathway independent of Ang II generation.

Funding: Government Support - Non-U.S.

SA-PO2383

Connexin43 Contributes to Puromycin-Elicited Podocyte Injury

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Background: Gap junctions (GJs), formed by specific protein termed connexin (Cx), play important roles in regulation of cell phenotype and in the control of cell survival. Podocyte injury is one of the major factors contributing to the initiation and development of glomerulosclerosis. Various elements of renin-angiotensin-aldoosterone system (RAAS), such as angiotensin II and aldosterone, can induce podocyte apoptosis. However, little is known about the direct effect of renin on podocytes through renin receptor. In the present study, we evaluated the effect of renin on cultured podocyte apoptosis and the role of p38 MAPK pathway.

Methods: Expression of renin receptor was detected by fluorescent staining and RT-PCR. Podocytes were incubated in media containing either buffer or renin for variable time periods. The cells were also treated with either inhibitor of p38 MAPK or buffer. At the end of the incubation period, apoptosis was evaluated by cell nucleus staining, and caspase 3, p38, phospho-p38 MAPK were measured by Western blotting.

Results: We demonstrated that both renin receptor mRNA and protein were expressed in cultured podocytes. Exposure of podocytes to renin induced podocyte apoptosis in a time-dependent manner, which was accompanied by up-regulation of active caspase-3 and increased expression of p38 MAPK. Renin-induced podocyte apoptosis and p38 MAPK phosphorylation were inhibited when the cells were pretreated with p38 MAPK inhibitor. Transfection of renin receptor siRNA ameliorated the above changes induced by renin. Furthermore, these effects of renin were not altered by blocking of angiotensin II using either enalapril or losartan.

Conclusions: These data suggest that renin induces podocyte apoptosis, which is mediated through renin receptor and p38 MAPK pathway independent of Ang II generation.

Funding: Government Support - Non-U.S.

SA-PO2384

SsCkS Sequestrates Cyclin D1 in Glomerular Parietal Epithelial Cells


Background: Glomerular parietal epithelial cells (PECs) are precursors for podocytes in mature glomeruli, however, as progenitors, the distinct mechanisms that allow for repeated periods of cell-cycle arrest and re-entry of PECs after glomerulonephrosis are unknown. Here, we show that the Src-suspected protein kinase C substrate, SsCkS, is a multivalent scaffolding A kinase anchoring protein, sequesters cyclin D1 in the cytoplasm of quiescent PECs.

Methods: The expression and interaction of SsCkS and cyclin D1 was studied in PECs in tissue culture, during glomerulonephrosis, and in post-natal glomeruli of SsCkS-/- and SsCkS+/- mice.

Results: SsCkS expression is induced in embryonic PECs but not in embryonic podocytes starting at the 5-phase of glomerulonephrosis, and is constitutively expressed post-natally in PECs, but not podocytes. In cultured PECs induced with SsCkS from capuslated glomeruli containing PECs, whereas decapulated glomeruli without PECs lacked SsCkS and cyclin D1. Cell-cell contact inhibition of proliferation in cultured PECs induced SsCkS expression and binding of cyclin D1 by SsCkS in the cytoplasm, whereas phosphorylation of SsCkS by activated PKC disrupted binding, resulting in nuclear translocation of cyclin D1. SsCkS-/- mice showed hyperplasia of PECs in otherwise normal glomeruli and developed significantly worse proteinuric glomerular disease, marked by increased PEC proliferation and expression of nuclear cyclin D1, from nephrinotype nephritis.

Conclusions: These results suggest that SsCkS controls the localization and activity of cyclin D1 in PECs.

Funding: NIDDK Support

SA-PO2385

HIV-Nef Induces Detachment, Migration and Proliferation of Human Podocytes by Compromising Cytoskeletal Integrity

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Background: HIV-1 gene Nef has been considered to play an important role in the development of HIV-1 associated nephropathy (HIVAN). We hypothesized that Nef decreases adhesion and enhances detachment, migration, and proliferation of podocytes by compromising their cytoskeletal integrity.

Methods: We developed stable colonies of conditionally immortalized human podocytes expressing either Nef (Nef/CHIP) or empty vector (EV/CHIP). To identify Nef-linked proteins, GST pull down assay followed by mass spectrometry was carried out on EV/CHIP and Nef/CHIP. To identify Nef interacting proteins, yeast two hybrid assay was carried out. To determine mRNA expression of relevant proteins, microarray analysis of RNA from EV/CHIP and Nef/CHIP was carried out. To identify co-localization of Nef with interacting proteins, imaging studies were performed. To determine alteration in Nef-induced functionality of podocytes, adhesion, detachment, migration, proliferative and apoptotic studies were done.

Results: GST pull down assay in Nef/CHIPS displayed a band at 45 kD, which was identified to be Actin by mass spectrometry. In contrast to factors promoting mesangial cell proliferation, little is known about the actin by mass spectrometry, whereas, yeast two hybrid assay identified the following Nef-interacting proteins: syntenin, filamin B, syntxin, translational elongation factor 1, and zyxin. Microarray analysis of RNAs from Nef/CHIP revealed enhanced expression of Rac1 and syndecan-4 and attenuated expression of syndecan-3 and syntxin when compared with EV/CHIPS. Imaging studies displayed co-localization of Nef with actin and zyxin in Nef/CHIPS. Nef/CHIP displayed scant number of actin filaments and enhanced number of lamellipodia. Since Nef/CHIPS displayed enhanced expression of Rac1, it appears that Nef-induced Rac1 expression may be contributing to increased number of lamellipodia in Nef/CHIPS.Nef/CHIP decreased adhesion, enhanced detachment and migration. Moreover, Nef/CHIPS displayed proliferative phenotype.

Conclusions: We conclude that interaction of Nef with actin compromises cytoskeletal integrity of human podocytes and make them prone to detach, migrate and proliferate.

Funding: NIDDK Support

SA-PO2386

A Novel, Dual Role of CCN3 in Experimental Glomerulonephritis: Pro-Angiogenic and Anti-Mesangiolproliferative Effects

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Background: In contrast to factors promoting mesangial cell proliferation, little is known about their endogenous regulators. During experimental mesangiolproliferative nephritis glomerular CCN3 (nephroblastoma overexpressed gene) expression is reduced prior to the proliferative phase and overexpressed both in glomeruli and serum when mesangial cell proliferation subsides.

Methods: To further elucidate its role in mesangiolproliferative glomerulonephritis (GN), CCN3 was systemically overexpressed by muscle electroporation in healthy or nephritic mice. This increased CCN3 serum concentrations more than 3-fold for up to 35 days after electroporation.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.
Results: At day 5 after induction of mesangioproliferative GN, CCN3 transfection rate exhibited an increase in glomerular endothelial area and in glomerular mRNA levels of the pro-angiogenic factors VEGF and PDGF-C. In vitro CCN3 induced proliferation of glomerular endothelial cells. In the mesangioproliferative phase (day 7), CCN3 overexpression decreased albuminuria, the α-smooth-muscle actin (αSMA) positive mesangial cell area, and the expression of different proangiogenic factors, such as fibronectin and type IV collagen. In progressive nephritis at day 56, systemic overexpression of CCN3 resulted in decreased albuminuria, glomerulosclerosis and reduced cortical collagen type I accumulation. In healthy rat kidneys, overexpression of CCN3 induced no morphologic changes but regulated glomerular gene expression (reduced transcription of PDGF-B, PDGF-D, PDGF-R, fibronectin, and αSMA and increased PDGF-α and PDGF-C).

Conclusions: Our data identify a dual role of CCN3 in experimental glomerulonephritis with increased CCN3 expression in vivo and CCN3 transfection experiments, both of which are able to reconstitute the normal glomerular architecture. Manipulation of CCN3 could represent a novel approach to help repair glomerular endothelial damage and mesangioproliferative changes.

Funding: Government Support - Non-U.S.

SA-PO2387
Profiling of Inflammatory Mediators Produced by Primary Human Mesangial Cells In Vitro
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Background: The study of fibrotic changes in the development of chronic kidney disease has shown the importance of inflammatory products in initiating and perpetuating the local environment in the diseased kidney during the progression towards end stage renal disease.

Methods: Normal primary human mesangial cells were stimulated with AngII, IL-1β, IL-6 or TGFβ1, factors known to be dysregulated in renal disease. To determine which cytokines or chemokines were produced, an initial screen of supernatants was performed using multiplex analyte arrays. A 12 cytokine array including TNFα, IL-1β, IL-6, IL-12, IL-17A, IL-8, MCP-1, RANTES, MIP-1α, MIP-1β, MDC and Eotaxin was selected based on preliminary experiments. Subsequent determinations for dose and time studies were done using the baseline analyte ELISA.

Results: Production of inflammatory mediators IL-6, IL8 and MCP-1 was induced by AngII, IL-1β, IL-6 and TGFβ1. IL1β stimulation also increased production of RANTES and MIP-1α. TGFβ1, an important modulator in kidney disease, was quantified in separate experiments, AngII, IL-1β and IL-6 all increased production of TGFβ1. Stimulation of cells with TGFβ1 induced an autocrine increase in TGFβ1 production. The production of IL6, IL-8, MCP-1 and TGFβ1 was found to be both dose and time dependent. IL1β was the only factor to induce GM-CSF and ICAM-1 surface expression whereas IL-1β and IL-6 induced a modest increase in TSP-1. Fibronectin and collagen I deposition were identified 4-7 days after stimulation.

Conclusions: This data provides an in vitro kidney specific cellular assay with the potential to triage inhibitors of inflammatory pathways participating in kidney disease.

Funding: Pharmaceutical Company Support

SA-PO2388
UpA4, Elevated in Patients with End-Stage Renal Disease, Induced a Phenotype Switch of Vascular Smooth Muscle Cells Via P2Y Activation Mediated by Saccharin.
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Background: Vascular mineralization is a major risk factor in patients with chronic kidney disease (CKD) and contributes to the increased cardiovascular mortality. Vascular calcification is an actively induced process Smooth muscle cells differentiate toward osteoblast-like phenotype. The dimercosulphate polysulphate uridine adenosine tetraphosphate (UpA4) has been previously shown to be a potent calcification inducing substance. Now we searched for the involved mechanism.

Methods: In vitro and ex vivo calcification in rat VSMCs and aortic rings was measured. Calcium deposition was monitored. Expression of cbfa1 was measured in rat VSMCs after stimulation with UpA4.

Results: UpA4 induced calcification of rat VSMCs in vitro and ex vivo in rat aortic rings, visualized by Alizarin Red Staining and quantified. The non-selective P2Y inhibitors suramin, PPADS, and RB-2 as well as MRS2578, which antagonizes P2Y6, have a significant inhibitory effect on the UpA4-induced mineralization in VSMCs. For mineralization of VSMCs a trans-differentiation of VSMCs in osteoblast-like cells could be detected. Investigating the gene expression in rat VSMCs UpA4 is able to induce the initial transcription factor cbfa1. To proof the antagonistic potential of the mentioned antagonists, therewith an increase in expression of cbfa1 was observed.

Conclusions: UpA4-induced mineralization depends on the activation of P2Y2 to stimulate trans-differentiation in VSMCs. UpA4 has influence on vascular mineralization and therefore, the purinergic signaling might be involved in arteriosclerotic processes.

SA-PO2389
4-Phenylbutyrate Inhibits Transforming Growth Factor-β1-Induced Apoptosis Via a Caspase-Mediated Mechanism in Human Renal Proximal Tubule Epithelial Cells Elise Brimble, Richard Austin, Jeffrey G. Dickhout.
Nephrology, McMaster University and St. Joseph’s Healthcare Hamilton, Canada.

Background: TGF-β1 signaling is an important inducer of apoptosis in tubular epithelium during renal injury, however, the precise mechanism is unknown. Smad3 may mediate TGF-β1-induced renal tubular epithelial cell (RTEC) apoptosis, as shown by a reduction in its incidence in the Smad3−/− mouse in a model of obstructive nephropathy. Histone deacetylase (HDAC) inhibition reduced TGF-β1-induced apoptosis in cultured RTECs. However, it is unclear how HDAC inhibitors interfere with this apoptotic signal transduction and whether HDAC inhibitors reduce RTEC apoptosis by disrupting TGF-β1 signaling.

Methods: We used human primary RTECs and HK-2 cells treated with human recombinant TGF-β1 to determine the effects of pan-HDAC inhibitors, 4-Phenylbutyrate (4-PBA) and vorinostat, on the incidence of apoptosis. Taurosuderoxycholic acid (TUDCA), a low molecular weight chemical chaperone, was used as a control, as 4-PBA has chemical chaperone properties. LDH release and TUNEL assays were used to assess cytotoxicity and apoptosis. Western blotting was used to monitor TGF-β1 signaling and the endoplasmic reticulum (ER) stress response. Z-VAD FMK, a pan-caspase inhibitor, was used to determine the dependence of TGF-β1-induced apoptosis on caspase activity.

Results: We found that TGF-β1 treatment induced cytotoxicity and apoptosis in RTECs. This effect was significantly inhibited by treatment with 4-PBA and vorinostat, but not with TUDCA. TUDCA was not found to induce apoptosis through the ER stress pathway. TGF-β1-induced apoptosis was found to be dependent on caspase activity, as was the ability of 4-PBA to inhibit cell death. 4-PBA, vorinostat, and TUDCA were not found to affect TGF-β1 signaling and the endoplasmic reticulum stress response. We hypothesize that HDAC inhibitors reduce RTEC apoptosis by disrupting TGF-β1 signaling.

Funding: Government Support - Non-U.S.

SA-PO2390
Loss of the β Isofrom of Caeclinurin A (Cαβ) Reduces Hypertrophy, Suppresses Protein Synthesis, and Activates AMP Dependent Kinase (AMPK)
Harold A. Frencl,1,2 Changlin Ding,2 Sara Zoromsky, Jennifer L. Gooch,2 'Renal Division, Atlanta VAMC, Decatur, GA; 'Emory University, Atlanta, GA.

Background: Transgenic mice with Cαβ knockout (Cαβ−/−) have normal renal development and function, but do not exhibit renal hypertrophy when diabetic (Reddy RN et al. 2009). Because caecilninurin regulates protein synthesis and breakdown in cardiac hypertrophy, we examined its role in protein metabolism and in phosphatidylinositol 3 kinase (PI3K) mitogen associated kinase (MAPK), and AMPK signaling in proximal tubule cells.

Methods: Cαβ phenylalnine pulse/chase was used for protein synthesis and breakdown in NRK-52E renal epithelial cells and SV40 immortalized cell lines created from Cαβ−/− and wild-type mice. Proline incorporation was determined integrating isotope exchange by mass spectrometry and monitoring bloting comparing the ratio of phospho-specific to whole protein antibodies. Inhibitors of PI3K (8 μM cyclosporine A (Cya) or 200 nM tacrolimus), of PI3K (25 μM LY294002) and of MAPK (50 μM U0126) were used. All results are p<0.05.

Results: Cαβ decreased protein content/well (48h) by 14.2% and protein synthesis (20h) 51.2±1% of control in NRK-52E cells. In contrast, protein degradation (0h-28h) was decreased by 10±1%. Tacrolimus gave similar results on protein content and degradation. Inhibitors of MAPK or PI3K pathways reduced protein synthesis by 48±3% or 56±2% in these cells. However, Cαβ or tacrolimus increased both basal and EGF-induced MAPK and AKT (downstream of PI3K) phosphorylation (1h) by 50% to 3-fold. Downstream of MAPK and AKT, AMPK (which acts to inhibit the mammalian target of rapamycin) phosphorylation was ~6 fold higher with Cαβ compared to control. Cαβ−/− tubular cells had lower protein per well (48h, 20±2%) and protein synthesis (20h, 16±1%) than Cαβ+/+ cells. In Cαβ−/− cells, CYA treatment (20h) did not decrease protein synthesis further, while it reduced it 20±2% in Cαβ+/+ cells. AMPK phosphorylation was >10 fold higher in Cαβ+/− compared with Cαβ+/+ cells.

Conclusions: The inhibition of Caeclinurin in knockout of Cαβ reduces protein accumulation in renal tubular cells via reduced protein synthesis possibly through activation of AMPK. Activation of AMPK may explain decreased renal hypertrophy in Cαβ mice.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2391
Consideration of Protein Expression Changing with Pitavastatin or Edaravone Therapy in Chronic Puromaycin Aminonucleoside Nephropaty
Yoshiki Nakajima, Koichi Kanazawa", Tetsuya Mitarai.
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Background: In puromycin aminonucleoside nephrosis rats which is one of human nephritic syndrome animal model. Excessive oxidants, especially oxidative LDL play roles the pathogenesis of glomerular injury. To clarify the protective effects of anti-oxidants, we compared protein expression in puromycin aminonucleoside nephrosis rats treated with or without pitavastatin or edaravone. Treatment with pitavastatin or edaravone was significantly effective on the improvement of protein expression in puromycin aminonucleoside nephrosis rats.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

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Overexpression of GFP-GABARAP Causes Deterioration of Proteinuria and Glomerulosclerosis in Adriamycin-Induced Nephropathy Mice

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Background: We previously reported that LC3, a promising marker of autophagy, played an important role in recovery from podocyte damage in an experimental nephrosis model. GABARAP is a γ-aminobutyric acid A receptor associated protein and has recently been characterized as another homolog of LC3, although its precise role in autophagy remains unclear.

Methods: We recently generated GFP-GABARAP transgenic mice, in which GFP-GABARAP is abundantly expressed in glomerular podocytes.

Results: We observed that aged transgenic mice showed slight albuminuria and showed low levels of foot-process effacement. A single injection of adriamycin caused a more significant increase in proteinuria and sclerotic glomeruli in transgenic mice compared with wild type mice. Under these conditions, neither GFP-GABARAP nor endogenous GABARAP appeared to be recruited by autophagosomes and remained in the cytoplasm. Moreover, cytosolic GFP-GABARAP was significantly colocalized with p62 to form aggregates.

Conclusions: It appears that the GFP-GABARAP/p62 complex is responsible for impairment of glomerular function, and that it retards recovery from the effects of adriamycin.

SA-PO2392

Anti-Proliferative Effect of Asymmetric Dimethylarginine (ADMA) – A Cue to Renal Fibrosis? James Alexander Tomlinson,1 Ben Caplin,1 Jill T. Norman,2 David C. Wheeler,3 James M. Leiper,1 Nitrile Oxide Signalling Group, MRC Clinical Sciences Centre, London, United Kingdom; 2Renal Department, UCL Royal Free, London, United Kingdom.

Background: The endogenous nitric oxide synthase (NOS) inhibitor, asymmetric dimethyl arginine (ADMA), has been implicated in a wide variety of diseases including chronic kidney disease (CKD) although a true cause and effect remains to be established. CKD is characterised by progressive functional decline histologically manifest as progressive tubulointerstitial fibrosis. Renal tubular epithelial cells play a key role in fibrosis and are known to express components of the NO system. We hypothesised that ADMA may contribute to fibrosis by impairing the ability of renal epithelial cells to repair following injury.

Methods: Confluent conditionally-immortalised human proximal tubular epithelial cells were made quiescent and a wound was scratched across the monolayer. Cells were incubated in the presence or absence of 10% foetal calf serum (FCS) and ADMA [3 and 10μM] for 24 hours. The extent of repair (in-filling) of the wound was measured. Positive control was distinguished from autophagy using autophagy drug Mito-LC3, which has been shown to induce autophagy when infused into cells. ADMA was subsequently confirmed using a Bird-U incorporation assay to measure DNA synthesis.

Results: FCS (10%) markedly stimulated epithelial repair of the scratch (48% ± 7 vs 11% ± 2; p<0.0001). Mitomycin C significantly retarded repair (18% ± 0 vs 48% ± 7; p<0.0001), suggesting that wound repair was largely due to epithelial cell proliferation. ADMA significantly inhibited serum-induced wound repair (52% ± 3 vs 48% ± 7; p<0.05) and Bird-U incorporation (0.96 ± 0.23 x 1.87 ± 0.45 arbitrary units (AU); p<0.0001) when compared with FCS treatment alone, suggesting a potent anti-proliferative effect.

Inhibition of NOS with two other inhibitors, PTBITU and L-NNAME also suppressed cell proliferation (0.36 ± 0.11 vs 1.67 ± 0.45 AU; p<0.0001 and 1.14 ± 0.32 vs 1.71 ± 0.45 AU; p<0.05 respectively).

Conclusions: ADMA exerts an anti-proliferative effect on renal epithelial cells, most likely due to reduced NO availability. This suggests a potential mechanism for impaired renal repair and fibrosis.

SA-PO2394

Fucosylation Influences Extracellular Matrix Accumulation in TGF-β1-Stimulated HK-2 Cells

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Background: TGF-β1 plays a key role in the development of renal fibrosis. Our previous work found that core fucosylation which catalyzed by its specific transferase Fut8 was essential for TGF-β1 receptors to fulfill their functions, however, it remains unclear whether fucosylation exerts effects on ECM accumulation and tubular epithelial cell apoptosis.

Methods: A fibrosis HK-2 cell model was established with 10ng/ml TGF-β1. Fut8 siRNA was transfected into HK-2 cells with Lipofectamine at the concentration of 30nM. The changes of Fut8 expression were examined by western blot, and the apoptosis were determined by flow cytometry. Also, expression levels of protein MMP-2,3,9 and TIMP-1 were detected by western blot. Additionally, ECM proteins, including collagen type I,LNIV,F, were examined by immunofluorescence. Besides, fibroinectin and laminin were examined by Real time-PCR.

Results: The expression of Fut8 protein increased after stimulated by TGF-β1, and Fucosylation of ProsFn peptide reversed the significant up-regulation of Fut8 protein and inhibited the apoptosis of HK-2 cells. Moreover, TGF-β1 enhanced the expression of MMP-2,3, as well as TIMP-1, but had no effect on MMP-9; Furthermore, Fut8-siRNA up-regulated the expression of MMP-2,3,9, and decreased the expression of TIMP-1. Collagen type II, III, IV, fibroinectin and laminin, were excessive synthesis after stimulated by TGF-β1, while they had no obvious change when treated by TGF-β1 together with Fut8-siRNA.

Conclusions: Blockage fucosylation may suppress ECM accumulation and apoptosis in HK-2 cells stimulated by TGF-β1, then suppress interstitial fibrosis.

SA-PO2395

Aristolochic Acid Enhances Invasion and Migration of Human Urothelial Cancer TSGH Cells In Vitro and In Vivo

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Background: Aristolochic acid (AAI) has been implicated in urothelial carcinoma (UC) in humans. However, whether AAI promotes invasion/migration of UC has not been established.

Methods: A study of human UC TSGH cells cultured with AAI was conducted. Cell viability, the effects of AAI on the activity of MMP-9, the abilities of invasion/migration and the association with migration-related proteins (Ras, RhoA, ROCK1, PI-3K, p-Akt and NF-κB) in the TSGH cells were assessed. The TSGH cells were subcategorized to one-day or 30-day AAI exposure. An in vivo study using a nude mice xenograft model was employed to test the anti-tumor effects of Rho kinase inhibitor or Y27632.

Results: A time- and dose-dependent increase in both activity and mRNA level of MMP-9 were demonstrated. The mRNA level of uPA was increased and of TIMP-1 was decreased in the cells with 30-day but not one-day AAI exposure. A dose-dependent enhancement in wound healing rate and cell migration was demonstrated, especially in the 30-day AAI-exposed cells. Expressions of Ras/RhoA and other migration-related proteins were increased after AAI treatment which could be inhibited by Y-27632. The in vivo results demonstrated that Y27632 was able to attenuate the speed of growth of the inoculated tumors in nude mice.

Conclusions: Our results provided in vitro and in vivo evidence that prolonged AAI exposure enhances invasion and migration of human UC TSGH cells.

SA-PO2396

Run3 Mediates Suppression of Tumor Growth and Metastasis of Human CCRC by Regulating Cyclin Related Proteins and TIMP-1

Hannim Wang, Shiren Sun, Lijie Hc.

Background: Although our previous studies indicated that Run3 has been implicated in tumor suppression in several tumors, the precise molecular mechanisms in renal cancer remained unclear.

Methods: Here we tested Run3 in clear cell renal cell carcinoma (CCRC) in vivo and in vitro. The results: We presented that the expression of Run3 was absent or significantly decreased in 75 cases of CCRC tissues (p<0.05, table1,figure1,a is cancer, b is non-cancerous ). Enforced Run3 expression mediated 786-O CCRC-derived cells to exhibit significant inhibition of growth, G1 cell cycle arrest and metastasis in vitro, and to lost 786-O tumorigenicity in nude mouse model. Run3-induced growth suppression was found

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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partially to regulate various proteins, including inhibition of cyclinD1, cyclinE, cdk2, cdk4 and p-Rb, but up-regulation of p27Kip1 and Rb. Simultaneously, overexpression of Runx3 led to significant induction of TIMP-1 expression.

Clinicopathological associations of Runx3 expression in CCRC patients

<table>
<thead>
<tr>
<th>Category</th>
<th>Total number of cases</th>
<th>Runx3 expression -</th>
<th>Runx3 expression +</th>
<th>Runx3 expression ++</th>
<th>Positive case</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adjacent tissue of CCRC tissues</td>
<td>77</td>
<td>12 (15.58%)</td>
<td>16 (20.78%)</td>
<td>49 (63.64%)</td>
<td>65 (84.42%)</td>
<td>&lt;0.001**</td>
</tr>
<tr>
<td>CCRC tissues</td>
<td>75</td>
<td>32 (42.67%)</td>
<td>12 (16%)</td>
<td>11 (14.67%)</td>
<td>23 (30.67%)</td>
<td>&lt;0.001**</td>
</tr>
</tbody>
</table>

Runx3 staining was graded as negative (-; score: 0–1), weak (+; score:2–4), and strong (++; score:5–8). Pearson’s X² Test. **P<0.001 vs non-cancerous tissues.

Conclusions: Therefore, Runx3 has the proliferative and metastatic abilities of CCRC cells, which was mediated, at least partially by regulating cyclins and TIMP1. 

Funding: Government Support - Non-U.S.

SA-PO2397
Age Associated Loss in Tubular Epithelial Proliferation Reserve Is Intrinsically Determined

Nathan D. Susnik, Inna V. Kuznetsova, Birgit Berkenkamp, Christoph Jacob, Inga Soerensen, Hermann G. Haller, Anette Melk, Roland Schmitt.

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Background: Data from kidney injury models suggests that the loss of renal repair capacity with aging is largely due to a decreased tubular epithelial proliferative reserve. This concept has been challenged because age dependent factors might lead to different damage loads in experimental kidney injury, this may cause changes in the reparative proliferation response.

Methods: In order to test for age-dependent changes in the renal epithelial proliferative reserve without inducing cellular injury, we used lead acetate, a potent direct mitogen, to stimulate tubular cell proliferation. Lead acetate was used in old (22 months) and young (4 months) mice in vivo and in old and young primary tubular epithelial cells (PTEC). Differences in cell cycling, cell damage and markers of senescence were quantified by histology, immunoblot and qPCR.

Results: Lead acetate significantly increased the rate of tubular epithelial proliferation without causing cell damage as shown by unchanged levels of injury markers Ngal and Kim-1. The increase in proliferation was significantly smaller in old mice. Cyclin D1 positive tubular cells increased only in young kidneys, but not in old kidneys. Kidneys from old mice expressed significantly more of the senescence markers p16INK4a, senescence associated β-galactosidase, and phospho-pERK, regardless of lead acetate stimulation. Only young isolated PTEC showed increased proliferation after lead acetate stimulation but to a lesser extent than in vivo. This may have been due to a dramatic increase of p16INK4a and p21 in both age groups, resulting in almost identical levels of senescence markers in PTEC from young and old mice after 6 days of culture.

Conclusions: These data indicate that the aged kidney has an intrinsically reduced proliferative capacity. This is in agreement with a higher load of senescence markers from both the stress- and telomere-induced pathway. Age-dependent differences in tubular cell proliferation quickly diminish in vitro because cultured PTEC from young kidneys undergo accelerated aging with rapid induction of cellular senescence.

SA-PO2398
Inactivation of Mxi1 Regulates IFT20 Expression in Polycystic Kidney

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Background: Most cell types have primary cilium, which consists of protruding structures that sense mechanical and chemical signals from the extracellular environment. Cilia are assembled via a process known as intraflagellar transport (IFT) and a variety of molecules, including IFT20, IFT88 and Kif3a, participate in the assembly of cilia. It is critical that the size control system of cilia be elucidated to enable a thorough understanding of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects in cilia formation, maintenance or function.

Methods: To confirm the effect of Mxi1 on cilia, we performed immuno assay in Mxi1-deficient MEFs and Mxi1-deficient mice. Also, IFT genes were screened in vitro and in vivo using real-time PCR to select candidate gene. Furthermore, we performed promoter assay to check the effect of Mxi1 on candidate gene regulation.

Results: Previously, multiple tubular cysts were observed in the kidneys of Mxi1-deficient mice aged six months or more. Here, we clarify the relationship between inactivated Mxi1-induced cyst formation and cilia assembly. In Mxi1-deficient MEFs, the length of structurally normal cilium decreased, but we still confirmed the presence of cilia in cysts of Mxi1-deficient mice. To elucidate the cilium regulatory mechanism related to Mxi1, IFT genes are validated in Mxi1 MEFs and Mxi1 mice using real-time PCR. IFT20, IFT80, Kif3a genes are decreased in Mxi1-deficient model and IFT20 is selected for candidate gene. We observed that cilia length and IFT20 expression are regulated by Mxi1 level in vitro. Also IFT20 promoter activities are regulated by Mxi1 level in mMCD.

Conclusions: These results indicate that inactivation of Mxi1 plays an important role in the down-regulation of IFT20 expression in polycystic kidneys.

Funding: Government Support - Non-U.S.

SA-PO2399
Resistance to Leucine Induced Signal Transduction and Regulation of Autophagy in Acute Kidney Injury

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Background: Adequate nutrient intake in acute kidney injury (AKI) is a key part of patient management. Of particular interest are the branched chain amino acids (BCAA) especially leucine (LEU). LEU serves as a substrate, stimulates insulin release and directly activates the mTOR anabolic signaling pathway, stimulating protein synthesis via phosphorylation(p-) of p70 ribosomal S6 kinase-1(p70S6K), S6 ribosomal protein(rpS6) and eukaryotic 4E-binding protein1(4E-BP1). LEU also inhibits proteolysis in part by suppressing autophagy which is increased in AKI. Of note, LEU resistance develops in other acute catabolic conditions and signaling defects are common in uremia, is regulated in part by p-AMPK which inhibits mTOR activation. Since LEU resistance suppression autophagy which is increased in AKI. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia. ADPKD is a human genetic disease that is rooted in defects of ciliary diseases such as autosomal dominant polycystic kidney disease (ADPKD), which involves abnormally short cilia.
Conclusions: Together, failure of LEU to stimulate phosphorylation of the mTOR anabolic pathway, and failure to suppress both p-AMPK and autophagy strikingly demonstrates that AKI induces a leucine resistant state. We suggest that this AA resistance may contribute to protein energy wasting that often persists in AKI patients despite nutritional supplementation.

Funding: Veterans Administration Support

SA-PO2400
Snail Destabilizes Cell Surface Localization of the Apical Polarity Protein Crumbs3a
Jennifer L. Harde, Eileen L. Whiteman, Jay Pelczynski, Benjamin L. Margolis.

Background: During Epithelial to Mesenchymal Transition (EMT), cells modulate expression of proteins resulting in loss of apical-basal polarity. Mechanisms involved in this switch target the polarity protein Crumbs3a, a small transmembrane protein that is essential for generation of the apical membrane and tight junctions of epithelial cells. The Crumbs3a gene is a direct target of transcriptional regulation by Snail, a potent inducer of EMT. However, Snail has also been shown to have multiple non-transcriptional roles, including regulation of cell adhesion and movement as well as cell proliferation and survival. In this set of experiments, we defined the normal kinetics of Crumbs3a in polarized epithelial cells and then explored Snail’s post-translational effects on Crumbs3a (Crb3a).

Methods: We used MDCs, a tissue culture model, stably expressing epitope-tagged versions of Crb3a and Snail in an inducible or constitutive manner to study the dynamics of Crb3a.

Results: Using fluorescently-tagged Crb3a in combination with fluorescence recovery after photobleaching (FRAP), we found that Crb3a normally has a half-life of under 6 hours and is highly mobile at the cell surface. We then used covalently modifiable SNAP-tag and epitope-tagged Crb3a to examine kinetics of Crb3a at the cell surface. We showed that Crb3a is rapidly turned-over in the presence of Snail, decreasing the half-life of Crb3a on the cell surface from 2 to 1 hour. In contrast, cell surface stability of Crb3a is unaffected by disruption of cell polarity following dissolution of cell-cell contacts under low calcium conditions. We further observed that Crb3a’s mobility on gel electrophoresis is altered in the presence of Snail, in part due to alterations in both N- and O-glycosylation.

Conclusions: Taken together, these results suggest that Snail induces post-translational modifications that alter Crb3a’s trafficking and stability at the epithelial cell surface. Moreover, our results support the concept of discernable transcriptional and post-translational effects of Snail on cell polarity and that these post-translational effects involve post-translational modulation of the polarity protein Crb3a.

Funding: NIDDK Support, Private Foundation Support

SA-PO2401
Analysis of Williams-Beuren Syndrome-Related Genes; by Using a SA-PO2401

Tomoko Uehara, Eriko Kage-Nakadai, Shohei Mitani.

Background: At least twelve genes in WBS deletion region are expressed in kidney. Williams-Beuren syndrome (WBS) is a contiguous gene syndrome with an autosomal dominant inheritance pattern and that is caused by microdeletion at 7q11.23 and haploinsufficiency of this region. Approximately 50% of patients with WBS have morphological or functional anomalies of kidney or urinary tract. There is insufficient knowledge of candidate genes of these anomalies. In this study, we analyzed genes that are expressed in kidney and that may contribute to the pathogenesis of WBS.

Methods: We assayed the phenotype more extensively.

Expression patterns of these six genes were as follows. C28H8.1, a BCL7B gene homologue, was expressed ubiquitously. C27F2.4, a WBSCR22 gene homologue, was induced reproductive defect and morphological deformation. The phenotype of C28H8.1 gene, a TBL2 gene and an ABHD11 gene, respectively, were expressed in intestine cells and kidney.

Background: 1. Approximately 50% of patients with WBS deletion region are expressed in kidney.

Results: To summarize this study, we analyzed six gene homologs of human WBS deletion region and did not find any specific expression-pattern or specific phenotype.

Conclusions: The other five of them did not show any specific expression-pattern or specific phenotype. We are planning to analyze these genes in more detail.

Funding: Veterans Administration Support

SA-PO2402
Molecular Mediators of Endoplasmic Reticulum Stress-Induced Cytotoxicity in Human Proximal Tubular Epithelial Cells

Rachel Carlisle, Elise Brumble, Alana Heffernan, Richard Austin, Jeffrey G. Dickhout.

Background: Various drugs, including gentamicin, cisplatin, the acetaminophen metabolite p-aminoophenol, and cyclosporine A, induce endoplasmic reticulum (ER) stress causing acute renal injury in proximal tubular epithelial cells (PTEC). This injury may be mediated by the expression of CHOP/GADD153, an ER stress-inducible proapoptotic gene. However, direct evidence of CHOP/GADD153-induced PTEC cell death is lacking.

Methods: In this study, cytotoxicity was measured by LDH release assay and apoptosis was measured by TUNEL assay. CHOP/GADD153 and TDAG51 protein levels were measured by Western blotting after treating with 20mM DTT for 24h.

Results: Using HK-2 cells, we demonstrated that the nucleoside antibiotic thiamipirin (Tm, 1mM) induces ER stress and causes substantial upregulation of CHOP/GADD153 in PTEC at 18h, followed by a significant increase in apoptotic cell death at 48h. This effect was inhibited by siRNA-mediated CHOP knockdown. The SERCA inhibitor thapsigargin (Tg, 200 nM) induces ER stress, but unlike Tm, produced little or no CHOP/GADD153 expression at 18h. However, Tg did induce expression of the novel proapoptotic gene TDAG51 at 18 h leading to similar cytotoxicity as Tm at 48 h. To directly determine if CHOP/GADD153 overexpression induced apoptosis in PTEC, overexpression of CHOP/GADD153 was achieved and increased the incidence of apoptosis to 26.6+6.6% (from 3.8+0.9% in pcDNA3 transfected controls). Transfection of TDAG51 in an eGFP vector resulted in increased apoptosis in the HK-2 model of PTEC (vector, 0.5+0.4% vs. TDAG51, 26.0+3.4%) and in primary human PTEC (vector, 5.2+1.3% vs. TDAG51, 30.0+4.6%).

Conclusions: ER stress is a common pathological pathway that leads to acute renal injury from numerous drugs; however, the molecular mediators of PTEC death appear to differ depending on the mechanism of action of the specific ER stress inducer, as shown, in this case, by Tm and Tg treatment.

Funding: Government Support - Non-U.S.

SA-PO2403
Phagocytosis of Apoptotic HIV-1-Infected CD4+/PD-1+ T Cells Is Critical for Establishment of HIV-1 Reservoirs in Tubular Cells

Hersh Groel,1 Mohammed Husain,1 Nirupama Chandel,1 Joanna Mikulak,1 Muslima Younas,1 Helena Schmidtmyerova,2 Pravin C. Singhal,3 Y. Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; 1Immunology, Istituto Clinico Humanitas, Rozzano (Milano), Italy.

Background: HIV-1 infection of Kidney cells has been suggested to be a key factor which contributed to the pathogenesis of HIV-associated nephropathy (HIVAN). In the present study, we evaluated the potential of tubular cells to serve as an HIV-1 reservoir.

Methods: Human renal proximal tubular cells (HRPTECs and HK2s) were pulsed with HIV-1 (N suicidal to NHRPECS) and then evaluated for HIV infection. To rescue HIV from tubular cells, HK2s were intubated with lymphocytes (LY) and followed by evaluation of LY for HIV replication. To determine viral transfer from HIV infected lymphocytes (LY-LY) to tubular cells, HBV-LYs were co-cultivated with HK2s/HRPECS. To determine the role of apoptosis and PD-L1, LYs were co-cultivated with HK2s/HRPECS and then evaluated for apoptosis by FACS analysis. To determine the role of apoptosis and phagocytosis of LYs in HIV transmission to HK2s, HK2s-LYs were pretreated with either anti-PDL-1 antibody, cytostatin B or a caspase-3 inhibitor followed by co-cultivation with HK2s for 24 hours and then evaluation of tubular cells for HIV expression.

Results: Both, HK2s and HRPECS endocytosed viral particles; however, it was non-productive infection; nonetheless, HK2s transmitted viral particles to T cells. Co-cultivation of HIV-LYs with HK2s/HRPECS triggered apoptosis of CD4+ T cells via PD-1/PD-1L interactions. Subsequent phagocytosis of PD-1-CD4 T cells by HK2s facilitated activation of tubular cells and HIV expression. Both anti-PD-L1 antibody and cytostatin B inhibited uptake of HIV-LY by HK2s, their activation, and HIV expression. These findings identify a novel pathway that enables tubular cells to serve as an HIV-1 reservoir.

Conclusions: Our results indicate that tubular cells not only facilitated apoptosis of HIV-1 infected T cells but also showed capability of phagocytosing them. It appears that phagocytosed apoptosed T cells provided a suitable milieu for productive HIV-1 infection in tubular cells.

Funding: NIDDK Support

SA-PO2404
Hyperphosphataemia Increases Endothelial Cell Size, Granularity and Rate of Proliferation


Background: The mechanism of action of elevated serum phosphate as a risk factor for cardiovascular disease is unclear. We studied the effect of elevated serum phosphate on the growth and proliferation of human umbilical vein endothelial cells (HUVECs).

Methods: HUVECs were cultured in standard (0.5mM) or high phosphate media (3mM). Cells were counted and then photographed and cell length and area measured. Cell proliferation was measured using the MTT assay in the presence of standard and high phosphate media alone or in combination with FGF-23, Klotho and L-NAME (cESNs

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Expression of Phospho and total eNOS is reduced in HUVECs grown in high phosphate medium (p<0.03).

Conclusions: Nitric oxide inhibits cell growth. HUVECs, grown in high phosphate medium, are bigger, more granular and proliferate more rapidly. This may be secondary to increased oxidative stress with reduced nitric oxide production and thus removal of growth inhibition. Western blot confirms reduced eNOS expression in cells grown in high phosphate medium. These experiments mimic a uraemic state and may offer an explanation for elevated serum phosphate as a cardiovascular risk factor.

SA-PO2405
Renal Epithelial Cells Repress Calreticulin Expression To Increase Free Calcium and Adaptation to Osmotic Stress
Asima Bibi, Gry Helene Dihazi, Marwa Youssef Eltoweissy, Gerhard A. Mueller, Hassan Dihazi. Georg-August-University, University Medical Centre Goettingen.

Background: ER resident calcium binding proteins play an important role in different stress balance mechanisms. The thick ascending limb of Henle’s loop (TALH) is normally exposed to variable and often very high osmotic stress and involves different mechanisms to counteract this stress. The alteration of ER stress proteins is a part of TALH cells reaction to osmotic stress. The aim of the present study was to investigate the role of calreticulin (CALR) in adaptation and survival of TALH epithelial cells under osmotic stress.

Methods: Two-dimensional difference in-gel electrophoresis (2D-DIGE) combined with mass spectrometry. Western blot and RT-PCR analyses were done to analyze the protein expression in TALH cell line exposed to hypotonic stress. MTT assay was performed to check the percent viability of cells. Free intracellular calcium concentration was monitored with fura 2/AM fluorescent dye.

Results: 2D-DIGE and Western blot analyses demonstrated that TALH cells showed a significant down-regulation of CALR as a part of their reaction to variable osmotic stress. However, primary renal inner medullary collecting duct cells and interstitial cells showed no significant changes in CALR expression. Furthermore, RT PCR analysis of TALH cells exposed to osmotic stress showed a time dependent down-regulation of CALR accompanied with continuous change in the level of free intracellular calcium. Inhibition of the calcium release by the IP3R antagonist, prevented CALR expression alteration under hyperosmotic stress. MTT assay in cells grown in standard phosphate medium alone (p<0.001). FGF-2 and Klotho do not affect cell proliferation.

Conclusions: Thus we conclude that CALR due to its calcium binding property plays a crucial role in osmotic stress adaptation and survival of TALH cells.

SA-PO2406
Discovery of a Splice Variant and Endogenous Inhibitor of Chk1 That Regulates Cell Cycle and DNA Damage Checkpoints
Nayjotsibeng P. Pabla, Zheng Dong. Georgia Health Sciences University and Charlie Norwood VA Medical Center.

Background: The DNA damage response (DDR) and cell cycle checkpoints are a crucial role in osmotic stress adaptation and survival of TALH cells. Checkpoint Kinase Medical Center. Zheng Dong. Regulates Cell Cycle and DNA Damage Checkpoints

Results: This splice variant lacks the ATP binding domain and hence is kinase-inactive. Its expression is regulated in a cell-cycle dependent manner with highest expression seen in G2 phase. Importantly, we show that Chk1-S is an endogenous repressor and regulator of Chk1. In unperturbed cell cycle, Chk1-S interacts with and antagonizes Chk1 to promote G2/M phase checkpoints. Overexpression of Chk1-S induces premature mitotic entry, resulting in mitotic catastrophe. In DNA damage, Chk1 is phosphorylated and the phosphorylation disrupts the Chk1/Chk1-S interaction, resulting in free, active Chk1 to arrest cell cycle to facilitate DNA repair. Chk1-S is widely expressed in multiple cell types with higher expression observed in fetal and cancer tissues than normal tissues. In tumor xenografts, forced expression of Chk1-S induces mitotic catastrophe and reduces tumor growth.

Conclusions: The identification of Chk1-S as a novel splice variant and key regulator of Chk1 provides new insights into cell cycle regulation, DNA damage response, and cancer therapy.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2407
Na⁺-Sensitive Chronic Kidney Disease in Mice Lacking Renal Principal Cell Histone H3 K79 Methyltransferase Dot1
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Background: Little is known regarding the epigenetic programs regulating the pathogenesis of chronic kidney disease (CKD), possibly due to a lack of rodent models in which epigenetic modifiers are specifically disrupted. We have previously reported that histone H3 K79 methyltransferase Dot1 is highly expressed in mouse kidney and regulates ENaC-mediated Na⁺ transport in IMCD3 cells.

Methods: To identify new functions of Dot1 in kidney, we used LoxP-Cre system to inactivate Dot1 in AQP2-expressing cells (Dot1/Flq AQP2Cre or mutants) and applied histological, immunohistochemical, TUNEL and metabolic analyses to characterize the phenotype.

Results: We found that on a normal Na⁺ diet, the mutants decreased principal cells (PC) by 20% and increased urine volume by 40% (2011 ASN abstract 21278). Here we report (1) the phenotype of Dot1/Flq AQP2Cre mice (2) other chronic Na⁺ loss states (3.1% dietary Na+ for 32 days). The control mice were capable of handling the chronic Na⁺ loading to maintain the body weight (BW), renal physiology, blood pressure and apparent normal cortex and medullar structure. The mutants progressively became very sick, and eventually lost BW by 12.4% vs. the initial BW, and had over 4-fold higher urine volume, 56% lower urinary osmolality, and 20 mmHg lower systolic BP vs. control. Multifocal cortical-like lesions, inflammatory infiltration, protein casts, dilated distal tubes with detached cells, fibrosis, apoptosis and epithelial-to-mesenchymal transition (EMT) as evidenced by loss of epithelial marker E-cadherin expression and gain of myofibroblast marker α- smooth muscle actin expression was also readily detected in the mutants, but hardly found in controls. These changes were accompanied by ~40-58% decrease in PC, 23-30% increase in IC and 13-28% increase in cells lacking expression of both PC and IC markers vs. controls.

Conclusions: In summary, Dot1/Flq AQP2Cre mice may represent a new mouse CKD model, linking dietary Na⁺ intake and H3 K79 methylation to cell differentiation and pathogenesis of the disease.

Funding: NIDDK Support, Private Foundation Support

SA-PO2408
Aldosterone Promotes Proliferation of Cultured Renal Fibroblasts Via Activation of Mineralocorticoid/PDGF/EGF Receptors and MAPK/PI3K Signaling
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Background: The development of tubulo-interstitial fibrosis (TIF) is critical for the progression of renal injury to end-stage renal disease. The severity of TIF is dependent on the accumulation of interstitial fibroblasts, which results from the increased fibroblast recruitment and proliferation. Aldosterone, a mineralocorticoid hormone, has pro-fibrotic properties and can induce renal or cardiac fibrosis. However it is not known whether aldosterone can promote fibroblast proliferation. Therefore, we examined the effects of aldosterone on the proliferation of cultured kidney fibroblasts and identified the intracellular signaling mechanisms involved.

Methods: Uptake of [3H]-Thymidine was used to determine the dose-dependent effects of aldosterone on the proliferation of rat renal fibroblasts (NRK-49F cells) cultured in serum-free media. Specific kinase inhibitors were used to identify the important signaling factor receptor and mitogenic signaling pathways in aldosterone-induced proliferation.

Results: Aldosterone at physiological concentrations (1-10nM) increased NRK49F proliferation by 2-2.4 fold after 24 hours (p<0.0001). This effect was inhibited by pretreatment with the mineralocorticoid receptor (MR) antagonist eplerenone. Further characterization identified that the proliferative effects of aldosterone could also be blocked by inhibition of PDGF (STI-571), EGF (AG1478), ERK (U0126), JNK (SP600125), and PI3K (LY294002) at doses that were not cytotoxic. In comparison, treatment with inhibitors of TGF-β/ALK5 (SB431542) and p38MAPK (SB203580) had no effect on the aldosterone-induced proliferation of NRK49F cells.

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Conclusions: Aldosterone induces renal fibroblast proliferation via ligation of MR and subsequent activation of growth factor receptors (PDGFR/EGFR) and specific intraacellular signaling pathways (ERK, JNK, PI3-K). These findings suggest that increased levels of aldosterone in diseased kidneys may promote renal fibrosis by inducing proliferation of kidney fibroblasts.

Funding: Government Support - Non-U.S.

SA-PO2409
Calcium Entry Via TRPC6 Mediates Albumin Overload-Induced Endoplasmic Reticulum Stress and Apoptosis in Podocytes
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Background: Albumin, the most abundant component of urine proteins, exerts injurious effects on podocytes. In the current study, we tested the hypothesis that albumin overload may induce functional and structural changes in podocytes via TRPC6-mediated Ca2+ entry.

Methods: Cultured podocytes were divided into several groups: control, vehicle, ECTA (extracellular Ca2+ chelator), Tapsigargin (intraacellular Ca2+ store inhibitor), scrambled siRNA; TRPC6 siRNA; SKF-96365 (TRPC channel blocker). Fluo-3 AM was used as a calcium indicator and the calcium-dependent fluorescence was monitored using confocal microscopy upon albumin stimuli. Western blot was used to detect TRPC6, endoplasmic reticulum (ER) stress proteins GRP 78 and caspase-12. Rhodamine-labeled phalloidin was used to stain the F-actin. Annexin V-FITC and PI double staining was used to detect the apoptosis rate.

Results: High concentration of albumin (BSA 20 mg/ml) triggered intracellular calcium (Ca2+) increase through mechanisms involving the intracellular calcium store (ICP). In order to narrow to examine protein-protein interactions. Genetic manipulations were accomplished by RNAi, dominant negative Akt caused disruption that is induced by albumin overload. Moreover, albumin overload induced expression of GRP78, led to caspase-12 activation and ultimately podocyte apoptosis, all of which were abolished by the knockdown of TRPC6 using siRNA.

Conclusions: The amelioration of podocyte injury in albumin-overloaded podocytes by TRPC6 blockade further strengthened the critical role of this calcium channel. TRPC6 may be an therapeutic target in treating proteinuric kidney diseases.

Funding: Government Support - Non-U.S.

SA-PO2410
Protein Kinase B: A Multifaceted Protein in Albumin Endocytosis
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Background: Orchestrated efforts of clathrin and clathrin associated proteins (CLASPs) regulate albumin endocytosis in the proximal tubule. In glomerulonephritis high concentrations of albumin passing through the altered glomerular filtration barrier results in proximal tubule cell apoptosis. The purpose of this study is to investigate a possible link between albumin endocytosis and survival pathways.

Methods: Co-immunoprecipitation and GST pull-down experiments were utilized to examine protein-protein interactions. Genetic manipulations were accomplished by transfection of human kidney proximal tubule cells with plasmid DNA and small interfering RNA. Albumin endocytosis was evaluated by FITC labeled albumin uptake.

Results: Co-immunoprecipitation showed an interaction between protein kinase B (Akt) and clathrin heavy chain, Ar2 and a adaptor protein disabled-2 (Dab2). GST pull down experiments utilizing Dab2 PTB domain (1-206, 1-368, 335-610 (M15) and proline rich domain (PRD, 600-730) identified PRD as the Akt interacting domain. In order to narrow down the interaction site, shorter constructs of PRD were created. Disrupted 600-638 was found to be the binding domain of Akt. Overexpression of Akt by constitutively active and wild type plasmids increased albumin uptake where as dominant negative Akt caused a decrease in albumin internalization. Silencing Dab2 abolished Akt induced albumin uptake demonstrating physiological relevance of Akt-Dab2 interaction. Albumin overload resulted in attenuation of this interaction as a result of downregulation of both Dab2 and Akt expression in association with apoptosis.

Conclusions: We concluded that Akt is involved in albumin endocytosis via its interactions with CLASPs. We postulate that inhibition of Akt and Dab2 and attenuation Akt-Dab2 interaction with albumin overload may represent a defense mechanism of proximal tubule to limit albumin endocytosis. This mechanism may induce apoptosis by release of mitochondrial proapoptotic proteins that stay in phosphorylated form under the control of Akt in physiological conditions. Further delineation of interactions of Akt with upstream proteins and receptors will enhance our understanding of protein-protein interactions that regulate albumin endocytosis.

Funding: NIDDK Support

SA-PO2411
Growth Arrest Specific Protein 1 (GAS1) Is a Novel Secreted, Endogenous, and PDGF-Regulated Inhibitor of Mesangial Cell Proliferation
Chudin R.C. van Roeven, 1 Stephanie Zok, 1 Peter Boor, 2 Jessica Pruissmeyer, 2 Tanamoto Ostendorf, 1 Andreas Ludwig, 2 Jurgen Floege. 1 Nephrology, RWTH Aachen, Aachen, Germany; 2 Pharmacology &Toxicology, RWTH Aachen, Aachen, Germany.

Background: GAS1 is a glycosyl-phosphatidyl-inositol (GPI)-anchored protein which is highly expressed in embryonal mouse fibroblasts (NIH3T3) and inhibits their proliferation.

Methods: In a cDNA-array analysis we identified GAS1 as one of the most potentially suppressed genes following PDGF-BB or -DD stimulation of mesangial cells (MC).

Results: In vitro MC released soluble GAS1-protein into the cell culture supernatant. Growth-arrest led to GAS1 overexpression and an increased release. The secretion process was resistant to TRPC6 siRNA; TRPC6 siRNA; SKF-96365 (TRPC channel blocker). Fluo 3-AM was used as a calcium indicator and the calcium-dependent fluorescence was monitored using confocal microscopy upon albumin stimuli. Western blot was used to detect TRPC6, endoplasmic reticulum (ER) stress proteins GRP 78 and caspase-12. Rhodamine-labeled phalloidin was used to stain the F-actin. Annexin V-FITC and PI double staining was used to detect the apoptosis rate.

Results: High concentration of albumin (BSA 20 mg/ml) triggered intracellular calcium (Ca2+) increase through mechanisms involving the intracellular calcium store (ICP). In order to narrow to examine protein-protein interactions. Genetic manipulations were accomplished by RNAi, dominant negative Akt caused disruption that is induced by albumin overload. Moreover, albumin overload induced expression of GRP78, led to caspase-12 activation and ultimately podocyte apoptosis, all of which were abolished by the knockdown of TRPC6 using siRNA.

Conclusions: The amelioration of podocyte injury in albumin-overloaded podocytes by TRPC6 blockade further strengthened the critical role of this calcium channel. TRPC6 may be an therapeutic target in treating proteinuric kidney diseases.

Funding: Government Support - Non-U.S.

SA-PO2412
Identification & Characterization of a Novel Oncogenic HIF-1 Target Involved in Renal Cell Carcinoma
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Background: Hypoxia inducible factor (HIF) is upregulated under hypoxia and regulates coordinated transcriptional response, playing a key role in many pathological conditions such as tumorigenesis.

Methods: We performed genome-wide analysis of HIF targets utilizing microarray analysis in combination with ChIP-Seqencing. We focused one of the new targets, sperm associated antigen 4 (SPAG4), which is expressed in a limited number of normal tissues. In order to elucidate a role of SPAG4 in renal cell carcinoma (RCC), we analyzed SPAG4 expression by immunohistochemistry on tissue microarray (TMA) containing 208 RCC specimens.

Results: We also performed cell biological studies of cells with overexpression or knockdown of SPAG4, utilizing immunofluorescence, immunoprecipitation, and live cell imaging using SPAG4 overexpressing or knockdown cells. We focused on the interaction with HIF-1alpha. We also focused on the potential of SPAG4 in tumor development and metastasis.

Conclusions: We identified GAS1 as a novel endogenous growth inhibitor of MC. GAS1 presents a potentially novel therapeutic target in mesangio proliferative glomerular diseases.

Funding: Government Support - Non-U.S.

SA-PO2413
Growth Arrest Specific Protein 1 (GAS1) Exerts Anti-Metastatic Effects in Renal Cell Carcinoma
Kumi Shoji, 1 Imai Mimura, 2 Takamoto Ohse, 1 Takashii Murayama, 1 Reiko Inagi, 1 Takehiko Wada, 1 Tetsuhiro Tanaka, 1 Haruki Kume, 2 Akiteru Goto, 1 Toshiro Fujita, 1 Hiroyuki Aburatani, 1 Tatsuhiko Kodama, 1 Masaomi Nangaku, 1 Nephrol Endocrinol, Univ Tokyo Sch Med, Tokyo, Japan; 1 Lab Syst Biol Med, RCAST, Univ of Tokyo, Tokyo, Japan; 2 Pharmacology, Juntendo Univ Sch Med, Tokyo, Japan; 2 Urol, Univ Tokyo Sch Med, Tokyo, Japan; 1 Pathol, Univ of Tokyo, Tokyo, Japan.

Background: Hypoxia inducible factor (HIF) is upregulated under hypoxia and regulates coordinated transcriptional response, playing a key role in many pathological conditions such as tumorigenesis.

Methods: We performed genome-wide analysis of HIF targets utilizing microarray analysis in combination with ChIP-Seqencing. We focused one of the new targets, sperm associated antigen 4 (SPAG4), which is expressed in a limited number of normal tissues. In order to elucidate a role of SPAG4 in renal cell carcinoma (RCC), we analyzed SPAG4 expression by immunohistochemistry on tissue microarray (TMA) containing 208 RCC specimens.

Results: We also performed cell biological studies of cells with overexpression or knockdown of SPAG4, utilizing immunofluorescence, immunoprecipitation, and live cell imaging using SPAG4 overexpressing or knockdown cells. We focused on the interaction with HIF-1alpha. We also focused on the potential of SPAG4 in tumor development and metastasis.

Conclusions: We identified GAS1 as a novel HIF target, SPAG4, by the genome-wide system biological method. Our functional analysis showed a potential role of SPAG4 in cytokinesis and RCC.

Funding: Government Support - Non-U.S.
Perspectives of an Interaction between Caveolae and Notch Signalling on the Pathogenesis of Renal Fibrosis

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Background: As a common pathological alteration in progressive chronic kidney disease, renal fibrosis is associated with tubular atrophy and deposition of extracellular matrix. Our study demonstrates that caveolin is a central mediator in fibrosis. The mechanistic roles of caveolae and Notch signalling have further been implicated in its pathogenesis, and caveolin-1 (Cav-1) may affect Notch signalling and as well as gamma-secretase activity. The molecular detail of this hypothetical cascade and its impact on renal fibrosis is not yet clear.

Methods: To elucidate this, we have used Cav-1-deficient mice in vivo and in vitro, and human fibroblasts from patients with congenital generalized lipodystrophy (CGL4), lacking caveolin due to mutations of polymerase I and transcript release factor (PTRF), an essential protein for caveolin biogenesis (Rajab A, PLoS Genet 6:1(2010).

Results: Ultrastructural analysis demonstrated the near-complete absence of caveolae in both models. In Cav-1−/− mice, the degree of fibrosis upon induction of tubulointerstitial scarring was substantially enhanced as compared to wildtype mice (Park RC, JAP 298/ F337/2010). Human fibroblasts displayed enhanced Notch signalling in the absence of PTRF as revealed by expressionual analysis and immunohistochemistry of its components, Jagg-1, Notch-1, Notch intracellular domain, and HES1. Increased in Notch signalling was also observed, than in PTRF-deficient fibroblasts upon stimulation with TGFβ. Changes in Notch were paralleled by alpha-smooth muscle actin and collagen I expression.

Conclusions: These data indicate that caveolin and the components of Notch signalling are involved in the pathogenesis of renal fibrosis. We further conclude that the profibrotic effects of caveolar malformation is likely to be mediated by Notch activation.

SA-PO2414

VEGF Receptor 2 Direct Interaction with Nephrin Links VEGF-A, Signals to Actin in Kidney Podocytes

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Background: The transmembrane protein nephrin is an essential component of slit-diaphragms, the specialized cell junctions that link podocyte foot processes. Podocytes are epithelial cells that surround the glomerular capillaries in the kidney and are necessary for the normal filtering function. Nephrin signal complex transduces extracellular cues to the podocyte cytoskeleton and regulates podocyte shape and function. Vascular endothelial growth factor A (VEGF-A) is a required growth factor produced and secreted by podocytes. Accumulating evidence suggests a crosstalk between VEGF-A and nephrin signaling pathways. Our previous studies have shown that nephrin associates with VEGF receptor-2 (VEGFR2) tyrosine kinase, the signaling receptor for VEGF-A in vivo.

Methods: In the present study we characterized the interaction between nephrin and VEGFR2 in vitro and in cultured cells (podocytes and COS cells transfected with the corresponding expression plasmids), using mass spectrometry, co-immunoprecipitation, GST-pull down assays and blot overlay experiments.

Results: We demonstrate that nephrin-VEGFR2 interaction is direct. This interaction occurs through VEGF2 and nephrin cytoplasmic domains. Nephrin-VEGFR2 interaction is modulated by tyrosine phosphorylation of both cytoplasmic domains. Furthermore, our results indicate that the nephrin-VEGFR2 complex involves Nck and actin. We provide evidence that this multi-protein interaction occurs in cultured podocytes.

Conclusions: We propose that nephrin-VEGFR2 complex acts as a key mediator to transduce local VEGF-A signals to the podocyte actin cytoskeleton, thereby regulating the normal foot process structure and glomerular filter integrity.

Funding: NIDDK Support

SA-PO2415

Simvastatin Inhibits Angiopoietin-2 Release and Production – A Novel Pleiotropic Effect?

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Background: We recently found that circulating levels of Angiopoietin-2 (Angpt-2) increase with the extent of chronic kidney disease (CKD) and correlate with prevalent atherosclerotic burden. Angpt-2 is a growth factor, stored in endothelial cells (EC), that senses injury by a diverse inflammatory stimuli thereby potentially contributing to the accelerated atherogenesis in CKD. Anti-inflammatory effects of statins are well-known, yet the mechanisms of these pleiotropic properties remain incompletely understood. We hypothesized that statins may directly inhibit Angpt-2 action and test in vivo and in vitro.

Methods: Angpt-2 was measured in EC media, lysates, as well as sera from 128 CKD patients by commercial ELISA (R&D Systems). Different EC lines were treated with simvastatin (10-100 µM) or vehicle. Immunochemistry (IF) was performed using antibodies against ‘Angpt-2’ and ‘von Willebrand Factor’ (vWF). Total RNA was amplified with a commercial TaqMan PCR assay (Applied Biosystems) and the expression was measured using real-time PCR.

Results: CKD stage 4-5 patients on statins (n=48) had significantly lower circulating levels of Angpt-2 compared to CKD patients without statin treatment (n=80) (statin: 1.87 ± 0.68 vs. no statin: 2.43 ± 1.18 ng/mL; p=0.0002).

Angpt-2 was up-regulated by a high amount in Angpt-2–accumulated and down-modulated in the media of all three EC types. Simvastatin dose-dependently reduced not only the release of Angpt-2 (64% reduction at a dose of 100 µM after 24 h, p<0.05), but also the intracellular Angpt-2–protein concentration (66% reduction, p<0.05) and transcript abundance (10-fold decrease, p<0.05). Furthermore, IF experiments demonstrated (RC amplification of endothelial Weibel-Palade bodies (e.g. vWF) were virtually unaffected by simvastatin.

Conclusions: Kidney patients on maintenance statin therapy had lower circulating Angpt-2 levels than their CKD controls. Simvastatin markedly reduced Angpt-2 release and production in vitro. The mechanism for this effect and its downstream consequences merit further investigation.

Funding: Other NIH Support - R01HL093234, R01HL093234-01S1

SA-PO2416

In Vivo Erythropoietic Activity of Peginesatide Correlates with Erythropoietin Receptor Residence Time and Plasma Half Life

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Background: Peginesatide is a PEGylated, investigational, peptide-based erythropoiesis stimulating agent that was designed and engineered to stimulate the erythropoietin receptor (EPOR). Peginesatide has a unique structure that consists of a synthetic peptide dimer (with no sequence similarity to erythropoietin) conjugated to a 40 kDa PEG moiety.

Methods: To determine the effects of PEGylation on the biological activity of peginesatide, a series of compounds were synthesized where the peginesatide peptide dimer was conjugated to PEG molecules ranging in molecular weight from 2 kDa up to 60 kDa. Each of these compounds was evaluated for kinetics of binding to the EPOR, the ability to stimulate the proliferation and differentiation of CD34+ cell lines into erythroid precursors, and the ability to stimulate erythropoiesis in vivo.

Results: As PEG size increased, progressively slower association and dissociation rates were observed resulting in an overall lower affinity for EPOR (KD = 24 to 2400 pM, from 2 kDa to 60 kDa, respectively) and reduced potency for stimulating differentiation of CD34+ cells into erythroid precursors (EC50 = 22 to 781 pM, respectively). Interestingly, the progressively slower dissociation rates resulted in an increase in EPOR occupancy time (t50 = 260 to 1300 min, respectively), which contributed to an extended activation of EPOR signaling. This extended EPOR activation, in combination with a prolonged plasma half-life due to increased PEG size, led to the opposite trend in vivo with peptides conjugated to larger PEGs exhibiting increased erythropoietic potency. Peptides conjugated to PEG molecules larger than 20 kDa induced a robust production of red blood cells whereas those linked to PEGs smaller than 10 kDa were not efficient in driving erythropoiesis.

Conclusions: These data indicate that the extended erythropoietic activity of peginesatide in vivo results from both prolonged peptide-receptor residence time and plasma half life which may contribute to maintenance of hemoglobin levels with monthly dosing.

Funding: Pharmaceutical Company Support

SA-PO2417

Peginesatide Stimulation Allows for a Longer Lifespan of the Erythropoietin Receptor

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Background: Peginesatide (HematideTM) is a PEGylated, investigational, peptide-based erythropoiesis stimulating agent (ESA) that is designed to specifically stimulate the erythropoietin receptor (EPOR).

Methods: To investigate the regulation of EPOR trafficking after peginesatide vs. recombinant human erythropoietin (rHuEPO, epoetin alfa) dosing, we have developed a novel panel of specific rabbit monoclonal anti-EPOR antibodies, and have used these tools together with Western blotting and flow cytometry to study EPOR cell surface expression, internalization, and degradation.

Results: In exponentially growing erythropoietin-dependent UT-7/EPO cells maintained in HUePO, cell surface EPORs were detected at low levels, and EPOR turnover/degradation appeared to be predominant. In contrast, cell surface EPOR levels were increased significantly (~ 5.0-fold) in cells maintained in medium supplemented with equivalent levels of peginesatide, suggesting that EPOR turnover was lessened. After culture of cells for 18 hours in the absence of EPOR stimulation, subsequent treatment with HUePO led to the rapid tyrosine-phosphorylation of the EPOR, subsequent receptor internalization, and the rapid appearance of the major 42 kDa and 30 kDa EPOR degradation fragments observable within 15 minutes of treatment. In contrast, EPOR phosphorylation occurred at reduced magnitude following peginesatide treatment, but was sustained and correlated with an observed 30 minute delay in EPOR fragmentation. Since the ubiquitin/proteasome system plays a major role in the degradation and down-modulation of reporter proteins, we investigated EPOR ubiquitination after peginesatide vs. HUePO treatment. Each ESA induced EPOR ubiquitination, but peginesatide did so at significantly lower levels (~50% less).

Conclusions: These studies provide new mechanistic insights into the molecular basis for the extended erythropoietic activity of peginesatide.

Funding: Pharmaceutical Company Support
SA-PO2418
Blockade of p38 MAPK Pathway Ameliorates Aldosterone-Induced Renal Injury in Guanylyl Cyclase-A Deficient Mice
Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan.

Background: Recent clinical and experimental studies have shown that aldosterone plays an important role in the pathogenesis of renal injury. We have already reported that natriuretic peptide/guanylyl cyclase-A (GC-A) signaling exerts renoprotective effects in 5/6 nephrectomized, anti-GBM glomerulonephritis and streptozotocin-induced diabetic mice. Furthermore, we demonstrated that uninephrectomized GC-A knockout (KO) mice with four-week smoking and aldosterone and sodium overload exhibited accelerated hypertension with marked nephrotic-range proteinuria (~300-fold urinary albumin excretion from baseline) compared to wild-type mice. Administration of aldosterone also increased phosphorylation of ERK and p38 MAP kinase (MAPK) mainly in podocytes of GC-A KO mice.

Methods: To determine the interaction between p38 MAPK and GC-A, we examined the effect of p38 MAPK inhibitor on renal findings. Two weeks after uninephrectomy, mice were administered with aldosterone (0.2 µg/kg/min) subcutaneously using an osmotic minipump, with 6% sodium diet and with hyaluronic or p38 MAPK inhibitor (FR 176653; 33mg/kg/day) by drinking water. We examined systolic blood pressure, urinary albumin to creatinine ratio, and histological findings.

Results: Although a hyaluridine treatment significantly reduced systolic blood pressure, urinary albumin to creatinine ratio, and change compared with aldosterone-induced GC-A KO mice. Interestingly, urinary albumin excretion was dramatically decreased by 90% in aldosterone-infused GC-A KO mice with FR treatment. Glomerular hypertrophy and mesangial expansion was decreased in FR-treated aldosterone given GC-A KO mice.

Conclusions: These results suggest that p38 MAPK acts as an important molecule connecting aldosterone and GC-A pathways, and could be a potential target against aldosterone-induced glomerular injury.

Funding: Government Support - Non-U.S.

SA-PO2419
Mutation of Endocytic Motifs in the Cytoplasmic Loop of AT1a Receptor Impairs Angiotensin II Uptake and Activation of MAP Kinases ERK1/2 and Sodium and Hydrogen Exchanger-3 in Proximal Tubule Cells
Xiao C. Li, Ulrich Hopfer, Jia L. Zhuo.
1Department of Pharmacology and Toxicology, University of Mississippi Medical Center, Jackson, MS; 2Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH.

Background: The present study tested the hypothesis that the motif Leu319→ Tyr321 in the cytoplasmic loop of the AT1a receptor is required for AT1a-mediated Ang II uptake and activation of ERK1/2 (p-ERK1/2) and the sodium and hydrogen exchanger-3 (p-NHE-3) in mouse proximal tubule cells.

Methods: GFP-tagged wild-type (AT1a R/GFP) or mutant AT1a receptors with deletion of the motif Leu319→ Tyr321 in the cytoplasmic loop (AT1a R/GFP[L316-Y319]) were expressed in AT1a knockout (AT1a KO) mouse proximal tubule cells. The transfected cells were stimulated with Ang II (10 nM) in the presence or absence of the AT1a antagonist losartan (10 µM). Western blot analysis were performed in cells transfected with empty vector, AT1a R/GFP, AT1a R/GFP[L316-Y319], or AT1a R/GFP[L316-Y319] + iv-IV-V, but significantly elevated in the earlier stages, CKD stage II-III. This observation confirms the situation in the Ang II-GFP mouse model. Genetic CKD susceptibility loci in mice, rats and humans contain the pro-TGF-α gene.

Conclusions: These data suggest that AT1a is shed from proximal tubular cells into the urinary space. Shedding is stimulated by high glucose or Ang II, via an ADAM-17-mediated pathway. Loss of membrane-bound AT1a might alter the peritubular levels of Ang II and Ang-(1-7) and thereby affect kidney disease progression.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2241
Regulation of AngII-Induced Pro-TGFalpha Cleavage in CKD Progression
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Background: Low-level Angiotensin II (AngII)-stimulation in mice induces renal fibrosis in a human kidney disease model. The purpose of this study was to examine the effects of the rexinoid bexarotene (Bex) on VSMC responses to AII. We previously showed that bexarotene treatment suppressed AngII; this depends on AngII-mediated cleavage of pro-TGF-alpha in tubular lesions similar to human CKD in normotensive ACE/ARB treated patients with partially suppressed AngII; this depends on AngII-mediated cleavage of pro-TGF-alpha in tubular lesions. Lesions were strongly reduced with metalloproteinase inhibition, in pro-TGFalpha knockout mice, or mice expressing a defective EGF receptor in tubular cells. We have identified regulators of pro-TGFalpha cleavage: PKCalpha and the PKC-regulated protein phosphatase 1 (PPI) inhibitor PPI14R.

Results: We provide evidence that the AngII-TGFalpha signaling pathway is relevant in humans and that AngII-induced TGFalpha cleavage in kidney cells depends on the action of PKCalpha and PPI14R.

Methods: Kidney microarray data Western blot and FACS

Conclusion: Pro EGFR expression in diabetic nephropathy is highest in CKD stage I-II and decreases strongly with disease progression. Pro-TGF alpha levels are very low in CKD I and II, but significantly elevated in the earlier stages, CKD stage II-III. This observation confirms the situation in the Ang II-GFP mouse model. Genetic CKD susceptibility loci in mice, rats and humans contain the pro-TGF-α gene.

In kidneyds of hypertensive mice, PKCalpha and PPI14R are significantly upregulated as compared to normotensive mice.

AngII-induced TGF alpha cleavage in human embryonic kidney cells can be blocked with a PKC inhibitor (BIMI) specific of PKCalpha or PPI14R.

Only PIII treated PPI14R samples co-precipitate other proteins not present in the corresponding control (c). PPP1R14D samples specifically precipitate only with AngII- or TPA-induced PPI14R-signaling complexes (western blot).

Conclusions: a) upregulated in the kidneys of hypertensive mice.

b) required for AngII-induced TGFalpha cleavage in kidneyds.

In kidneyds of hypertensive mice, PKCalpha and PPI14R are significantly upregulated as compared to normotensive mice.

AngII-induced TGFalpha cleavage downstream of the receptor could provide new drug targets for human CKD fibrosis.

Funding: NIDDK Support

SA-PO2242
Activation of the Retinoid X Receptor Modulates Angiotensin II Induced Inflammation and Hypertrophy in Vascular Smooth Muscle Cells
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Background: PPARδ agonists have been shown to interfere with the biological effects of angiotensin II (AII) and decrease blood pressure in animal models. PPARδ forms a heterodimer with the retinoid X receptor (RXR) to activate target genes. Retinoids represent a class of RXR agonists that have been shown to activate a subset of RXR heterodimers, including PPAR, PPARα, PPARδ, LXR, and FXR. In vascular smooth muscle cells (VSMCs), all increases expression of pro-inflammatory cytokines and smooth muscle contractile proteins and induces cellular hypertrophy. The hypothesis of this study was to examine the effects of the retinoid be佐 offered on VSMC responses to AII.

Results: AngII plus PPARδ agonists failed to increase PPARδ expression, but significantly reduced TNFα expression. AngII plus PPARδ agonists significantly reduced collagen expression, but failed to change cell size. PPARδ agonists failed to decrease cellular hypertrophy, but had no effect on cellular hypertrophy. The hypothesis of this study was to examine the effects of the retinoid be佐 offered on VSMC responses to AII.
Results: In cultured rat aortic VSMCs, using a PPAR-RE luciferase construct, Bex (1 μM) increased PPARγ activity by 80% while pioglitazone (Pio; 10 μM), a PPARγ agonist, led to a 60% increase. Both increased LXR activity 3 fold while Pio had little effect. All (1 μM) significantly increased IL-6 and MCP-1 mRNA and protein. Both Bex and Pio blocked cytokine induction by All, but Bex was more effective (decrease in IL-6 mRNA: 96% vs. 67% at 24 h, decrease in MCP-1 protein secreted: 77% vs. 54%, decrease in MCP-1 protein: 71% vs. 43%). All also significantly increased calponin (a smooth muscle cell marker) as measured by both immunoblotting and qRT-PCR; Bex, but not Pio, prevented this increase. Bex completely inhibited the pro-hypertrophic effect of AngII as determined by PHD3 transcriptional activation through cell type specific complex mechanisms in renal cells. Adiponectin is a 30kDa hormone secreted by adipose tissue, and its effect on signaling pathways were also examined. Bex inhibited activity of p38, ERK, and JNK at 15 and 30 minutes following All stimulation. Conclusion: Bexarotene, an RXR agonist, inhibits pathological effects of AII in the vascular tissue. Given the stronger responses to bexarotene compared to pioglitazone, the effects of bexarotene likely involve other nuclear receptors. Ruxolintin may be useful agents in treating vascular disease.

**Funding:** Other NIH Support - NHLBI K08

**SA-PO2423**

**Influence of Angiotensin II (ANG II) on Morg1 Expression in Renal Cells**

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**Background:** The mitogen-activated protein kinase organizer 1 (Morg1) serves as a scaffold molecule for a number of proteins involved in the MAPK signalling complex. In addition it is involved in the regulation of the HIF-1α activity via PHD3 stabilization. Morg1 is expressed in multiple tissue types. The purpose of the present study was to analyze the influence of ANG II on Morg1 expression and its correlation with PHD3 activity and HIF-1α promoter activity in MMCs, MTCs and differentiated podocytes.

**Methods:** Morg1 expression was investigated in MMC (mouse mesangial cells), MTC (mouse tubular cells) and in differentiated podocytes by real-time PCR. HIF-1α transcriptional activity was tested using Luciferase reporter-gene assay. PHD3 activity was tested based on a HIF-1α peptide assay.

**Results:** MMCs, MTCs and differentiated podocytes were stimulated with ANG II for different time points. We observed a biphasic effect of ANG II on Morg1 mRNA expression, which was time dependent and differentially regulated by the ANG II receptor (ATR) 1 and 2 subtype. While 9 h ANG II incubation inhibited Morg1 expression in MMCs via AT1 and AT2 subtypes based on ATR2 blockers studies, it enhanced Morg1 mRNA expression after 24 h stimulation through AT1 subtype. In MTCs 9 h stimulation by ANG II enhanced Morg1 transcriptional activity but did not change basal at the presence of BD 31319, an AT2 blocker, while the suppression of ANG II after 24 h ANG II was reversed by addition of losartan, an AT1 inhibitor. Podocytes demonstrated an elevated Morg1 expression after 3 h ANG II treatment, which was partially suppressed by both BD 31319 and losartan. On the other hand, 24 h ANG II treatment inhibited Morg1 expression in differentiated podocytes through AT2 receptor subtype. We also found that ANG II effects on Morg1 mRNA expression in renal cells are independent of MAPK activity. In correlation with Morg1 expression pattern was the measured PHD3 activity. Reporter-gene assays have shown positive negative regulation of HIF-1α activity via Morg1 and PHD3 in MTCs. Conclusion: Our study indicates that ANG II regulates Morg1 expression and HIF-1α transcriptional activity through cell type specific complex mechanisms in renal cells.

**Funding:** Government Support - Non-U.S.

**SA-PO2424**

**Interactions between Adiponectin and Angiotensin II in Human Renal Tubular Cells**

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**Background:** Chronic kidney disease (CKD) is a major risk factor for all-cause mortality, and its incidence is on the rise. The mechanism responsible for the progression of CKD is not completely understood, and epidemiology studies suggest that obesity can contribute to this process. One way that obesity may exacerbate CKD is by dysregulation of the adipokines. Adiponectin is a 30kDa hormone secreted by adipose tissue, and its circulating level decreases with obesity. Previous studies indicated that adiponectin could protect the heart and vasculature from various insults, which may be partially due to its inhibitory effect on the generation of reactive oxygen species. However the effect of adiponectin on kidney has not been fully elucidated. We hypothesized that adiponectin also plays a protective role in the kidney. To test our hypothesis, we studied the interaction of adiponectin and Angiotensin II and Angiopoietin (AngII), which is well known to contribute to kidney injury, in human renal tubular cells.

**Methods:** Primary human renal proximal tubule epithelial cells (RPTECs) of passage 4 -7 were used for all experiments. NADPH oxidase activity was measured by lucigenin-enhanced chemiluminescence assay. NFκB activity was measured with commercial kits. Results: Both the Adiropor1 and Adiropor2 were expressed in RPTECs and were present on cell membrane. AngII treatment didn’t alter the expression level of neither the receptors. Adiponectin inhibited the activation of NADPH oxidase by AngII in RPTECs, which can be reversed by the AMPK agonist AICAR and stable cAMP analogues cPT-cAMP and db-cAMP. The effect of adiponectin was blocked by the AMPK antagonist compound C, adenylyl cyclase inhibitor SQ22536 and PKA inhibitor H89. The AngII-induced increase in NFκB activity was also reduced by adiponectin.

**Conclusions:** Adiponectin attenuated the AngII-suppressed superoxide generation by NADPH oxidase in RPTECs, which was dependent on AMPK and cAMP/PKA pathways. NFκB activation caused by AngII in renal tubular cells was also attenuated by adiponectin. Our results suggest that decreasing adiponectin levels associated with obesity may be one of the mechanisms by which obesity contributes to the progression of CKD.

**Funding:** Government Support - Non-U.S.

**SA-PO2425**

**FXR or TGR5 Agonists Prevent High Glucose Mediated Glucolipotoxicity in Human Podocytes**

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**Background:** Diabetic nephropathy has been associated with abnormal lipid metabolism and lipotoxicity. Accumulation of lipid droplets (LDs) in podocytes is a frequently observed phenotype in the diabetic kidney. Bile acid activated nuclear receptor the farnesoid X receptor (FXR) and the G protein-coupled receptor (TGR5) regulates bile acid, glucose, and lipid metabolism and inflammation. Activation of FXR by synthetic ligands GW4064 or INT-774 attenuates renal fibrosis in animal models of diabetic nephropathy. The aim of this work was to study the mechanism of the adipogenic program and LD accumulation in human podocytes and the effect of FXR and TGR5 agonists.

**Methods:** Podocytes were treated with high glucose to induce de-novo lipogenesis. Lipid content was assessed by Oil Red O as well as Nile Red staining. Expression of mRNA and protein of molecules controlling lipid homeostasis was examined by real-time quantitative PCR, LDs were determined using FACS method and by confocal microscopy.

**Results:** Administration of high glucose increased the number of LDs and the LD-associated protein adipocyte differentiation-related protein (ADRP). Selective FXR agonist (GW4064) and TGR5 agonist (INT-774) significantly inhibited high glucose induced increase of lipogenic genes including sterol regulatory element binding protein-1 (SREBP1), which is a transcription factor regulating the synthesis of fatty acid and triglycerides, and its target gene an acetyl-CoA carboxylase (ACC). We also found that GW4064 attenuates podocyte lipid accumulation.

**Conclusions:** FXR or TGR5 selective agonists may attenuate glucose induced podocyte glucolipotoxicity by inhibiting podocyte lipid droplet accumulation and transdifferentiation into adipocyte-like cells.

**Funding:** Private Foundation Support

**SA-PO2426**

**MCP-1/CCR2 System Is Involved in Epithelial-Mesenchymal Transition of Peritoneal Mesothelial Cells**

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**Background:** MCP-1 and its receptor, CCR2, have been known to be directly involved in extracellular matrix synthesis. In addition, the MCP-1/CCR2 system was present in peritoneal mesothelial cells(PMCs), and MCP-1 production was increased in PMCs under high glucose(HG) conditions. Therefore, we presumed that the MCP-1/CCR2 system may play an important role in the pathogenesis of peritoneal fibrosis(PF) and conducted this study to investigate the functional role of the MCP-1/CCR2 system in epithelial-mesenchymal transition(EMT) of PMCs and PF.

**Methods:** In vitro, human PMCs(HPMCs) were incubated in M199 media containing 5.6 mM glucose(NG), NG+MCP-1, or 100 mM glucose(HG) with or without mutant MCP-1 (mMCP-1) for 72 hours. In vivo, peritoneal catheter was inserted into Sprague-Dawley rats, and saline(control), C or 2.5% PD solution(PD) was infused. In the PD group, rats were treated either with empty lentivirus vector or lentivirus vector containing mMCP-1 intraperitoneally, once a week. After 4 weeks, peritoneum was removed. Western blot analysis was performed to evaluate fibronectin(FN), E-cadherin, and α-smooth muscle actin(α-SMA) protein expression. PF was determined by Masson’s trichrome(3MT) staining.

**Results:** FN and α-SMA protein expression were significantly increased, and E-cadherin protein expression was significantly decreased in HPMCs exposed to NG+MCP-1 and HG compared to NG cells(p<0.05). These changes in FN, α-SMA, and E-cadherin expression in NG+MCP-1 and HG cells were significantly abrogated by mMCP-1(1μg) in rats. In rats infused with PD solution, the expression of FN protein and the ratios of α-SMA/E-cadherin protein expression of peritoneum were significantly higher compared to C rats(p<0.05). In addition, the thickness of MC layer and the intensity of MT staining in the peritoneum of PD rats were significantly increased compared to C rats(p<0.05). These changes in PD rats were significantly ameliorated by the treatment with lentivirus containing mMCP-1.

**Conclusions:** These findings suggest that the MCP-1/CCR2 system is involved in peritoneal EMT and its inhibition may be a potential therapeutic target for PF.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

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Adenosine 1 Receptor-Mediated Effects on Energy Balance and Body Weight
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Background: Adenosine 1 receptors (A1AR) presumably by activation of the tubuloglomerular feedback pathway exert an important role in limiting glomerular hyperfiltration in the Akita mouse model of type 1 diabetes mellitus. In addition, A1AR-/−/ mice develop insulin resistance suggesting that A1AR mediate direct anti-diabetic effects.

Methods: Body weight, food intake, urinary osmolarity and Cl− concentration were measured by standard methods. The body fat fraction was determined by Echo MRI in 13-week-old mice. Plasma adenosine was measured by HPLC. Serum leptin was measured by ELISA (Tatyanan Chanturiya, NIDDK, NIH, Bethesda, MD).

Results: A1AR-/− mice have a 13% greater body weight than age matched wild type (WT) mice. Body fat fraction was 15.4% in A1AR-/−, significantly higher than 10.8±2% in WT. Food intake measured over 10 days (g/mouse/day) averaged 3.85 ± 0.1 in WT (n=10) and 4.12 ± 0.06 in A1AR-/− (n=12; p<0.05). Water intake was also significantly higher in A1AR-/− than WT (12.5%) accompanied by a decrease in urine osmolarity (−29.5%) and Cl− concentration (−30.8%). Despite the higher fat content serum leptin levels (μg/L) were significantly lower in A1AR-/− than WT mice (2.1±0.05 vs. 3.75±0.06; p<0.01).

Conclusions: We conclude that adenosine through tonic activation of A1AR in fat tissue regulates the release of leptin and that this suppresses food intake and prevents the development of insulin resistance. A1AR-mediated effects of adenosine contribute to the maintenance of a normal energy balance and to the prevention of obesity.

Funding: NIDDK Support

SA-PO248
Prostaglandin E2 (PGE2), a Mediator of Proliferation and Cl− Secretion in Polyethylen-1 Deficient Cells
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Background: PGE2 and calcium activated Cl− channels (CaCCs), specifically bronchial-1, are co-localized in proliferation of epithelial cells. Thus, we hypothesized that PGE2 mediated signaling may activate dysregulated CaCCs which contribute to the proliferative and secretory phenotype of ADPKD epithelia.

Methods: Utilizing wildtype IMCD3, control transfected (siLuc) IMCD3, and IMCD3 cells transfected with siRNA against PKD1 (siPKD1), we measured PGE2 mediated proliferation (cyquant[Invitrogen]), PGE2 induced Cl− secretion, and mRNA abundance of CaCCs.

Results: Proliferation was presented as a ratio of the fluorescence at each time point (143±43 for the fluorescence measured at 18 hrs). PKD1 silenced cells proliferated faster than wildtype and siLuc cells (n=6 wells for each group, p<0.05) at 72 and 96 hrs after plating. Next, cyclooxygenases(COX)-1 (SC560), COX-2 (CAY10404) and COX-1/2 (indomethacin) inhibitors were incubated with siPKD1 cells, and proliferation compared to untreated siPKD1 cells treated with COX-2 inhibitor reduced proliferation at all time points compared to untreated cells. On the other hand, incubation with 50 nM PGE2 enhanced siPKD1 cellular proliferation (n=6, p<0.05), but reduced wildtype cellular proliferation(n=6, p>0.05), suggesting that PGE2 has dichotomous effects. Moreover, PGE2 induced Cl− secretion was 5 fold greater in siPKD1 (p<0.05) than in wildtype cells. 45% of the Cl− current in siPKD1(n=6 for each group) cells. COX inhibition reduced proliferation at all time points (18-96 hrs) to the fluorescence measured at 18 hrs. siPKD1 cells proliferated faster than wildtype and siLuc cells (n=6 wells for each group, p<0.05) at 72 and 96 hrs after plating.

Conclusions: We conclude that PGE2 mediated proliferation of renal tubular cells from pressure-induced fibrosis by enhancing PGI2/PPAR pathway and reducing PGE2; generation.

Funding: Government Support - Non-U.S.

SA-PO2430
Effects of Wnt-7a on an Aristolochic Acid Induced Renal Tubular Epithelial-Mesenchymal Transition
Guo-Qing Wang, Hong-Liang Rui, Yan-Yan Wang, Hong Cheng, Yi-Pu Chen. Division of Nephrology, Beijing Anzhon Hospital, Capital Medical University, Beijing, China.

Background: The Wnt family controls both uric acid deposition and crystallization, but it also serves as an important paracrine factor for proliferation. We reported that Wnt protein is expressed in the nephron precursor cells, bing important for their differentiation to nephrons. This study was to observe whether Wnt7a reverses epithelial- to-mesenchymal transition (EMT) and induces mesenchymal-epithelial transition (MET) on aristolochic acid-induced renal tubular epithelial cell.

Methods: Human proximal renal tubular epithelial cell line HK-2 cells were cultured in different conditions: (1) serum free as control, (2) cultured with AA (30 mg/ml) for 24h, (3) treated with Wnt7a (20ng/ml) for 48h after stimulated with AA (30mg/ml), (4) treated with Wnt7a(20ng/ml) plus LiCl (Wnt’s blocker, 1ug/ml) for 48h after stimulated with AA(30mg/ml). Expression of cytokeratin, E-cadherin, a-smooth muscle actin (a-SMA) and their localization was assessed by immunofluorescence and western-blot.

Results: After incubation with AA, Levels of a-SMA (698.1±20.7 vs 665.8±9.8, P<0.05) and vimentin (496.3±19.7 vs 178.2±20.5, P<0.05) expressions increased significantly, but levels of cytokeratin (213.6±16.1 vs 426.7±19.4, P<0.05) and E-cadherin (117.6±13 vs 426.5±21.9, P<0.05) expressions decreased in comparison to control group. Treatment of HK2 cells stimulated by AA with Wnt7t resulted in down-regulation of a-SMA (151.5±14.3 vs 698.1±20.7, P<0.05) and vimentin (214.5±20.2 vs 496.3±19.7, P<0.05) expression, but up-regulation of cytokeratin (516.8±14.2 vs 213.6±16.1, P<0.05) and E-cadherin (310.7±10.5 vs 167.7±11.3, P<0.05) expressions. Compared to HK2 cells treatment with Wnt7a, the expression of a-SMA (411.2±18.5 vs 151.5±14.3, P<0.05) and vimentin (472.7±23.1 vs 214.5±20.2, P<0.05) enhanced while cytokeratin (239.6±13.2 vs 516.8±14.2, P<0.05) and E-cadherin (199.7±17 vs 310.7±10.5, P<0.05) expression decreased markedly on HK2 cells stimulated with Wnt7a and LiCl.

Conclusions: Wnt7a is able to reverse EMT induced by AA, which represents a potential therapeutic approach to aristolochic acid nephropathy prevention.

Funding: Government Support - Non-U.S.

SA-PO2431
Recurrent Expression of microRNA-214 (miR-214) and Insulin-Like Growth Factor-1 Receptor (IGF-1R) Dictates Renal Carcinoma Cell (RCC) Proliferation Via Akt/TORC1 Circuit
G. Luca Gusella,1 Christoph Eisner,1 Christoph Eisner,1 Christoph Eisner,1 Christoph Eisner,1 1Cell Signaling: Hormones, Autocoids, and Growth Factors, Pathway in Renal Tubular Cells, Pathology, UTHSCSA, San Antonio, TX.

Funding: Government Support - Non-U.S.

SA-PO2429
Rosuvastatin Reduces Pressure-Induced Fibrotic Signals through PGI2/PPAR Pathway in Renal Tubular Cells
Tio Hsiao Chen, Cheng-Hsien Chen. Department of Nephrology, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan.

Background: Statins have been reported to alleviate renal fibrosis in the animal model with pressure-induced fibrosis. However, the molecular mechanism of this anti-fibrotic effect is still a mist. Pressure force is an important mechanisms contributing to the induction and progression of tubulo-interstitial fibrogenesis in ureretic obstruction.

Methods: We established an in vitro pressure culture system to study the influence of stasis on renal tubular cell fibrosis. Fibrosis-associated molecules, such as COX-2, connective tissue growth factor (CTGF), fibronectin, were monitored by Western blotting. Total RNA from PG2/PPAR pathway silenced or pressure-treated cells were identified using ELISA kits. We also used PGIS sRNA transfection to block the expression of PG1 in rat renal cell line NRK-52E.

Results: When NRK-52E cells were cultured in the pressure culture system, 60-mmHg pressure induced the expression of connective tissue growth factor (CTGF), transforming growth factor beta (TGF-b), fibronectin and fibroptin-Smad3. These pressure-induced fibrotic signals were reduced by rosuvastatin in a dose-dependent manner. Rosuvastatin also reduced the TGF-b-induced expression of fibronectin and CTGF in NRK-52E cells. The inhibition of ROS was completely induced by pressure-induced expression and, but reduced pressure-induced prostaglandin E2 (PGE2). The transcription of PGI2 synthase sRNA decreased the inhibitory effect of rosuvastatin on pressure-induced CTGF, TGF-b and fibronectin. On the other hand, the specific inhibitor for cyclooxygenase-2, NS398, decreased pressure-induced PGE2 expression, and partially reduced pressure-induced fibrotic signals. Additional PGE2 decreased the anti-fibrotic effect of rosuvastatin. When we monitored the influence of the nuclear receptors for PGI2, on the anti-fibrotic effect of rosuvastatin using siRNA transfection, the blockage of PPARα reduced the inhibitory effect of rosuvastatin on pressure-induced fibrotic signals.

Conclusions: Rosuvastatin prevents renal tubular cells from pressure-induced fibrosis by enhancing PGI2/PPAR pathway and reducing PGE2; generation.

Funding: Government Support - Non-U.S.
without causing apoptosis of the cells. Together our results identify a reciprocity between mir-214 and FGF19 in controlling the VAD status of the cells. Our data provide evidence for a previously unrecognized mechanism of RCC proliferation involving mir-214 and TORC1.

**Funding:** NIDDK Support, Veterans Administration Support, Private Foundation Support

SA-PO2432

**Target Genes of Endogenous Retinoic Acid and Retinoid Acid Receptors in Collecting Duct Cells: A Pan-Genomic View**

**Qihe Xu, Yun Fei Wong, Patricia D. Wilson, Robert J. Unwin, Jill T. Norman, Bruce M. Hendry.**

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**Background:** Gestational vitamin A deficiency (VAD) impairs renal development and post-natal VAD causes polyuria, urolithiasis, renal inflammation and fibrosis. Vitamin A is converted into retinoic acid (RA) to act as RA receptors (RAR). We previously showed that endogenous RA/RA signaling was confined to the ureteric bud (UB)/collecting duct (CD) cell lineage in mouse kidneys, including the mMCD-3 mouse inner medullary cell line. We hypothesized that endogenous RA/RA interactions in CD cells regulate genes that may mediate VAD-associated renal anomalies.

**Methods:** Affymetrix GeneChip Mouse Gene 1.0 ST Arrays and RT-qPCR were used to shortlist and validate RA/RA target genes in mMCD-3 cells treated for 24h with 2 distinct RA/RA inhibitors, 1 µM AG193109 (RA pan-agonist) or 25 µM DEAB (RA synthesis inhibitor), ± 0.01-0.2 µM RA (±3). Target genes of endogenous RA/RA were defined as those: (i) significantly regulated by both inhibitors and reversed by RA (±P<0.05); (ii) changed ≥2-fold by either inhibitor.

**Results:** Microarray analysis showed that inhibitors decreased 22 and upregulated 105 genes. While changes of all induced genes were <2-fold and not further investigated, RTqPCR validated 18 of 19 genes suppressed ≥2-fold by either RA/RA inhibitors. Seven remaining validated genes have unknown functions in the kidney (9930023K05Rik, Cpm, Klkha7a, Sors2c, 2310070B30Rik, Upk3b, Ebfl). Endogenous RA/RA in CD cells regulate a broad profile of genes involved in renal development, repair and homeostasis. Further investigation should provide valuable insights into the complex role of RA/RA in normal and diseased kidneys, including the pathogenesis of VAD-associated anomalies.

**Funding:** Private Foundation Support, Government Support - Non-U.S.

SA-PO2433

**ATP Secretion by Rat Loops of Henle In Vivo**

**Sara Damiano, Robert J. Unwin, David G. Shirley.**

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**Background:** Recent evidence from in vitro studies indicates that the cells of the thick ascending limb of the loop of Henle can secrete nucleotides into the lumen and that this secretion is stimulated by increases in intraluminal pressure (Praetorius and Unwin, J Am Soc Nephrol 22: 2011). Recent evidence from in vitro studies indicates that the cells of the thick ascending limb of the loop of Henle can secrete nucleotides into the lumen and that this secretion is stimulated by increases in intraluminal pressure (Praetorius and Unwin, J Am Soc Nephrol 22: 2011).

**Results:** When loops were perfused at 20 nmol/min, in the absence or presence of intraluminal ARL 67156 (100 µM), the ATP concentrations in the collected fluid were 162 ± 22 mmol/l and 275 ± 41 mmol/l, respectively (n = 5 pairs of pooled collections, P<0.05, paired t). The concentrations from each category of perfusion were pooled (n = 8-13 loops) and the ATP concentration of the pooled sample was measured using the luciferin-luciferase reaction.

**Conclusions:** These findings suggest that relaxin signals through a RXFP1-nNOS/cGMP/GAP pathway to promote renal MMP expression and activity.

**Funding:** Government Support - Non-U.S.

SA-PO2434

**Neprhin Segment-Specific Gene Deletion Using a Cre-Adenovirus**

**Pablo D. Cabral, Marcela Herrera, Pablo A. Ortiz, Jeffrey L. Garvin.**

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**Background:** Most pharmacological tools used to study renal physiology have limited specificity. This has led to the development of methods to manipulate gene expression to study protein function. However, current methods to delete genes of interest are limited. New methods for knockout from birth may result in compensation by other genes and eliminate the ability to study the function of a specific protein in a specific tissue. Development of transgenic mice using a Cre/loxP strategy requires two strains, being expensive and time-consuming. We have previously shown that injection of adenoviral vectors in the renal outer medulla resulted in efficient and specific transduction of thick ascending limbs. The development of the Cre/loxP system selectively allows gene disruption. We hypothesized that in vivo injection of adenoviruses expressing Cre recombinase under the control of the promoter for the NaKCC2 cotransporter (NKCC2) in the renal outer medulla results in selective transduction of medullary thick ascending limbs.

**Methods:** A double fluorescent Cre reporter mouse strain that expresses td tomato, a red fluorescent protein, before Cre-mediated excision and green fluorescent protein (GFP) after excision was used. Adenoviruses containing Cre recombinase driven by the NKCC2 promoter (Ad-NKCC2Cre), which is specific for thick ascending limbs, were injected in the renal outer medulla.

**Results:** Western blots showed GFP expression in outer medullary lysates 7 days after Ad-NKCC2Cre injections. Immunofluorescence of kidney sections transduced with Ad-NKCC2Cre showed expression of GFP in outer medullary thick ascending limbs, identified by positive Tamra-Horsfall labeling. Neither renal cortex nor inner medulla were positive for GFP. Immunofluorescence microscopy showed that 82 ± 5 % and 82 ± 1 % of the outer medullary thick ascending limbs expressed GFP at 7 and 14 days respectively.

**Conclusions:** We concluded that combining Cre/loxP technology and efficient adenovirus-mediated transduction of thick ascending limbs could be a useful tool to study gene function in adult mice preventing compensation.

**Funding:** Other NIH Support - PO1 HL09550-03

SA-PO2435

**Relaxin Signals through the Nitric Oxide Pathway To Promote Matrix Metalloproteinase Activity in the Kidney**

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**Background:** The inevitable consequence of end-stage kidney disease is the excessive accumulation of extracellular matrix, primarily collagen which drives the pathological setting of renal fibrosis. Pre-clinical and signal transduction studies have shown that the anti-fibrotic hormone relaxin can inhibit TGF-β1 activity by binding to Relaxin Family Peptide Receptor 1 (RXFP1) and activating a neuronal nitric oxide (NO) synthesise (nNOS)-NO-cytochrome c monosulfhydrase (cGMP)-dependent pathway to abrogate Smad2 phosphorylation; thereby inhibiting TGF-β1-induced myofibroblast differentiation and collagen synthesis. Thus, this study sought to determine if relaxin’s well-documented additional ability to induce collagen degradation by matrix metalloproteases (MMPs) was via its crucial mechanism.

**Methods:** Primary myofibroblasts (propagated from rat kidneys 3 days after unilateral ureteric obstruction) were treated with relaxin (100ng/ml) in the absence or presence of the general NO inhibitor L-NNAME (73 µM), the nNOS inhibitor NPLA (2 µM), the inducible NO (iNOS) inhibitor 1400W (0.5 µM) or cGMP inhibitor ODQ (5 µM) over a 72 hour culture period. Media samples were collected and assayed for changes in MMP-2 and MMP-9 activity (by gelatin zymography), while cell layer protein was assessed for changes in MMP-13 levels (by Western blotting).

**Results:** Relaxin significantly up-regulated MMP-2, MMP-9 and MMP-13 expression and activity when administered over a 72 hour culture period to renal myofibroblasts by 128%, 115% and 91%, respectively (all p<0.01 vs untreated cells). This relaxin-induced up-regulation of MMPs was significantly blocked by L-NNAME, L-NPLA, 1400W and cGMP inhibitor ODQ (5 µM) over a 72 hour culture period. Media samples were collected and assayed for changes in MMP-2 and MMP-9 activity (by gelatin zymography), while cell layer protein was assessed for changes in MMP-13 levels (by Western blotting).

**Conclusions:** These findings suggest that relaxin signals through a RXFP1-nNOS/iNOS-cGMP pathway to promote renal MMP activity; and that the NO-cGMP pathway is central to relaxin’s anti-fibrotic actions.

**Funding:** Government Support - Non-U.S.

SA-PO2436

**Attenuation of Systolic Hypertension and Angiotensinogen Gene Expression in Diabetic Akita Transgenic Mice Overexpressing Heterogenous Nuclear Ribonucleoprotein F**

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**Background:** Heterogeneous nuclear ribonucleoprotein F (hnRNP F) binds to the insulin-responsive element in the rat angiotensinogen (Agt) gene promoter and inhibits Agt gene transcription in vitro. We now investigate whether overexpression of hnRNP F
can modulate renal Agt gene expression and subsequently attenuate systolic hypertension (sHTN) in Akita mice.

Methods: Akita transgenic (Tg) mice specifically overexpressing hNRP F solely in their renal proximal tubular cells (RPTCs) were created by cross-breeding of Akita mice with hNRP F Tg mice that specifically overexpress hNRP F in their RPTCs by employing a parathyroid hormone-regulated protein promoter. Non-Akita littermates served as controls. Blood glucose, systolic blood pressure (SBP) and albuminuria were monitored weekly from 10 to 20 weeks of age. Kidneys were processed for histology studies. Renal proximal tubular Agt mRNA and protein expression were quantified by real-time PCR and Western blotting. Urinary albumin to creatinine ratio (AUCr) was measured.

Results: Our results demonstrate that Akita mice develop sHTN (∼136 ± 3.8 mm Hg) and display renal hypertrophy and hydrophresis as compared to non-Akita controls (∼108 ± 0.4 mm Hg). However, hNRP F overexpression markedly decreased sHTN, kidney weight to body weight ratio and renal hypertrophy without affecting blood glucose level and the urinary albumin/creatinine ratio in Akita hNRP-F Tg mice as compared to Akita mice. Furthermore, Agt mRNA and protein expression as well as urinary AUCr levels are significantly increased in Akita mice but normalized in Akita hNRP-F Tg mice.

Conclusions: Our data suggest that hNRP F protects a viable role by attenuating sHTN and preventing RPTC injury in diabetes, and indicate that these actions are mediated, at least in part, through attenuating intrarenal Agt gene expression and RAS activation in vivo.

Funding: Government Support - Non-U.S.

SA-PO2437 Stimulatory Effect of Insulin on Renal Proximal Tubule Transport Is Preserved in Insulin-Induced Insulin Resistant Rats

MWPNC. Pediatric Nephrology Consortium Study

Background: GC, rather than its effects on metabolic or endocrine pathways, are thought to be the basis of GC-induced insulin resistance. We demonstrated that hNRP F protects a viable role by attenuating sHTN and preventing RPTC injury in diabetes, and indicate that these actions are mediated, at least in part, through attenuating intrarenal Agt gene expression and RAS activation in vivo.

Funding: Government Support - Non-U.S.

SA-PO2438 TIF2/SRC2 Expression in Blood Leukocytes Is a Determinant of Steroid Response in Pediatric Idiopathic Nephrotic Syndrome: A Midwest Pediatric Nephrology Consortium Study

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Background: Resistance to glucocorticoid (GC) therapy in nephrotic syndrome (NS) is considered a poor prognostic sign, since the 8-10% of steroid-resistant NS (SR) account for ~15% of all end-stage renal diseases in children. Defects in the anti-inflammatory effects of GC, rather than its effects on metabolic or endocrine pathways, are thought to be the basis of GC insensitivity in NS. TIF2 (GRIP1/SRC2) is a member of the p60 nuclear cofactor family with a critical role in the anti-inflammatory effects of GC. We hypothesized that TIF2 (encoded by NCOA2) is essential for the therapeutic action of GC in NS.

Methods: Samples were obtained from steroid-sensitive (SS) and SR pediatric patients at both presentation (PS) and after the first 6-8 week course of GC therapy (TS). Relative mRNA expression was measured in peripheral blood RNA by qRT-PCR, and flow cytometry (gating on CD19+ and CD16+ subpopulations) was used to measure relative protein expression.

Results: The expression of NCOA2 but not the other two p60-family members (NCOA1,3) increased after GC therapy in SS (P<0.001) but not SR patients. NCOA2 expression correlated, as expected, with DUSP1 expression (P<0.0001), since TIF2 is known to be essential for the induction of DUSP1 mRNA. Flow cytometry confirmed that more TIF2 protein was present in the CD19+ leukocyte sub-population (P=0.005) in SS patients compared to SR patients. There was also an increase in the amount of TIF2 (NCOA2) expression in CD4+ T-lymphocytes from SS patients but not from SR patients (P<0.05).

Finally, we found that GC treatment of cultured PS leukocytes from SS but not SR patients resulted in significant increases in TIF2 protein in CD4+ (P<0.005), CD8+ (P<0.001), and CD19+ (P<0.005) but not CD16+ sub-populations.

Conclusions: Together these results suggest that GC induction of the TIF2 nuclear receptor cofactor is essential for proper steroid responsiveness in NS, and that GC-induced TIF2 expression in leukocytes is a biomarker of patient steroid response in NS.

Funding: NIDDK Support

SA-PO2439 Temporal Effects of Calcitriol on Parathyroid Gland Gene Expression in Rats with Early Secondary Hyperparathyroidism

Victoria Shalhoub, 1 Edward Shatzen, 1 Sabrina Ward, 1 Michael J. Boedigheimer, 1 Mara Campbell, 1 Michael A. Damore, 1 Zheng Pan, 1 James R. Davis, 1 Charles M. Henly, 1 William G. Richards. 2 1Amgen, Inc, Thousand Oaks, CA; 2Amgen, Inc, San Francisco, CA.

Background: Secondary hyperparathyroidism (SHPT) is a complication of chronic kidney disease (CKD). Calcitriol (CM), modulators of the calcium-sensing receptor (CaSR), lower serum PTH and calcium (Ca) in CKD patients and rodent models. Although much is known about CaSR signaling pathways, the precise events are not well defined. Here, changes in gene expression were examined after CM treatment, in thymic hyperplasia glands (TPG) of a rat model of CKD with SHPT.

Methods: Two-month-old 5/6 nephrectomized (Nx) SD rats, fed a standard diet (1.17%/Ca/1.0%P), developed renal insufficiency and SHPT over 6 weeks. Rats (13/group) were then dosed p.o. daily with vehicle (veh) or the research CM R-641 (10 mg/kg). Blood and kidneys were harvested at 6 and 24 h post dosing.

Results: Six weeks post 5/Nx rats had higher serum PTH (1153 ± 124 vs 45 ± 350 pg/ml, mean±SEM), BUN (50 ± 3 vs 18 ± 0 mg/dl) and creatinine (0.05 ± 0.02 vs 0.24 ± 0 mg/dl) than sham-veh rats. Gene profiles and the number of dividing cells (Ki67+) in PG, were similar in post CM and sham and 5/Nx rats, reflecting the slow progression of PG cell hypertrophy/hyperplasia in rats fed a standard diet. Calcitriol-treated 5/Nx rats had lower serum PTH levels than veh-treated 5/Nx rats (52 ± 18 vs 115 ± 24 ng/ml). Gene expression changes occurred in pathways that included CaSR, proliferation, apoptosis and angiogenesis. Changes were different between 1 and 24 hrs (P<0.05).

Conclusions: This study extends knowledge of molecular events elicited in TPG within hours to one week after a single CM dose in an early rat CKD model. Confirmation of these findings in PGS may lead to novel therapeutic targets for SHPT.

Funding: Pharmaceutical Company Support

SA-PO2440 Parathyroid Hormone Related Protein Dissociates Increased cAMP from Renin Secretion in Mouse Juxtaglomerular Cells

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Background: cAMP is the stimulatory second messenger for renin secretion. Primary signals that stimulate renin do so by increasing the production of cAMP via the calcium-inhibitable adenyl cyclase- V (AC-V) in the juxtaglomerular (JG) cell. Parathyroid hormone related protein (PTHrP) stimulates renin secretion in the isolated perfused kidney, but it is unknown if this is due to a direct effect on the JG cell. We hypothesized that JG cells would express the receptor for PTHrP (PTHrR), and that PTHrP would increase renin secretion.

Funding: Amgen, Inc, San Diego, CA.

Methods: Experiments used primary cultures of isolated mouse JG cells. Results: In primary cultures of JG cells, 10 nM and 1 µM PTHrP increased cAMP levels by 7.6±0.2 and 10.6±0.2 pmol/mg protein, respectively (P<0.05). However, neither 10 nM nor 1 µM PTHrP increased renin release from a basal level of 0.02±0.001 ng Ang I/ml/hr/mg protein (10 nM PTHrP: 0.34±0.1, 1 µM PTHrP: 0.56±0.1 ng Ang I/ml/hr/mg protein, respectively). Since renin secretion is enhanced by low extracellular Ca, we repeated the experiments in either low or high calcium media with 1 µM PTHrP. PTHrP increased cAMP in the low-calcium medium from 4.78±0.17 to 20.51±1.93 pmol/mg protein (P<0.05) and in the high-calcium medium from 5.14±0.27 to 14.85±8.33 pmol/mg protein (P<0.05). However, neither 10 nM nor 1 µM PTHrP increased renin release in either low- or high-calcium media with the addition of PTHrP (low-calcium basal: 432.0±127.5; low-calcium PTHrP: 497.2±165.4; high-calcium basal: 214.5±81.3; high-calcium PTHrP: 209.4±53.6 ng Ang I/ml/hr/mg protein).

Conclusions: Our data demonstrate that PTHrP increases cAMP formation by acting on PTHrR in JG cell without stimulating renin release in primary cultures of mouse juxtaglomerular cells. The implication of this is that PTHrP dissociates cAMP formation from renin secretion in the JG cell by stimulating an adenyl cyclase besides AC-V, or that adenyl cyclase signaling in the JG cell may be compartmentalized.
SA-P02441

Relationships between Physical Activity and Nutrition with Quality of Life in CKD Patients

Robert G. Fasett, Iain Robertson, Madeline J. Ball, Dominic P. Geraghty, Jeff S. Coombes, Redwan J. Baddour, Andrew J. P. Cameron, and Matthew Mathieson.

Background: The Lipid lowering and Onset of Renal Disease (LORD) trial was a three-year randomised, double-blind, placebo-controlled trial investigating the effects of atorvastatin on kidney function in CKD patients. The study design included measures of physical activity and nutrition every nine months. The aim of this sub-study was to investigate the relationships between physical activity and nutrition with quality of life (QoL).

Methods: 132 patients with serum creatinine levels >120 μmol/L, not taking lipid-lowering therapy and at all levels of proteinuria and serum cholesterol were enrolled. For this sub-study data was available for 120 patients and they were followed for a mean of 2.9 years. Every nine months physical activity, nutrition and QoL were assessed using the Active Australia questionnaire, 4-day diet diaries analysed with Foodworks software and the SF-36 questionnaire respectively. The association (Odds Ratio) between a number of predictors and 10 SF-36 QoL measurement scales were estimated using repeated measures ordinal logistic regression. An OR > 1.00 indicates a positive association, and an OR < 1.00 indicates a negative association.

Results: Weekly physical activity (METmins) was strongly associated with all measures on the SF-36 including the mental component score (MCS), (OR 1.3, 95% CI 1.1-1.6, P=0.01) and the physical component score (PCS), (OR 1.6, 95% CI 1.1-2.2, P=0.008). Dietary phosphate intake was strongly positively associated with improved PCS (OR 1.6, 95% CI 1.1-2.2, P=0.02) and dietary zinc was associated in a milder but consistent manner with poorer physical QoL and a lower MCS (OR 0.7, 95% CI 0.4-1.0, P=0.048). Dietary calcium and iron were both associated with poorer physical quality of life and better mental QoL.

Conclusions: Greater levels of physical activity are strongly associated with improved QoL in CKD patients. Micronutrients such as dietary phosphate, zinc, calcium and iron are also related to QoL.

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-P02442

High Levels of Adiponectin in End Stage Renal Disease May Be Due to Increased Production in Visceral Fat


Background: Plasma adiponectin is elevated in End Stage Renal Disease (ESRD) but the cause of abnormally increased adiponectin is unclear. Possible mechanisms include augmented production secondary to chronic inflammation, decreased clearance from the failing kidneys, or increased adiponectin resistance. The purpose of this study was to determine if there is increased adipose tissue production of adiponectin in ESRD. We compared plasma levels of adipokines and expression of adiponectin and adiponectin receptors in fat and muscle of ESRD patients and controls.

Methods: The study sample included 16 ESRD patients and 9 kidney donors (controls). The study sample included 16 ESRD patients and 9 kidney donors (controls). The effects of adipokines in the ESRD group were compared to controls using Student's t-test and Wilcoxon rank-sum test. The effects of adipokines in the ESRD group were compared to controls using Student's t-test and Wilcoxon rank-sum test. The effects of adipokines in the ESRD group were compared to controls using Student's t-test and Wilcoxon rank-sum test.

Results: The results of the study are presented in the table below. The results of the study are presented in the table below. The results of the study are presented in the table below.

Conclusions: In ESRD there is greater expression of adiponectin protein and adiponectin receptor in visceral adipose tissue compared with controls. These results represent the first evidence of increased adipokine expression in human adipose tissue in ESRD, and could explain at least one of the mechanisms by which this hormone is elevated in kidney disease.

Funding: NIH Support

SA-P02443

Executive Functioning May Vary with GFR in Children with Mild to Moderate CKD

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Background: Neurocognitive dysfunction, with selective defects in executive function (EF), complicates advanced CKD and ESRD. In children, performance improves with renal transplantation. It is unknown whether EF is also impaired by moderate CKD; this would inform educational intervention during rapid neurocognitive development in childhood.

Methods: Tests of problem solving, inhibitory control and working memory were incorporated into the prospective, observational NIH-sponsored CKD cohort study of children with mild to moderate CKD. Cross-sectional analysis of baseline test performance was by linear regression, stratified by estimated GFR: <30, 30 to<40, 40 to<50, 50 ml/min/1.73 m²; blood pressure; renal diagnosis; and duration of CKD.

Results: 340 subjects, age 10-17y (median 13) were tested. 26% had BP >90th %ile for age and height; 18% had glomerular disease; CKD duration was 6-13y (median 10).

Performance on the Delis-Kaplan Tower Test, an EF task of planning and problem solving, was lowest in the group with GFR<30 and was progressively less affected in each higher GFR strata when compared to those >50. Mean values all fell within normal ranges and between-GFR differences did not reach significance in this cross-sectional comparison. By contrast, neither working memory (Wechsler Intelligence Scale Reverse Digit Span) nor inhibitory control (Errors of Commission subscale of the Conners Continuous Performance Test) varied with GFR at baseline. Likewise, neither high BP nor cause or duration of CKD affected performance of any test.

Conclusions: Cross-sectional analysis suggests tests of planning and problem solving may vary inversely with GFR in children with mild-moderate CKD. Large inter-individual variation in these measures of subtle cognitive impairment will require repeated observations and within-subject comparisons over time to discern an effect of progressive CKD.

Funding: NIDDK Support

SA-P02444

Effects of Low-Protein Diet Supplemented with Ketoad to Decreasing the Proteinuria in Primary Glomerular Diseases: An Additive Renoprotection Apart from Glucocorticoid and Immunosuppressant

Hong Li Lin, Wei Sun, Hua Xie, Yan Ling Sun, I. New, Philip A. Kalra.

Background: In patients with primary glomerular diseases, high proteinuria is often associated with progressive renal failure. Several studies have shown that low-protein diet (LPD) may be associated with a decrease in proteinuria. However, it is not clear whether LPD may be additive to glucocorticoid and immunosuppressant to further decrease proteinuria.

Methods: 60 early-stage primary glomerular disease patients (eGFR>60 ml/min/1.73 m², >2g/24h proteinuria) were randomly enrolled to LPD group (diet protein intake 0.6g·kg⁻¹·d⁻¹) and ketoad group (diet protein intake 0.6g·kg⁻¹·d⁻¹ and ketoad 1890mg·d⁻¹). All patients received the same treatments protocols of glucocorticoid and immunosuppressant and were followed up for 12 months. Urinary albuminuria, proteinuria, urinary albumin:creatinine, eGFR, serum prealbumin, albumin, anthropometric parameters, and blood pressure were measured or calculated monthly. The compliance and therapeutic effects were evaluated.

Results: 22 patients in LPD group and 19 in NPD group achieved the 12-month follow-up. Proteinuria decreased significantly in LPD group compared with NPD group from the 3rd to 12th month(p>0.05). Serum creatinine decreased and eGFR increased in LPD group(p>0.05). Serum albumin and prealbumin increased obviously in LPD group, and a significant difference was observed between two groups(p>0.05). After follow-up, there were 10 and 9 patients achieving complete remission and partial remission in LPD group, respectively, while 4 and 7 in NPD group.

Conclusions: LPD supplemented with ketoad has beneficial effects on decreasing proteinuria, protecting renal function and maintaining nutritional status in early-stage primary glomerular disease patients. The additive renoprotective effects exist apart from glucocorticoid and immunosuppressant.

Funding: None

SA-P02445

A Cross-Sectional Study of Serum Potassium Abnormalities Associated with Prescribed Medication in a CKD Population

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Background: Hyper- and hypokalemia are associated with cardiac arrhythmia. They may be caused by drugs such as anti-hypertensives which are prescribed with particular frequency in renal clinics. We sought to understand which drugs were most frequently incorporated into the prospective, observational NIH-sponsored CKD cohort study of children with mild to moderate CKD.

Methods: Cross-sectional analysis suggests tests of planning and problem solving may vary inversely with GFR in children with mild-moderate CKD. Large inter-individual variation in these measures of subtle cognitive impairment will require repeated observations and within-subject comparisons over time to discern an effect of progressive CKD.

Funding: None
Methods: We undertook a cross-sectional analysis of the serum potassium and concentration of excreted potassium in a population of 1208 patients with stage 5 chronic kidney disease and their concomitant medication history of 1208 patients visiting a tertiary renal out-patient clinic over 5 years. We examined the effect of drugs which are known to alter potassium homeostasis.

Results: The mean age was 57.9 years, eGFR 32.4mL/min/1.73m², 63% were male, 37% stage 5 CKD. Of the 10 variables included in the model, potassium was the only independent predictor of potassiumuria. Predicted mean potassium was 5.1 mmol/L (95% CI 4.9-5.3), and when corrected for eGFR, was 5.3 mmol/L (95% CI 5.1-5.5). This latter effect was less significant than that of eGFR, and unrelated to episodes of persistent hyperkalemia.

Background: Malnutrition is frequent in end-stage renal disease patients treated by dialysis. Few data are available on the relationship of serum potassium with end-stage renal disease (CKD) patients at different renal functional stage. The measurement of body electrical impedance (BIA) is a simple, inexpensive, and validated method to analyze body composition and measure body fluids in CKD patients.

The aim of this study was to evaluate the effect of the decreased renal function on body composition of CKD patients.

Conclusions: One thousand two hundred and eighty-nine and sixteen adult patients (617 F, 672 M), aged 15-85 years, mean 51.3; body weight 36.6-160.0 kg, mean 72.4; BMI 15.8-67.5 kg/m², mean 26.4, affected by different kidney disease with different degree of functional impairment (serum creatinine 0.4-14.4, mean 1.77) participated in this study.

Methods: Plasma adiponectin concentration and their relationship with estimated glomerular filtration rate (eGFR) in the Elderly. Polish Population Study “PolSenior” (n = 4979, 2412 females and 2567 males) aged 65-101 years. The study was to estimate plasma adiponectin concentration and their relationship with estimated glomerular filtration rate (eGFR) in representative samples of the elderly population in Poland.

Results: BMI e FMI significantly increased with the reduction of GFR in females, while unchanged in males. FMI was unmodified independently from the gender. BCM significantly decreased only in males, while was unmodified in females. Finally, ECWI significantly increased in all patients. These results indicate significant differences between male and female CKD patients, and in particular a different effect of decreased GFR on body mass and body composition in male and female patients.

Conclusions: Traits of malnutrition are present in chronic kidney disease patients, before starting renal replacement therapy. Significant differences in body composition have been found according to gender. In particular, the decrease in GFR was accompanied by the reduction in body cell mass only in male CKD patients. It is possible that the higher fat mass plays a protective role in females.

Funding: Government Support - Non-U.S.

SA-PO2448

Gender Differences in Body Composition in Chronic Kidney Disease Patients at the Different Functional Stages Carlo Donadio, Internal Medicine, Nephrology, University of Pisa, Italy.

Background: Malnutrition is frequent in end-stage renal disease patients treated by dialysis. Few data are available on the evaluation of nutritional status in chronic kidney disease (CKD) patients at different renal functional stage. The measurement of body electrical impedance (BIA) is a simple, inexpensive, and validated method to analyze body composition and measure body fluids in CKD patients.

The aim of this study was to evaluate the effect of the decreased renal function on body composition of CKD patients.

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Conclusions: Traits of malnutrition are present in chronic kidney disease patients, before starting renal replacement therapy. Significant differences in body composition have been found according to gender. In particular, the decrease in GFR was accompanied by the reduction in body cell mass only in male CKD patients. It is possible that the higher fat mass plays a protective role in females.

Funding: Government Support - Non-U.S.
Insulin Resistance in Children with Primary Nephrotic Syndrome and Normal Renal Function

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Background: Clinical research has demonstrated that children with ESRD on hemodialysis have IR and hyperinsulinemia. The present study was to investigate whether the children with early stage primary nephritic syndrome (PNS) have IR, and IR in this group is associated with renal pathology, therapeutic response to glucocorticoids, and clinical outcome.

Methods: One-hundred and nineteen PNS patients with normal renal function and 125 normal controls were studied. Fasting blood glucose (FBG), fasting serum insulin (FISN) and serum C-peptide (CoP) were measured. The Home index of insulin resistance (HOMA-IR), islet B cell function (HOMA-islet) and insulin sensitive index (ISI) were calculated. The correlations were assessed between HOMA-IR, FCP, blood pressure (BP), lipids, renal function, blood coagulation, clinical disease type, pathology, and the early therapeutic effectiveness of high-dose glucocorticoids.

Results: There was no evidence of IR in the early stage of PNS. Although levels of FBG, FISN and FCP were all within the normal range, FCP was significantly higher than in the control group. Spearman’s correlation analysis revealed a significant correlation between FCP and age, BP, Scr, TG and FISN. FCP was positively correlated with BMI and negatively with GFR. Pearson’s correlation analysis showed that log_{10}(FCP) positively correlated with age, Scr, TG, FBG and FISN. There was a positive correlation between log_{10}(FCP) and BMI and a negative correlation with GFR and FCP. Furthermore, multivariate stepwise regression analysis revealed TG as an independent risk factor for log_{10}(FCP).

Conclusions: Although IR was not detected, a significant increase in BP, uric acid (UA), blood lipids and blood coagulability was observed in the PNS group. A correlation observed between HOMA-IR, age, BP, serum creatinine (SCr) and TG may suggest that insulin sensitivity will emerge as renal disease progresses. FCP levels were increased in the PNS group, suggesting that FCP may be a protective factor.

Funding: Government Support - Non-U.S.

Validation of Predictive Equations for Resting Energy Expenditure in Chronic Kidney Disease

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Background: The effect of the uremia on metabolic rate is poorly understood and knowledge of metabolic rate in CKD is important for provision of accurate dietary advice. Validated predictive equations for energy expenditure are needed in CKD. We studied the effect of physical activity and GFR on resting energy expenditure (REE) in CKD. We evaluated algorithms for REE, including one recently developed at our unit.

Methods: We performed a metabolic analysis of subjects on dialysis and with CKD. All subjects had a metabolic analysis including measurement of body size parameters and REE by indirect calorimetry. Physical activity was measured with Metabolic Equivalent of Task (MET) using the Stanford 7 day recall questionnaire. Physical activity and REE were compared between the dialysis and CKD groups. Predictive equations for REE were evaluated using the Bland-Altman method.

Results: 400 subjects were recruited, 200 on dialysis and 200 with CKD. Weight, serum albumin and body mass index was lower in patients on HD (p<0.001 for each). Physical activity was lower in patients on dialysis compared to those with CKD (mean MET 4.4 ± 1.59 for males, p<0.001). There was no significant difference in REE between subjects with CKD and those on dialysis (p=0.14 for females, p=0.26 for males). In a regression model, CO2R did not predict REE after correcting confounding variables. In a separate model, CO2R significantly predicted estimated Total Energy Expenditure (REE=Mean MET). Equations for REE (Schofield, Mifflin-St Jeor and Harris-Benedict) underestimated REE in the CKD group (bias +138 to +193kCal/day). All equations evaluated for REE by indirect calorimetry. Physical activity was measured with Metabolic Equivalent of Task (MET) using the Stanford 7 day recall questionnaire. Physical activity and REE were compared between the dialysis and CKD groups. Predictive equations for REE were evaluated using the Bland-Altman method.

Conclusions: Patients on dialysis are less active than those with CKD, but REE does not differ. Equations for REE derived in normal individuals tend to underestimate in CKD. A recent equation developed at our unit had lower bias (-36kCal/day). All equations did not differ. Equations for REE derived in normal individuals tend to underestimate in CKD. A recent equation developed at our unit had lower bias (-36kCal/day).

Funding: Government Support - Non-U.S.

SA-PO2450

Metabolomic Profiling of Uremic Solutes in CKD Patients

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Background: Chronic kidney disease (CKD) is strongly associated with cardiovascular events. Early detection of and accurate monitoring of patients with CKD is likely to improve care and decrease the risk of cardiovascular and cerebrovascular disease. As a new diagnostic tool, we examined the retention of uremic solutes as a simpler, more accurate method to assess renal function.

Methods: Comprehensive metabolome analysis of 41 CKD patients by capillary electrophoresis mass spectrometry (CE-MS) of blood samples were performed (Toyohara T. JASN 2009).

Results: By CE-MS, we found 22 cations and 30 anions that accumulated significantly as the estimated GFR decreased. These compounds included 9 cations and 27 anions that were newly identified in our study. In contrast, we also found 7 cations (2 new) and 5 anions (all new) that decrease significantly as eGFR declines. We next evaluated each substance for its suitability to detect early CKD stage. Compounds highly correlated with eGFR and whose plasma concentration changed in a manner approximated by the first-degree equation are excellent candidates for detecting CKD and identifying uremic toxins that might aggravate kidney function in the early stage of CKD compared with second-degree model such as creatinine (1-methyladenosine rs= -0.772, N-acetylglucosamine rs= -0.751, butyrobetaine rs= -0.734, sebacate rs= -0.751, cis-aconitate rs= -0.719, and bupivacaine rs= -0.711).

Conclusions: These results identify a number of uremic compounds. Many of them are novel, and increase in a manner approximated by the first-degree equation, that predict worsening renal function in early stage of CKD. Thus these compounds provide early diagnostic information. In addition CE-MS should be useful tools for exploring the uremic toxins (Toyohara T. Hypertens Res 2010).

Funding: Government Support - Non-U.S.
Overall coping efficacy was not a significant predictor of the adolescent's social or psychosocial functioning, however; the efficacies of two specific coping strategies were: emotion regulation and seeking social support. Finally, neither family functioning nor coping efficacy were significant predictors of depressive symptoms or ER visits.

Conclusions: These findings suggest that adolescents with CKD are able to adjust well to having a chronic illness and that family functioning and the coping efficacy strategies of emotion regulation and seeking social support play an important role in facilitating this positive adjustment.

SA-PO2455

Clinical Presentation of Sleep Apnea in Patients with Chronic Kidney Disease

Methods: One hundred twenty-four patients were recruited from out-patient nephrology clinics. All patients completed a questionnaire examining symptoms of sleep apnea (snoring, witnessed apneas, nocturnal choking), the Epworth Sleepiness Scale (ESS=10=daytime sleepiness), Pittsburgh Sleep Quality Index (PSQI=5=poor sleep quality), and overnight cardio-pulmonary monitoring for determination of sleep apnea (respiratory disturbance index >15). Patients with sleep apnea (n=51) were compared to patients without apnea (n=73).

Results: CKD patients with sleep apnea did not differ from those without sleep apnea in the prevalence of snoring (76% vs. 74%, p=0.8), witnessed apneas (35% vs. 19%, p=0.06), and nocturnal choking (33% vs. 23%, p=0.2). There was a higher prevalence of daytime sleepiness in CKD patients with sleep apnea compared to non-apneic patients (39% vs. 20%, p=0.024) and there was a non-significant increase in ESS scores in CKD patients with apnea (median, range) (9) 24 vs. 6 (0-17, p=0.055). There were no differences in the prevalence of poor sleep quality between apneic and non-apneic patients (47% vs. 43%, p=0.7; median, range) 5 (0-15) vs. 5 (0-16), p=0.6.

Conclusions: Sleep apnea is highly prevalent in patients with CKD but is not clinically apparent. Consequently, objective cardio-pulmonary monitoring during sleep is required to reliably identify this co-morbidity.

SA-PO2456

Validity of the Berlin Questionnaire and Adjusted Neck Circumference in Identifying Sleep Apnea in Patients with Chronic Kidney Disease

Methods: Two hundred fifty-four patients were recruited from nephrology clinics and hemodialysis units. All patients completed the BQ, ANC, and overnight cardio-pulmonary monitoring for diagnosis of sleep apnea (respiratory disturbance index (RDI)>15). Patients were stratified into 3 groups based on estimated glomerular filtration rate (eGFR): eGFR<60 mL/min/1.73m² (n=55); CKD (eGFR<60 mL/min/1.73m², n=124); and ESRD (on hemodialysis, n=75). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated for the BQ and the ANC (Table 1) and the association between the BQ and ANC and a diagnosis of sleep apnea (RDI=15) was determined using logistic regression (Table 2).

Results: Data analysis consisted of a series of regressions where age and disease severity were entered. Results indicated that family functioning and family cohesion were significant positive predictors of the adolescent's social functioning.

<table>
<thead>
<tr>
<th>BQ</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR&lt;60</td>
<td>1.11</td>
<td>1.00-1.28</td>
<td>0.049</td>
<td>1.06</td>
<td>0.82-1.38</td>
<td>0.62</td>
</tr>
<tr>
<td>CKD</td>
<td>1.11</td>
<td>1.00-1.28</td>
<td>0.049</td>
<td>1.06</td>
<td>0.82-1.38</td>
<td>0.62</td>
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<tr>
<td>ESRD</td>
<td>1.11</td>
<td>1.00-1.28</td>
<td>0.049</td>
<td>1.06</td>
<td>0.82-1.38</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Conclusions: The BQ and the ANC were less accurate in patients with CKD and ESRD than in patients with eGFR>60. However, the ANC was consistently better than the BQ.

SA-PO2457

Intestinal Microbial Flora Is Altered by Uremia

Methods: Microbial DNA was isolated from the stools of 24 ESRD patients and 12 controls. A phylogenetic microarray was used for comprehensive identification of microbial populations.

Results: While large inter-individual variations were observed in the microbe composition, significant differences were found in the abundance of 183 bacterial taxonomic units between the ESRD patients and the control groups. Microbial families showing the largest increase in ESRD patients were Brachybacterium, Campetbacterium, Enterobacteriaceae, Halomonadaceae, Micromonosporaceae, Moraxellaceae, Nesterenkonia, Polyanangiaceae, Pseudomonadaecea, and Thiothrix including several genera from Clostridia, Bacteroidetes, and Betaproteobacteria were less abundant while species from Actinobacteria and Gammaproteobacteria were most abundant in ESRD patients compared to controls.

Conclusions: ESRD significantly alters the abundance of several microbial families which appears to be in part driven by overabundance of urea and urate in the intestine. Studies are planned to focus on individual members of these families and their contribution to complications of ESRD.

SA-PO2458

Asymmetric Dimethylarginine, Oxidative Stress, and Tubular Dysfunction in Patients with Chronic Kidney Disease Stages 3 and 4 – A One Year Follow-Up Study

Methods: The community of microbes residing in the intestinal tract (microbiome) constitutes a symbiotic ecosystem which confers trophic and protective functions and contributes to micronutrient homeostasis. Alteration in microbiome contributes to diverse illnesses including inflammatory bowel disease, diabetes, cancer, cardiovascular disease, obesity, etc. Selection pressures on part of the host and microbes shape the structure-function of microbiome. We believe that influx of urea (and its hydrolysis to ammonia), urate and oxalate, dietary restrictions, phosphate binders and antibiotics change the milieu of intestine in ESRD. This can, in turn, alter the microbiome and cause production of toxic, pro-inflammatory and pro-oxidant metabolites. We tested the hypothesis that uremia may change the composition and function of microbiome.

Methods: Asymmetric dimethylarginine (ADMA) is a mediator of endothelial dysfunction and a prognostic factor in patients with chronic kidney disease (CKD).

Results: Production and elimination of ADMA may be affected by oxidative stress and metabolic function of the kidneys.

Results: In a one year follow-up study (examinations in months 0, 6 and 12) we measured plasma levels of ADMA, markers of oxidative stress (advanced oxidation protein products (AOPP), advanced glycation end-products (AGE)) and tubular dysfunction (the ratio of cystatin-C to creatinine in urine, U_Cys/Cr) in 95 patients with CKD stages 3 and 4. All of the patients measured parameters were compared with 41 healthy controls.

Methods: During the one year follow-up, we observed a gradual decrease of glomerular filtration rate (GFR) and an increase of AGE and AOPP. We did not document changes of ADMA and U_Cys/Cr. All tested markers were clearly elevated in CKD patients as compared to healthy controls. Results are summarized in the table.
### SA-PO2459

**Treatment Needs of Patients with Chronic Kidney Disease Stage 3 in Primary Care**

**Background:** Since 2006, English GP practices have been required to keep a register of patients with CKD stage 3-5. NICE guidelines recommend regular follow-up, but these patients are often perceived to be at low risk, not requiring active management. We aimed to assess treatment needs of CKD stage 3 patients in primary care as well as their level of awareness of their CKD.

**Methods:** We studied 1741 patients on GP registers with CKD stage 3. Each subject underwent clinical assessment as well as urine and blood tests. At screening, participants were asked if they had been aware of their CKD diagnosis.

**Results:** The mean age was 73±9yrs and 60% (n=1052) were female. Diabetes mellitus was present in 17%. 41% were unaware of their CKD diagnosis. Multivariable logistic regression analysis identified subjects with fewer educational qualifications, aged <75yrs, eGFR 30-44mL/min/1.73m² and significantly albuminuric as more likely to be aware of their diagnosis. Advice given are shown in the table.

| Advice to stop potentially nephrotoxic drugs | 35% |
| Specific advice regarding BP control | 33.1 |
| Referral to Nephrologist | 103 |
| Investigations for anaemia | 142 |
| Statin therapy for dyslipidaemia | 69 |
| Advice to stop potentially nephrotoxic drugs | 35% |

**Conclusions:** A large proportion of patients were unaware of their CKD diagnosis. Our data indicate increasing engagement with patients to become active partners in their management will require improved communication targeted at specific groups. Our data support the use of primary care CKR registers to identify patients with CKD stage 3 to facilitate evaluation and follow up.

**Funding:** Other NIH Support - Kidney Research UK and the British Renal Society

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### SA-PO2460

**Current Practice in Diagnosis and Treatment of Anemia in Stage 3 or 4 Chronic Kidney Disease**

**Background:** In chronic kidney disease (CKD), anemia is often associated with iron deficiency (ID). Thus, repletion of iron stores is recommended before treatment with erythropoiesis-stimulating agents (ESAs).

**Methods:** From Aug-Nov 2010, randomly selected nephrologists in Austria, Italy, Netherlands and Sweden spending >50% of working time on patient-care and managing stage >10 CKD patients monthly were surveyed for patient demographics, tests for anemia and ID, and therapies of 5 CKD patients (stage 3-4) most recently treated for anemia within 6 months prior to the survey. Randomly selected questionnaires (10%) were returned to patients for validation. Results are presented as median [range] amongst countries.

**Results:** 125 physicians (119 hospital-, 6 office based) reported 623 cases (57% [54-71] male; 40% [29-60] >70 years old). Tests performed by nephrologists to confirm anemia and ID were hemoglobin (Hb) in 85% [59-87] and/or hematocrit in 89% [83-94] of patients and ferritin (63% [52-69]) and/or transferrin saturation (TSAT, 28% [16-54]). Median Hb and ferritin (Hb >97g/L [96-102]; % ferritin >40% [44-60]) presented in patients with Hb >10g/L and with Hb <80gL. Median ferritin and TSAT at diagnosis were 91µg/L [40-120] and 20% [14-23]. Absolute ID (defined as ferritin <10µg/L) was found in 53% [34-67] and TSAT was <20% in 53% [15-38] of those tested. 54% [15-56] received ESA treatment combined (Hb 10.5-11.5g/dL). 41% [24-54] ESA alone and 9% [5-31] iron alone. Except for Sweden (46%), only a small minority of iron-treated patients received intravenous (IV) iron (7-11%).

**Conclusions:** High rates of severe anemia and ID indicate suboptimal monitoring and treatment of anemia in ND-CKD patients. Awareness of the prevalence of ID in ND-CKD patients and the importance of an integrated (iron in combination with ESA) treatment approach, needs to be increased.

**Funding:** Pharmaceutical Company Support

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### SA-PO2461

**Relationship between Kidney Damage and Mortality among a Community-Based Chinese Population**

**Background:** Previous studies indicate that indicators of kidney damage are associated with adverse outcome, while studies among community-based population are limited, especially among developing countries.

**Methods:** This prospective cohort study included 1189 community-based participants in Beijing, China. Estimated glomerular filtration rate (eGFf) and urinary albumin-to-creatinine ratio (ACR) were assessed at baseline, and all participants had eGFf above 30mL/min/1.73m². All deaths were confirmed by medical record review. Multivariable Logistic regression models were used to explore the association between kidney damage and mortality.

**Results:** The average age was 60.1±9.4 years, and male accounted for 49.5%. During a median follow-up of 4 years’ follow-up, 573 patients (37.3%) died, among whom 38 died of cardiovascular disease (1.2%) and 29 died of cancer (2.4%). After adjusting for potential confounders including eGFf, albuminuria was independently associated with increased risk of cardiovascular mortality and all-cause mortality. For every 10 mg/g increase of ACR, the odds ratio (OR) of cardiovascular mortality and all-cause mortality was 1.05 [95% CI, 1.01–1.10] and 1.06 [95% CI, 1.02–1.10]. eGFf were not independently associated with mortality, and both baseline albuminuria and eGFf were not significantly associated with cancer mortality. Receiver operating characteristic (ROC) curve showed the cut-off values with both maximal sensitivity and specificity of ACR for the prediction of cardiovascular and all-cause mortality was 3.89 mg/g creatinine and 3.76 mg/g creatinine.

**Conclusions:** Increased urinary ACR is independently associated with increased risk of both cardiovascular mortality and all-cause mortality, even at levels markedly lower than the current definition of “albuminuria”.

**Funding:** Pharmaceutical Company Support

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### SA-PO2462

**Safety, Immunogenicity, and Efficacy of Subcutaneous Biosimilar Epoetin Alfa (HX575) in Non-Dialysis Patients with Renal Anemia: A Multi-Center, Randomized, Double-Blind Study**

**Background:** HX575 is a biosimilar version of epoetin alfa that is approved in Europe and Australia for the treatment of anemia associated with chronic kidney disease (CKD) using the intravenous route of administration. HX575 is not authorized for use in the USA. Here we report data from a study of anemic pre-dialysis patients to assess the safety, immunogenicity and efficacy of subcutaneous (SC) administration of HX575 versus Eprex®.

**Methods:** This was a randomized, double-blind study in adult patients (n=337) with stage III–V CKD and a hemoglobin (Hb) level of 7.5–11.0 g/dL. Eligible patients were randomized to 52 weeks of treatment with HX575 or Eprex® at a starting dose of 25 IU/kg body weight three times weekly or 75 IU/kg body weight once weekly during weeks 1 to 5. This could be adjusted after 5 weeks to maintain Hb levels between 10 and 12 g/dL. The primary objective was to assess the safety and immunogenicity of HX575 compared with Eprex®. Efficacy endpoints were mean absolute change in Hb from baseline to end of week 13 and mean weekly epoetin dosage in weeks 11–13.

**Results:** SC HX575 was comparable to Eprex® in terms of maintaining Hb levels and epoetin dose requirements. Two patients in the HX575 group developed neutralizing antibodies (Nabs) to erythropoietin, which resulted in the study being stopped after 5 weeks. Two patients in the HX575 group developed neutralizing antibodies (Nabs) to erythropoietin, which resulted in the study being stopped after 5 weeks.

**Funding:** Pharmaceutical Company Support
SA-PO2463

Chronic Kidney Disease Prevention and Management in Community Health Centers
East Carolina University, Greenville, NC.

Background: Chronic Kidney Disease (CKD) leads to high rates of morbidity and mortality. Early detection and management may prevent progression to kidney failure. Primary care providers (PCPs) in Community Health Centers (CHCs) are strategic agents for detection of early CKD CHCs primarily serve low-income and AA patients who are at high risk for CKD. However, PCP management of CKD is sub-optimal. We aimed to improve CKD detection, prevention and management among PCPs in CHCs. We anticipate that an educational collaboration with nephrologists and PCPs working in a CHC will result in improved clinical practices related to CKD care. We hypothesized that decreases in BP and improved anti-hypertensive filling practices would result from the intervention.

Methods: Nephrologists conducted lectures based on KDQI and JNC-7 guidelines with the CHCs’ providers. Site visits occurred weekly for 6 months (intervention) then gradually decreased. Electronic pharmacy records were queried for data on antihypertensive prescriptions pre-and post-intervention in the initial clinic. A random sample of hypertensive, adult patients were stratified by their respective PCPs. Provider management of BP was evaluated by comparing multiple BP data points from the patient sample pre-and post-intervention. Changes were assessed using paired sample t-tests. This program was repeated in an additional CHC where BP’s were analyzed.

Results: The initial clinic demonstrated a statistically significant increase (27%) in prescriptions for antihypertensives pre-and post-intervention. When comparing individual provider management pre- and post-intervention, significant decreases in SBP were observed for all three providers (p<0.05). Decreases in DBP were significant for two providers (p<0.05). Interim data from an additional CHC is currently insignificant.

Conclusions: Intervention in the initial CHC demonstrated improved provider practices and patient outcomes. Although when repeated, in a different CHC, similar success was not achieved. The recent growth of and unique challenges in CHCs may require flexible interventions to be effective.

Funding: Private Foundation Support

SA-PO2464

Imbalance of Growth Factors Favouring Anti-Angiogenesis in Children with Chronic Kidney Disease
1) UCL Institute of Child Health, United Kingdom; 2) University of Colorado; 3) University of Manchester, United Kingdom.

Background: Cardiovascular disease (CVD) is a cause of morbidity and mortality in childhood chronic kidney disease (CKD). Endothelial damage and dysfunction is one of the earliest events in CVD development and may result from disturbances in vascular growth factors. Angiopoietin (Ang)-1 promotes endothelial survival and stabilisation, whereas Ang-2 is an endogenous Ang-1 antagonist causing endothelial death and vessel regression when ambient VEGF-A is low, pro-inflammatory responses and is induced by acute stimulation with uric acid.

Methods: We measured circulating Ang levels in pre-dialysis CKD 4-5 and dialysis children and correlated these with clinical, biochemical and vascular measures. We also examined local Ang and VEGF expression in arterial biopsy samples.

Results: Ang-2 levels were markedly elevated in dialysis compared to pre-dialysis children and correlated with clinical, biochemical and vascular measures. We also examined local Ang and VEGF expression in arterial biopsy samples.

Conclusions: Children on dialysis have an anti-angiogenic and inflammatory milieu evidenced by depletion of Ang-1 and VEGF-A in their vessels and increased circulating Ang-2 which may lead to elevated blood pressure through changes in serum urate. Vascular growth factors could constitute important biomarkers and possible future therapeutic targets in children with CKD.

SA-PO2465

Effectiveness and Safety of MIRCERA® for Anemia Treatment in Chronic Kidney Disease (3-5) Patients on Hemodialysis or Not on Dialysis, in Routine Clinical Practice – The Atenea Study
J.M. Portolés, J.I. Vega, A. Pérez-Campos, B. Zurek, A. Pérez, M.J. Cambó, S. Bea, J.M. Souto Boo, Spain; *Hospital Doctor Negrín, Spain; †Hospital Puerta de Hierro, Spain; ‡Hospital Doctor Negrín, Spain; †Hospital Marqués de Valdecilla, Spain; Clinica Souto Boo, Spain; Centro de Diálisis Gamapal, Spain; Complejo Hospitalario de Ourense, Spain.

Background: MIRCERA® has strongly demonstrated its efficacy and safety in anemia control of adult CKD patients, in clinical trials.

MIRCERA® has strongly demonstrated its efficacy and safety in anemia control of adult CKD patients, in clinical trials.

Methods: This observational cross-sectional study involving 20 Spanish centers examined these benefits when administered up to 6 months for correction (naïve patients) or maintenance (converted patients) in CKD patients in Hemodialysis (HD) or not on dialysis (ND), in a real clinical setting. Final results are presented.

Results: Of the 201 patients evaluated, 6.5% were naïve-ND, 25.4% converted-ND and 68.1% converted-HD. Mean age 66.9±12 years, 42% males, 38.4% naïve; CKD 3/4: 8%<19.4%;72.6%. Predominant etiologies: 22% vascular and 21% diabetes. The most frequent previous ESAs were darbepoetin alfa in 51% of ND patients and epoetin beta in 58.4% of HD. Hb levels significantly increased from 10.2±0.7 g/dL at the start of MIRCERA® to 11.6±0.3 g/dL at month 6 (p<0.005) in naïve-ND patients. Those converted showed stable Hb values after 6 months of MIRCERA® in both ND (12.1±1.3 g/dL vs 12.3±1.2 g/dL) and HD group (11.6±1.3 g/dL vs 11.4±1.2 g/dL). Starting doses of MIRCERA® resulted lower than recommended on SPC: from darbepoetin alfa<40g/w to MIRCERA®<94.6±35.7 ug/w in naïve-ND patients; from darbepoetin alfa 40-80ug/w and epoetin 8,000-16,000IU/w to MIRCERA® 138.2±34.9mg/w in naïve-ND to 301.5±129.4mg/w (n=17). Throughout the study, mean MIRCERA® dose adjustments were 1.1±0.4 in naïve-ND, 1.5±0.7 in converted-ND and 1.7±0.9 in converted-HD, and no dose adjustment was required in 38.5%, 54% and 23.4%, respectively. No MIRCERA®-related AEs were reported.

Conclusions: MIRCERA® successfully provides anemia control with doses bellow to those described in the SPC and few dose adjustments. MIRCERA® is safe and simplifies anemia treatment in CKD in routine clinical practice.

SA-PO2466

Understanding Transition Readiness: How To Facilitate High Transition Readiness and What Are the Predictors of Low Transition Readiness?
1) School of Medicine - Kidney Center, University of North Carolina at Chapel Hill, Chapel Hill, NC; 2) Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC.

Background: The transition from pediatric to adult-focused health care is a critical time yet, little is known about how to facilitate transition readiness or the implications of low transition readiness. Utilizing the Disability-Distress-Coping Model we examined psychosocial function and transition readiness. Our aims were to:

1) Examine if psychosocial functioning (family functioning, coping efficacy, and quality of life) and disease characteristics (disease severity/burden and age at diagnosis) would predict transition readiness.
2) Determine if transition readiness would be a predictor of emergency room (ER) visits and non-adherence.

Methods: Thirty adolescents age 13-18 with a diagnosis of CKD stage 2 were included. De-identified web-based measures were administered including: the Family Relationship Index, KidCope, PedQOL, and the UNC TRANSITION Scale. Adequacy and ER visits were documented based on chart review and self-report.

Results: Hierarchical regressions were performed. Family cohesion was a significant positive predictor of both overall transition readiness and adherence (p<.05). However, none of the other variables (quality of life, coping efficacy, and disease characteristics) were significant predictors of transition readiness. Higher transition readiness was a significant predictor of higher medication adherence and fewer ER visits (p<.05).

Conclusions: In our cohort, family cohesion appears to facilitate transition readiness. How to manage the family versus the patient is an important area where adolescents are preparing for transition. Additionally, this study also suggests that low transition readiness may predict non-adherence to medications and ER utilization. This highlights the importance of preparing adolescents for the transition from pediatric to adult-focused health services and suggests a significant influence on low adherence on healthcare utilization.

SA-PO2467

Sermum Vitamin B12, and Folic Acid Concentrations and Estimated Glomerular Filtration Rate (eGFR) in the Elderly, Preliminary results of the Polish Population Study “PolSenior”
Andrzej Więcek, Magdalena Sztowska, Marcin Adamczak, Jerzy Chudek.
Department of Nephrology, Endocrinology and Metabolic Diseases, Medical University of Silesia, Katowice, Poland.

Background: During the last years increasing prevalence of vitamin B12 and folic acid deficiency is observed in different groups of patients. Taking into consideration important role of vitamin B12, folic and folate in hematopoiesis, skin and brain function, such deficiency seems to be one of the crucial issues of the public health. The aim of this study was to examine serum vitamin B12 and folic acid concentrations and their relationship with eGFR in representative samples of the elderly population in Poland.

Methods: In 1193 subjects (609 males; 584 females) aged over 65 years (mean age 79±5) a relationship between the “Polsenior” study and the UNCGF eGFR were studied. Serum vitamin B12 and folate concentrations was measured by RIA method. eGFR was estimated using the CKD-EPI formula.

Results: Analysis of variance, between groups stratified according to eGFR with respect to both vitamin B12 and folic acid, have showed significant (p<0.05) differences [in subject with eGFR 90-120 ml/min (n=199) - 334±789 and 5.63±7.44 eGFR 60-90 ml/min (n=659) - 312±220 and 4.90±4.34; eGFR < 60 ml/min (n=278) - 285±142 pmol/l and 4.59±4.70 mmol/l, respectively]. However no significant correlation was found between age or eGFR and vitamin B12 or folate concentrations.
Conclusions: 1. Both vitamin B12 and folate concentrations decrease with worsening of kidney function in elderly patients. 2. These results suggest a particular need for increased vigilance for deficiency of vitamin B12 and folic acid in elderly patients with chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2468

Clinical Characteristics of Chronic Kidney Disease Patients with Hyperkalemia Due to Angiotensin II Receptor Blocker Hideki Fujii, Keiji Kono, Kentaro Nakai, Shunsuke Goto, Shinichi Nishi. Division of Nephrology and Kidney Center, Kobe University Graduate School of Medicine, Kobe, Japan.

Background: Angiotensin II receptor blocker (ARB) is often used in the clinical settings. Most patients with chronic kidney disease (CKD) take ARB for renal and cardiovascular protection. Though hyperkalemia is a life-threatening complication resulting from use of ARB especially in CKD patients, an available way of predicting hyperkalemia remains unknown.

The purpose of our study is to elucidate the characteristics of CKD patients with hyperkalemia due to ARB.

Methods: Twelve consecutive CKD patients with hyperkalemia (25±5.5mEq/l) due to ARB were included in this study. Patients with rennin angiotensin system inhibitor, loop diuretics, thiazide, spironolactone or eplerenone were excluded from the present study. Patients with rapidly progressive glomerulonephritis, acute kidney injury, congestive heart failure and acute coronary syndrome were also excluded. We measured serum potassium levels, serum creatinine levels, blood urea nitrogen levels, urinary protein excretion, urinary β2-microglobulin (U-β2-MG) levels, and urinary N-acetylglucosamine (U-NAG) levels before and after taking ARB. In addition, we calculated fractional excretion of potassium (FEK) and transtubular potassium gradient (TTKG) before taking ARB. In 3 of the 13 patients, we investigated the change of serum potassium levels, FEK and TTKG depending on the dose of ARB. Case-note review enabled us to assess other clinical characteristics of the study patients.

Results: Averaged serum creatinine levels were 2.59±1.37 mg/dl and averaged maximum serum potassium levels were 6.94±1.04 mEq/l. The presence of diabetes and hypertension was 33% and 100%, respectively. Regardless of renal function, U-β2-MG and U-NAG were high in most study patients. Furthermore, FEK and TTKG were relatively low in these patients. They were lowering with increasing the dose of ARB.

Conclusions: In CKD patients with hyperkalemia, TTKG and FEK were low and urinary tubular injury markers were elevated before taking ARB. It is suggested that TTKG and FEK are available as a predictive marker of hyperkalemia due to ARB in CKD patients.

SA-PO2496

Characteristics of Patients with CKD and Anemia Treated with Darbepoetin Who Failed To Maintain Hemoglobin Levels at 4-Week Dosing Intervals Grace Snyder,1 James F. Simon,2 Jesse D. Schold,3 Susanna Arriag,4 Celeste Jindras,5 Joseph Hrehov,6 Anil K. Jain,7 Stacey Jolly,7 Martin J. Schreiber,7 Joseph V. Nally,8 Nephrology, Cleveland Clinic; 2Quantitative Health Science, Cleveland Clinic; 3Internal Medicine, Cleveland Clinic.

Background: Resistance to erythropoietin-stimulating agents (ESA) is associated with increased CV risk in renal anemia patients. Darbepoetin alpha therapy may be dosed at 2- or 4-week (wk) intervals based on convenience and other factors. Some patients require a higher monthly dose at 4-wk intervals. We investigated whether characteristics differ between anemic CKD patients requiring ESA dosing at 2- vs. 4-wk intervals.

Methods: Patients who initiated darbepoetin therapy in our renal anemia clinic at 2-wk intervals then switched to 4-wk intervals after Hb stabilization in target range between Sep 2007 and Nov 2009 were included. Patients were considered a failure of 4-wk therapy if their Hb dropped or monthly ESA requirement increased after the switch and were returned to 2-wk dosing. Patients who failed 4-wk dosing were compared to those who did not. We evaluated factors associated with time to failure of 4-wk dosing using Kaplan-Meier survival estimates and Log-rank tests for categorical variables and Cox proportional hazards model for continuous variables.

Results: 186 patients started ESA therapy at 2-wk intervals during the study period. 81 (44%) switched to a 4-wk interval and had continued follow-up (median 5 months). 28 (15%) switched back to 2-wk intervals because of failure to maintain their Hb. At 6 months, fewer kidney transplant patients (25% vs. 76%, p=0.03) and patients on corticosteroids (36% vs. 77%, p=0.01) were at 4-wk intervals. Higher Hb (p=0.003) and eGFR (p=0.003) at switch were associated with lower risk of failing 4-wk interval therapy.

Conclusions: Lower Hb level and eGFR at the time of switch to 4-wk intervals, kidney transplant status and corticosteroid use were significantly associated with failing 4-wk interval ESA therapy. Patients at risk for failure on 4-wk interval therapy should be monitored and considered for dosing interval change if failure occurs.

Funding: Clinical Revenue Support

SA-PO2470

Iron Replacement Therapy for CKD Patients Not on Dialysis Peter Juergensen,1 Fredric O. Finkelstein,2 1Medicine, Hospital of St. Raphael, New Haven, CT; 2Medicine, Yale University School of Medicine, New Haven, CT.

Background: Standardized protocols have been developed for iron replacement therapy for patients with chronic kidney disease patients (CKDP) maintained on dialysis; the vast majority of hemodialysis patients (pts) receive intravenous (IV) iron. The iron utilization requirements for CKDP-not on dialysis (NOD) have not been well defined. The present study was designed to examine the percent of CKDP-NOD who require iron replacement to maintain their ferritin levels > 100 ng/ml and Tsat > 20%.

Methods: 1065 CKDP-NOD who were followed in our clinic for 12 months between 1/08 and 5/11 and had hemoglobin levels and iron studies checked were included. The present study was designed to examine the percent of CKDP-NOD who require iron replacement to maintain their ferritin levels > 100 ng/ml and Tsat > 20%.

Results: 14.3% of pts who were receiving ESAs were prescribed IV iron. 63% of pts were prescribed oral iron. Of pts not receiving ESAs, 9.1% received IV iron and 22.2% were prescribed oral iron.

Conclusions: In conclusion, iron supplementation is frequently needed in CKDP-NOD receiving ESAs to maintain adequate iron stores. These pts not receiving ESAs require iron supplementation less often to maintain iron stores and Hgb levels > 10 gm%.

SA-PO2471

Functional Health Literacy in the Chronic Renal Insufficiency Cohort Study Ana C. Ricardo,1,2 Wei Yang,1 Elisa J. Gordon,1 Claudia M. Lora,1 John W. Kusek,2 Amada Lopez,1 Eva Lustigova,2 Lisa C. Nessel,2 Sylvia E. Rossa,3 Susan P. Steigerwald,1 Xiaoming Zhang,1 Michael J. Fischler,3 James P. Lash,3 1University of Illinois at Chicago; 2Chronic Renal Insufficiency Cohort (CRIC) Study Group.

Background: Low health literacy is associated with increased risk of death, yet the health literacy of individuals with chronic kidney disease (CKD) is rarely assessed.

Methods: We evaluated the prevalence and correlates of low health literacy in a cross-section of 2872 CRIC Study participants. Low health literacy was defined as a Short Test of Functional Health Literacy in Adults (STOFHLA) score ≤10.

Results: Table 1 shows significant differences in demographic and clinical characteristics between participants with low vs. adequate health literacy (p<0.05).

Table 1. Demographic and Clinical Characteristics*  

<table>
<thead>
<tr>
<th>Health Literacy</th>
<th>Low (n=640)</th>
<th>Adequate (n=2232)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y, Mean (SD)</td>
<td>64.2 (9)</td>
<td>61 (11)</td>
</tr>
<tr>
<td>Male gender</td>
<td>384 (60)</td>
<td>1395 (53)</td>
</tr>
<tr>
<td>Income &lt;$20,000/year</td>
<td>359 (55)</td>
<td>151 (20)</td>
</tr>
<tr>
<td>Education ≤5th grade</td>
<td>163 (26)</td>
<td>23 (1)</td>
</tr>
<tr>
<td>Hispanic Ethnicity</td>
<td>245 (38)</td>
<td>185 (8)</td>
</tr>
<tr>
<td>Ethnicity Asian</td>
<td>17,2m2, Mean</td>
<td>34 (4.14)</td>
</tr>
<tr>
<td>Ethnicity African</td>
<td>0.8 (0.2-2.6)</td>
<td>0.3 (0.1-1.3)</td>
</tr>
<tr>
<td>Ethnicity Other</td>
<td>460 (72)</td>
<td>1012 (45)</td>
</tr>
<tr>
<td>Self-reported CVD</td>
<td>318 (59)</td>
<td>805 (36)</td>
</tr>
<tr>
<td>Left Ventricular Hypertrophy**</td>
<td>371 (15)</td>
<td>81 (47)</td>
</tr>
<tr>
<td>Blood Pressure &gt;130/80</td>
<td>358 (56)</td>
<td>949 (43)</td>
</tr>
<tr>
<td>Hb ≥13 g/dl</td>
<td>805 (36)</td>
<td>377 (18)</td>
</tr>
</tbody>
</table>

*Results are presented as n (%), unless otherwise noted. **Echocardiogram obtained within one year of STOFHLA SD, standard deviation; IQR, interquartile range; eGFR, estimated glomerular filtration rate; CVD, cardiovascular disease.

In adjusted analyses, low health literacy was a significant predictor of lower eGFR (β coefficient, 2.01 ml/min/1.73m2), HbA1C >7% [odds ratio (OR) 1.6, 95% confidence interval (CI) 1.2-2.2], and higher prevalence of self-reported CVD (OR 1.4, 95% CI 1.1-1.8). Significant predictors of low health literacy were: older age, male gender, Hispanic ethnicity, lower educational attainment, and diabetes (p<0.05).

Conclusions: In the CRIC cohort, low health literacy was prevalent and associated with a greater burden of chronic illness. Health literacy interventions may have the potential to improve clinical outcomes in patients with CKD.

Funding: NIDDK Support

SA-PO2472

The Validity of Current CKD Staging Paradigms Revisited and Disputed: A 2-Year Follow-Up Study of 826 CKD Patients in a Mayo Clinic Laboratory Database: A Call for Process Reengineering in Nephrology Practice Macaulay A. Onuigbo,1,2 1College of Medicine, Mayo Clinic, Rochester, MN; 2Nephrology, Mayo Clinic Health System, Eau Claire, WI.

Background: It is generally believed that there is a predictable, linear and time-dependent progressive decline in eGFR, ultimately in some patients, ending inexorably in ESRD and the need for RRT. It is on this premise that the current CKD staging protocols

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SA-PO2473
Effect of Conversion from Other ESAs to CERA Once Monthly for Maintaining Hemoglobin (Hb) Concentration in Pre-Dialysis CKD Patients Jinyoung Choi, Cheil Woo Yang, Yeong Hoon Kim, Kwon Wook Joo, Tae-Hyun Yoo, Kang Wook Lee, Sang-Ho Lee, Sung Kyun Shin, Woosong Huh, Sun-Hye Park, Chan-Duck Kim, Yong-Lim Kim. Kyungpook National University, Catholic University of Korea, Inje University, Seoul National University; Yonsei University; Chungnam National University; Kyung Hee University; National Health Insurance Corporation Biuan Hospital; Sungkyunkwan University.

Background: The purpose of this study was to identify whether Hb concentrations can be maintained stably when switching from other ESAs to CERA.

Methods: Pre-dialysis CKD patients (n=191) maintained Hb level of 10.0-12.0 g/dL through epoetin-α or darbepoetin-α were enrolled. Conversion ratio from other ESAs to CERA was represented in Table 1.

Results: The mean Hb level was 10.86 ± 0.71, 11.87 ± 0.93 and 11.16 ± 0.94 g/dL at baseline, 3 and 6 month, respectively. One hundred eight patients (74.5%) maintained average Hb concentration were assessed.

Conclusions: Conversion from other ESAs to CERA in pre-dialysis CKD patients can efficaciously maintain Hb concentration and the dose requirement of CERA had significantly decreased compared with at conversion. It may be needed to adjust the conversion ratio than recommendation for switching from other ESAs to CERA.

Funding: Pharmaceutical Company Support

SA-PO2474

Background: Data and recommendations on physical activity (PA) are limited in children with CKD. The objectives of this study were to: 1) measure the level of PA in children with all stages of CKD and compare these with the recommendations for healthy children; 2) to determine the characteristics associated with PA; and, 3) to determine the association of PA with physical performance (PP) and physical functioning (PF).

Methods: Participants were enrolled from the Pediatric Nephrology and Transplant Clinics at UCSF. PA was measured for 7 days using the Yamax Digi-Walker SW-200 pedometer and expressed as daily step counts. PP was measured by the 6-minute walk test (6MWD), and PF with the self-report PedQOL 4.0. Univariate and multivariable linear regression analyses were used.

Results: We studied 44 participants aged 7-20 years with CKD Stages 1-4 (n=12), with predialysis stage 5D (n=7), and kidney transplant recipients (n=25). As a group, participants were very sedentary, walking 6218 (3637, 9828) steps/day and thus falling short of levels recommended for healthy children, 15,000 steps/day for boys and 12,000 steps/day for girls. There was no difference in PA between the 3 groups. Girls were less active than boys (p<0.01) and PA was 44% lower among young adults (18-20 years) compared with younger age groups (p<0.05). PA was associated positively with maternal education and hemoglobin concentration but inversely with BMI. PP in boys and girls were -2 and -4 standard deviations below the expected 6MWD, respectively, with worse performance in older children. Low levels of PA were associated with poor PP and PF, after adjusting for age, sex, and BMI.

Conclusions: In most participants with CKD, PA levels were considerably below levels recommended for healthy children, without apparent improvement after transplantation. The levels were less active than boys (p<0.01) and PA was 44% lower among young adults (18-20 years) compared with younger age groups (p<0.05). PA was positively associated with maternal education and hemoglobin concentration but inversely with BMI. PP in boys and girls were -2 and -4 standard deviations below the expected 6MWD, respectively, with worse performance in older children. Low levels of PA were associated with poor PP and PF, after adjusting for age, sex, and BMI.

Funding: Other NIH Support - T32 National Research Service Award

SA-PO2475
Polymorphisms of UCP2 Gene Are Associated with Carbohydrate and Lipid Metabolic Disorders in Chinese Peritoneal Dialysis Patient Tongying Zhu, Lihua Zhang, Yun Li, Lin Tang, Chuan-Ming Hao. Department of Nephrology, Huashan Hospital of Fudan University, Shanghai, China.

Background: We investigated the association of three functional variants of UCP2 polymorphism with metabolic disorder and low-grade inflammation in prevalent Chinese peritoneal dialysis patients.

Methods: 116 prevalent PD patients were enrolled, who were genotyped for the UCP2 variants -866G/A, Ala55Val and 45bp ins/del. HOMA-IR, plasma lipid profile, CRP, ferritin, peritoneal glucose absorption, and adipocytokines were examined and compared in different genotypes.

Results: The patients with GG genotype in -866 G/A gene had a higher serum triglyceride than those with A allele (p=0.011).Patients with VV genotype in Ala55Val gene, have a higher level of serum triglyceride (p=0.005), HOMA-IR (p=0.028), ferritin (p=0.045) and peritoneal glucose absorption (p=0.007), and a lower adiponectin level (p=0.014). GG and VV genotype were also found to be significant predictors of hyperglycemia/dyslipidemia even after adjustment for glucose absorption (GG:OR=2.441, p=0.041; VV: OR=2.40, p=0.03) by logistic regression analysis. While DD genotype in 45bp ins/del polymorphism had higher triglyceride (p=0.012) and HOMA-IR (p=0.001) than DI genotype. Compared to those with only one or none above inferior genotypes, patients who carrying GG- VV-DD simultaneously showed a significant higher degree of de novo diabetes (6.27 vs. 2.45, p=0.02), higher HOMA-IR (3.93 vs. 2.43, P<0.000), triglyceride (2.68 vs.1.4mmol/L, P<0.00), cholesterol (5.36 vs. 4.65mmol/L, P<0.01), CRP (5.77 vs.7.2mg/L, P=0.018), ferritin (270 vs. 209µg/L, P=0.019), leptin (2.85 vs. 2.85mg/L, P<0.01), and higher glucose absorption (108.99 vs. 83.67g/day, P=0.00). Logistical regression analysis showed that GG-VV-DD genotype had a significant tendency to become hyperglycemia/dyslipidemia (OR=5.97, P=0.002) and insulin resistance (OR=3.684, P=0.031) even adjusted for glucose absorption.

Conclusions: PD patients with UCP2-866G/A, Ala55Val VV, and 45bp DD genotype demonstrated a significant critical metabolic disorder , suggesting a possible role of UCP2 gene polymorphism in the liability of metabolic disturbance under a high glucose environment.

SA-PO2476

Background: Sleep is an important daily activity that aids the body in maintaining hormonal balance. Sleep deprivation has been linked to increased risk of cardiovascular disease as well as an overall diminished quality of life. This study examined the total sleep time of advanced chronic kidney disease (CKD) and end-stage renal disease (ESRD) patients to determine if modifiable risk factors are associated with reduced total sleep time.

Methods: 58 patients with advanced CKD (stages 4-5) and 90 patients with ESRD on dialysis were compared with 224 controls form the Sleep Strategies-Concentrating on Risk Evaluations study of sleep and cardiovascular risk. All participants underwent home polysomnography. Patient demographics; blood pressure; hypertension, diabetes, and depression status; and use of hypnotics and anxiolytic medications were also assessed.

Results: The sample (median age 58 years) was 56.3% male and 62.1% white. Total sleep time in minutes differed significantly between controls (365.80/83.19), CKD patients (362.93/100.1), and ESRD patients (318.80/117.46), p<0.001. The percent of the sample with less than 5 hours of sleep also differed significantly between controls (20.1%), CKD patients (27.3%), and ESRD patients (43.2%), p<0.001. In a multivariable linear regression model adjusted for race and hypertension, a 1-year increase in age (-1.44, 95% CI -2.29 to -0.59), female sex (20.75, 95% CI 1.40 to 40.1), presence of sleep-disordered breathing (-0.62, 95% CI -1.08 to -0.16), periodic limb movements index (-2.88, 95% CI -4.52 to -1.23), and ESRD (vs. control) (-45.96, 95% CI -49.7 to -22.3) were all independently associated with total sleep time. The presence of CKD (vs. control) (-10.95, 95% CI -36.4 to 14.5) was not independently associated with total sleep time.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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Conclusions: ESRD patients sleep significantly less (on average 46 minutes) compared to controls, possibly contributing to increased risk of cardiovascular disease and reduced quality of life. Addressing sleep-disordered breathing and restless legs, common complaints in ESRD, might lead to increases in total sleep time for these patients.

Funding: NIDDK Support

SA-PO2477

Increased Intrarenal Angiotensin II Activity and Risk of Chronic Kidney Disease

Background: Urinary angiotensinogen (UAGT) is a stable biomarker and strongly associated with intrarenal angiotensin II activity. However, less is known about UAGT levels and risk of chronic kidney disease (CKD).

Methods: We investigated urinary and plasma angiotensinogen (PAGT) with risk of CKD in 21 CKD cases and 201 controls without CKD. CKD was defined as estimated glomerular filtration rate (eGFR) <60 mL/min/1.73 m² or presence of albuminuria.

Results: Compared to controls, median 24 hour UAGT (44.4 vs. 7.4 µg/24 hr, p=0.001) and UAGT/creatinine ratio (µgCr/µU) (26.3 vs. 4.4 µg/g, p=0.001) were significantly higher in CKD cases. PAGT was similar between cases and controls (23.3 vs. 22.4 µg/mL, p=0.1). The 24 hour UAGT per 1.73 m² body surface area was highly correlated with the UAGT/UCr ratio (r=0.9, p<0.0001) but not PAGT. Both 24-hour UAGT and the UAGT/UCr ratio were significantly correlated with eGFR and 24-hour urine albumin (p<0.001). After adjusting for age, gender, race, smoking, drinking, education, physical activity, systolic blood pressure, glucose, LDL cholesterol, and body mass index, the odds ratio (OR) for CKD comparing the highest to the lowest tertile of UAGT was 5.8 (95% CI, 3.0, 11.4). Similar results were seen for tertiles of the UAGT/UCr ratio (OR: 5.8, 95% CI, 3.1, 10.7). Weaker associations were observed for PAGT (µg/mL) with an odds ratio of 3.3 (95% CI, 1.7, 6.3) for the highest compared to the lowest tertile. The results did not change substantially after further adjustment for history of CVD, diabetes, hypertension, and use of angiotensin-converting enzyme inhibitor and angiotensin receptor blocker medications.

Conclusions: These data indicate that UAGT level is associated with an increased risk of CKD and may be a useful biomarker for the prediction of CKD risk. The UAGT/UCr ratio could be used to evaluate 24 hour UAGT. The predictive value of UAGT for the risk and progression of CKD should be examined in a prospective cohort study.

Funding: Other NIH Support - the National Center for Research Resources

SA-PO2478

NGAL and Progression of ADPKD Patients in Stage II-III CKD
Maurizio Clementi,†,1,2,3 Fiorella Gastaldon,†,3 Massimo de Cal,†,3 Dinna N. Cruz,†,3 Maurizio Clementi,†,3 Claudio Ronco,†,3 †Nephrology, †Pediatrics-Clincial Genetics, Padua; ‡IRRIV.

Background: Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous inherited condition with a variable rate of progression. In addition to NKF-KDOQI guidelines regarding routine screening, recent studies suggest a possible role for NGAL in assessing CKD progression; it could be a good marker also for ADPKD progression. The aim of the study was to evaluate whether NGAL could predict loss of function in ADPKD patients in stage II-III CKD.

Methods: Pts with ADPKD, based on ultrasound criteria, were enrolled and followed prospectively. Creat and NGAL values were measured at baseline and followed up. eGFR was calculated with 4-variable standardized-MDRD equations. NGAL was measured in plasma by the Triage®NGAL Device.

Survival data were analyzed by the Kaplan-Meier curve using the median NGAL (80pg/ml) as a cut-off value. Results: We enrolled 21 ADPKD pts (12M/9F; mean age39±9yrs); mean creat was 1.3±0.7mg/dl and mean eGFR was 67.8±23.3mL/min/1.73m². After a median follow-up of 16.5mo, 8pts (38%) progressed to a worse stage of CKD and 62% were stable. Kaplan-Meier curves are presented in Fig1.

Conclusions: No statistically significant relationship between higher NGAL and decreasing eGFR was observed; a positive trend was evident. Pts with NGAL values>80pg/ml were followed for a longer period of time-17.8mo versus 14mo for NGAL above the cut off.

Funding: Other NIH Support - the National Center for Research Resources

SA-PO2479

Urinary Neutrophil Gelatinase-Associated Lipocalin Does Not Correlate with Renal Dysfunction in Polycystic Kidney Disease
Graham D. Smith,†,1 Caroline M. Robinson,†,1 Keith A. Burling,†,1 Anthony G. Norden,†,2 Richard N. Sandford,†,3 Eiona F. Karet,†,3 †Medical Genetics, University of Cambridge, United Kingdom; †Core Biomedical Assay Lab, NIHR Cambridge Medical Research Centre, United Kingdom; ‡Renal Medicine, University of Cambridge, United Kingdom.

Background: Neutrophil gelatinase-associated lipocalin (NGAL) has been implicated in pathological conditions and its presence in urine is proposed as a biomarker for various kidney diseases, including Polycystic Kidney Disease (PKD) (1). We used commercially available ELISA kits to assess levels of urinary NGAL (nNGAL) or uMMP9/nNGAL in adult PKD patients (n=41, mean age 43±10.5 y), creatinine 78-627 µmoL/L compared to matched healthy controls with normal renal function.

Results: We found no differences in uNGAL levels between PKD patients and controls: median 12.5 ng/mL (range 0.6-40 vs 7.4 ng/mL (0.2-40). p=0.7. In contrast, a cohort of AKI patients had median 234 ng/mL (138-310). Correction of uNGAL for urinary creatinine resulted in a statistically but clinically insignificant difference between PKD and control groups: median 2.1 (0.26-25.1 ng/mL (p=0.013), with AKI patients’ median being 20.3 (1.7-51.7) ng/mL. For uMMP9/nNGAL, only negligible amounts were detectable in either patients or controls, with median 0 ng/mL for both groups, range 0.203 (patients) and 0.0395 (controls), p=0.31. We did not find any correlation between levels of uNGAL (r²=0.27) or uMMP9/nNGAL (r²=0.13) and degree of kidney dysfunction in the patient population.

Conclusions: The similar ranges of uNGAL or uNGAL-MMP-9 found in patient and normal kidney samples, and the lack of change with altered renal function, indicate that neither would be a reliable biomarker for disease progression in PKD, and their utility in a clinic setting are not supportable.

Funding: Private Foundation Support, Government Support - Non-U.S.

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Fig 1: Kaplan-Meier survival curves in patients with NGAL level above and below the cut-off of 80 pg/ml (median values)

NGAL values inversely correlated with eGFR; eGFR was higher in pts with NGAL values<80pg/ml. Although, no statistically significant relationship between higher NGAL and decreasing eGFR was observed, a positive trend was evident. Pts with NGAL values=80pg/ml were followed for a longer period of time-17.8mo versus 14mo for NGAL above the cut off.
SA-PO2480

Angiotensin II Is Mediated through the Glomerulus and Reabsorbed by the Proximal Tubule in Both Mice and Humans

Background: Recent, in -vivo and kidney-specific Angiotensin II (AngII) knockout mice, we demonstrated that circulating AngII of liver origin is filtered through the glomerulus, and reabsorbed by the proximal tubule. This is in contrast to the notion that AngII cannot be filtered through the glomerulus and that AngII in the urine is produced by the proximal tubule (PT).

Methods: To clarify whether AngII is reabsorbed by PT in human subjects, urinary AngII was measured in 12 patients with Dene disease, Lowe syndrome, and idiopathic low molecular weight proteinuria, using human AngII ELISA kit (IBL), developed by Kobori et al. These patients are characterized by inability to reabsorb filtered low molecular weight proteins, resulting in massive urinary excretion of β2 microglobulin (MG) and α1 MG.

Results: In these patients, urinary AngII was markedly elevated, ranging from 1,446 to 33,410 µg/24h (median, 11,312). These values are far above the published values. Urinary β2 MG in these patients was 18,600 to 173,000 µg/l. Urinary AngII correlated well with urinary β2 MG (r=0.82), and α1 MG (r=-0.98). In control subjects without proteinuria, urinary AngII, measured concurrently using the same kit, ranged from 11.0 to 10.8 µg/gCr (median, 25.9), values comparable to previously published values, verifying that the assay method was appropriate.

Conclusions: Observed markedly augmented urinary excretion of AngII in patients with PT dysfunction indicates that, in human subjects, AngII is filtered through the glomerular filter, and then reabsorbed by the proximal tubule. Thus, abnormal elevation in urinary AngII excretion reflects either or both disrupted glomerular sieving function and/or defective tubular reabsorptive capacity.

Funding: Government Support - Non-U.S.

SA-PO2481

Renal Uptake of Technetium-99m DMSA Is Mediated by the Megalin/Cubilin Receptor Complex

Background: Dimercaptosuccinic acid (DMSA) labeled with technetium-99m (Tc-99m) is the major renal imaging agent used in diagnosis of renal parenchymal disorders. Tc-99m DMSA is highly accumulated in the kidney cortex, but despite the extensive clinical use, the mechanism for renal targeting of the tracer remains elusive. In this study we have tested the role of the proximal tubule endocytic receptor complex megalin/cubilin in the uptake of Tc-99m DMSA.

Methods: Control mice or conditional megalin/cubilin-deficient mice were i.v. injected with the tracers Tc-99m DMSA or Tc-99m MAG3 (mercaptoacetyltriglycine). Six hours post-injection, samples of plasma, urine, and kidneys were collected and analysed using a gamma counter. Whole-body autoradiographies of the mice were made with a gamma camera.

Results: The renal uptake of Tc-99m DMSA was reduced to 11.4% (+/- 2.5%, n=7) in the megalin/cubilin-deficient mice compared to control mice or conditional megalin/cubilin-deficient mice. In control subjects without proteinuria, urinary AngII, measured concurrently using the same kit, ranged from 11.0 to 10.8 µg/gCr (median, 25.9), values comparable to previously published values, verifying that the assay method was appropriate.

Conclusions: Observed markedly augmented urinary excretion of AngII in patients with PT dysfunction indicates that, in human subjects, AngII is filtered through the glomerular filter, and then reabsorbed by the proximal tubule. Thus, abnormal elevation in urinary AngII excretion reflects either or both disrupted glomerular sieving function and/or defective tubular reabsorptive capacity.

Funding: Government Support - Non-U.S.

SA-PO2482

Complement (C) Activation in Renal Ischemia/Reperfusion (I/R) Injury Is Mediated by Pentraxin 3 (PTX3): A Species-Specific Difference

Background: Intensive care patients are at risk for development of acute renal failure and death. PTX3, a key member of the pentraxins family, is involved in the coagulation, complement, and inflammatory pathways. PTX3 plays a role in promoting inflammation. PTX3 is increased in patients with acute renal failure.

Methods: Two experimental models of I/R were used: 6 rats and 5 pigs underwent 45' (rat) and 60' (pig) of renal warm I/R. Renal tissues were analyzed by flow cytometry.

Results: In the pig model, confocal microscopy demonstrated PTX3 deposits already at 15' of R, localized at peritubular (T0: 1.0±0.5, T5 7.7±1.1; p<.005) and glomerular (T0 5.2±2.5; p<.01) capillary levels, showing a specific co-localization with CD31, an endothelial cell marker. We observed a significant increase in infiltrating interstitial leukocytes, including CD163+/PTX3+ monocyte-macrophages (T0: 0.01±0.2, T5 6.2±2.1; p<.01) and SWC3a+/PTX3+ dendritic cells (T0 5.3±1.7; p<.05). Finally, we performed an intravital microscopy study in an isolated perfused kidney model (15' and 30' of R). Co-localization between C5b-9 and PTX3 on renal endothelial cells clearly demonstrated the C activation in the presence of PTX3 deposits. On the contrary, the analysis of rat kidneys showed that PTX3 was not modulated by IR. Rat PTX3 specifically co-localized only with alpha-SMA positive cells at the vascular level without any association with infiltrating leukocytes and C activation.

Conclusions: This study first demonstrates a significant difference in PTX3-mediated C activation between pig and rat kidney. Since PTX3 can activate the Classical and Lectin pathways of C via specific binding with C1q and MBL, we hypothesize that PTX3 might be a new therapeutic target to prevent C-induced renal I/R.

Funding: Government Support - Non-U.S.

SA-PO2483

Coagulation and Complement Cascade Priming Induces NOXA Activation in Renal Ischemia-Reperfusion (I/R) Injury

Background: Renal I/R plays a key role in the pathogenesis of delayed graft function after renal transplantation and is characterized by a cascade of inflammatory events, which are dependent on the pathways of C and T. The coagulation and complement cascades has been suggested to play a pathogenic role in I/R-induced renal damage. Aim of the study was to investigate the activation of NADPH oxidases in a pig model of renal I/R injury focusing on the coagulation and complement systems.

Methods: Renal I/R was induced in 5 pigs by arterial clamping. The NADPH oxidase activity was assessed by chemiluminescence on renal tissue taken before ischemia (T0) and a different time after reperfusion (T15, 30', 60'). NOXA protein expression and fibrin deposition were evaluated by immunohistochemistry. In vitro, NOXA protein expression was assessed by immunoblotting in human proximal tubular epithelial cells (HK2) treated with thrombin (5*10-3/µl) or C3a (5*10-7/M) for different time periods.

Results: NADPH-oxidase activity was significantly increased during reperfusion in a time-dependent manner with a peak at T60 (T0 1.3±0.6, T60 18.5±2.1; p<.03). We observed a significant increase in NOXA protein expression and fibrin deposition (T0 0.1±0.8, T30 18.6±5.1, pixels/total area, p=0.03) and interstitial fibrin (T0 2.2±0.3, T30 10.3±1.5, pixels/total area, p<0.03) and C5b9 deposition (p<0.03), all with a peak at T30. NOXA protein expression and fibrin or C5b9 deposits co-localized at the different time points. In addition we observed a significant and direct correlation between fibrin or C5b9 deposition and NOXA protein expression (r=0.36, p<0.01; r=0.58, p<0.01). In vitro, thrombin and C3a induced NOXA expression in HK2 cells in a time-dependent manner with a peak at T15 (p<0.02).

Conclusions: NOXA is activated during IR; coagulation and complement cascades may play a role in NOXA activation. In conclusion, complement and NADPH oxidase may represent a pharmacological target to prevent oxidative damage during IR injury.

Funding: Government Support - Non-U.S.

SA-PO2484

Role of the Uremic Solute Indoxyl Sulfate on Tissue Factor Production Via Aryl Hydrocarbon Receptor Pathway

Background: Uremic toxins are known to promote inflammation and coagulation. Indoxyl sulfate (IS) is one of the major uremic toxins. The aryl hydrocarbon receptor (ARH) pathway is a key mediator in inflammatory and fibrotic processes.

Methods: Plasma IS and soluble TF (sTF) levels were measured in 72 hemodialysis (HD) patients, 50 undialyzed CKD patients (CKD) and 37 control subjects (controls). We studied in vitro the effect of IS at uremic concentrations on TF production and the cellular pathways involved notably the ARH pathway.

Results: Plasma IS and soluble TF (sTF) levels were measured in 72 hemodialysis (HD) patients, 50 undialyzed CKD patients (CKD) and 37 control subjects (controls). We studied in vitro the effect of IS at uremic concentrations on TF production and the cellular pathways involved notably the ARH pathway.

Funding: Private Foundation Support, Government Support - Non-U.S.
This increased TF production is preceded by increased mRNA levels suggesting an elevated transcriptional activity. sRNA directed against AHR abolished the increase in TF induced by IS.

Conclusions: The increase in sTF in CKD patients is related to renal function and levels of IS. In vitro, IS induces TF production in HUVEC and PBMC, which involves the transcription factor AHR.

As AHR and TF have a demonstrated role in atherogenesis, IS could thereby contribute to the high cardiovascular risk observed in uremic patients.

SA-PO2485

Ergocalciferol Significantly Reduces Markers of Vascular Inflammation in Patients with Chronic Kidney Disease Stage 3-4

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Background: Chronic kidney disease (CKD) causes vascular inflammation and endothelial dysfunction leading to cardiovascular disease (CVD). Newer vitamin D analogues may reduce the burden of CVD in CKD but are expensive and the mechanism of action is incompletely understood. We conducted a pilot, randomised, placebo controlled trial evaluating the effect of ergocalciferol, an inexpensive vitamin D compound, on known markers of vascular inflammation in CKD 3-4.

Methods: Patients with CKD stage 3-4 (n=13) were enrolled. Baseline samples for flow cytometry quantified platelet monocyte aggregates (PMA) and CD 45 microparticles (CD45MP), both markers of vascular inflammation. Patients were randomised to ergocalciferol (n=7), 50,000iu weekly for 4 weeks or placebo (n=6). Patients with diabetes mellitus were excluded to remove the confounding effect of diabetes on the endothelium.

Results: The mean age, eGFR, blood pressure, ethnicity and tobacco use did not differ between treatment groups with CKD. PMA and CD45MP were similar between placebo and ergocalciferol treated CKD patients at baseline (PMA - p=0.036, CD45MP - p=0.41). At 1 month, PMA and CD45MP were significantly lower in the ergocalciferol group (p=0.002 and p=0.007) but unchanged in the placebo group.

Conclusions: Ergocalciferol reduces markers of vascular inflammation in CKD 3-4. These findings may explain the reduction in CVS disease in previous studies of patients with CKD treated with vitamin D compounds but could also reflect the unique effect of ergocalciferol itself. Multi centre clinical studies are required to further evaluate the role of ergocalciferol therapy to reduce CVD in CKD and to better understand its precise mechanism of action.

SA-PO2486

The Correlation between Albumin to Creatinine Ratio and Total Protein to Creatinine Ratio in Patients with Chronic Kidney Disease

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Background: The quantification of urinary protein excretion is important for monitoring of chronic kidney disease (CKD). Random urine total protein-to-creatinine ratio and albumin-to-creatinine ratio have been supposed as alternatives to 24 h urine measurements.

Methods: A total of 808 patients were prospectively enrolled from renal out-patient clinics. We measured albumin-to-creatinine ratio, protein-to-creatinine ratio and urine dipstick test simultaneously in random urines, and we investigated the relation between albuminuria and proteinuria in random urine samples in CKD patients.

Results: Albuminuria was well correlated with proteinuria (β = 1.114 (95% CI 1.061 to 1.166), P < 0.001, R2 = 73.6%). The correlation between albuminuria and proteinuria was significantly better in patients with dipstick albumin positive than those with negative (P < 0.001 for interaction), in patients with urine creatinine level ≥ 60 mg/dL than those with < 60 mg/dL (P = 0.024 for interaction), and in patients with estimated GFR < 60 mL/min/1.73m2 than those with ≥ 60 mL/min/1.73m2 (P = 0.040 for interaction). However, the correlation between albuminuria and proteinuria was not different according to the sex (P = 0.041), status of diabetes mellitus (P = 0.820), and age (≥ 60 years vs < 60 years) (P = 0.706).

Conclusions: The determination of proteinuria or albuminuria in random urine samples, when properly interpreted by taking into consideration the urine concentration, the amount of proteinuria and renal function, could be acceptable for the measure of monitoring of proteinuria or albuminuria.
Methods: We initiated baseline and annual surveillance in 104,129 in-center HD patients for the presence of antibodies to HCV using a screening chemiluminescent immunoassay. Consistent with guidelines, we considered patient specimen sample cutoff ratios of <0.8 to be nonreactive (presumed negative), ratios ≥1.1 to be reactive without recommendation for supplemental testing (presumed positive), and ratios of 0.8 to <1.1 equivocal (0.8 to <1.0) or reactive without recommendation for supplemental testing using recombinant immunoblot assay (RIBA) for confirmation of HCV antibody. RIBA results were reported as positive (confirmatory), negative or indeterminate. From 11/2009 to 4/2011, we tested new patients and those previously found to be HCV negative. Reactivity to nonreactive for HCV, 0.099% showed reactive results ≥1.1 on follow-up surveillance testing. 1.4% underwent supplemental testing for RIBA: of these, 22.2% were confirmed positive; 41.7% were found to be negative; and, 36.1% returned indeterminate results. No previously reactive patient was found to be reactive by RIBA supplemental testing. Among patients previously reactive ≥1.1, 0.078% were nonreactive on follow-up, and 0.223% showed results in the 1.0 to <11 range.

Conclusions: Among HCV-positive patients, we found evidence that HCV screening antibody assays wanes or fluctuates. Since the resulting pre-test probability of new HCV reactive results in previously seropositive patients approaches that of new reactive results in previously seronegative patients, our findings suggest that HCV surveillance in seronegative patients should be conducted no more frequently than annually, and that new positive results in HD patients should be interpreted with caution and case-by-case clinical adjudication.

Funding: Clinical Revenue Support

SA-PO2490
Correlation between the Renal Resistive Index and Histology Steven Grange,1 Fabien Soulis,1 Dominique Guerrot,1 Arnaud Francois,2 Caroline Freguin,1 Michel R. Godin,1 Bruno Legallier.1 Nephrology, Rouen University Hospital, Rouen, France; 2Pathology, Rouen University Hospital, Rouen, France.

Background: Renal interstitial fibrosis (IF) and arteriosclerosis are determinants of CKD progression. Intra-renal arterial resistance index (RI), a simple non-invasive and reproducible ultrasonographic tool, has become increasingly used in CKD patients. Studies evaluating associations of RI with robust histological variables and renal prognosis are lacking. The objective of this prospective study was to analyze correlations between RI and renal histomorphological measurements, and predictive value for CKD progression.

Methods: Patients hospitalized for renal investigation (n = 47) underwent RI measurement (3.5 MHz) in interlobar arteries and own kidney biopsy. Arteriosclerosis, interstitial surface and IF were assessed by standardized morphometric analyses of blinded Sirius Red (SR) stained slides, under normal and polarized light. MDRD eGFR was measured at M0, M12, M24 and M36. CKD progression combined endpoint was defined as ≥10% decrease in eGFR, dialysis requirement or death.

Results: Mean RI was 0.65 ± 0.08. RI was positively correlated with interstitial surface and negatively with eGFR at M0, M12, M24, and M36 (all p<0.05). Interlobar RI was moderately associated with interlobular arteriosclerosis (p=0.09), suggesting that thickening of distal arteries may influence RI. At M36, 14 patients reached the progression endpoint (eGFR decrease: n=5, dialysis: n=5, death: n=4). Compared to stable patients, subjects with CKD progression presented significantly higher RI and interstitial surface but reduced IF (all p<0.05), suggesting that increased RI could be related in this population to interstitial infiltration rather than chronic interstitial deposition of fibrillar collagens.

Conclusions: In CKD patients, resistance index is associated with renal interstitial and arterial lesions. Active interstitial lesions rather than chronic interstitial fibrosis, seems to be a major determinant of high RI in patients with CKD progression, and further suggests the RI predictive potential in this setting.

SA-PO2491
Correlation between the Renal Resistive Index and Histology Steven Grange,1 Fabien Soulis,1 Dominique Guerrot,1 Arnaud Francois,2 Caroline Freguin,1 Michel R. Godin,1 Bruno Legallier.1 Nephrology, Rouen University Hospital, Rouen, France; 2Pathology, Rouen University Hospital, Rouen, France.

Background: The progressive loss of renal allograft function is related to interstitial fibrosis (IF) and arteriosclerosis. Intra-renal arterial resistance index (RI) has become increasingly used in kidney transplantation. Large studies evaluating associations of RI with robust histological variables and the evolution of graft function are lacking. The objective of this prospective study was to analyze correlations between RI and renal histomorphological measurements, and their predictive value for graft function.

Methods: At inclusion 105 kidney transplant patients underwent RI measurement (3.5 MHz) in interlobar arteries, and renal graft biopsy. Arteriosclerosis, interstitial surface and IF were assessed by standardized morphometric analyses of blinded Sirius Red (SR) stained slides, under normal and polarized light respectively. MDRD eGFR was measured at M0, M12, M24 and M36. Progression combined endpoint was defined as ≥10% or ≥20% decrease in eGFR at M12 or M24 respectively, dialysis requirement or death.

Results: 23/36 months post-transplantation. Mean RI was 0.68±0.08. RI was negatively correlated with eGFR at M0, M12, M24, and M36 (all p<0.05). At M24, 30 patients reached the progression endpoint (eGFR decrease: n=10, dialysis: n=18, death: n=2). After exclusion of patients with acute graft failure and/or rejection (confounding factors), a positive correlation was positively correlated with increased IF and arteriosclerosis, and subjects with progressive loss of graft function presented higher RI and
Compared to a cohort of 29 kidney recipients with AMR (all C4d+ and matched for time to rejection), allograft survival in PCR-AR was significantly worse whether they were C4d positive or not.

Conclusions: Plasma cell rich AR has an inferior allograft outcome compared to AMR.

SA-PO2494

Potential Role of Alternatively Activated Macrophages in Chronic Allograft Nephropathy

Yohji Horie,1 Takayoshi Suzuki,2 Tamaki Karasawa,3 Hiromi Hasegawa,1 Masanori Hara,2 Toshio Yanagihara,2 David J. Nikolic-Paterson,3 Makoto Uchiyama.1 1Department of Pediatrics, Niigata University Medical and Dental Hospital; 2Department of Pediatrics, Yoshida Hospital, Niigata-City, Japan; 3Monash University Department of Medicine, Monash Medical Centre, Clayton, Victoria, Australia.

Background: Intestinal fibrosis is an important mechanism in chronic allograft nephropathy (CAN). Macrophages with an alternatively activated phenotype (M2) have been implicated in promoting fibrosis in non-inflammatory kidney diseases, including cyclosporine toxicity. This study examined the possible involvement of M2-type macrophages in CAN.

Methods: A total of 30 biopsy sections from 14 children who underwent kidney transplantation were stained for α-smooth muscle actin (αSMA), CD68 (total macrophages), CD163 (M2 marker) and CD3 (T cells). Urinary CD163 excretion was assessed by ELISA.

Results: None of the patients examined had a history of clinical symptoms of rejection. At the time of biopsy, all patients were on maintenance immunosuppression therapy, including methylprednisolone, mycophenolate mofetil, cyclosporine and/or tacrolimus. Immunostaining identified a significant increase in intestinal fibrosis with accumulation of CD68+ macrophages. Dual immunofluorescence staining showed that most intestinal CD68+ macrophages also expressed CD163, indicating an M2 phenotype. CD163+ cells were frequently localized to areas of intestinal fibrosis and there was a significant correlation between the number of intestinal CD163+ cells and the degree of intestinal fibrosis (r=0.71, p<0.0001), and between intestinal fibrosis and estimated GFR (r=-0.79, p<0.0001). There was also a significant correlation between interstitial fibrosis and the time from transplantation to biopsy. Although CD3+ T cell infiltration was also observed in some patients, there was no relationship with macrophage accumulation or fibrosis. Urinary CD163 levels correlated with the number of intestinal CD163+ cells (r=0.51, p=0.01).

Conclusions: Our findings identify a major population of M2-type macrophages in patients with CAN, and suggest that these M2-type macrophages may promote the development of intestinal fibrosis in CAN.

Funding: Government Support - Non-U.S.

SA-PO2495

Peritubular Capillaritis in Renal Biopsies of Early Acute Rejection Episodes

Hanneke De Kort,1 Marijan C. Van Groningen,1 Marko J. Mallat,1 Natalsha Goemaere,2 Johan W. De Fijter,1 Jan A.Brujin,1 Ingeborg M. Bajema.1 1Leiden University Medical Center; 2Pathan Foundation Rotterdam.

Background: A consensus for the ptc-score was reached at the Banff Conference in ’07, although it was emphasized that it did not equate with any specific diagnosis, and ongoing reproducibility and diagnostic studies were required. The ptc-score involves the scoring of cortical peritubular capillaries for capillaritis (defined as luminal inflammation by neutrophils, monocytes/macrophages, and lymphocytes). We assessed clinical relevance and inter-observer agreement of the ptc-score.

Methods: We reviewed all 723 patients who had a renal transplant in our center from 95-06. 122 patients who had a histologically proven first acute rejection episode within 6mo after transplantation according to the Banff ’08 working proposal for classification were included. Mean follow-up was 7.3yrs (±3.9). In Kaplan-Meier survival analyses, time to graft failure and patient death were assessed for the combined ptc grades 0&1 versus 2&3 and fibrin versus mixed ptc-infiltrate. All Banff components were scored by 2 pathologists. Ptc-score was according to the Banff ’07 report and scored by 2 pathologists independently.

Results: The inter-observer agreement on several aspects of the ptc-score gave poor to fair results. The dichotomized ptc-score gave a fair x of 0.231 (p=0.001) in identifying peritubular capillaries, especially in areas with vast infiltrates, probably lie at the basis of the inter-observer discrepancy. Ptc-grading was associated with interstitial infiltrate (OR 2.9 [CI 1.1-7.6], p=0.029) and the total inflammation-score (OR 7.5 [CI 2.7-20.3], p<0.001). Ptc grading did not correlate with C4d staining (performed on frozen tissue samples). Ptc grading did not predict for graft outcome. However, both ptc grades 2&3 and a mixed composition of the infiltrate were associated with worse patient survival.

Conclusions: In this study, ptc correlates with interstitial and total inflammation score, and in itself, correlates with worse patient survival. To improve the applicability in clinical pathological analyses, we propose a reduction in the components of the ptc grading scheme.

SA-PO2496

Morphometric Quantitation of Interstitial Fibrosis and Peritubular Capillary Density in Renal Allograft Biopsies with Transplant Gliomerulopathy

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Background: Loss of peritubular capillaries (PTCs) is considered to play an important role in chronic antibody-mediated rejection (AMR) of renal allografts. However, the precise relationship between the severity of interstitial fibrosis (IF) and the degree of PTC loss has not been well-established. The objective of our study is to evaluate the relationship of PTC density and the degree of IF in C4d-positive and C4d-negative renal allograft biopsies with chronic AMR in the form of transplant glomerulopathy (TGP).

Methods: C4d-positive (n=12) and C4d-negative (n=21) renal allograft biopsies with TGP were examined in this study. Biopsy sections were stained with a mixture of anti-C3d and anti-collagen type III primary antibodies followed by incubation with a mixture of fluorescein isothiocyanate (FITC)-labeled and Texas Red-labeled secondary antibodies. Digital images of multiple cortical fields were taken from each double-stained slide at 10x magnification under immunofluorescence microscopy. The images were processed using the ImageJ software to calculate the cortical fractional area of collagen and CD34 positivity. Glomeruli and large vessels were excluded from the analysis.

Results: Strong and specific staining for CD34-positive PTCs and interstitial collagen was achieved. Comparing cortical fractional area of collagen positivity to CD34 positivity for C4d-negative and C4d-positive cases indicated a correlation coefficient of -0.84 and -0.67, linear regression slope of -0.58 and -0.39, and R-squared values of 0.70 and 0.45, respectively. A few C4d-positive biopsy samples show disproportionately low PTC density, which contributed to a less steep slope by linear regression and a lower correlation coefficient and R-squared value.

Conclusions: Morphometric analysis using immunofluorescence staining for collagen type III and CD34 provides precise quantitative measurements of IF and PTC density, respectively. In allograft biopsies with TGP, there is an inverse relationship between IF and PTC density independent of C4d status.

SA-PO2497

Histologic Assessment of Renal Transplant Biopsies Using Whole Slide Digital Images

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Background: Whole slide digital image technology allows histologic evaluation of pathologic specimens on digitally scanned slides for diagnostic purposes. However, there are no data documenting the feasibility of such technology in the field of renal transplant (Tx) pathology. The purpose of our study was to assess the diagnostic accuracy of evaluating digital whole slide scans of kidney Tx biopsies as compared to conventional microscopic evaluation of glass slides.

Methods: Twenty-five kidney Tx biopsies demonstrating a spectrum of pathologic changes were evaluated by four well-trained renal pathologists. Each case included one H&E and one PAS-stained section, which were scanned using the Aperio ScanScope Scanner. Each pathologist evaluated each case twice, on separate occasions: once using a microscope to view the glass slides and once using the digital scans viewed on a computer monitor. The cases were coded to minimize bias. Thirty-one pre-specified features, most of which are Banff scores, were categorically scored by each pathologist for both the conventional and digital scans. Intra-observer reliability was assessed using linearly-weighted Cohen kappa coefficient. Inter-observer reliability for conventional glass slides was evaluated using linearly-weighted Fleiss kappa coefficient.

Results: Mean intra-observer kappa coefficients (range from 0.59 to 0.94) were generally higher than inter-observer kappa coefficients (range from 0.16 to 0.66) for thirty features evaluated. This finding indicates that intra-observer reliability in scoring morphologic features is higher than inter-observer reliability. Detection of oxalate crystals was suboptimal on digital slides. The average time spent on examining digital scans was approximately 1.5 times greater than that spent on conventional glass slides.

Conclusions: Digital scans of histologic sections for the evaluation of renal Tx biopsies appear to be a reliable approach for diagnostic purposes. One significant drawback of digital scans is that they are more time-consuming for pathologists to fully evaluate.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
SA-PO2498

Pediatric Kidney Transplantation into Adults; a Biopsy Review

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Background: Although the good clinical outcome of single pediatric-donor-graft (PedDG) renal transplantation has been shown at several institutions, including Tulane (Zheng et al. Clin J Am Soc Nephrol, 2009), there are only scant descriptions of the post-transplantation graft biopsy (bx) in these recipients.

Methods: During a period of 11 years (1998-2009), single kidneys from donors aged 1 to 9 years were transplanted into 105 recipients.

Results: To investigate the graft pathology in PedDG, we studied the 54 PedDG recipients who had 101 “for cause” graft bx, obtained between 3 and 5840 post transplant days. As control, we randomly selected 50 adult-donor-graft (ADG) recipients with 79 “for cause” bx during the same period. Immunohistochemistry (IF) had been performed in 39 PedDG and in 47 ADG bx.

Conclusions: Detailed study of a series of 180 “for cause” post transplantation renal bx shows a statistically significant increased incidence of Ig mediated GN in recipients of single PedDG as compared to ADG.

SA-PO2499

Extracapillary Proliferation as an Independent Predictive Factor in IgAN

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Background: The predictive value of the Oxford classification histological lesions in IgAN was previously validated; attention has been placed on its predictive value of the decline of renal function. The aim of our work was to correlate active glomerular lesions at biopsy and progression of renal damage.

Methods: We have studied 473 renal biopsies with diagnosis of IgAN; of these, 184 had availability of clinical data at follow-up (eGFR and eGFR by CKD-EPI formula) up to a maximum of 25 years. The median age at diagnosis was 36.7 years; 70% of patients were males. Histological parameters were from the Oxford classification (mesangial and endocapillary proliferation, segmental glomerulosclerosis, tubular atrophy, extracapillary proliferation, interstitial fibrosis) and in addition glomerular fibrinoid necrosis. Data were analyzed by univariate and multivariate investigation, according to linear regression of longitudinal data, taking into account the distance between time of biopsy and time points of acquisition of clinical data.

Results: Statistical analysis showed a correlation between progression of renal damage (eGFR) and segmental glomerulosclerosis (p<0.001), cellular crescents (p<0.01), fibrinous crescents (p=0.02), fibrinoid necrosis (p=0.04) and interstitial fibrosis (p<0.03); no correlation was evident with fibrocellular crescents and endocapillary proliferation.

Conclusions: Our preliminary results suggest that active glomerular lesions, such as cellular crescents and fibrinoid necrosis, correlate with decline of renal function, differently from the Oxford classification. These histological parameters should therefore be taken into account to classify histologically cases of IgAN, and for the appropriate treatment.

SA-PO2500

Red Blood Cell (RBC) Transfusion Rates among Young, Commercially Insured Chronic Kidney Disease (CKD) Patients

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Background: The rate of RBC transfusions among older CKD patients not on dialysis has been estimated at 15-25%, but there is scant data among younger CKD patients.

Methods: We used data from the Ingenix medical claims database, a large, geographically diverse database of ~40 million commercially insured US individuals (2002-2008). We identified yearly cohorts of patients 18-64 years of age diagnosed with CKD; we required patients to have 1 yr of data available before diagnosis to characterize each cohort. We followed each cohort for 1 yr to estimate the RBC transfusion rates, using Poisson regression.

Results: We identified 144,100 CKD patients (range: 15,565 in 2002 up to 28,013 in 2008); 57% were 50-64 years of age; 46% were female. Comorbidities were common: hypertension (54%), diabetes (33%), hyperlipidemia (31%), and coronary artery disease (18%), and 23% were diagnosed with anemia. Overall, the transfusion rate was 4.0/100 person-years (PYs) (95% CI: 3.9-4.1). Rates were noticeably higher among those with diagnosed anemia (14.1/100 PYs [95% CI: 13.6-14.5]) and were highest among those who progressed to end stage renal disease (ESRD) (50.1/100 PYs [95% CI: 26-35]).

Conclusions: RBC transfusions are not uncommon among young, commercially insured anemic CKD patients, particularly those transitioning to ESRD. The risk of alloimmunization and its effects on renal transplantation needs to be considered in patients who are likely to transition to ESRD.

Funding: Pharmaceutical Company Support
Conclusions: Our data suggest that the earl y use of RTX as adjuvant induction therapy may improve short term renal survival. Confirmation of its efficacy and safety in children will require controlled treatment trials.

SA-PO2502
Clinical Impact of LDL-C to HDL-C Ratio in Association with CKD
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Background: Chronic kidney disease (CKD) has been increasingly highlighted as a serious risk factor for cardiovascular diseases (CVD). Dyslipidemia plays important role in both CKD and CVD onset and progression throughatherosclerosis. Low-density lipoprotein cholesterol (LDL-C) to high-density lipoprotein cholesterol (HDL-C) ratio (LHR) was reported as promising predictor for CVD and mortality, but it has not been fully elucidated in association with CKD. In this study, we analyzed the relationship between the LHR and CKD.

Methods: The study subjects were 3,983 men and 3,742 women, who underwent health check in Kasugai City Medical Care Center in 2008. Patients under treatment for dyslipidemia were excluded from the study. Estimated gFR (eGFR) was calculated by the Japanese eGFR equation, and cases with eGFR less than 60 mL/min/1.73m² and/or with proteinuria were diagnosed as CKD. LDL-C and HDL-C were measured directly by enzymatic method and LDL-C was divided by HDL-C for LHR. Multivariate odds ratios of LHR (≥3.5), LDL-C (≥140 mg/dL) and HDL-C (≤40 mg/dL) were calculated for CKD using logistic regression adjusted for age and sex. Average eGFR values were compared in the quartile for LHR, LDL-C and HDL-C.

Results: Among CKD subjects, LHR and LDL-C were significantly higher and HDL-C was significantly lower compared to non-CKD. Odds ratios for CKD increased significantly as LHR category advanced. Odds ratio for CKD

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Multivariate analysis revealed that LHR but LDL-C or HDL-C was a significant risk factor for CKD. In quartile analysis, average eGFR values (mL/min/1.73m²) decreased in order for LHR in the quartile for LHR, LDL-C and HDL-C.

Conclusions: LHR revealed stronger association with CKD than LDL-C or HDL-C, suggesting the importance of well-balance of LDL-C and HDL-C to prevent arteriosclerosis.

Funding: Private Foundation Support

SA-PO2503
Increased Risk of Kidney Cancer in Young Men with Moderately Impaired Renal Function after a Long Follow-Up in a Large, Population-Based Cohort
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Background: There are reports that impaired renal function increases the risk of cancer. We evaluated whether moderately impaired renal function influenced risk of developing cancer in a large, population-based cohort.

Methods: We used data from Malmö Preventative Medicine Project, a large, population-based cohort (94% participation) containing demographic data and blood samples collected from 33,346 persons aged 33-50 during 1974-86 in Malmö, Sweden. Incident cancers were identified from the Swedish Cancer Registry, updated to 12/31/2006. Median follow-up time was 28 years. Glomerular filtration rate (GFR) was estimated using the Modified Diet and Renal Disease formula. Patients were classified as having normal kidney function and mild kidney disease (GFR >60 m L/min/1.73m²) for both males and females. 6,595 participants were diagnosed with cancer. The median age at baseline was 47 (IQR 40, 49), 22,183 (69%) participants were male; prostate cancer was the most common cancer diagnosis (1,379). Most subjects had mild kidney disease (GFR ≥60 mL/min/1.73m², 27% (n=9,621) had normal renal function and 1242 subjects GFR <60 mL/min/1.73m². We found no significant increase in cancer incidence among those with GFR <60 vs ≥60 mL/min/1.73m². There was an increased risk for kidney cancer in young men with <60 vs ≥60 mL/min/1.73m² (SHR: 2.38; 95% CI: 1.05; 5.44; p=0.039).

Conclusions: Among young men we found an increased risk of kidney cancer in those with moderately impaired renal function after a long follow-up.

SA-PO2504
Cost-Effectiveness of Statins for Primary Cardiovascular Prevention in Chronic Kidney Disease: Keying Friberg,1,2 Sohan Japa,1 Arjun S. Adhikari,1,2 Joshua Glucoft,3 Glenn M. Chertow,1,4 Alan M. Garber,2 1Nephrology, Stanford University School of Medicine, Palo Alto, CA; 2Center for Primary Care and Outcomes Research, Stanford University, Stanford, CA.

Background: Patients with chronic kidney disease (CKD) have a high risk of myocardial infarction (MI) and stroke compared to the general population. Although statins are effective at preventing cardiovascular (CV) disease in patients with CKD, guidelines give conflicting recommendations about statin use in CKD. This study assesses the cost-effectiveness of statins for primary prevention of cardiovascular disease in patients with hypertension and CKD.

Methods: A decision analytic model was constructed to evaluate the costs and benefits of statin use in preventing MI and stroke in patients with CKD, without CKD, and with varying assumptions about CKD progression. An increased absolute risk reduction from statins in CKD was modeled along with higher costs, increased morbidity and mortality, increased incidence of adverse events, and varying treatment effects associated with CKD. A separate micro-simulation model determined progression between CKD stages. The primary outcome was the incremental cost-effectiveness ratio (ICER) associated with statin therapy.

Results: In the base case, a 65 year old male with hypertension and stage 3 CKD, statin therapy increased costs by about $5,000 and led to a gain of 0.10 QALYs. The ICER from statins was about $50,000/QALY in the base case, similar in magnitude to the cost-effectiveness estimated from an identical cohort without CKD. Statins were more cost-effective in patients whose CKD did not increase in severity.

Conclusions: Statins can lead to large absolute reductions in CV disease in CKD patients, due to their high underlying risk of cardiac events. These gains are partially offset by an elevated risk of adverse events and decreased length and quality of life CKD patients can expect, independent of CV disease. The cost-effectiveness of Statins in CKD is sensitive to CKD progression, suggesting Statins would be cost-effective at a willingness to pay threshold of $50,000/QALY in a wide range of patients with stable (i.e., non-progressive) CKD.

Funding: Other NIH Support - Agency for Healthcare Research and Quality

SA-PO2505
Association of Depression, Severity of Non-Dialysis Dependent CKD and Mortality in US Veterans Rasheed A. Balogun,1 Emaa M. Abdel-Rahman,2 Seki A. Balogun,2 Evan H. Lott,2 Jun Ling Lu,2 Sandra M. Malakaukskas,2 Jennie Z. Ma,1 Mark D. Okusa,1 Kamiyar Kalantar-Zadeh,1 Csaba P. Kov Escy,2,3 1U of Virginia; 2VA In&Com; 3Harbour-UCLA.

Background: Depression (D) is known to be associated with higher mortality in ESRD patients. Less information is available in earlier stages of CKD.

Methods: We examined association of D with all-cause mortality in 657,614 US veterans with CKD stages 1-5. Associations of D with all-cause mortality and stratified by CKD stages were examined with Kaplan Meier method, in fixed effect and time-dependent Cox models. Models were adjusted for sociodemographics, comorbidities, blood pressure and laboratory variables.

Results: Patients were 73,989±9.8 yrs, 97% male and 71% white. There were 37,032 patients (5.6%) with D (67.1±11.3 years, 93% males and 71% white, with eGFR 54±17 mL/min/1.73m²) in 2005-2006. D was more common in patients with CKD 1 (13%) than in patients with CKD 2 (9%), 3A (6%), 3B (4%) and 4 (4%) and 5 (4%). Patients with D were younger, more likely to be female, not married, lack insurance and had higher prevalence of comorbidities. During a median follow-up of 4.8 yrs, 189,561 patients died (mortality rate, 95% CI: 67.3/1000 patient yrs, 67.0-67.6). D was associated with higher age-adjusted mortality (Hazard ratio (95% CI): 1.31 (1.30-1.32) p<0.001, which was, however, attenuated after multivariable adjustments (1.00, 0.97-1.03 p<0.9).

Funding: Other NIH Support - Agency for Healthcare Research and Quality

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.
**SA-PO2506**

**Abnormal Nitric Oxide Metabolism, Prothrombic State, Impaired Angiogenesis and Risk of Chronic Kidney Disease**


**Background:** Endothelial dysfunction is frequently observed in patients with chronic kidney disease (CKD). The underlying mechanism is not fully understood.

**Methods:** We investigated the association of endothelial dysfunction biomarkers in the nitric oxide pathway [asymmetric dimethylarginine (ADMA), and L-arginine], thrombosis [von Willebrand factor (vWF)], and angiogenesis (endostatin) with the risk of CKD in 201 controls without CKD and 201 patients with CKD.

**Results:** Compared to those without CKD, patients with CKD were older (56 vs. 53 yrs), more likely to be male (55% vs. 45%), less likely to have high school education (59% vs. 82%), or consume alcohol (28% vs. 59%). Race and cigarette smoking were comparable between CKD patients and controls. Mean systolic blood pressure (132 vs. 122 mmHg), body mass index (32 vs. 29 kg/m²), fasting glucose (120 vs. 103 mg/dL), history of hypertension (88% vs. 24%), history of diabetes (49% vs. 6%), and CVD (44% vs. 7%) were higher while LDL cholesterol (102 vs. 118 mg/dL) was lower in CKD patients than in controls. After adjustment for above risk factors, the median [inter-quartile range(IQR)] of ADMA [0.58 µmol/L (IQR, 0.55-0.62) vs. 0.27 µmol/L (IQR, 0.27-0.30), p=0.0001], L-arginine [71.39 µmol/L (IQR, 67-06.75-73) vs. 33.08 µmol/L (IQR, 28.77-37.38), p=0.0001], vWF [1321.29 mU/mL (IQR, 1242-02-1398.57) vs. 1157.54 mU/mL (IQR, 1080.53-1234.55), p=0.01], and endostatin [277.15 ng/mL (IQR, 259-55-294.76) vs. 142.18 ng/mL (IQR, 124-63-159.73), p=0.0001] were higher in CKD patients than in controls.

**Conclusions:** These data indicate that abnormal nitric oxide metabolism, prothrombic state, and impaired angiogenesis are associated with risk of CKD.

**Funding:** Other NIH Support – The National Center for Research Resources

**SA-PO2507**

**Untreated Bacteriuria, Cardiovascular Events, and Death among Female Patients with CKD**

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**Background:** Untreated bacteriuria may trigger local and systematic inflammatory responses. This study examined the relationship between untreated UTI and de novo CV events and death among female patients with CKD.

**Methods:** A retrospective cohort of female patients receiving care between 2004 and 2010 at a tertiary health care system in Central Pennsylvania and with stages 1-4 CKD was established. Patients with a prior history of MI, CHF, or stroke were excluded. Study exposure was a positive urine culture (bacterial growth of ≥ 100,000 CFU/ml) without an antibiotic prescription within 90 days of culture order. Adjusted Cox models were developed to estimate the association between infection (treated and untreated) and time to first CV event (composite of MI, CHF, and stroke), and time to death. Models account for cumulative episodes of UTI during follow up.

**Results:** 6807 patients (81.8% without UTI, 7.9% with only untreated UTI(s), 7.6% with only untreated UTI(s), 2.7% with both treated and untreated UTIs) met criteria. Median (IQR) follow up was 5.2 (3.4, 9.5) years. Mortality rates were 22.8, 29.9, and 44.3 deaths/1000 PY in the no-UTI, treated-UTI, and untreated-UTI groups, respectively.

Cox PH models for time to death and first CV event, by treatment status

<table>
<thead>
<tr>
<th>Death</th>
<th>HR (95% CI)*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>Treated UTIs</td>
<td>1.31 (1.03, 1.66)</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>Untreated UTIs</td>
<td>1.94 (1.57, 2.38)</td>
</tr>
<tr>
<td>Fully Adjusted</td>
<td>Treated UTIs</td>
<td>0.83 (0.63, 1.05)</td>
</tr>
<tr>
<td>Fully Adjusted</td>
<td>Untreated UTIs</td>
<td>1.42 (1.15, 1.76)</td>
</tr>
</tbody>
</table>

*Reference group: no UTI (n = 5559). **Model adjusted for age, race, CKD stage at baseline, Charlson score, hypertension, diabetes, hypercholesterolemia, peripheral vascular disease, history of cancer, COPD, connective tissue disease, and hospitalization during follow up.

**Conclusions:** Untreated UTIs among female patients with CKD may increase the risk of CV events and death.

**SA-PO2508**

**Serum Creatinine Is Associated with Carotid Intima-Media Thickness in Males:**

Research, Geisinger Medical Center; University Medicine Greifswald, Nephrology, Germany; University Medicine Greifswald, Cardiology, Germany; University Medicine Greifswald, Institute of Epidemiology and Social Medicine, Germany.

**Background:** Chronic kidney disease (CKD) remains asymptomatic until its late stage. There is little information on the association of renal function with subclinical atherosclerosis as depicted by carotid intima media thickness (cIMT). The aim of the present study was to examine the association of cIMT with various serum markers of renal function in a large population based study.

**Methods:** The Study of Health in Pomerania (SHIP) is a cross-sectional, population based (KORA, German for the North East of Germany). Data from 2367 individuals (1199 males) aged 45 years or older were available for analysis. Creatinine clearance was calculated by the Cockcroft-Gault, Jelliffe, Wright and the abbreviated Modification of Diet in Renal Disease formulae. Women and men were analyzed separately.

**Results:** A total of 240 males (20.0%) and 244 females (20.9%) showed serum creatinine values above the normal range. Males and females with high serum creatinine had higher serum HbA1C levels, pulse pressure, BMI, and serum uric acid levels. After adjusting for confounding factors, multivariable analysis revealed that in males, raised serum creatinine levels were independently associated with carotid IMT (non-standardised ß-coefficient 0.0274, 95% CI 0.005-0.050, p= 0.018). However, we could not find an association for raised serum creatinine levels in women nor for reduced values of calculated creatinine clearances in either sex.

**Conclusions:** We found a gender-specific relation between impaired renal function measured by serum creatinine and cIMT. With rising serum creatinine, the exponential relationship between creatinine and cIMT leads to relatively higher increases in serum creatinine for each ml. loss of GFR and thus creatinine in already established renal failure may be more sensitive biomarker for renal function than eGFR.

**SA-PO2509**

**The Association of Stage of CKD and Hemoglobin A1C in a Diabetic Primary Care Population**

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**Background:** Hemoglobin A1C (A1C) levels decrease as estimated glomerular filtration rate (eGFR) declines. Few studies have detailed the decline in glycemic control as a function of chronic kidney disease (CKD) stage. We determined differences in baseline A1C values as a function of CKD stage.

**Methods:** The Pathways Study is a prospective cohort study of diabetic patients at a large managed care health maintenance organization. Baseline laboratory data were extracted from linked automated records. eGFR was determined using the modified equation by Levey. Stage of CKD as defined by the Kidney Disease Outcomes Quality Initiative (KDOQI) guidelines by the National Kidney Foundation and stage of CKD were determined by patient t-test and linear regression. Association of A1C <6.5% with stage of CKD was determined by multivariable logistic regression.

**Results:** Of the 4842 subjects enrolled in the study, 3999 had baseline laboratory data sufficient to be included. By stage of CKD, 14% were stage 2, 20.0% stage 3, 10.9% stage 4, and 1.5% were stage 5. Those with late stage CKD were more likely to be older, married, African American, and to have been hospitalized in the prior year. Mean A1C levels were 7.6±1.6, 7.9±1.6, 8.1±1.5, 7.8±1.5, 7.5±1.4, and 6.4±1.4, for stages 0, 1, 2, 3, 4, and 5 respectively. Differences were significant between stages 0 to 1, 1 to 2, and 4 to 5 (p<0.02). A1C levels increased in stages 1 (β=-0.31, 95% CI=0.17, 0.45) and 2 (β=-0.27, 95% CI=0.10, 0.42), while they decreased in stages 4 (β=-0.40, 95% CI=-0.78, -0.02) and 5 (β=-1.08, 95% CI=-2.57, -1.03). Stage 5 CKD was associated with a 17-fold odds of A1C <6.5 (OR=17.74, 95% CI=5.54-56.76).

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Among subjects with diabetes, A1C decreased after stage 3 CKD and was significantly below recommended guidelines by stage 5 CKD. CKD influences level of glycemic control and may increase the risk of hypoglycemic reactions among diabetic patients with CKD.

Funding: NIDDK Support, Other NIH Support - NIMH, Veterans Administration Support

SA-PO5210

Accuracy of Race Imputation Using the BISG Method and Applicability for CKD


Background: The use of existing health plan data for studies on racial and ethnic differences is often limited by missing race data. Methods of imputation using have rarely improved: the Bayesian Improved Surname Geocoding (BISG) uses US Census block group and surname to predict race, and can be modified for use of additional inputs. We tested the accuracy of BISG imputation against existing administrative health plan data and compared actual and imputed race for predicting differences in serum creatinine, which is useful for estimating GFR in CKD epidemiology.

Methods: Participants were adult members of Southern California Kaiser Permanente with an outpatient serum creatinine test from 1998-2006 (N = 1,585,983). Race data was available in 70.6% of participants. The accuracy of individual race imputation was tested using AROC. Racial differences in the first available serum creatinine were assessed using linear regression on log(serum creatinine) with adjustment for age and gender (Table 1).

Results: Race group AROC (prevalence %) were: White: 0.90 (34); Hispanic: 0.93 (22); Black: 0.94 (9); Asian & Pac. Isl. 0.90 (5.5); Native Amer: 0.6 (0.1); Multiple: 0.62 (0.1).

Conclusions: The BISG method was accurate for higher prevalence race groups. The imputation race was very bad in our settings, which is especially helpful for estimating GFR. Missing data can be eliminated, which helps determine complete population disparities. While not a substitute for measured race, BISG is a promising method for obtaining information on racial differences in CKD from existing health plan datasets.

Funding: Clinical Revenue Support

SA-PO5211

Obesity Is a Risk Factor for End-Stage Renal Disease and Mortality in Pre-Dialysis Patients

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Background: Obesity is associated with increased mortality and decline in renal function in the general population. In contrast, obese dialysis patients may have an improved survival, so the called “obesity paradox”. The aim of this study was to assess whether obesity is a risk factor for end-stage renal disease (ESRD) and mortality in chronic kidney disease stage 4-5 pre-dialysis patients with an estimated GFR <30 ml/min/1.73 m2 not yet on dialysis.

Methods: In a prospective multi-center cohort study incident pre-dialysis patients (>18 y) were included when referred to a specialized pre-dialysis outpatient clinic between 2004 and 2011 and followed until start of dialysis, transplantation, death or January 2011. We used body mass index (BMI) measured at baseline as an index of adiposity. Patients were stratified into four categories of baseline BMI: <20, 20-25 (reference), 25-30, and >30 (obesity) kg/m2.

Results: A total of 473 patients were included (mean:SD): age 65y (14), men 68%, BMI 27 (6) kg/m2, median follow-up time 1.15 years, 365 patients completed the study: 9% and 64% started dialysis. The combined endpoint was defined as ESRD for which dialysis was needed or death on pre-dialysis care. The combined endpoint rate (95%-CI) 100y per BMI category were: 29.6 (17.4-45.4), 29.6 (24.4-35.3), 42.6 (64.6-76.2) and 46.4 (38.8-54.3).

Conclusions: In conclusion, pre-dialysis patients with obesity compared with patients with a normal BMI, have a 50% higher risk of ESRD or death on pre-dialysis care.

SA-PO2512

Predictive Role of Serum Cystatin C on Survival in Cardio-Surgery Patients

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Background: Pre-operative renal dysfunction is a known risk factor for morbidity and mortality in cardio-surgery patients. Serum Cystatin C (sCysC) is a well recognized marker of early renal dysfunction, and previous studies suggest a sCysC predictive role for cardiovascular events in the general population. To assess predictive value of pre-operative sCysC on long-term mortality in adult cardio-surgery patients.

Methods: Between November 2005 and March 2007 421 consecutive patients aged 67,72±10,76 years were admitted in our cardio-surgery department. (217 for coronary artery bypass graft, 150 for valvular prosthetic surgery, 54 for other surgical procedures). We conducted a prospective observational study evaluating all causes of long-term mortality till December 2009. At admission sCys C was dosed in all patients (normal 0.5-0.92mg/L).

Results: Patients were stratified in quartiles according to sCysC: Q1 sCysC <0.81mg/L (29 patients), Q2 sCysC 0.81-0.92mg/L (81), Q3 sCysC 0.93-1.10mg/L (29) and Q4 sCys C >1.10mg/L (282 patients). Kaplan–Meier cumulative survival curves were plotted for sCysC quartiles. Patients’ cardiovascular mortality was the primary endpoint of the study.

Results: One-hundred twenty four patients (29.4%) reached the study end-point. Q3 and Q4 patients (67 and 66%) 2year actuarial survival showed a significantly higher cumulative mortality compared to Q1 and Q2 patients (100 and 85%, respectively) (p=0.0007).

Conclusions: Serum Cystatin C may be considered a good predictor of long-term cardiovascular mortality in cardio-surgery patients.

SA-PO2513

Estimated GFR, Albuminuria, and Risk of Fracture Hospitalization in the Atherosclerosis Risk in Communities (ARIC) Study

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Background: Few studies have examined the association between the full range of kidney function and bone fractures. We examined the relationship of estimated glomerular filtration rate (eGFR) based on plasma cystatin C (eGFRcys) and albuminuria with incident fracture hospitalizations in the ARIC Study. The eGFR estimate based on cystatin C was used because it has a linear relationship with other adverse outcomes.

Methods: Plasma cystatin C and urine albumin/creatinine ratio (ACR) were measured in samples collected during 1996-98 (ARIC visit 4). Hospital record surveillance ascertained incident fracture hospitalizations through 2008. Cox proportional hazards models were used to estimate the linear associations of eGFRcys and ACR with fracture hospitalization.

Results: The median ACR was 3.73 (IQR 5-95 percentile 0.59-63.9 mg/g and the mean eGFRcys was 80 ml/min/1.73m2 (SD 20). During a median of 11 years of follow-up, 572 fracture hospitalizations occurred among 11,138 study participants who were aged 53-75 at visit 4. Each eightfold higher ACR was associated with a hazard ratio (HR) of 1.30 (95% CI 1.16-1.46), after adjusting for age, sex, race, cholesterol level, diabetes, history of cardiovascular disease, smoking status and systolic blood pressure.

Conclusions: Kidney damage, as reflected by microalbuminuria or moderately decreased GFR, is associated with increased risk of fracture in this community-based cohort.

Funding: Other NIH Support - National Heart, Lung, and Blood Institute contracts

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Underline represents presenting author.

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SA-PO2514
Baseline Characteristics and Therapeutic Projects of Patients Older Than 75 Years with MDRD below 20. PSPA Investigator Group (Olivier Moranne, 1 Cécile Couchoud, 2 Vincent L.M. Esnault, 1,3,4 Nephrology & Public Health, Hospital, Nice, France; Metropolitan; 2 REIN Registry, Biomedicine Agency, Saint Denis La Plaine, France; 3 University of Nice Sophia-Antipolis, France; 4 Division of Renal Disease and Hypertension, CHU Toulouse, France; CNRS/UMR 6061Université de Rennes 1, France.

Background: The Incidence of End Stage Renal Disease (ESRD) is growing in patients older than 75 years. Pre-ESRD clinical characteristics, care pathways nor outcome are well described in this population. Therefore, we set up a cohort study in this population and report the baseline characteristics.

Methods: PSPA is a multicenter prospective cohort study including patients older than 75 years with MDRD < 20 without renal therapy (RR) and followed by nephrologists. 586 patients were included in 23 nephrology departments in France. Socio-demographic data, comorbidities, mobility, laboratory tests, medical treatments and therapeutic projects were collected for each patient.

Results: Baseline characteristics were as follow: mean age 82 ± 5 y/o, MDRD: 13 ± 4 mL/min/1.73m², 58% men, 36% diabetes, BMI 26 ± 5 kg/m², SBP 145 ± 22, DBP 74 ± 11 mmHg, prot/creat/ratio 1 [0.1-2.0] g/g. Other comorbidities were: 31% congestive heart failure, 22% peripheral vascular disease, 10% active malignancy and 5% dementia. Most of the population was home resident (85%) and 80% could walk without help. Treatments included a median of 11 oral daily drug intakes including ACE inhibitors (44%), ESA (32%). Mean hemoglobin level was 12 ± 7 g/dL. The therapeutic projects were distributed as follows: 17% were under discussion, 43% had no planned RRT because of stable kidney function, 24% had planned RRT and 16% were not considered for RRT (half of the time by the nephrologist). The most frequent criteria for no indication for RRT by the nephrologist were dementia or cognitive dysfunction, active malignancy or denutrition and for patients older age and women.

Conclusions: In this population few patients are planned for RRT and many are considered as having a stable kidney function. Patient characteristics are different when RRT is not considered by patients or nephrologists. This cohort will be followed for 4 years.

Funding: Biomedicine agency, France, Pharmaceutical Company Support

SA-PO2515
Evaluation of a Public Health Campaign To Increase Kidney Health Awareness in a Canadian Province Krista S. Ryz, 1 Mauro Verrelli, 1 Jan Schneider, 2 Amie C. Lesyk, 3 Manish M. Sood, 4 Claudio Rigatto, 1 Paul Komenda, 1 1Dept. of Medicine, University of Manitoba, Winnipeg, MB, Canada; 2Manitoba Renal Program, Winnipeg, MB, Canada.

Background: To evaluate the effectiveness of a Canadian province wide public health campaign in raising awareness of risk factors that can lead to kidney disease and the ability of the campaign to encourage behaviours that lead to early detection.

Methods: A multi-faceted public health campaign was undertaken in urban and rural/remote Manitoba, Canada in March, 2011. The March 2011 campaign was built on a similar advertising platform that had been executed the previous year. A variety of media were employed to reach the target audience including radio, television, newspaper, web based efforts, postcards with prescriptions, and advertising on the side of buses. We performed a pre and post campaign telephone interview omnibus survey of Manitobans 18 years of age and over in February and April 2011 respectively. Respondents were selected by random digit dialing and a resident was asked to determine awareness of the campaign, if aware, their understanding of the message. Weighting was applied to data correcting for differences between the demographics of the sample and the 2006 census data on Manitoba’s population.

Results: In pre-campaign sampling, there were a total of 3,751 co-operative contacts, of which 804 completed interviews. In post-campaign sampling a total of 3,231 co-operative contacts were completed. In pre-campaign sampling, 7% of respondents were familiar with the campaign, increased from 7% to 25%. 30% of urban respondents that were familiar with the campaign increased from 8% to 26%.

Conclusions: The cross-sectional association between sodium intake and CKD was 0.66 (95% CI, 0.54 to 0.82; p=0.0001) for quartiles 1 through 4, respectively. In this cohort, 14.2% had CKD (7.3% had an eGFR <60 mL/min/1.73m² and 8.4% had albuminuria > 30 mg/g). After adjustment for age, sex, race, diabetes, hypertension and diuretic usage there was a significant association between higher quartiles of sodium intake and decrease of CKD, with adjusted odds ratios of 3.0, 0.85 (95% confidence interval [CI], 0.71 to 1.01;p=0.06), 0.77 (95% CI, 0.64 to 0.91; p=0.003), and 0.66 (95% CI, 0.54 to 0.82; p=0.0001) for quartiles 1 through 4 respectively.

Conclusions: In this population-based cohort study, higher salt intake is associated with a lower risk of CKD. Clinical trials are needed to evaluate if dietary salt intake is a modifiable risk factor for CKD or kidney disease progression.

SA-PO2517
Low Dietary Potassium Intake Is Associated with an Increased Risk of Chronic Kidney Disease in Adults Shaleenda Sharma, 1 Kim McCann, 1 Anna Jeanette Jovanovich, 1 Michel B. Chonchol, 2 Jessica B. Kendrick, 6 1Division of Renal Disease and Hypertension, University of Colorado Denver, Aurora, CO; 2Denver Health Medical Center, Denver, CO.

Background: Potassium intake is inversely related to blood pressure levels but the relationship between potassium intake and chronic kidney disease (CKD) has not been examined.

Methods: We performed a cross-sectional study using the National Health and Nutrition Examination Survey (2001-2006). 13,917 adult participants with dietary data were included in the analysis. Dietary potassium intake was calculated from 24-hour dietary recall. Potassium intake was examined in clinically significant categories [Low <2000, normal: 2000-4000 and high >4000 mg/day] and in quartiles 1-4 (≤ 1373, 1738-2455, 2456-3341, > 3342 mg/day). The primary outcome was CKD defined as a eGFR <60 mL/min or eGFR >60mL/min with albuminuria (~30 mg/g). Multivariate logistic regression models were used to examine the association between potassium intake and CKD.

Results: The mean age and eGFR (SE) of participants was 45.0 (± 4.8) years and 88.0 ± (0.6) mL/min/1.73m². The mean (SE) potassium intake was 2790 ± 22 mg/day. After adjustment for age, sex, race, diabetes and hypertension, low potassium intake (<2000 mg/day) was associated with an increased risk of CKD (OR= 1.35, 95% CI: 1.14-1.59; p=0.005) when compared to normal intake group. High potassium intake (>4000 mg/day) was nearly significantly inversely related to CKD (OR = 0.78, 95% CI: 0.61-1.00, p = 0.05). When potassium intake was evaluated in quartiles, subjects in the first quartile had a 55% increased odds of CKD compared to subjects in the 4th quartile (OR = 1.55, 95% CI 1.25-1.93; p<0.0001). We also examined the relationship of combinations of potassium with sodium intake with CKD. Median intake of potassium and sodium were used to determine, 1 high and low potassium and sodium intake. Subjects with high potassium and high sodium intake had a decreased odds of CKD compared to subjects with low potassium and low sodium intake (OR=0.66, 95% CI 0.57-0.78; p<0.0001).

Conclusions: Low dietary potassium intake is associated with an increased risk of CKD. Studies are needed to examine effects of potassium on kidney disease.

SA-PO2518
Association of Common GFP-Associated SNPs with Albuminuria in Individuals of European Descent Conall M. O'Seaghdha, 1 Ming-Huei Chen, 2 Meredith C. Foster, 1 Carsten A. Böger, 3 Ian H. de Boer, 1 Anna Kotgen, 4 Afshin Parsa, 4 Murielle Bochud, 5 Wen Hong Linda Kao, 7 Caroline S. Fox, 1 NHLBI's Framingham Heart Study; 2Boston University; 3Regensburg University Medical Center, Germany; 4University of Washington, USA; 5Freiburg University, Germany; 6University of Maryland; 7Centre Hospitalier Vaudois, Switzerland; 8Johns Hopkins University.

Background: Albuminuria is an important marker of prognosis in chronic kidney disease (CKD). The presence of albuminuria predicts CKD progression and the development of end-stage renal disease. We hypothesized that single nucleotide polymorphisms (SNPs) recently identified as associated with estimated glomerular filtration rate (eGFR) and CKD might also be associated with albuminuria.

Methods: We investigated 16 eGFR/CKD-associated SNPs in a cohort of 32,324 individuals of European ancestry for their association with the urinary albumin-to-creatinine ratio (UACR) using regression analyses, adjusting for age and sex. We performed meta-analysis of results from 13 contributing cohorts using an inverse variance weighted method. We accounted for multiple testing using a false discovery rate adjustment.

The minor allele of rs171311 in the SLC20A1 gene, which is associated with lower eGFR, was associated with lower UACR levels (β-coefficient for UACR -0.034, p-value 0.0002). While several additional eGFR/CKD SNPs demonstrated nominal statistical association with UACR at DAB2, UBE2Q2, STC1 and GCKR, none were statistically significant after correction for the rate of association with UACR 0.06 – 0.30). Furthermore, alleles associated with lower eGFR tended to be associated with lower UACR levels (DAB2, UBE2Q2, and STC1) and an allele associated with higher GFR was nominally associated with higher UACR levels (GCKR).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.
Conclusions: Apart from the SHROOM3 locus, we observed no robust associations between eGFR-associated SNPs and uAACR. These findings suggest distinct genetic components to these traits.

Funding: Other NIH Support - NHLBI

SA-PO2519

Health Literacy and Blood Pressure Control among Hispanic Americans with Chronic Kidney Disease: A Report from the Paso del Norte Kidney Disease Study (PNKDS)

Jimena A. Blandon, Jinyi Ling, Tarek Alhamad, German T. Hernandez.

Background: Health literacy (HL) refers to a patient’s ability to comprehend medical instructions. Adequate HL may be important in achieving blood pressure control in CKD patients. We assessed HL levels among Hispanic patients with CKD stages 2-4. We evaluated the cross-sectional relationship between adequate levels of HL and BP control.

Methods: We enrolled patients with CKD stages 2-4 attending our nephrology clinic in El Paso, TX. Patients underwent an interview & exam. The patients’ HL was measured using the short-form Test of Functional Health Literacy in Adults (s-TOFHLA) in either English or Spanish. HL was classified as inadequate for s-TOFHLA scores 0-16, marginal for 17-22, and adequate for 23-36. BP control was defined as <130/80 mmHg.

We performed logistic regression with BP control as the outcome, comparing subjects with adequate HL to subjects with inadequate/marginal HL. Analyses were stratified by gender & diabetic status and were adjusted for age, language, health insurance, income & eGFR.

Results: 245 patients were enrolled, 51% female, 91% self-identified as Hispanic, 73% had an annual income <$20,000, 61% had diabetes, the mean eGFR was 44 ml/min, the mean BP was 135/75 mmHg. 36% had a BP <130/80 mmHg. The mean HL score was 22; 52% had adequate HL & 46% had inadequate/marginal HL (8% missing).

After adjusting for age, language, insurance, income & eGFR, there was no relationship between HL adequacy & BP control among diabetic men, non-diabetic men, and non-diabetic women. Among women with diabetes (n=69), the adjusted odds ratio for BP control was 3.33 (95% CI: 1.01-11.01; p=0.048) for women with adequate HL compared to those with marginal/inadequate HL.

Conclusions: Among Hispanics with CKD, there is a high prevalence of inadequate or marginal HL and poor BP control. Among diabetic females with CKD, adequate HL is associated with a higher odds of BP control. Treating providers should be aware of patients with poor HL as they may be at risk for uncontrolled BP.

Funding: Private Foundation Support

SA-PO2520

Outcome of Patients Attending Low Clearance Clinics in Northern Ireland

Anil Kumar Xavier, Ying C. Kuan.

Background: Low clearance clinics (LCC) are designed to focus multidisciplinary care on patients with advanced renal disease, particularly in preparation for renal replacement therapy (RRT). Although studies have highlighted the high mortality in this cohort, few studies have specifically looked at the outcomes of patients entered into LCC. This retrospective study looks at the outcome of patients, specifically at RRT or death, in LCC in Northern Ireland.

Methods: Patients who entered LCC between 2006-10 were included, and followed up until 1st April 2011. Duration for RRT commencement or death was calculated. Analysis of likelihood of either outcome, depending on age category was then analysed using Kaplan-Meier method and Cox proportional hazards model.

Results: Data of 910 patients (478 males, 432 females) were analysed. The age range was 18-98 years (median age -75). At the end of the follow up period, 324 (36%) patients had commenced RRT and 170 (18%) patients had died without receiving RRT. Using Cox hazard analysis, the risk of death increased with age (p<0.0001), and conversely, likelihood of RRT requirement correlated negatively with age (p<0.0001). Kaplan Meier survival chart of outcome related to probability of requiring RRT is as below.

Conclusions: 1. The risk of dialysis requirement was highest amongst those in the youngest age group, and decreased with advancing age.
2. Conversely, the risk of death without initiation of RRT is highest amongst those in the oldest age group.
3. LCC’s are beneficial for overall care of this vulnerable population group, and identified many patients at high risk of requiring RRT. Further studies in this population may allow for better targeting of therapy and resources to optimise outcome.

SA-PO2521

Fluid Balance and Patient Outcomes in AKI: Analysis of the Renal Study Participants

Martin P. Gallagher, Rinaldo Bellomo, Alan Cass, Louise Cole, Joanne Y. Lee, Serigne N. Lo, Shay McGuinness, John A. Myburgh, The George Institute for Global Health, Sydney, Australia; Austin Hospital, Heidelberg, Australia; University of Sydney, Kingwood, Australia; Auckland City Hospital, Auckland, New Zealand; St George Hospital, Kogarah, Australia.

Background: Fluid resuscitation is considered beneficial in critically ill patients with AKI, but there is little evidence to support it. Some evidence from observational studies suggests liberal fluid administration may be harmful. Using data from the RENAL Study we sought to examine the relationship between fluid balance (FB) and clinical outcomes in critically ill patients with acute kidney injury (AKI) requiring dialysis.

Methods: Fluid balance and clinical outcomes data from all participants in the study, a randomised trial of dialysis dose intensity in the setting of AKI in intensive care patients, was analysed using multivariable logistic regression, Cox proportional hazards, time dependent analysis and repeated measure analysis models.

Results: Data on 1464 participants was analysed. During study treatment, mean daily FB among survivors was -201 ml/day compared with +476 ml/day among non-survivors (p<0.001). Mean cumulative FB over the same period was -1294 vs. 311 ml (p<0.001). A negative mean daily FB during study treatment was independently associated with a decreased risk of death at 90 days (OR: 0.32, 95%CI: 0.24-0.43; p<0.001) and with increased survival time (P<0.001). In addition, a negative mean daily FB was associated with significantly increased renal replacement (RRT) free days (p<0.002), ICU free days (p<0.001) and hospital free days (p<0.01). These findings included adjustment for co-morbidities and patient acuity, and were unaltered after the application of different statistical models.

Conclusions: In RENAL Study participants, a negative fluid balance was consistently associated with improved clinical outcomes. Future interventional studies targeting fluid replacement may be one means of improving the poor outcomes of patients with AKI.

Funding: Government Support - Non-U.S.

SA-PO2522

Distribution of CKD and Albuminuria in Shropshire, U.K

R. Diwakar, Katherine Richmond, Kevin Eardley.

Department of Nephrology, Royal Shrewsbury Hospital, Shrewsbury, England, United Kingdom; Department of Biochemistry, Royal Shrewsbury Hospital, Shrewsbury, England, United Kingdom.

Background: In the elderly (> 65 years) non-diabetic population there is ongoing debate about using eGFR as a screening tool for diagnosing Chronic Kidney Disease (CKD). CKD in Shropshire involve predominantly this age group. The Department of Health Quality and Outcomes Framework (QOF) recommends annual assessment including testing of Urine Albumin Creatinine ratio (UACR) to be performed. This is an expensive strategy the benefits of which is unclear in elderly non-diabetics with CKD Stage 3A without albuminuria. Hence we decided to analyse what proportion of nondiabetic CKD patients in Shropshire is above 65, has CKD stage 3A with no albuminuria.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: Shropshire is a county of about 500,000, 000 people. All samples sent by general practitioners in one 2 laboratory. Data was collected retrospectively from the computer systems of these laboratories of nondiabetic adult patients (age > 18) who had eGFR of < 60 ml/min (Stage 3 CKD or above) between October 2009 to September 2010.

Results: 9333 patients were found to have a GFR of < 60 ml/min (Stage 3a 6582, Stage 3b-2256, Stage 4- 454 and stage 5- 41). Among these 9333 patients UCR data was missing in 142 patients. Data from the remaining 9191 patients (1372 ≤ 65, 7819 > 65 years of age) were analysed further. ACR analysis revealed macroalbuminuria (UACR < 3 g/g creatinine) in 1819 patients. Total proteinuria (UACR > 30) was observed in 1753 patients (1581 > 65 years), overt proteinuria (UACR > 30) in 307 patients (254 > 65 years). In those > 45 years 4377 patients had CKD stage 3a and normalalbuminuria, 1409 patients had stage 3b CKD and normalalbuminuria. In the 9191 patients with CKD stage 3- 5 in Shropshire (in whom UACR data was available), 47.6 % were elderly patients with CKD Stage 3A with no albuminuria. Yearly assessment including UACR in this group is probably not beneficial as it neither predicts progression of renal failure nor is it predictive of increased cardiac risk compared to general population. Recognising this in future QOF guidelines to General Practitioners in the UK could represent considerable savings.

SA-PO2525
Aortic Valve Calcification Predicts Coronary Artery Disease in Patients with Chronic Kidney Disease
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Department of Internal Medicine, Pusan National University School of Medicine, Busan, Republic of Korea.

Background: Aortic valve calcification (AVC) is common in chronic kidney disease (CKD) patients and the prevalence of coronary artery disease (CAD) in CKD patients is higher than other disease populations. But little is known about the association between AVC and CAD in CKD patients.

Methods: We retrospectively reviewed the medical records of 408 patients who underwent both transthoracic echocardiography (TTE) and coronary angiography in our university hospitals. The patients were divided into 2 groups according to their estimated glomerular filtration rate (eGFR), CKD group (n=131, eGFR 15-59 ml/min/1.73m²) and non-CKD group(n=277, eGFR ≥ 60 ml/min/1.73m²). TTE reports were reviewed to evaluate the AVC and the Gensini score was used to evaluate the severity of CAD.

Results: The CKD group showed higher prevalence of AVC (32% vs. 11%, P<0.001), CAD (61% vs. 24%, P<0.001) and more severe CAD (Gensini score, 25.86 ± 30.76 vs 15.83 ± 27.39, P<0.001) than non-CKD group. In multivariate logistic regression analysis, AVC was the predictor of CAD only in CKD group (OR=11.079, P<0.001), whereas there was no statistical significance in non-CKD group (OR=1.655, P=0.228).

Conclusions: The severity and prevalence of CAD were higher in CKD group compared to non-CKD group. AVC seemed to predict the CAD only in CKD group. Identifying the presence of AVC by non-invasive TTE could be useful in predicting the CAD in patients with CKD.

SA-PO2526
Prevalence and Predictors of Fatigue in CKD and ESRD
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Department of Medicine, University of Maryland School of Medicine, Baltimore; 1Umed University, Sweden.

Background: Fatigue is a common debilitating symptom in patients with advanced chronic kidney disease (CKD), but there are no studies comparing the severity of fatigue using validated measures among non-dialysis dependent CKD and end-stage renal disease (ESRD) patients. The Functional Assessment of Chronic Illness Therapy-Fatigue (FACT-F) is a 13-item fatigue scale, which has been shown to be reliable and responsive to change in fatigue in patients with cancer. The aim of this study is to examine the prevalence and severity of fatigue, and identify predictors of fatigue in a cross-sectional survey of 173 CKD and ESRD patients.

Methods: We examined fatigue in 87 CKD stage 4-5 patients and 86 ESRD patients using the FACT-F and SF-36 scales. The participants were divided into two groups: (1) ESRF patients ≤ 24 versus (≤ 24) based on the mean score among anemic cancer patients. Predictors of worse fatigue at baseline were determined using linear regression model.

Results: The mean FACT-F score among all participants was 34.5 ± 11.0 and was similar among the CKD and ESRD groups (p=0.73). The FACT-F score correlated closely with the SF-36 vitality score (Pearson correlation coefficient 0.81). After adjusting for age, race, gender, group (CKD versus ESRD), and medication use (benzodiazepine, antidepressant), the presence of cardiovascular disease, low serum albumin, depression, poor subjective sleep quality, and excessive daytime sleepiness were found to be independent predictors of worse fatigue at baseline.

Conclusions: Patients with CKD stage 4-5 and ESRD experience profound fatigue. Interestingly, the severity of fatigue is greater in these two groups. The FACT-F is a useful scale to assess the severity of fatigue in this population and correlates highly with the SF-36 vitality scores. Depression, poor sleep and low albumin levels may be targets for interventions to improve fatigue in patients with advanced CKD.

SA-PO2527
A Comparison between Patients Referred and Not Referred to an Innercity Nephrology Clinic
Anjil Achariya,1, Sophie Kwock,1 Venu Gopal Kankani,2 Rakesh Malhotra. 1Nephrology, Jacobi Medical Center, Bronx, NY; 2Scarsdale, NY.

Background: The prevalence of chronic kidney disease (CKD) is increasing. Ethnic minorities are at high risk, constituting a majority of the population in Bronx inner-city hospitals. There is a high prevalence of diabetes mellitus (DM) and hypertension in this population, which places them at a high risk for CKD. Early detection of CKD and renoprotection are crucial in this group.

Conclusions: Patients with CKD stage 4-5 and ESRD experience profound fatigue. Interestingly, the severity of fatigue is greater in these two groups. The FACT-F is a useful scale to assess the severity of fatigue in this population and correlates highly with the SF-36 vitality scores. Depression, poor sleep and low albumin levels may be targets for interventions to improve fatigue in patients with advanced CKD.
Objective: To compare the baseline characteristics, comorbid conditions and stage of CKD of patients at referral to a nephrology clinic versus those not referred.

Methods: We conducted a retrospective chart review of all patients seen in the primary care clinic from July 1, 2009 to December 31, 2009 who had a serum creatinine (Scr) of 1.5mg/dl or greater. Data was collected on demographics, comorbid conditions and stage of CKD for those referred to nephrology as well as for those not referred.

Results:

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Nephrology Consults n (%)</th>
<th>No referee Consults n (%)</th>
<th>Pvalue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>42 (59.2)</td>
<td>161 (63.6)</td>
<td>.001</td>
</tr>
<tr>
<td>Female</td>
<td>29 (40.8)</td>
<td>92 (36.4)</td>
<td>.17</td>
</tr>
<tr>
<td>Age</td>
<td>59.7±10.72</td>
<td>60.7±11.74</td>
<td>.19</td>
</tr>
<tr>
<td>BMI</td>
<td>30.1±6.5</td>
<td>31.4±10.0</td>
<td>.68</td>
</tr>
<tr>
<td>Smoker, n(%)</td>
<td>13 (18.3)</td>
<td>79 (31.3)</td>
<td>.17</td>
</tr>
<tr>
<td>Insurance, n(%)</td>
<td>68 (95.8)</td>
<td>243 (97.2)</td>
<td>.17</td>
</tr>
<tr>
<td>Condrombidity</td>
<td>Stage 1</td>
<td>Stage 4</td>
<td></td>
</tr>
<tr>
<td>DM, n(%)</td>
<td>34 (47.9)</td>
<td>149 (58.9)</td>
<td>.002</td>
</tr>
<tr>
<td>Hypertension, n(%)</td>
<td>10 (60.5)</td>
<td>212 (65.8)</td>
<td>.005</td>
</tr>
<tr>
<td>CAD, n(%)</td>
<td>34 (47.9)</td>
<td>74 (29.2)</td>
<td>.001</td>
</tr>
<tr>
<td>CHF, n(%)</td>
<td>21 (29.6)</td>
<td>43 (17)</td>
<td>.009</td>
</tr>
<tr>
<td>HIV, n(%)</td>
<td>9 (12.7)</td>
<td>34 (13.4)</td>
<td>.88</td>
</tr>
<tr>
<td>Hepatitis C, n(%)</td>
<td>5 (7)</td>
<td>17 (6.7)</td>
<td></td>
</tr>
</tbody>
</table>

Comorbidity:

- DM, n(%)<0.05
- Hypertension, n(%)<0.05
- CAD, n(%)<0.05
- CHF, n(%)<0.05
- HIV, n(%)<0.05
- Hepatitis C, n(%)<0.05

Conclusions:

- 10.7% of the patients in the non-referral group were in stage 4 CKD.
- 58.9% of those with an SCr greater than 1.5mg/dl and not referred for nephrology consultation had DM.
- Despite early stage of CKD in 80% of those referred, CAD was present in almost 48% of patients.
- 10.7% of the patients in the non-referral group were in stage 4 CKD.
- Education of primary care physicians for early nephrology referral of a high-risk population for progression of CKD should be a priority.

SA-PO2528

Effect of Imputing Non-Ignorable Missing Data on Serial Measures of Renal Function: The Strong Heart Study

Nawar M. Shara,1,2 Hong Wang,1,2 Barbara V. Howard,1,2 Jason G. Umans1,2 MedStar Health Research Institute; 1Georgetown-Howard University Clin & Translational Sci Ctr.

Background: Kidney and cardiovascular disease (CVD) co-progress over time. However, it is challenging to simultaneously study interactions between progressive CKD and CVD in longitudinal studies because their morbidity and mortality lead to missing data which are likely not missing at random (NMAR). We have previously assessed the choice of imputation methods to best account for MAR renal function data in prediction of CVD (Kidney Int, 2007). We now explore approaches for cases in which data are NMAR and when exclusion of the missing data would likely bias relationships.

Methods: A cohort of 4549 American Indians from the Strong Heart Study with complete serial measures of serum creatinine data was considered. This cohort has a high burden of obesity, DM, CKD, and CVD. We used 4 different approaches to derive renal data subsets with MAR or NMAR data using the following algorithms: 1. missing completely at random 2. autoregressive MAR data 3. autoregressive MAR augmented with age and sex 4. and empirical non-ignorable missing data. These four subsets with missing data were used to examine the performance of five imputation methods: 1. listwise deletion (LD) 2. mean of serial measures 3. adjacent value (AV) 4. multiple imputation (MI) and 5. pattern-mixture (PM). One-hundred bootstrap samples were obtained with replacement to ascertain the accuracy of the point estimates.

Results: The hazard ratios (HRs) from each of the imputed sets were contrasted with HRs obtained from complete data set and compared across the different models. HRs generated by the PM method were closest to those using complete data. For NMAR data, the mean Scr imputed by PM differed less from that in the full dataset than did mean Scr using any of the of the imputation methods. The PM and AV imputation methods provided HRs for CVD risk which were similar to those using the complete data. By contrast, the PM overestimated the mean of Scr and underestimated the hazard ratio for CVD risk when data were MAR.

Conclusions: Pattern-mixture imputation best accounted for non-random missing renal function data in a large study of progressive CKD and CVD.

Funding: Private Foundation Support

SA-PO2529

Lack of Specialized Renal Care in Patients with Chronic Kidney Disease: The Implicate Study

Patrick Saudag,1 Marangon Nicola, Chantal Martinez, Catherine Stoermann, Belen Ponte, Sophie M. De Seigneux, Pierre-Yves F. Martin. Nephrology, Geneva University Hospitals, Geneva City, Geneva, Switzerland.

Background: We undertook a prospective randomised trial to determine the impact of specialised care by nephrologists compared to guidelines-directed management by primary care physicians (PCP) on prognosis, planning of RRT and patient satisfaction in CKD patients.

Methods: Single center prospective randomised study. Inclusion criteria: CKD patients with an eGFR < 45 ml/min, aged 18-80 years old and enrolled during a hospitalization. Exclusion criteria: AKI or ESRD, estimated life expectancy < 2 yrs, refusal or inability to sign writing consent and patients previously known by nephrologists. The primary composite endpoint is death and/or hospitalisation during the 24 months after inclusion. The secondary endpoints are initiation of urgent RRT, decline of renal function and quality of life. Study design: Patients are randomised in two arms: - Combined management PCP and nephrologists (4 nephrology visits/year) - Management by PCPs with the help of written instructions and consultations being provided by our unit if requested by PCPs. Quality of life will be assessed every year.

Results: At the end of May 2011, 289 patients have been eligible, of whom 69 patients refused to participate and 70 (24%) were already followed by a nephrologist. One hundred and fifty patients have been randomised. Mean age is 66 ± 9 yrs, mean baseline eGFR is 31 ± 9 ml/min and mean Charlson comorbidity score is 4.7. Out of the 289 patients, 219 had either diabetes or nephrosclerosis, 19 had chronic glomerulonephritis (CGN) and 51 other diagnoses of CKD. Follow-up for patients with CGN rises to 89% and falls to 17% in patients with CKD due to diabetes or nephrosclerosis.

Conclusions: These preliminary results indicate that there is a lack of renal care for patients with CKD, especially for those whose renal impairment is due to diabetes or nephrosclerosis. The Implicate study may demonstrate whether regular renal care in these patients brings substantial benefits in terms of survival, hospitalisation rate and decline of renal function. ClinicalTrials.gov: NCT00929760

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO2530

Chronic Kidney Disease Is A Risk Factor for Complications of Peripheral Artery Disease

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Background: Peripheral artery disease (PAD) coexists in patients with chronic kidney disease (CKD) and is often associated with significant morbidity and mortality. During the 1-year follow up period after diagnosis of PAD, we identified risk factors associated with composite end point of gangrene, death, or re-hospitalization for PAD.

Methods: In this retrospective study, we obtained data from veterans who were evaluated for PAD and who had abnormal ankle-brachial index (0.9> ABI > 1.3), digital pressure (<50) or abnormal qualitative waveform. Veterans who had prior history of PAD were excluded. MDRD GFR was estimated. Veterans were followed for one year after index vascular evaluation. Primary end point was composite of gangrene, ischemic ulcer, death or re-hospitalization for PAD symptoms.

Results: A total of 597 veterans underwent vascular evaluation and after excluding normal studies and veterans with prior history of PAD, our study group included 346 veterans. During the 1 year follow up, 27 veterans (group A) reached composite endpoint and 319 did not (group B). On comparing the two groups, patient characteristics including history of diabetes (56% vs. 50%), hypertension (89% vs 92%), smoking (70% vs 67%), hyperlipidemia (70% vs 80%), ischemic heart disease (30% vs 39%) were statistically similar.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Group A (n=27)</th>
<th>Group B (n=319)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>70±10</td>
<td>66±10</td>
<td>.03</td>
</tr>
<tr>
<td>Vascular Imaging Study</td>
<td>12 (44%)</td>
<td>146 (46%)</td>
<td>.05</td>
</tr>
<tr>
<td>Severe PAD (ABI ≤ 0.7)</td>
<td>20 (73%)</td>
<td>153 (48%)</td>
<td>.013</td>
</tr>
<tr>
<td>Revascularization</td>
<td>3 (11%)</td>
<td>56 (18%)</td>
<td>.05</td>
</tr>
<tr>
<td>CKD (eGFR &lt;60ml/min)</td>
<td>14 (52%)</td>
<td>90 (28%)</td>
<td>.01</td>
</tr>
</tbody>
</table>

As shown, risk factors for veterans reaching composite endpoint include older age, severe PAD and stage III or worse CKD.

Conclusions: Chronic kidney disease appears to be associated with an increased risk of PAD-related complications and death. Further study is needed to see if earlier screening for PAD in patients with CKD can lead to earlier diagnosis and management and reduce these complications.
SA-P02531
Effect of Angiotensin Receptor Blockers on Serum Phosphate and Calcium and Its Association with Renal and CV Outcomes
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Background: Previous studies have shown that high serum phosphate and low calcium are associated with increased risk of renal and cardiovascular outcomes. Angiotensin Receptor Blockers (ARB) delay the progression of renal disease and provide cardiovascular (CV) protection. We assessed the effects of ARB treatment on phosphate and calcium and investigated to what extent short-term effects of ARBs on these parameters translate into long-term renal and CV outcomes.

Methods: In a combined analysis of the Reduction of Endpoints in non insulin dependent diabetes mellitus with the Angiotensin II Antagonist Losartan (RENAAL) and Irbesartan Diabetic Nephropathy (IDNT) trials, we first determined the effect of ARB therapy on phosphate and calcium by testing the one year change versus placebo, secondly determined the association of these changes with long-term outcomes and finally adjusted the effect of ARB treatment on long-term outcomes for change in calcium and phosphate in Cox regression analyses.

Results: Compared to placebo, ARB treatment increased calcium levels with 0.07 mg/dL (95%CI 0.03 to 0.11; p<0.001) at year 1, but did not change phosphate (0.00 mg/dL (95%CI -0.05 to 0.08; p=0.64). Each 0.1 mg/dL increment in calcium in the first 12 months resulted in a reduction of long term renal risk of 7% (95%CI 5 to 8; p<0.001). No association was observed between the one year change in calcium and cardiovascular outcome. Adjustment of the renoprotective effect of ARBs for the observed one year change in calcium attenuated the effect of ARB treatment from 21% (95%CI 9 to 31) to 7% (95%CI 1 to 18), suggesting that part of the renoprotective effect is attributable to its effect on serum calcium. Essentially similar results were obtained when the analyses were repeated in the trials separately.

Conclusions: ARB treatment induces an increase in serum calcium, which explains part of its long-term renoprotective effect in diabetic patients with nephropathy.

Funding: Government Support - Non-U.S.

SA-P02532
Patterns of Progression in Chronic Kidney Disease (PoPe) Study: Baseline Data
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Background: The factors influencing the rate of CKD progression remain unclear. eKiDNA is a National collaborative CKD research group assessing CKD patients referred to nephrology practices in Australia. We present baseline data from the first 3105 patients entered into the study. We aim to assess referred Australian CKD patients longitudinally for rates of progression of CKD, and to explore factors that may influence that such as demographics, biochemistry, therapies and co-morbidities.

Methods: This is an observational cohort study. Data were collected on referred CKD patients attending nephrology practices in Australia using Audit 4 software. The data collected included demographics, diagnosis and stage of CKD.

Results: The data on the first 3105 patients seen and entered into the Audit 4 database are presented. The study is ongoing. The average age of the cohort was (mean/SD) (68/14.9) years. CKD Stages were represented as follows: 1-2: 11%, 3: 39%, 4:21%, 5: 25%. Hypertension may be over represented as it is recorded as a co-morbidity and may have been recorded in all stages of CKD. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%. Diagnoses recorded for all stages of CKD were: Hypertension 51%, Diabetic Nephropathy 25%, Chronic Kidney Disease 62:38%

Conclusions: We have initiated a CKD research group of Audit 4 users investigating CKD longitudinally in Australia. Baseline data shows a large number of stage 3 CKD patients with mainly hypertension and diabetic nephropathy. Longitudinal data will enable assessment of factors determining the rate of CKD progression, adequacy of therapy models of CKD care and resource utilization.

SA-P02533
Use of Coenzyme Q10-Depleting Medications by Chronic Kidney Disease Status in the U.S.
Vanessa Grubbs,1 Laura C. Plantinga,1 Delphine S. Tsui,1 Neil R. Powe,1,2 1University of California, San Francisco; 2San Francisco General Hospital.

Background: Many commonly used medications deplete body stores of coenzyme Q10 (CoQ), which is believed to have antioxidant effects. This depletion may contribute to oxidative stress and subsequent renal dysfunction. The use of CoQ-depleting medications in the U.S. by CKD status is unknown.

Methods: Using 1999-2008 National Health and Nutrition Examination Survey data, we examined reported use of CoQ-depleting medications among 21,169 non-pregnant adults (age 20+ years). CoQ-depleting medications included: beta blockers, clonidine, gemfibrozil, statins, hydralazine, thiazide diuretics, sulfonylureas, thricyclic antidepressants, and phenothiazine derivatives. CKD stage 1/2 was defined by urinary albumin/creatinine ratio ≥30 mg/g with eGFR ≥60 ml/min/1.73 m2 and CKD stage 3/4 by eGFR 15-59 ml/min/1.73 m2. The prevalence and odds of taking a CoQ-depleting medication by CKD status were estimated via multivariable logistic regression, weighted to the U.S. population.

Results: Overall, an estimated 20.7% of participants reported taking at least one CoQ-depleting medication, but prevalent use was significantly higher among those with greater CKD severity: 16.3%, 33.2%, and 53.0% of those with no CKD, CKD stage 1/2, and CKD stage 3/4, respectively (p<0.001). Among 10,388 participants without diabetes (self-reported or glycosylated hemoglobin >6.5) or hypertension (self-reported or blood pressure >140/90), conditions for which most CoQ-depleting medications are indicated, prevalent use followed a similar pattern: 5.4%, 8.4%, and 24.8% of those with no CKD, CKD stage 1/2, and CKD stage 3/4, respectively (p<0.001). After adjustment for demographics, co-morbid disease, and healthcare visits, those with advanced CKD were 1.5-fold more likely to be taking a CoQ-depleting medication than those without CKD (stage 1/2: OR 1.0, 0.93-1.29; stage 3/4: OR 1.5, 1.30-1.80, vs. no CKD).

Conclusions: Greater CKD severity is associated with higher use of CoQ-depleting medications. The extent to which CoQ-depleting medications contribute to CKD development and progression and whether CoQ dietary supplementation may ameliorate this effect is worthy of investigation.

Funding: NIDDK Support

SA-P02534
Referral Patterns for CKD to Nephrology; the Implications in the Management of the Renal Patient in an Academic Internal Medicine Practice
Alejandro Solano Bayardo,1 Tiffanier M. Lowy,2 Leela M. Mathew.1 1Internal Medicine Department, Unity Health System, Rochester, NY.

Background: Our objective was to determine the rate of referral and primary care physicians' patterns of referral to nephrologists in patients with CKD.

Methods: We conducted a retrospective review through NexGen EMR and Unity Faculty Partners, an academic ambulatory Internal Medicine clinic in Rochester, NY. We analyzed all patients with an ICD-9 code for CKD from 2007-2010, encompassing a total of 166. Out of this, 37 were excluded based on ESRD, those who had expired or lost to follow-up. Data was collected from the remaining 129 patients and graphed based on GFR, age and attending physician.

Results: CKD Referral to Nephrology

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
SA-PO2535
Analysis of Cause of Death and Level of Active Medical Management in a Conservative Care Programme for End Stage Renal Disease. Linda H. Bissel,1 Maria Fish,2 Vicky Hinton,2 Linda Evans,3 Christine Porter,2 Mark A.J. Devolden.1,2 1 Renal and Transplant Unit, Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; 2 School of Clinical Sciences, University of Nottingham, United Kingdom.

Background: In end stage renal disease (ESRD), renal replacement therapy (RRT) does not always improve quality or duration of life. Nottingham Renal Unit has an established conservative care (CC) programme which advises on symptom control and management of complications of CKD. We analyzed our 10 year prospective database to determine impact of the CC team on patients who have died. We determined the proportion of deaths attributed to ESRD and the proportion where bone disease or anaemia were being actively managed.

Methods: We analyzed data from patients who died on the CC programme, assessing demographics, CKD stage, changes in eGFR and laboratory parameters from start of CC to death. The proportion of deaths due to cause of death were coded by EDTA-ERA classification.

Results: At analysis 269 patients had died on the programme. 53% male, 93% Caucasian and median age 81. 37% had diabetes mellitus. Aetiology of CKD was uncertain in 42%. 43% were CKD stage 5 at start compared with 70% at death. Median number of days to death was 315. Table 1 illustrates median age, eGFR and laboratory parameters. Serum albumin and bicarbonate were lower at death. At start 7% pts were prescribed erythropoietin (EPO) therapy compared with 54% at death. Most common documented cause of death was therapy ceased at 38%, respiratory 17% and cardiac disease in 16%.

Conclusions: Classification of ‘therapy ceased’ in this cohort is likely to mean death from ESRD, though there might be some exceptions to this (currently under analysis). The diagnosis of patients who did not die of renal failure. Median eGFR at start of programme is higher than expected from published data due to the inclusion of CKD stage 3 and 4. The CC programme at Nottingham Renal Unit offers patients an important alternative to RRT. Active medical management from the CC team appears to be important, regardless of whether patients subsequently die of renal failure or another pathology.

SA-PO2536
Evaluation of Chronic Kidney Disease Detection among High Risk Populations Using Automated Estimated Glomerular Filtration Rate Reporting

Methods: We identified 2077 hypertensive and 1042 diabetic patients. Patient characteristics were comparable before and after eGFR reporting. Mean age was 58±9 years old, 60% females, and 55% Caucasian. The one year cumulative incidence of CKD detection was 0.09 for hypertensive patients and 0.12 for diabetics. Hypertensive and diabetic patients seen after eGFR reporting had a higher 1-year risk of CKD detection than before eGFR reporting, (Relative risk (RR):1.66, 95% confidence interval (CI):1.01-2.73) and (RR=1.29, 95%CI:1.01-2.71). Respectively. After adjusting for age, race, and gender, the 1-year risk of CKD detection among those seen after automatic eGFR reporting was 77% higher among hypertensive patients (RR=1.77,95%CI:1.03,2.77) and 25% higher among diabetics (RR=1.25, 95%CI:0.75,2.10), than the 1-year risk of CKD detection among those seen before automatic eGFR reporting.

Conclusions: Increased detection of CKD occurred after eGFR reporting among high risk populations. These data suggest that automatic eGFR reporting promotes earlier detection of CKD among high risk populations. Future studies should examine whether earlier detection leads to changes in management of CKD.

Funding: NIDDK Support

SA-PO2537
Comparing eGFR Equations in the Elderly

Methods: The BIS is an ongoing prospective population-based cohort studying CKD in people ≥70. Sampling was done stratified for age and gender. Confirmatory analysis was done with t-test and chi²-test. Agreement between formulas was assessed using Bland Altman and kappa statistics.

Results: The characteristics of the study population and subpopulations (eGFR<60 ml/min/1.73m²) are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total</th>
<th>eGFR&lt;60 ml/min/1.73m²</th>
<th>eGFR&lt;60 ml/min/1.73m²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1967</td>
<td>1240</td>
<td>727</td>
<td></td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td>80</td>
<td>78</td>
<td>0.001</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td>52</td>
<td>53</td>
<td>0.09</td>
</tr>
<tr>
<td>BMI</td>
<td></td>
<td>27</td>
<td>26</td>
<td>0.28</td>
</tr>
<tr>
<td>Art. Hypertension</td>
<td></td>
<td>77</td>
<td>71</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diabetes mel</td>
<td></td>
<td>23</td>
<td>21</td>
<td>0.27</td>
</tr>
<tr>
<td>MI</td>
<td></td>
<td>14</td>
<td>19</td>
<td>0.064</td>
</tr>
<tr>
<td>Stroke</td>
<td></td>
<td>8</td>
<td>7</td>
<td>0.02</td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td>52</td>
<td>23</td>
<td>0.24</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.0</td>
<td>0.8</td>
<td>1.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cystatin C (mg/l)</td>
<td>1.2</td>
<td>1.04</td>
<td>1.54</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>eGFR&lt;60 ml/min/1.73m²</td>
<td>65</td>
<td>56</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>ACR &gt;500</td>
<td></td>
<td>26</td>
<td>20</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Data are presented as ‘n’ and ‘%’.

Conclusions: Significant discrepancies of eGFR can be observed when using different estimating equations in the elderly. Comparison to GFR measurement is needed in people ≥70.

Funding: Private Foundation Support

SA-PO2538
Which Is the Best Estimating Equation for GFR in Adolescents?

Background: In 2009, Schwartz, et al. via the CKD study revised a commonly used equation (equation) to estimate GFR (eGFR) for children. The CKD-ID bedside (bCKD) equation tends to underestimate measured GFR (mGFR) in children over the age of 4 years, including adolescents compared to older patients. Adol patients(pts) may be closer to adult body size and physiology than to younger pts. We investigate whether the adult derived eGFR equations or the new bCKD equations are more accurate to estimate mGFR in adol pts.

Methods: Retrospective analysis of 140 pts 13 to 17 yrs with mGFR by isotalamic steady-state methodology at a tertiary care pediatric hospital. Diagnoses (Dx) include solid tumor pts both pre and post chemotherapy (chemo), children with chronic kidney disease (CKD) and with renal transplants (txp). Serum creatinine (SCr) was determined by enzymatic assay.

Results: Age range 13 to 17 yrs, mean age 14.9 (SD1.1). 49% male. The mean Scr for all pts was 0.76 mg/dl (SD 0.4). By Dx group, 15% were done pre-chemo, 50% were post-chemo, 16% had reached and 13% were txp pts.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
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The BCID eqn underestimated mGFR by 13%, while CKDEPI overestimates by 17% and MDRD 50%. The BCID eqn was more accurate in the youngest subjects and increasingly underestimated mGFR as age increased.

All three equations where more accurate for girls compared to boys. 

Conclusions: The BCID eqn is reliable in younger pts, but less reliable in older adols, particularly boys. The MDRD and CKDEPI eqns accuracy are similar to BCID eqn, but overestimating mGFR in the opposite direction. Stratifying by age and gender, both CKDEPI and MDRD have an advantage over the BCID eqn in pts over 14 yrs of age. Overall, the BCID eqn may benefit from an adjustment in adols for gender, similar to what is seen in the adult-based eqns. and the original Schwartz eqn.

SA-PO2539

Comparison of Creatinine-Based and Cystatin C-Based GFR Estimating Equations in Chinese Children

Xuebei Li, 1 Menghe Gong, 2 Xuewang Li, Hongmei Song, 2 Yan Qin, 3 Ke Zheng. 1. Nephrology, Peking Union Medical College Hospital, Beijing, China; 2. Internal Medicine, Peking Union Medical College Hospital, Beijing, China; 3. Pediatrics, Peking Union Medical College Hospital, Beijing, China.

Background: There is no data about glomerular filtration rate, measured through plasma or renal clearance of the exogenous markers, in the Chinese children. The objective of this study is to measure GFR and validate the GFR estimating equations in the Chinese children, aiming at selecting the equations with best performance in GFR estimation and CKD staging.

Methods: 85 children were enrolled in the study. Single-compartment plasma clearance of 125I-DTPA was measured using the radioactive counts of the two plasmas sampled after a bolus injection and transformed into two-compartment plasma clearance (mgGFR). mGFR was normalized (mgGFR) by body surface area. eGFR was calculated using 9 different equations, based on Creatinine (3), Cystatin C (3) or their combination (3). Creatinine and cystatin C were measured using kinetic Jaffe method and immunoturbidimetry, respectively. Correlation and agreement between eGFR and mgGFR and the accuracy of GFR estimation was compared to validate and select GFR estimating equations.

Results: Compared with the other six equations, original Schwartz equation, Filler equation and CKD equation produced the eGFR with better correlation with mgGFR. Pearson r was 0.78, 0.73 and 0.75, respectively, stronger explanation of variance in mgGFR (R² was 0.812, 0.716 and 0.730), smaller bias (bias was 0.2, -4.2 and 3.5), narrower 95% LOA (-61.63, [50.42] and [37.44]), better performance in Bland-Altman analysis, higher intraclass correlation coefficient (0.594, 0.724 and 0.741), higher ICC (0.592, 0.722 and 0.735), higher ratio of eGFR within mgGFR:10% (30.6%, 33.8% and 37.7%) and within mgGFR:30% (81.2%, 76.6% and 77.9%), higher ratio of correct CKD staging (65.9%, 68.8% and 75.3%), and better agreement in CKD staging between eGFR and mgGFR (Kappa value was 0.60, 0.61 and 0.65).

Conclusions: The selected equations used to estimate GFR in Chinese children were original Schwartz equation, Filler equation and CKD equation, while CKD equation had the best performance.

Funding: Government Support - Non-U.S.

SA-PO2540

Estimating GFR in Renal Transplantation: A Multicentric Evaluation of Cystatin C Performance

Ingrid Masson, 1 Nicolas Maillard, 2 Eric Alamartine, 2 Etienne Cavaleri, 3 Pierre Delaney, 2 Christopher R. Mariat. 1. Nephrology, University Hospital of St-Etienne, St-Etienne, France, Metropolitain; 2. Nephrology, University Hospital of Liege, Liege, Belgium; 3. Biology, University of Liege, Belgium.

Background: Serum cystatin C (SCy) might be a better GFR marker than serum creatinine (SCr) in renal transplant patients. The aim of our study was 1- to confirm or not the superiority of SCy in estimating renal graft function and 2- to determine the influence of demographic, gender, age, time post-tx, stages of CKD, diabetic status, proteinuria, IS regimen, usCRP and Albuminemia.

Results: Mean (+/-SD)ulin clearance, SCr and SCy were 50.6 (+/-19) mL/min/1.73m2, 129 (+/-52) µmol/l and 1.64 (+/-0.61) mg/l, respectively. P30 was 75%, 77%, 81% and 84% for the CKD-EPI(SCr) equation, the MDRD Study equation, the CKD-EPI(SCy) equation and the CKD-EPI(mix) equation, respectively (p<0.05 for the comparison between SCr-based and SCy-based equations). Aera under ROC for a GFR threshold of 60 mL/min/1.73m2 was 0.832 and 0.901 for SCr and SCy, respectively (p<0.0001). After adjustments for the different non-GFR determinants tested, SCy remained significantly superior to SCr in predicting renal graft function.

Conclusions: Our data, obtained in a large population of transplant patients and with a standardized method of SCy dosage, confirm the superiority of SCr over SCy. Whether this better predictive performance may clinically translate into a better management of the transplant patient remains to be determined.

Funding: Government Support - Non-U.S.

SA-PO2541

GFR Estimation in the Morbidly Obese Pre and Post Bariatric Surgery: One Size Does Not Fit All

Samra Abouachacra, 1 Ahmed Chaaban, 1 Nicole Gebran, 1 Qutaiba Abdulatif Daoud, 1 Bassam O. Bernihe, 1 Hanan Luay Al Omary, 2 Mohamed Ahmed, 1 Said Abuhansha. 1. Tawam Hospital, United Arab Emirates; 2. Baghdad University, Iraq.

Background: Hyperfiltration with increased glomerular filtration rate (GFR) is commonly associated with obesity. This is expected to improve post bariatric surgery. However formula-based GFR estimation in the obese is limited by body size confounders necessitating use of modification factors, the reliability of which remains uncertain.

Our aim was to compare GFR- estimating formulae in morbidly obese patients at baseline & post bariatric surgery.

Methods: Retrospective review of 220 patients (145 females), mean age 34.7±10 yrs with post-op followup over 6 months.

GFR was calculated using MDRD, CKD-epi- Usual & Body Surface Area (BSA)-adjusted; Cockcroft Gault (CG) & Lean Body Weight-corrected (CG-LBW) formulae.

Results: Significant reduction in weight & BP was achieved with data as shown.

Funding: Government Support - Non-U.S.

SA-PO2542

Renal Effects of Bariatric Surgery in Patients with Chronic Kidney Disease

Nephrology, Peking Union Medical College Hospital, Beijing, China.

Background: Smoking is a well-known risk factor for chronic kidney disease (CKD) and cardiovascular disease (CVD). Smoking is responsible for an estimated 10% of incident CKD cases. Smoking cessation is recommended for all patients with CKD, including those with stage 1-2 disease. However, the impact of smoking cessation on CKD progression is not well understood.

Methods: A retrospective analysis of 100 patients with stage 1-2 CKD who underwent bariatric surgery at the Department of Bariatric Surgery, Peking Union Medical College Hospital, Beijing, China, from January 2010 to December 2019. The patients were divided into two groups: the smoking cessation group (n=50) and the smoking continuation group (n=50).

Results: The smoking cessation group had a significantly lower prevalence of smoking-related comorbidities, including hypertension, diabetes mellitus, and dyslipidemia, compared to the smoking continuation group. The smoking cessation group also had a lower prevalence of CKD progression, as measured by a decrease in estimated glomerular filtration rate (eGFR) over the follow-up period. Furthermore, a significantly higher proportion of patients in the smoking cessation group achieved smoking cessation at 1 year after surgery compared to the smoking continuation group.

Conclusions: Smoking cessation is a feasible and effective strategy to improve outcomes in patients with stage 1-2 CKD who undergo bariatric surgery. These findings highlight the importance of smoking cessation as a component of comprehensive care for patients with CKD.

Funding: Government Support - Non-U.S.
**SA-PO2543**

Relationship between Glomerular Filtration Rate, Body Surface Area and Gender

Belen Redal-Baijzar,1 James G. Heaf,2 Knud Rasmussen,1
1Nephrology, Roskilde Hospital, University of Copenhagen, Roskilde, Denmark;
2Nephrology, Copenhagen University Hospital at Herlev, Herlev, Denmark.

Background: Normalisation of GFR with BSA with extreme body sizes might be misleading and this can have consequences in drug dosing and living donor kidney assessment.

Methods: Cross sectional study of 895 cancer patients, to investigate whether GFR indexed for BSA is reliable in extreme body sizes and whether this relationship is influenced by gender. GFR was investigated with [131I]iothalamate (reference test) in 895 patients with stage I or II cancer. GFR and BSA were calculated with the reference test and several GFR estimations: (1) Cockroft-Gault; (2) MDRD-4; (3) CKDEPI. Cystatin C-based formula: Larsson, Larsson modified, Grubb, Hoek.

Results: There were no significant differences between genders for BSA or GFR estimated with BSA. GFR and BSA correlation with GFR and GFR ml/min per 1.73 m². R values 0.57 (p < 0.001) and 0.69 (p < 0.001) for men and women respectively.

Conclusions: These results suggest that indices of GFR with indexed BSA may be misleading in extreme body sizes and should be interpreted with caution.

**SA-PO2545**

Accuracy of a Glomerular Filtration Rate Estimating Equation over Time in People with a Wide Range of Kidney Function

Smita Padala,1 Hocine Tighiouart,2 Lesley Stevens Inker,3 Gabriel Conteras,3 Julia Lewis,1 Michael Steffes,2 Roger A. Rodby,2 Christopher H. Schmid,4 Andrew S. Levey,1 Tufts Medical Center; 2University of Miami; 3Vanderbilt University Medical Center; 4University of Minnesota; 5Rush University Medical Center.

Background: The accuracy of GFR estimated from serum creatinine (Scr) over time is not well known. We examined the rate of change in measured GFR (mGFR), estimated GFR (eGFR) and the difference between mGFR and eGFR (error) over time in a large dataset with a wide range of kidney function.

Methods: GFR was measured using urinary clearance of [131I]iothalamate (reference test) in subjects with and without kidney disease and diabetes. GFR was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation (index test). The change over time was modeled using linear mixed models. We considered a change in error larger than ± 3 ml/min/1.73 m² per year as clinically significant.

Results: There were 13,708 GFR measurements in 3635 subjects over a mean follow up of 3.6 years. The mean mGFR, eGFR and error at baseline were 76, 76, and -0.3 ml/min per 1.73 m². The mean (standard error) change in mGFR, eGFR and error were -2.3 (0.12), -2.2 (0.09) and -0.1 (0.10) ml/min/1.73 m² per year (P < 0.001, < 0.001 and 0.6 respectively).

Conclusions: We found no significant change in the accuracy of eGFR over time. Changes in eGFR over time in most individuals were due to changes in mGFR rather than changes in non-GFR determinants of Scr. In the absence of recognized changes in non-GFR determinants of Scr, clinicians should interpret changes in eGFR as reflecting changes in mGFR.

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**SA-PO2544**

Cystatin C vs Creatinine-Based Equations Compared to 99m TcDTPA Scintigraphy for the Assessment of Glomerular Filtration Rate in Chronic Kidney Disease

Hernan Trimarchi,1 Nephrology, Hospital Britanico, Buenos Aires, Argentina.

Background: In chronic kidney disease (CKD) accurate estimation of the glomerular filtration rate (GFR) is essential. Gold standards are expensive and laborious. Different equations based on cystatin C have been proposed to estimate GFR. However, cystatin C levels are frequently far from reflecting real GFR. We compared creatinine- with cystatin C-based formulae, using [99mTc]DTPA scintigraphy as gold standard, and propose the best equation for each stage of CKD.

Conclusions: Despite the small sample size and short follow up, using CG-LBW formula, a significant increase in GFR was seen in obese patients with early CKD after bariatric surgery. Though similar changes were seen with CKD-Epi eGFR, this is clearly unreliable without BSA correction. When adjusted, an overestimation of GFR was observed with pre-operative eGFR.

**SA-PO2546**

Simple Cystatin C Formula Compared to Sophisticated CKD-EPI Creatinine & Cistatin C-Based Formula for Estimation of Glomerular Filtration Rate in Elderly Patients with Mild to Moderate Impaired Kidney Function

Sebastian Bevc,1 Radovan Hojs, Robert Ekart, Maksimiljan Gorenjak, Ludvik Puklavec. Dept. of Nephrology, Nuclear Medicine, Clinical Chemistry, UKC Maribor, Slovenia.

Background: Serum creatinine (Scr) concentration and Sccreatine-based formulas are the most commonly used markers to estimate glomerular filtration rate (GFR). Recently, serum cystatin C (Scys)-based formulas and the newer creatinine & cystatin C-based formula (The Chronic Kidney Disease Epidemiology Collaboration formula (CKD-EPI creatinine & cystatin C formula)) were proposed as new GFR markers. The aim of our study was to compare them with the more sophisticated CKD-EPI eGFR.

Conclusions: We found no significant change in the accuracy of eGFR over time. Changes in eGFR over time in most individuals were due to changes in mGFR rather than changes in non-GFR determinants of Scr. However, in controls and in early stages of CKD creatinine-based methods or equations may correlate better with [99mTc]DTPA.

Funding: NONE

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**SA-PO2548**

Cystatin C- vs Creatinine-Based Equations Compared to 99m TcDTPA Scintigraphy for the Assessment of Glomerular Filtration Rate in Chronic Kidney Disease

Hernan Trimarchi,1 Nephrology, Hospital Britanico, Buenos Aires, Argentina.

Background: In chronic kidney disease (CKD) accurate estimation of the glomerular filtration rate (GFR) is essential. Gold standards are expensive and laborious. Different equations based on cystatin C have been proposed to estimate GFR. However, cystatin C levels are frequently far from reflecting real GFR. We compared creatinine- with cystatin C-based formulae, using [99mTc]DTPA scintigraphy as gold standard, and propose the best equation for each stage of CKD.

Conclusions: Despite the small sample size and short follow up, using CG-LBW formula, a significant increase in GFR was seen in obese patients with early CKD after bariatric surgery. Though similar changes were seen with CKD-Epi eGFR, this is clearly unreliable without BSA correction. When adjusted, an overestimation of GFR was observed with pre-operative eGFR.
was to compare CKD-EPI creatinine & cystatin formula and simple Scys formula (100/Scys) against 51CrEDTA clearance in elderly patients with mild to moderate (GFR 90-30 ml/min/1.73m2) impaired kidney function.

Methods: 106 adult Caucasians patients older than 65 years (58 women, 48 men; mean age 72.5 years) were included. In each patient 51CrEDTA clearance, Scys (Jaffe – IMDS traceable method) and Scys (immunonephelometric method) were determined. GFR was calculated using the CKD-EPI creatinine & cystatin formula and simple Scys formula.

Results: The mean 51CrEDTA clearance was 52.2 ± 15.9 ml/min/1.73m2, mean Scya 1.6 ± 0.47 mg/dL, mean Scys 1.79 ± 0.6 mg/L. Statistically significant correlations between 51CrEDTA clearance and both formulas were found (P<0.0001). In the ROC curve analysis (cut-off for GFR 60 ml/min/1.73m2) no significant difference of diagnostic accuracy between CKD-EPI creatinine & cystatin formula and simple Scys formula was found (P=0.395). Bland and Altman analysis for the same cut-off value showed that CKD-EPI creatinine & cystatin formula (bias: -21.2 ml/min/1.73m2) underestimated and simple Scys formula (bias: 4.1 ml/min/1.73m2) overestimated measured GFR. All equations lacked precision. It was 8.7 ml/min/1.73m2 for CKD-EPI creatinine & cystatin formula and 9.8 ml/min/1.73m2 for simple Scys formula.

Conclusions: Our results indicate that simple Scys formula which requires just one variable (Scys concentration) is reliable marker of GFR in elderly patients with mild to moderate impaired kidney function and comparable to sophisticated CKD-EPI creatinine & cystatin formula.

SA-PO2547
Creatinine and Cystatin C: Different Performance in Staging Chronic Kidney Disease in the Elderly
Ana Rocha, Jorge Malheiro, Pedro V.A. Aguiar, António Manuel Nunes Cabrita.
Centro Hospitalar do Porto, Portugal.

Background: An older population with chronic kidney disease (CKD) has frequently a high cardiovascular (CV) comorbidity and a pro-inflammatory status. Several clinical and analytical variables in this context may interfere with serum creatinine (sCr) and cystatin C (sCys).

Methods: We proposed to analyse in a stage 1-4 CKD and over 60 years population the agreement between glomerular filtration rate (GFR) formulas based on sCr (MDRD) and sCys (Stevens) through Kappa statistics and Bland-Altman plot. The degree of difference between formulas and their association with clinical variables was analysed by spearman correlation. A multivariate linear regression model was used to determine the influence on sCr and sCys of prevalent clinical and analytic variables.

Results: We studied 163 subjects, mean age 74 years, 53.4% females and with 2 or more CV risk factors (hypertension, diabetes mellitus (DM), dyslipidemia) in over 80%.

The prevalence of CKD stages 3-4 was 81% by MDRD and 70% by Stevens formulas with more CV risk factors (hypertension, diabetes mellitus (DM), dyslipidemia) in over 80%.

The mean difference was -6.78 ml/min/1.73 m2, being significantly higher in younger age, male gender, milder CKD, in proteinuric and hypertensive patients. A multivariate adjusted model for age, gender, log-body mass index, DM, high ferritin status, albumin and HDL-cholesterol revealed a higher correlation with log-sCys (r=0.53) than log-sCr (r=0.38). DM and high ferritin were significant predictors of increased log-sCys and log-sCr. Higher log-sCys was also independently predicted by older age (p=0.005), female gender (p=0.02), lower albumin (p=0.005) and HDL-cholesterol (p=0.001).

Conclusions: In the elderly, a stronger association of sCys with nonrenal factors may limit its accuracy as determinant of GFR, but a higher correlation with these prevalent clinical and analytical variables can add clinical pertinence to it.

SA-PO2548
Glomerular Filtration Rate Via Plasma Iohexol in the Elderly – A Comparison of Slow and Fast Component
Peter Martin, Markus van der Giet, Natalie R. Ebert, Jens Gaedeke, Olga Jakob, Martin K. Kuhlmann, Elke Schaeffner.

Background: GFR measurement can be done using the clearance of Iohexol. We compare the modeling of Iohexol clearance using one or two components in a half logarithmic model.

Methods: We use a subsample (n=50) of the Berlin Initiative Study (BIS) with 10 measurement points of Iohexol concentration in the plasma at 10-300 min after infusion. The analysis of the slow and fast component of Iohexol clearance follows Schwartz et al (2006); fast component: 10 to 90 min, slow component: 120 to 300 min. GFR was calculated from the area under the curve using the slow (GFR(1)) or both components (GFR(2)). We assess bias and agreement using the method of Bland and Altman.

Results: Median age was 76 yrs (IQR 73-83 ys), 27 males, 23 females. Median body surface area was 1.81 m2(IQR 1.71-1.94). The area of the slow component (median 44.4 mg min/ml; IQR: 37.6 to 57.9) did not correlate with the additional area of the fast (median 4.9; IQR 3.7-5.9; r = -0.16). GFR (1) (median 71.4; 50-82.4) was about 10-15 % larger as compared to GFR (2) (median 62.6, 50-72.7). The correlation between GFR (1) and GFR (2) was 0.992. Bland Altman Plot shows moderate agreement (average difference 6.9 ml/min = 8.1 (2SD) per 1.73 m2) and larger differences for larger GFR values. After removal of systematic differences we could predict GFR(2) from GFR(1) with a precision of ∼3.7 ml/min per 1.73 m2.

Conclusions: In elderly subjects agreement between both approaches (slow vs. slow+fast component) was less compared to Schwartz et al (2006) probably due to smaller Iohexol measurements at 10 and 20 min after infusion in our sample. After including a bias correction the slow component was sufficient.

SA-PO2549
Glomerular Filtration Rates in a Healthy, Multi-Ethnic Asian Population
Boon Wee Teo, Evan J.C. Lee.
Medicine, Yong Loo Lin School of Medicine, National University of Singapore, Singapore.

Background: Measured glomerular filtration rates (GFR) in healthy Asians without chronic kidney disease (CKD) (Indians, Nephrorn physiol 2005 Barai et al; Chinese, AJKD 2010 Zuo et al) have reportedly been lower than in European populations. We assess the normal distribution of GFR in a multi-ethnic Asian population without kidney disease and the GFR estimated with the CKD-EPI equation.

Methods: We prospectively recruited 103 healthy participants (49.5% male, Chinese 33%, Malay 24.3%, Indian 23.2%, and others 19.4%). We measured Scr by an enzymatic method, and measured GFR (all GFR; in mL/min/1.73m2) using 3-sample plasma clearance of 99mTc-DTPA, normalized to body surface area (du Bois). We use linear regression to assess the association of demography with mGFR, and to develop a GFR prediction equation. We use demographic data (mean age) from previously published studies to predict the GFR of those populations. We estimate GFR (eGFR) using the CKD-EPI equation, and assess its performance using the bias (median difference of eGFR-mGFR), precision (IQR), root mean square error (RMSE), and the percentage accuracy of eGFR to within 15%, 30% and 50% of mGFR.

Results: Median age was 76 yrs (IQR 73-83 ys), 27 males, 23 females. Median body surface area was 1.81 m2(IQR 1.71-1.94). The area of the slow component (median 44.4 mg min/ml; IQR: 37.6 to 57.9) did not correlate with the additional area of the fast (median 4.9; IQR 3.7-5.9; r = -0.16). GFR (1) (median 71.4; 50-82.4) was about 10-15 % larger as compared to GFR (2) (median 62.6, 50-72.7). The correlation between GFR (1) and GFR (2) was 0.992. Bland Altman Plot shows moderate agreement (average difference 6.9 ml/min = 8.1 (2SD) per 1.73 m2) and larger differences for larger GFR values. After removal of systematic differences we could predict GFR(2) from GFR(1) with a precision of ∼3.7 ml/min per 1.73 m2.

Conclusions: In elderly subjects agreement between both approaches (slow vs. slow+fast component) was less compared to Schwartz et al (2006) probably due to smaller Iohexol measurements at 10 and 20 min after infusion in our sample. After including a bias correction the slow component was sufficient.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: Healthy Indians have significantly lower GFR compared to other ethnic groups, and the mean measured eGFR is lower than the mean GFR reported in Chinese in China, but our Indians had higher GFR than those in India. The CKD-EPI equation estimates GFR accurately in a multi-ethnic Asian population without CKD.

Funding: Government Support - Non-U.S.

SA-PO2550

The Significance of Cystatin C in Different Stages of Chronic Kidney Disease

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Background: To analyze the sensitivity of cystatin C in reflecting glomerular filtration rate (GFR) of chronic kidney diseases (CKD), estimate the scope of cystatin C in different CKD stages and different total GFR (TGFR) groups, assess the relevance of cystatin C with proteinuria and anemia.

Methods: 70 CKD patients were divided into five stages based on estimated GFR (eGFR), and four groups based on TGFR. 20 healthy persons were selected as control group. Ser, cystatin C, the quantity of 24-hour urinary protein, hemoglobin were measured, and TGFR was measured by kidney ECT, egFGR was calculated through Cockroft-Gault formula.

Results: There is statistical difference in the levels of serum cystatin C between the patients in CKD2-5 stages and control group (P < 0.05), as well as in different stages of the CKD2-5 patients. The concentration range of cystatin C in five stages of CKD are as follows: stage 1 is ≤ 0.17 mg/L, stage 2 is between 0.17 and 1.62 mg/L, stage 3 between 1.62 and 2.31 mg/L, stage 4 between 2.31 and 3.58 mg/L, and stage 5 is over 3.58 mg/L. The correlation of cystatin C in four TGFR groups are: in normal group cystatin C ≤ 0.11 mg/L, in slight injury group between 1.11 ~ 1.52 mg/L, in moderate injury group 1.52 ~ 2.58 mg/L, and in severe injury group >2.58 mg/L. Significant correlation coefficient exist in the cystatin C and the quantity of urinary protein in CKD 3 stage (R = 0.481, P < 0.05). There is correlation between cystatin C and the hemoglobin of 33 patients with anemia (R = -0.668, P < 0.001).

Conclusions: Cystatin C is more sensitive than serum creatinine in reflecting renal function. The quantity of urine protein have positive correlation with the level of cystatin C. And there is correlation between the concentration of cystatin C and the degree of anemia.

SA-PO2551

Screeing for Occult Renal Disease (SCORED) Is Useful Tool To Identify Individuals at High-Risk for Chronic Kidney Disease

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Background: The health burden of renal disease is high for patients and health services worldwide, and screening for chronic kidney disease (CKD) has been increasingly advocated. Population-based studies relating to the prevalence of CKD in the community are important. Our prospective study was aimed to stratify the risk scores of CKD patients based on the CKD-EPI equation.

Methods: The frequency of individuals at high-risk for CKD was determined using a cross-sectional study of 873 adult households in Palmas, Brazil, randomly selected using a stratified, cluster method. Age, gender, and race were similar to the entire Palmas' urban population.

Results: An estimated GFR <60 ml/min/1.73 m2 was present in 46 (5.3%) of participants studied, and the risk for having CKD was greater in women than in men, and it increased with age from 2.7% in the 18 to 44 yr age group to 19.0% in those 65 yr of age. The frequencies of CKD Stage 3, 4 and 5 were 4.8%, 0.5% and 0%, respectively. SCORED values included 224 (25.7%) patients with high SCORED values (≥24), and 649 (74.3%) subjects with low SCORED values. Subjects with higher SCORED values were at a significantly higher risk of having CKD compared with those who had lower SCORED values (12.9% vs 2.6%, χ2 = 35.58, p < 0.001). The sensitivity for predicting CKD by SCORED model was 63% and the specificity was 76%; the positive predictive value was 13%, whereas the negative predictive value was 76%.

Conclusions: High SCORED values were associated with a higher risk for having CKD in a general population-based sampling. This simple screening tool was a useful tool to identify individuals at high-risk for CKD.

Funding: Government Support - Non-U.S.

SA-PO2552

Comparison of Anticancer Drugs Dosing Recommendations for Chronic Kidney Disease Patients Based on Three Methods for Assessing Kidney Function

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Background: The aim of this study was to determine whether a difference exists when making anticancer drug dosage adjustments in patients with CKD and cancer based on estimation of GFR using the Chronic Kidney Disease Epidemiology Collaboration, Modification of Diet in Renal Disease (MDRD) simplified and Cockroft-Gault(CG) equation.

Funding: None.

SA-PO2553

Influence of Thyroid Function on Different Kidney Function Tests

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Background: The commonly used kidney function tests (KFT) have limitations, especially in thyroid dysfunction. Therefore we studied the most commonly used KFT in patients with hypo- and hyperthyroidism and after reaching euthyroidism.

Methods: Prospective case series in 16 patients with thyroid dysfunction. Serum creatinine (Cr), urea, uric acid, BUN, GFR calculated by Cockroft-Gault, estimated GFR (eGFR) by CKD-EPI equation, serum cystatin C (CysC), eGFR based on CysC, eGFR based on a combined (CysC and creatinine) formula and plasma NGAL were measured in hypo- and hyperthyroidism and after gaining euthyroidism.

Results: When Cr normalized in hypothyroid patients, creatinine decreased significantly (P<0.001) from 1.2/0.2 to 0.9/0.1 mg/dl and creatinine based eGFR increased significantly: 24 hour creatinine clearance (P<0.001), Cockroft-Gault (P<0.001) and CKD-EPI equation (P=0.001). The combined (CysC and creatinine) GFR formula increased significantly (P<0.01) compared with eGFR based on CysC decreased significantly (P=0.02). There was no significant change in NGAL levels (P=0.9).

When Cr normalized in patients with hyperthyroidism creatinine increased significantly (P<0.01) from 0.63/0.10 to 0.77/0.13 mg/dl and creatinine based eGFR decreased significantly: 24 hour creatinine clearance (P<0.03), Cockroft-Gault (P<0.01) and CKD-EPI equation (P=0.02). There was no significant change (P=0.76) for the combined (CysC and creatinine) GFR equation. In contrast CysC decreased significantly (P<0.01) and CysC based GFR increased significantly (P<0.001). There was no significant change in NGAL levels (P=0.61).

Conclusions: Thyroid function has a major influence on the vast majority of KFTs. CysC is strongly influenced by the thyroid function and should be avoided in thyroid disorders, but there was no effect of the thyroid function on the low NGAL levels.

The recommended KFT in thyroid dysfunction is a creatinine based GFR estimation. Furthermore kidney and thyroid function should always be used together to avoid misleading interpretations.

SA-PO2554

Vitamin D Insufficiency and Incident Chronic Kidney Disease in Australia: The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study

Matthew J. Damsniewicz, Diana M. Magliano, Robin M. Daly, Claudia Gagnon, Zhong X. Lu, Peter R. Ebeling, Steven J. Chadban, Robert C. Atkins, Peter G. Kerr, Jonathan E. Shaw, Keivan R. Polkinghorn, Robin M. Daly.

1Monash University, Australia; 2Baker IDI, Australia; 3Deakin University, Australia; 4Laval University, Canada; 5Melbourne Pathology, Australia; 6Melbourne University, Australia; 7Sydney University, Australia.

Background: 25-OH vitamin D (25D) insufficiency has been associated with CKD (albuminuria and impaired GFR) in cross-sectional studies, however this association is less clear in prospective studies. We aim to measure the 5-yr incidence of CKD in subjects with 25D insufficiency and assess 25D insufficiency as a risk factor for CKD.

Methods: The prospective AusDiab Study follow-ups 706 adults over 5 years. Incident CKD was defined as subjects negative at baseline but positive after 5 years for (1) eGFR <60 ml/min/1.73 m2 using the CKD-EPI equation (impaired GFR), or (2) spot urine
Vitamin D Insufficiency and Chronic Kidney Disease in Australia: AusDiab Study  Matthew J. Damsawiecz,1 Dianna J. Magliano,1 Robin M. Daly,1 Claudia Gagnon,2 Zhong X. Lu,3 Peter R. Ebeling,4 Steven J. Chadban,2 Robert C. Atkins,2 Peter G. Kerr,2 Jonathan E. Shaw,2 Kevan R. Polkinghorne,1 1Monash University, Australia; 2Baker IDI, Australia; 3Deakin University, Australia; 4Laval University, Canada; 5Melbourne Pathology, Australia; 6Melbourne University, Australia; 7Sydney University, Australia.

Background: Low 25D levels have been associated with albuminuria, however the association with GFR is less clear. We aim to determine the associations between 25-hydroxy vitamin D (25D) levels and an impaired glomerular filtration rate (GFR) in a population-representative cohort of Australian adults.

Methods: The study population was the baseline Australian Diabetes, Obesity and Lifestyle (AusDiab) Study cohort, surveyed from 1999–2000 (n=10,732). Estimated GFR (eGFR) was calculated using the CKD-EPI equation using enzymatic creatinine measurements, with CKD defined as <60ml/min/1.73 m2. Albuminuria was defined as a spot urine albumin to creatinine ratio of ≥2.5 mg/mmol for males and ≥3.5 for females. 25D insufficiency was defined as levels <50 nmol/L. Logistic regression models accounting for survey design were used to assess the effect of 25D insufficiency on albuminuria and CKD, adjusted for gender and age (model 1), and adjusted for multiple variables including age, gender, diabetic status, body mass index, cholesterol and triglyceride levels, smoking status, time of blood test (season), ethnicity, cardiovascular disease and systolic blood pressure, as well as eGFR or albuminuria, respectively (model 2).

Results: 30.6% of the study population had 25D level <50 nmol/L (95% CI 25.6-35.8%). 25D insufficiency was significantly associated with albuminuria in the unadjusted model (OR 2.05, p<0.0001), model 1 (OR 1.78, p<0.0001), and model 2 (OR 1.56, p<0.003). While 25D insufficiency was significantly associated with an eGFR<60 in the unadjusted model (OR 1.52, p<0.02), the association was not significant in the adjusted models (model 1: OR 0.95, p=0.78; model 2: OR 0.85, p=0.31).

Conclusions: 25D insufficiency was common in this population and levels of <50 nmol/L were independently associated with albuminuria, but not with impaired eGFR.

Uric Acid Is Associated with Chronic Kidney Disease and Hypertension in the Genetics of Coronary Artery Disease in Native Americans (GOCADAN)

Background: Uric acid has been associated with both prevalent and incident HTN and CKD, perhaps contributing to cardiovascular disease (CVD). No studies have explored these associations in Alaska Eskimos, a population with high rates of CVD but low prevalence of CKD and CVD.

Methods: Cross-sectional analysis of 89% (n=1078) of GOCADAN study participants with available lab data at baseline (2000–2004). CKD was defined by an eGFR <60ml/min/1.73m2. Albuminuria was defined as a urine albumin/creatinine ratio ≥30 mg/g. Using logistic regression models sequentially adjusted for age, sex, smoking, BMI, DM, triglycerides, systolic BP (or eGFR), and hsCRP to separately determine independent associations of uric acid with CKD and with HTN.

Results: The 7% (n=75) of GOCADAN participants with prevalent CKD were more likely to be older (63±4 y), have DM (9% v 3%), albuminuria (20% v 6%), or HTN (63% v 18%). Likewise, the 21% (n=230) with prevalent HTN were more likely to be older (57 v 39y), or have DM (13% v 4%), or albuminuria (17% v 4%) (All p<0.05). Uric acid was independently associated with prevalent CKD and with prevalent HTN (Table).

Table. Associations of Uric Acid and CKD in GOCADAN

<table>
<thead>
<tr>
<th>Model</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-univariate</td>
<td>1.8 (1.6–2.1)</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>2-age, sex, smoking</td>
<td>1.8 (1.5–2.1)</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>3-above + BMI, DM, systolic BP</td>
<td>2.0 (1.6–2.5)</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>4-above + BMI, DM, triglycerides, systolic BP (or eGFR) for the HTN model</td>
<td>2.0 (1.6–2.5)</td>
<td>≤0.0001</td>
</tr>
<tr>
<td>5-above + hS-creatinine</td>
<td>2.0 (1.6–2.6)</td>
<td>≤0.0001</td>
</tr>
</tbody>
</table>

Conclusions: Among Alaska Eskimos, uric acid is independently associated with both prevalent CKD and with prevalent HTN. Future studies are needed to determine whether uric acid contributes to increasing CVD in this population.

Funding: Other NHS Support - This analysis was supported by P30AG031057, U01HL06424, U01HL082490, U01HL082458, M01RR00047, UL1R3311975, MD-U54-DK064424-1061 and U1-RR031975 from the National Institutes of Health, Bethesda, MD.

Gout Risk Factors and Treatment among Chronic Kidney Disease Patients: A Hospital Based Cross Sectional Study

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Background: Gout has doubled in prevalence in the United States. Recent studies have proposed hyperuricemia and gout as a risk factor for progression of chronic kidney disease (CKD). This study investigated the prevalence gout in patients with CKD and compared the current practice patterns to recommendations and guidelines put forward by European League Against Rheumatism (EULAR).

Methods: A total of 1,827 patients admitted between 2009 and 2010 at St. Elizabeth Health Center with a diagnosis of chronic kidney disease were included by database search using ICD-9 codes. Patients were divided into two groups: CKD with gout and CKD without gout. Data collection included age, sex, and comorbid diagnosis. A subset of 50 patients was randomly selected to compare the treatment they received with EULAR guidelines.

Results: The prevalence of gout in the hospitalized patients with CKD was 251/1827 (13.7%; 95% CI 12.15%). Males exhibited higher rates of gout: 150/814 (18.4%) vs. 101/762 (13.3%), respectively (χ2=3.99, p=0.04). Both hyperuricemia and hypertension were higher in the CKD patients with gout: 46.2% versus 38.7% (p=0.004), and 82.5% versus 78.1%, respectively (χ2=2.20, p=0.14). Only 65% of CKD patients with gout were treated following EULAR guidelines.

Conclusions: This study reveals a high prevalence of gout in patients with CKD. Male sex, advanced age, CAD, hyperlipidemia, and hyperuricemia were significantly associated with gout among CKD patients. In contrast, the prevalence of diabetes was higher in CKD patients without gout. Treatment for gout was sub-optimal in the subset of examined CKD patients. Newer treatment modalities for gout may change these findings in the future. Greater awareness is needed to improve the management strategies for gout in CKD patients.

Association of Simple Renal Cysts by Computed Tomography (CT) with Kidney Function and Chronic Kidney Disease (CKD) Risk Factors

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Background: Simple renal cysts are common and can be better detected by CT scan than ultrasound, but the clinical relevance is not clear. The objective of this study was to determine if kidney function and CKD risk factors associate with cysts.

Methods: Contrast enhanced CT images of potential kidney donors were included in the study from 2000 to 2008 at Mayo Clinic. We reviewed the radiology reports for the presence of renal cysts. Clinical and laboratory data was abstracted from the medical record. Hypertension was defined by use of anti-hypertensive medications or blood pressure (BP) >140/90 mmHg. The association of kidney function and CKD risk factors with renal cysts was assessed with nominal logistic, ordinal logistic, and linear regression adjusting for age and sex.

Results: There were 1946 potential kidney donors, of which, 28% had at least one renal cyst. Simple renal cysts were 42% were male, and 15% had hypertension. Mean ± SD age was 43 ± 12 years, body surface area (BSA) was 1.98 ± 0.25 m², diastolic BP 74 ± 10 mmHg. 24-h urine albumin excretion was 8.2 ± 18 mg, measured glomerular filtration rate (GFR) was 102 ± 9 ml/min/1.73 m², and serum creatinine was 0.87 ± 0.17 mg/dl.

Conclusions: Simple renal cysts have been associated with both prevalent and incident HTN and CKD, perhaps contributing to cardiovascular disease (CVD). No studies have explored these associations in Alaska Eskimos, a population with high rates of CVD but low prevalence of CVD and CKD.
Diabetic Nephropathy: Prevalence and Progression (DNP) Study

**Methods:**

Prevalence and progression of Diabetic Nephropathy (DN) from Asian countries. In Singapore, population prevalence of diabetes of 11.3% and incidence of ESRF due to DN of 134 per million population are both very high. As majority of diabetes are managed in primary care setting, there is less chance of DN being detected in primary care. A review of literature shows that DN is a common healthcare cluster (National Health Care Group; NHG) by describing its prevalence and progression.

**Results:**

Prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81% prevalent patients within NHG with Type 2 Diabetes Mellitus [Chinese: 69%; Female: 52%, Mean (Age: 62 years; Duration of diagnosis: 7 years; HbA1c: 7.6%; BMI: 26.4), 81%

**Conclusions:**

Increased number and size of renal cysts showed some association with older age, male gender, larger BSA, diastolic BP, hypertension, increased albumin excretion, and hyperfiltration. These findings suggest simple cysts may be a marker of early kidney injury in relatively healthy adults.

**Funding:** NIDDK Support

**SA-PO2559**

**Diabetic Nephropathy: Progression and Prevention (DPP) Study**

**Methods:**

We tested the association between urinary 8-iso and subsequent CVD events in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes andCKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. 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We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD. We measured urinary 8-iso levels in a population-based study of 336 patients with type 2 diabetes and CKD.
Conclusions: There was no prospective association between baseline urinary 8-iso PGF_final and incident CVD over a median 15y follow-up in a population with high prevalence of DM, obesity and CKD and high risk of CVD.

Funding: Private Foundation Support

SA-PO2563
Factors Associated with Serum NGAL (neutrophil Gelatinase-Associated Lipocalin) in Patients with Chronic Kidney Disease
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Background: It is well known that serum or urinary levels of NGAL increases earlier than that of serum creatinine (Cr) in acute kidney injury (AKI), and that NGAL has been identified to be a useful biomarker of AKI. In addition, it has been reported that NGAL also increases in chronic kidney disease (CKD) with the property of inhibition of human erythropoiesis by stopping differentiation or apoptosis of erythroid precursors. The present study investigated the factors associated with serum levels of NGAL in patients with CKD.

Methods: We measured serum NGAL, using a ELISA Kit (Triage® NGAL, Biosite Incorporated, USA), in 103 patients with CKD stage 1-4 who are stable for more than three months. We also measured hemoglobin, hematocrit, serum creatinine and blood pressure. We also investigated the existence of comorbidity (hypertension, chronic heart failure, urinary protein, diabetes mellitus).

Results: Thirty three patients showed that serum NGAL levels were below 60ng/ml, which was upper limit of measurement. Mean levels of remaining seventy patients were 148.4±62.7ng/ml. NGAL levels are significantly positively correlated with age and negatively correlated with hemoglobin and 1Cr, respectively. In patients with chronic heart failure, Serum levels of NGAL were significantly increased, compared with those without it (200.4±63.4 vs 138.8±58.1ng/ml, respectively). Serum levels of Cr are only an independent factor related with NGAL in a multivariate analysis.

Conclusions: NGAL increases in correlation with residual renal function in CKD patients. Moreover, chronic heart failure is an important factor to increase serum levels of NGAL.

SA-PO2564
Cumulative Impact of Anemia, Albuminuria and Cystatin C on Adverse Prognosis in HIV-Infected Men
Minoru Ando,1 Naoki Yanagisawa,1 Ken Tsuchiya,2 Kosaku Nitta. 3Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; 2Department IV of Internal Medicine, Tokyo Women’s Medical University, Tokyo, Japan.

Background: The heightened risk for cancer and cardiovascular disease (CVD) in well-treated HIV-infected patients may be due to residual immunodeficiency and inflammation. The presence of anemia and chronic kidney disease (CKD) likely accelerates such ill linkage. We examined cumulative impact of anemia and CKD on prognosis in HIV-infected subjects.

Methods: A 3-year prospective cohort study was conducted in 520 HIV-infected men with undetectable HIV RNA level. All-cause death and incident cancer and CVD were considered critical events. Cumulative incidence of such events was analyzed by the Kaplan Meier method, stratified by presence or absence of microalbuminuria (ACR ≥30mg/g), high cystatin C (serum level ≥1.0 mg/L) or anemia (Hb <12 g/dL). Multivariate Cox proportional hazards analysis was used to calculate the HR of developing critical events for the combined impact of 3 variables. ‘Score 1’ was assigned to each variable, and the maximum score is a total of 3 points. The model was adjusted for age, CD4 counts and presence of comorbidities including viral hepatitis, hypertension and diabetes at baseline.

Results: The prevalence of microalbuminuria, high cystatin C, and anemia at baseline was 23.7%, 9.0%, and 5.0%, respectively. During the follow-up period, critical events developed in 34 subjects (6.5%). The cumulative incidence of events was significantly higher in the group with microalbuminuria, high cystatin C, or anemia than in each opposite. The HR for critical events increased 2.0-fold per one-point increase in the number of risk factors (95% CI, 1.72-3.25, P=0.0002).

Conclusions: The prevalence of microalbuminuria, high cystatin C and anemia may have a great synergistic impact on the incidence of critical events in well-treated HIV-infected men.

SA-PO2565
Renal Endogenous Angiotensin II as a Principal Source of Urinary Angiotensin II in Chronic Kidney Disease Patients
Xiaoquan Zhang, Xiaojing Ding, Jie Teng, Yi Fang, Jianzhou Zou. Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.

Background: It has been demonstrated that there was a significant relationship between UAGT and kidney Ang II content and renal Ang II immunostaining intensities. These results provide further evidence that UAGT may be a useful index of renal endogenous Ang II activity. This study analyzed the relationship of UAng II with circulating and intrarenal RAS activity in chronic kidney disease (CKD) patients to investigate the origin of UAng II in human.

Methods: 128 CKD patients who had not received ACEI or ARB during last 2 months were included in the study. Urinary and plasma renin activity, AGT, Ang II, aldosterone and type IV collagen were measured by RIA or ELISA. Urinary AGT, Ang II, aldosterone, and type IV collagen levels were expressed per 1 mg of urinary creatinine (mg Cr). Common logarithmic transformation for RAS components and urinary type IV collagen were performed because these variables did not exhibit normal distribution.

Results: Average UAng II in 128 CKD patients was 4.92 ± 0.70 pg/mg Cr). 54 (42.2%) patients were male, which was negatively correlated with UAng II (r = -0.36, P<0.01). Average proteinuria was 2.03 ± 2.66 g/24h, which was negatively correlated with UAng II (r= -0.20, P<0.05). Average plasma renin activity(PRA) was 162.10±1.94, 6.80±1.30 pg/ml/h, which was negatively correlated with UAng II (g=0.20, P<0.05). Average urinary angiotensinogen (UAGT) was 4.64 ± 1.27 mg/mg Cr), which was positively correlated with UAng II (g=0.29, P<0.01). Average urinary type IV collagen(UCol IV) was 5.19 ± 1.29 mg/mg Cr ) , which was positively correlated with UAng II (g=0.31, P<0.01). Multiple regression analysis indicated that male (P<0.01), low proteinuria (P<0.01) and high UAGT (P<0.01) were correlated significantly with high UAng II. There was no correlation of UAng II with plasma angiotensin II.

Conclusions: Renal endogenous angiotensin II may be the principal source of urinary angiotensin II in chronic kidney disease patients.

SA-PO2566
Body Mass Index Predictive Performance of Estimated Glomerular Filtration Rate: A cystatinC- Versus Creatinine-Based Formulas
Comparison
Jorge Malheiro, Isabel Fonseca, Maria Joao Carvalho Azevedo Rocha, Josefina Santos Lascasas, António Manuel Nunes Cabrita. Nephrology Unit, Centro Hospitalar do Porto, Porto, Portugal.

Background: Higher than normal body mass index (BMI) is a risk factor for incident chronic kidney disease (CKD). Serum creatinine and cystatin C are the two main endogenous markers of kidney function. The equations of high BMI (>25kg/m²) with CKD as estimated by creatinine- and cystatinC-based estimating equations were analyzed.

Methods: Stage 1-3 CKD patients from our ambulatory clinic with stable kidney function and simultaneous measurement of serum creatinine and cystatin C were randomly selected (n=179). Skewed data was log transformed. CKD was defined as estimated glomerular filtration rate (eGFR) <60ml/min/1.73m², using creatinine- or cystatinC-based formulas. Regression models evaluated the relationship between high BMI, CKD status (logistic) and eGFR (linear), adjusted for age, gender and cardiovascular risk factors (diabetes, hypertension, dyslipidemia).

Results: Patients sampled were mainly old (median age, 65 years), with a high prevalence of CKD (by cystatinC and creatinine-based formulas 59.8% and 51.4% respectively) and cardiovascular risk factors. Clinical comparison between patients with normal versus high BMI

<table>
<thead>
<tr>
<th>Normal BMI (n=67)</th>
<th>High BMI (n=112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years, median*</td>
<td>58</td>
</tr>
<tr>
<td>Male gender, %</td>
<td>67.2</td>
</tr>
<tr>
<td>Diabetes, %*</td>
<td>19.7</td>
</tr>
<tr>
<td>Hypertension, %*</td>
<td>74.1</td>
</tr>
<tr>
<td>Dyslipidemia, %*</td>
<td>59.7</td>
</tr>
<tr>
<td>CKD prevalence by creatinine-based eGFR, %</td>
<td>50.7</td>
</tr>
<tr>
<td>CKD prevalence by cystatinC-based eGFR, %</td>
<td>34.3</td>
</tr>
</tbody>
</table>

* P < 0.05

High BMI was not associated with CKD by creatinine-based eGFR (P=0.48). In contrast, high BMI was associated with CKD when defined using cystatinC-based equation in a multivariate-adjusted model (OR=2.20, P=0.03). In linear regression, BMI was also a predictor of eGFR by cystatinC with an estimated decrease of 0.9 ml/min/1.73m² per 1 Kg/m² increase in BMI (P=0.04). Linear regression between BMI and eGFR by creatinine was not significant (P=0.23).

Conclusions: High BMI status was associated with CKD when cystatinC-based eGFR was used, but not with creatinine-based eGFR. Influences of adiposity associated factors on serum cystatin C merit further investigation.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
709A
Iron Chelation with Deferoxamine for Diabetic Nephropathy in the db/db Mouse

**Background:** Catalytic iron (FeC₆F₆) being involved in redox cycling generates powerful reactive oxygen species and can contribute to the progression of diabetic nephropathy (DN) and activate the innate immune system mainly against microbial pathogens. But recently it has been demonstrated as proinflammatory and pattern-recognition receptors that play a key role in the innate inflammatory pathway remains elucidated. Toll-like receptors (TLRs) have been recognized as proinflammatory and pattern-recognition receptors that play a key role in the innate inflammatory pathway.

**Methods:** We tested the efficacy of the oral iron chelator deferoxamine in a prototypical mouse model of human diabetes mellitus type 2 and the beneficial effect of deferoxamine (FeC₆F₆) on renal and hepatic parameters.

**Results:** Compared with baseline (25.92 ± 0.49g vs. 30.75 ± 0.53g), weight was better preserved in the treatment group (59.2 ± 2.29g vs. 54.15 ± 8.81g) at euthanasia. Mean blood glucose concentration decreased in both groups (160.56 ± 10.74 vs. 345.69 ± 31.90mg/dL at week 6) with lower final values in the treatment group (345.69 ± 31.90mg/dL vs. 385.40 ± 89.49mg/dL at 12 weeks). The linear trend in the mean 24-hr Urinary FeC₆F₆ excretion (FeC₆F₆/Cr ratio) values was evaluated using a repeated measures analysis of variance (ANOVA) as a function of treatment, week, and their two-way interactions. Creatinine was significantly better preserved from the beginning (25.92 ± 0.49g vs. 30.75 ± 0.53g) and despite a considerable reduction in FeC₆F₆ excretion by the end of the study in the treatment group, this did not reach statistical significance.

**Conclusions:** FeC₆F₆ chelation using deferoxamine in a non-iron overload prototypical DN mouse model resulted in a significant reduction in proteinuria over a 6 month period.

**Funding:** Other NIH Support - Center for Clinical and Translational Research (CCTR) Award Number 1UL1RR028984 from the National Center For Forschung Resources.

**SA-PO2568**

The Effects of Antagonism of Toll-Like Receptors by GIT27 on Lipid Metabolism and Diabetic Nephropathy in High Fat-Fed Mice

**Background:** Metabolism of TLRs in the kidney, adipose tissue, and liver were significantly suppressed and urinary levels of triglyceride accumulation in both kidney and liver. In addition, lipid hydroperoxide levels of inflammation were significantly reduced. Therefore, we asked whether GIT27, an immunomodulatory agent interfering with the innate immune system mainly against microbial pathogens, can activate four individual adenosine receptors. At present, the role of adenosine in DN is unknown.

**Methods:** Methods: The efficacy of the oral iron chelator deferoxamine in a prototypical mouse model of human diabetes mellitus type 2 and the beneficial effect of deferoxamine (FeC₆F₆) on renal and hepatic parameters.

**Results:** Compared with baseline (25.92 ± 0.49g vs. 30.75 ± 0.53g), weight was better preserved in the treatment group (59.2 ± 2.29g vs. 54.15 ± 8.81g) at euthanasia. Mean blood glucose concentration decreased in both groups (160.56 ± 10.74 vs. 345.69 ± 31.90mg/dL at week 6) with lower final values in the treatment group (345.69 ± 31.90mg/dL vs. 385.40 ± 89.49mg/dL at 12 weeks). The linear trend in the mean 24-hr Urinary FeC₆F₆ excretion (FeC₆F₆/Cr ratio) values was evaluated using a repeated measures analysis of variance (ANOVA) as a function of treatment, week, and their two-way interactions. Creatinine was significantly better preserved from the beginning (25.92 ± 0.49g vs. 30.75 ± 0.53g) and despite a considerable reduction in FeC₆F₆ excretion by the end of the study in the treatment group, this did not reach statistical significance.

**Conclusions:** FeC₆F₆ chelation using deferoxamine in a non-iron overload prototypical DN mouse model resulted in a significant reduction in proteinuria over a 6 month period.

**Funding:** Other NIH Support - Center for Clinical and Translational Research (CCTR) Award Number 1UL1RR028984 from the National Center For Forschung Resources.

**SA-PO2569**

Losartan Reverts Glomerular Sclerosis Induced by Type 2 Diabetes by Reducing Mesangial Cell Transdifferentiation

**Background:** Losartan caused a significant increase in the expression of the capacity of the AT1 receptor inhibition by losartan to inhibit the expression of MFT-induced diabetes. The MFT-induced diabetes caused the transdifferentiation and reversion of fibroblasts to myofibroblasts. Losartan was showed that losartan was effective in reversing the progression of kidney disease in obese high fat-fed mice. Losartan treatment initiated after the nephropathy onset was able to significantly reduce these pre-existent fibrotic manifestations and reversed the MFT displayed by MC.

**Conclusions:** Losartan can be a potential strategy, not only to minimize the progression of the diabetic nephropathy, but also to reverse the fibrogenic processes by blocking cells back to their original phenotype.

**Funding:** Government Support - Non-U.S.
via A1AR pumps in wild type STZ-induced mice ameliorated the severity of apoptosis thereby indicating a protective role of extracellular adenosine by stimulating the A1BAR. Interestingly we could show an increase of the A2BAR in renal vessels during STZ-induced diabetes mellitus in an A2BAR reporter mouse by utilizing XGal staining.

Conclusions: In summary we show that adenosine generation by CD73 and stimulation of the extracellular A2BAR ameliorates renal damage occurring during diabetic nephropathy. We hope these studies will lay the groundwork for novel and specific therapeutic approaches in the treatment of DN, which are urgently needed for improving the outcome of Type 1 diabetic patients.

SA-PO2572

Defective Autophagy Promotes Podocyte Injury and Diabetic Nephropathy

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Background: Podocyte injury is one of the prominent features in the progression of chronic kidney diseases as well as in the condition of diabetic nephropathy. As terminally differentiated cell, the podocyte has the ability to maintain homeostasis under certain pathophysiological stress. Autophagy, a highly regulated lysosomal pathway in the recycling of cytosol portions and the removal of superfluous or damaged organelles, is essential for cell homeostasis. However, the role and mechanisms of autophagy in podocyte dysfunction under diabetic state remain largely unknown.

Results: In this study, we found that a progressive albuminuria and podocyte injury was accompanied by defective autophagy manifested as decreased volume and numerical densities of autophagosomes, along with, the downregulated expression of autophagy-related proteins including beclin-1, Atg12-Atg5, and LC3II/LC3I in kidneys from STZ-induced diabetic mice. Autophagy could be triggered in conditionally immortalized mouse podocytes in vitro with high glucose, advanced glycation end-products (AGEs), TGF-β1, Ang or bovine serum albumin (BSA) respectively. Blockade of autophagy with 3-Methylamphethamine (3-MA) or Beclin-1 siRNA promoted podocyte injury under both basal and autophagy-stimulating conditions. To investigate the correlation of ER stress with podocyte autophagy, we detected the abundance of CHOP, a sensor of severe ER stress, in podocytes treated with 3-MA or Beclin-1 siRNA. CHOP abundance was remarkably increased. Downregulation of CHOP with siRNA transfection protected podocytes from 3-MA or Beclin-1 siRNA-induced damage.

Conclusions: Taken together, these observations indicate that autophagy protects podocytes against damage through interfering ER stress and the defective autophagy may promote podocyte injury and proteinuria in diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2573

Nicorandil Ameliorates Podocyte Injury through the Activation of ATP-Dependent K+ Channel in Diabetic Nephropathy

Katsujiro Tanabe, Miguel A. Lanaspá, Wataru Kitagawa, Christopher J. Rivard, Richard J. Johnson, Takahiko Nakagawa. Division of Renal Diseases and Hypertension, University of Colorado Denver, Aurora, CO.

Background: Diabetic nephropathy is the leading cause for ESRD and it is urgent to find a therapeutic strategy. Unfortunately, RAS blockers are occasionally unsatisfactory, especially in patients with endothelial dysfunction. In the last ASN meeting, we reported that nicorandil ameliorated podocyte injury and reduced oxidative stress in diabetic endothelial nitric oxide synthase (eNOS) knockout (KO) mice. However, precise mechanism remains unclear. This reagent has dual actions; one is to release NO and the other is to open ATP-dependent K+ (KATP) channel. Here, we further examined a mechanism by which nicorandil prevented podocyte injury.

Methods: Podocyte injury was morphologically examined in eight week-old male eNOS-KO mice in which diabetes was induced by 50mg/kg of streptozotocin (p) for 4 consecutive days, and nicorandil (30mg/kg/day) was administered for 8 weeks. To study direct effects of nicorandil on the differentiated podocytes, cells were stimulated by normal or high glucose with/without 10^-5 M nicorandil for 72 hours.

Results: In diabetic eNOS-KO mice, an increase in urinary microalbuminuria and urinary 8-OHG excretion, both of which caused by diabetic condition, was significantly reduced by nicorandil. In the podocyte, podocin expression as well as number of WT-1 positive cells was less while oxidative stress was higher. However, nicorandil significantly blocked such podocyte injury. In addition, we found the expression of structural receptor-2 (SUR-2), which is the component of ATP-dependent K+ channel and importantly a binding site for nicorandil, in glomeruli, especially in podocytes. These data suggest that nicorandil could directly protect podocyte. Likewise, this assumption was proven by our in vitro evidence that high glucose caused an increase in ROS, as evidenced by the conversion from H2DCFDA to DCF, in cultured human podocytes whereas nicorandil significantly reduced ROS production.

Conclusions: These results suggest that nicorandil has protective effects by reducing ROS on podocytes through opening ATP-dependent K+ channel.

Funding: Pharmaceutical Company Support

SA-PO2574

Serum and Urinary ACE2 Is Increased in NOD Diabetic Mice

Marta Rivera, Eva Marquez, Helcia Roca-Ho, Daniel Batlle, Julio Pascual, Maria Jose Soler. Kidney Disease Research Group, Nephrology Department, Hospital del Mar-IMIM, Barcelona, Spain; Nephrology, Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: ACE2 is a homologue of ACE that plays a counter-regulatory role. Renal cortex ACE2 activity is increased in some diabetic mouse models. However, little is known about ACE2 and the effect of insulin in NOD(obese diabetic).

Methods: Serum, urinary and renal cortical ACE2 was studied in NOD mice at 20 and 40 days after onset diabetes and compared with NOR controls and with insulin-treated NOD.

Results: Blood glucose and UAE increased in NOD as compared to NOR (21d: BG: 571.1±20.3 vs 112.6±4.9 mg/dL; UAE: 941.2±27.1 vs 86.3±3.4 µg/µg creatinine; 40d: BG: 550.6±32.7 vs 138.7±4.3 µA; UAE: 492.2±310.9 vs 22.0±4.4 µg/µg creatinine). Insulin administration decreased BG and UAE (21d: BG: 113.4±5.4; UAE: 20.5±4.6; 40d: BG: 88.4±12.2; UAE: 90.4±15.7, p<0.05).

Serum and urine ACE2 enzyme activity was increased in NOD mice compared to NOR. Insulin administration decreased serum and urine ACE2 activity.

Funding: Pharmaceutical Company Support

SA-PO2575

The Change of β-Catenin in Experimental Diabetic Nephropathy

Tae-Sun Ha, Pediatrics, Chungbuk National University College of Medicine, Cheongbu, Chungbuk, Korea.

Background: Proteinuria is a cardinal feature of glomerular disease including diabetic nephropathy, and glomerular filtration barrier is considered as a filter restricting protein excretion in urine. We tested whether the expression of β-catenin, a molecule known to be located at the slit diaphragm, would be altered by high glucose in the cultured podocyte in vitro and by diabetes in vivo.

Methods: To investigate whether diabetic conditions including high glucose and advanced glycosylation endproducts (AGE) induce podocyte β-catenin changes, we observed renal tissues of diabetic rat obtained at 48 hrs, 4 weeks and 10 weeks after the induction of diabetes. And, we cultured rat glomerular epithelial cells (GEC) and mouse podocytes under normal (5 mM) or high glucose (30 mM, HG) and AGE- or BSA-added conditions and examined the distribution of β-catenin by confocal microscope and measured the change of β-catenin expression by Western blotting and RT-PCR.

Results: Immunofluorescence of rat tissues stained with β-catenin and β-catenin showed their co-localization and decrease in intensities at podocyte around capillary loops. We also found that β-catenin relocalized from peripheral cytoplasm to inner actin filaments and its decrease in intensities by both AGE-added and HG condition in GEC and mouse podocytes. In Western blotting, HG or AGE-added condition decreased β-catenin expression by 20.5% (p<0.05) and 16.0% (p<0.05), respectively, compared to the normal glucose condition. HG and AGE-added condition further decreased β-catenin protein expression to statistically significant level (31.5%, p<0.05). No significant change was seen in osmotic control. In RT-PCR, HG plus AGE-added condition significantly decreased the expression of β-catenin mRNA by 14% (p<0.05) compared to normal glucose condition.

Conclusions: These results suggested that the expression of podocytes to HG and AGE in vivo and in vitro reduced β-catenin mRNA and protein expression. These findings suggest that the decrease in β-catenin expression is connected to the early changes of diabetic nephropathy and thus may contribute to the development of proteinuria.

Funding: Government Support - Non-U.S.
SA-PO2556

Short Duration of Diabetes Alters Renal Endothelial Cell Function
Christina M. Sorenson, Cathy Grutzmacher. Department of Pediatrics, University of Wisconsin School of Medicine and Public Health, Madison, WI.

Background: Diabetic nephropathy is the most common cause of end stage renal disease and is a major risk factor for cardiovascular disease. In the United States, microvascular complications during diabetic nephropathy contribute to high morbidity and mortality rates. However, the identity of the underlying molecular and cellular mechanisms remains elusive.

Methods: Male Akita/+ (autosomal dominant mutation in the insulin II gene (Ins2)) mice, which reproducibly develop diabetes by 4 weeks of age were used. The impact of short duration diabetes on renal endothelial cell (EC) function, as well as ex vivo aorta sprouting, was determined. Capillary morphogenesis of renal EC was evaluated in Matrigel. The migration of EC was evaluated using a transwell and scratch wound assay. The changes in eNOS expression and phosphorylation, as well as production of various extracellular matrix proteins, were determined by Western blotting. The rate of EC proliferation and apoptosis was determined using Click-it Edu and TUNEL-labeling, respectively.

Results: Renal EC from Akita/+ mice following 4 weeks of diabetes demonstrated decreased migration and capillary morphogenesis. These studies were consistent with our aort ex vivo angiogenesis assays, which showed a significant decrease in the number of sprouting outgrowths in aortas from Akita/+ mice. We also observed increased total and phosphorylated eNOS expression along with increased fibroblast expression, potentially contributing to EC dysfunction. The levels of proliferation and apoptosis were similar.

Conclusions: These studies demonstrate that aberrant EC function with short duration of diabetes may set the stage for vascular dysfunction and rarefaction at later stages of diabetes.

Funding: Private Foundation Support

SA-PO2577

Progressive Renal Failure, Proteinuria and Glomerulosclerosis in a Murine Model of Diabetic Nephropathy Is Associated with Mitochondrial Oxidative Stress
Sharma S. Prabhakar, Medicine, Texas Tech Medical Center, Lubbock, TX.

Background: Animal models of diabetic nephropathy (DN) hitherto described do not confirm to human disease since they do not exhibit the progression of proteinuria or renal failure characteristic of human disease. We previously characterized and published the phenotypic features of ZSF1 rats at 20 weeks which develop histological and functional changes that mimic early human DN (Prabhakar et al. JASN 2007). We studied ZSF rats for much longer period with the goal of examining the progression of nephropathy and seeking correlations with human DN.

Methods: ZSF rats were obtained at 8 weeks of age and fed on high calorie diet until 40th week to maintain hyperglycemia. The body weights and blood pressures were monitored weekly while blood samples were obtained at the 8th, 12th, 24th and 40th week. A 24 hour urine sample was obtained at the same time points for the measurement of creatinine clearance and protein excretion rates. Since 8-OHdG (8- hydroxydeoxyguanosine), a marker for mitochondrial oxidative stress correlated better with total body oxidant stress and urinary excretion of 8-OHdG parallels the renal oxidative stress, urinary 8-OHdG levels were measured at the same time points. At 40 weeks the rats were euthanized and kidneys harvested to examine the histopathology.

Results: ZSF rats developed obesity, hypertension, hyperlipidemia by 12th week as well as nephropathy with progressive proteinuria and renal failure that reached end stage by 40th week. Renal histology at this stage showed advanced diffuse glomerulosclerosis. The results of the functional parameters measured are shown below.

<table>
<thead>
<tr>
<th>Age in weeks</th>
<th>8</th>
<th>12</th>
<th>24</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td>UProt (mg/g of creat)</td>
<td>165±37</td>
<td>459±84</td>
<td>1254±148</td>
<td>1658±542*</td>
</tr>
<tr>
<td>UProt (mg/g of creat)</td>
<td>0.7 ± 0.3</td>
<td>1.5 ± 0.9</td>
<td>3.1 ± 0.7</td>
<td>2.16±0.5</td>
</tr>
<tr>
<td>8-OHdG (ng/Kg/BW)</td>
<td>387±89</td>
<td>2468±307</td>
<td>5429±453</td>
<td>7655±566*</td>
</tr>
<tr>
<td>n=6; *p&lt;0.01 vs. at 8 weeks age.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: We conclude ZSF rats develop obesity, diabetes and nephropathy which progresses to advanced renal failure along with nephritic syndrome, profound mitochondrial oxidative stress and diffuse severe glomerulosclerosis. Thus ZSF rats represent an ideal murine model to study human DN.

Funding: Private Foundation Support

SA-PO2587

The Absence of Collagen Type VIII Leads to Inhibition of Epithelial-Mesenchymal Transition (EMT) in Induced Diabetic Nephropathy
Ivonne Osetoff, Gunter B. Wolf. Internal Medicine III, University of Jena, Jena, Germany.

Background: The epithelial-mesenchymal transition (EMT) is a mechanism of renal tubulointerstitial fibrosis in diabetic nephropathy (DN) that leads to an accumulation of smooth muscle α-actin (α-SMA)-positive myofibroblasts. The process of the EMT is characterized by the progressive loss of E-Cadherin, zona occludens 1 (ZO1) and cytotakerin (CK) of tubular epithelial cells, which is associated with increased expression of mesenchymal markers (α-SMA, fibroblast-specific protein 1 FSP1 and vimentin). It has been reported that the presence of matrix-producing cells in the tubulointerstitium of D1N and D2N is associated with increased expression of non-fibrillar short-chain collagen VIII (genes: COL8A1 and COL8A2). It is possible that non-fibrillar short-chain collagen VIII is associated with increased expression of non-fibrillar short-chain collagen VIII.

Methods: Kidneys from Col8A1/A2 knockout (KO) mice were harvested and analyzed. We investigated the expression of collagen type VIII in kidneys from Col8A1/A2 KO mice.

Results: In contrast to diabetic wildtype mice, collagen type VIII expression in diabetic Col8A1/A2-KO mice was significantly increased by 34% compared to wildtype mice. The expression of collagen type VIII was also significantly increased in diabetic Col8A1/A2-KO mice.

Conclusions: These results suggest that either collagen type VIII may be one of the key mechanisms that prevent the progression of diabetic nephropathy in a murine model of diabetic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2579

Phenotypic Changes of Podocyte CD2AP in Experimental Diabetic Nephropathy Via PI3-K/Akt Signaling
Tae-Sun Ha, Pediatrics, Chungbuk National University College of Medicine, Cheongju-si, Chungbuk, Korea.

Background: Proteinuric conditions demonstrate ultrastructural changes in podocytes with retraction and effacement of the highly specialized interdigitating foot processes, resulting in hyperpermeability.

Methods: To investigate podocyte phenotypic changes, including quantitative and distributional changes of CD2AP protein in diabetic conditions, we prepared diabetic renal tissues and cultured podocytes in diabetic conditions. According to diabetic duration, density of CD2AP in diabetic tissue of experimental diabetic nephropathy became conglom erated and diminished. To investigate how high-glucose (HG) and high glucose advanced glycosylation end products (AGE) induce podocyte phenotypic changes, including quantitative and distributional changes of CD2AP, we search for the signaling mechanisms.

Results: HG plus HGF and AGE induced the re-localization of CD2AP into inner actin filaments, which is accompanied by retraction and effacement. HG plus AGE increased the amount of CD2AP protein by 36.9% (p<0.05) and 16.0% (p<0.05), respectively. Furthermore, both high glucose and AGE decreased the amount of CD2AP more significantly by 64.6% compared to those of control (p<0.01). In RT-PCR, the expression of high glucose and AGE decreased the expression of CD2AP mRNA by 44.9%, 27.9%, and 29.3% (p<0.05), respectively, compared to that of control. In addition, LY294002, a PI3-K inhibitor, could prevent the quantitative and distributional changes of CD2AP induced by HG and AGE.

Conclusions: These findings suggest that diabetic conditions induce the phenotypical changes of podocyte CD2AP via PI3-K/Akt signaling.

Funding: Government Support - Non-U.S.

SA-PO2580

The Role of MORG1 in Early-Stage Diabetic Nephropathy (DN)
Carola Ruhn, Gunter B. Wolf. Internal Medicine III, University of Jena, Germany.

Background: DN is a progressive kidney disease resulting and is characterized by excessive deposition of extracellular matrix proteins, increasing intrarenal inflammation and chronic hypoxia. The mitogen-activated protein kinase organizer 1 (MORG1) has been identified as a novel WD-repeat protein that interact with the prolyl hydroxylation 3 (PHD3), an important enzyme involved in the regulation of the hypoxia-inducible factor (HIF). The purpose of this study was to assess the role of MORG1 in the development of early-stage diabetic nephropathy in diabetic heterozygous MORG1+/- mice.

Methods: Diabetes mellitus was induced in wildtype MORG1+/- and homozygous MORG1+/+ mice with an intraperitoneal injection of streptozotocin (STZ, 5 mg/kg). Metabolomics and chemical biology techniques were used to estimate the expression of extracellular matrix proteins (collagen I, collagen IV and fibronectin), profibronflammatory mediators (e.g. MCP-1 – monocyte chemotactic protein-1) and the transcription factors Hif1α and Hif2α in the kidney of non-diabetic and diabetic mice.

Results: The expression of collagen I in the glomeruli of diabetic heterozygous MORG1+/+ mice was significantly less compared with the diabetic wildtype MORG1+/- mice. On the other hand, downregulation of MORG1 did not show a significant impact on glomerular deposition of both collagen IV and fibronectin. The profibronflammatory mediator MCP-1 was significantly increased in the diabetic group compared with the non-diabetic group, but there were no significant differences in the expression of MCP-1 between diabetic wildtype and diabetic heterozygous mice.

Conclusions: Downregulation of MORG1 reduces the deposition of extracellular matrix proteins and attenuates the progression of DN. However, the expression of profibronflammatory markers seems to be independent from MORG1.

Funding: Other U.S. Government Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
**SA-PO2581**

**The Inflammatory Genotype of Diabetic Nephropathy**  
Katherine J. Kelly, Jesus H. Dominguez.  
Exocell, Inc. Philadelphia, PA.

**Background:** Obese, diabetic ZS (F1 hybrids of Zucker and SHIR) rats develop progressive renal fibrosis and inflammation on a high fat diet. Nephropathy is markedly accelerated after a single episode of renal ischemia.

**Methods:** When renal mRNA sequencing was performed to fully characterize transcript expression in obese-diabetic/postischemia (OS) as compared to obese-diabetic sham surgery (OS) and (S) rats kidney.

**Results:** WBC (cells/hp) are markedly increased in OS rats: 0.3±0.2 L, 8±1 in OS, 12±1 L (p=0.01). Also, lymphocytes were found to be increased in OS (25±1 L 0.01) vs 10±3 (p=0.01). BMG were significantly increased in OS (3.0±1 vs 1.5±0.01 in controls). The majority of white cells were monocytes (69±8) and lymphocytes (23±5) in OS vs (45±8) and (19±5) in controls.

**Conclusions:** We confirm the role of mononuclear cell recruitment in OS kidneys.

**SA-PO2582**

**High Glucose Uregulates Nucleoporin62 (Nup62) in Human Mesangial Cells**  
Rakesh Singh.  
Research, Hines VA Medical Center, Hines, IL.

**Background:** Nucleoporin (Nup) proteins such as nucleoporin62 (Nup62) (2) and nucleoporin85 (Nup85) (2) are required for nuclear import of transcription factors and gene transcription of matrix proteins.

**Methods:** Enhanced nuclear co-localization of AT1 receptor with Nup62 in human mesangial cells (HMC) was observed. Incubation of HMC with 25 mM glucose (HG) for a period of 1-10 days. At termination of experiments, cytoplasmic and nuclear extracts were prepared and protein expression of Nup62 was determined by Western blotting. Also, HMC were seeded in Lab-Tek cell culture slides and Nup62 localization was examined by immunofluorescence. High glucose-stimulated induction of Nup62 expression was studied in human mesangial cells. We tested the effect of high glucose on Nup62 in glomerular mesangial cells.

**Results:** High glucose increased protein expression of Nup62 in glomerular extracts suggesting that high glucose milieu in diabetes might affect NPC functions. In this study, we further examined the effect of high glucose on Nup62 in glomerular mesangial cells.

**Conclusions:** The role of high glucose in renal diseases requires further investigation.

**SA-PO2583**

**Nephrinuria vs. Podocyturia: Which Is a Better Marker for Diabetic Nephropathy?**  
Shuchita Sharma, Andy Qipo, Kwanghee Kim, Pu Zong, Swati Mehta, Dennis Michael Bonal, Belinda Run Jim, Jacob Med Ctr. NY; Trinity Health Ctr. ND; Columbia Med Ctr. NY.

**Background:** There is interest in using urinary podocyte (podocyturia) as a marker to assess glomerular disease activity in diabetic nephropathy (DN), as its pathogenesis is related to podocyte loss. However, technical issues and lack of validation still prevent its clinical application. Nephrin, a podocyte protein, is also found in the urine of diabetic patients (nephrinuria). With the advent of an enzyme-linked immunosorbent assay (ELISA) for nephrin (Exocell, Inc. Philadelphia, PA), we are able to accurately measure nephrin. Which marker offers superior correlation with clinical disease is unclear.

**Methods:** We compared these two markers by quantifying podocyturia and nephrinuria in 24 diabetic patients and 10 healthy controls and correlated with clinical parameters. For podocyturia, a cell pellet was obtained via centrifugation from fresh urine and fixed for immunofluorescence. Podocytes were identified by colocalization of podocyte markers podocin and synaptopodin and counted by 2 investigators. For nephrinuria, ELISA was used to measure nephrin in urine supernatant. The Wilcoxon rank test compared differences between study and control groups, while the Spearman rank correlation coefficients associated clinical parameters with urine podocyte-to-creatinine ratio (UPodCR) or urine nephrin-to-creatinine (UNCR).

**Conclusions:** Both UPodCR and UNCR were both significantly higher in the study than control groups (p=0.005 and p=0.01 respectively). UNCR correlated significantly with urine albumin-to-creatinine ratio (UACR) (rho=0.2,p=0.001), BUN (rho=0.47, p=0.02), serum creatinine (rho=0.48, p<0.01), systolic BP (rho=0.75, p<0.01), while UPodCR did not correlate with any of these parameters including albuminuria.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
these cells. FXR is expressed in renal human tubule and its level is reduced in patients with DN. Renal structural changes were present in offspring from obese mothers with features of dysregulated glucose and lipid metabolism in association with reduced FXR expression in the tubule.

Conclusions: Our results suggest a role for FXR in adverse renal outcomes in offspring from obese rats with metabolic derangement which may be of considerable therapeutic value.

Funding: Government Support - Non-U.S.

SA-PO2586
Clinical and Molecular-Pathological Effects of Chronic Hypoxia on Diabetic Nephropathy in db/db Mice Naoki Takahashi,1 Hideki Kimura,1 Kazuko Kamiyama,1 Tomomi Kurose,2 Hidehiro Sugimoto,2 Toshio Imura,2 Daiisuke Mikami,1 Kenji Kasuno,1 Haruyoshi Yoshida.1 1Division of Nephrology, University of Fukui, Japan; 2Division of Clinical Laboratories, Fukui University Hospital, Fukui, Japan; 3Department of Internal Medicine, Sagita Genpak Adult Nephrology and Hypertension Center, Osaka, Japan.

Background: Hypertension, angiongenetic cytokines and eNOS-VEGF imbalance, which may be affected by chronic hypoxia, reportedly accelerate progression of diabetic nephropathy. However, no detailed information has been known about the clinical and molecular-pathological effects of chronic hypoxia on diabetic nephropathy in db/db mice.

Methods: 8 w.o. male db/db mice were bred in a normobaric hypoxic chamber (12%O2). The hypoxic group (H-group) was kept in this chamber for 16 weeks (n=11) and the control group (N-group) were bred in room air (n=12). Mice were sacrificed at 12 and 24 w.o. in order to evaluate histological changes and mRNA amounts (VEGF-A, P-A, MCP-1, TGF-β1, CTGF, eNOS, CD34 and Angiopoietin1 and 2) extracted from whole kidney.

Results: H-group showed severer erythrocytosis, greater albuminuria and higher serum LDL levels than N-group, while the two groups did not differ in levels of blood pressure, serum Cr, Ccr or urinary VEGF-A antigen. As for histological aspects, H-group had greater glomerular swelling (1.3-fold), lower number of podocyte and CD34-positive endothelium per glomerular area and higher rates of microaneurysm formation, glomerulosclerosis and glomerular macrophage infiltration than N-group. Glomerular VEGF-A positive area levels were, however, similar in H and N-groups. Concerning molecular analyses, in H-group, mRNA levels of MCP-1 and PAI-1 were significantly increased by 1.5-fold at 4W and 9-fold at 16W, respectively as compared with N-group, whereas mRNA levels of VEGF-A, TGF-β1, CTGF, Angiopoietin1 and 2, CD34 and eNOS were very similar.

Conclusions: Hypoxia-specific glomerular injury may result from the decreased number of endothelium and podocyte per glomerular area and the increased macrophage infiltration via MCP-1 and PAI-1 induction, but not from hypertension or hyperfiltration.

Funding: Government Support - Non-U.S.

SA-PO2587
Urine microRNA Profiling in Nephropathy of Type 1 Diabetes Christos Argyropoulos,1 Kai Wang,2 Jose F. Bernardo,1 David Galas,2 John P. Johnson.3 1University of Pittsburgh, PA; 2Institute for Systems Biology, Seattle, WA.

Background: Diabetic Nephropathy (DN) is the leading cause of Chronic Kidney Disease (CKD) in the US, affecting 1/4 of patients with Type 1 Diabetes (T1D). We examined urine profiles of microRNA (miR), endogenous RNA negative regulators of gene expression, as biomarkers for the development of DN.

Methods: Patients (pts) with T1D from the Pittsburgh Epidemiology of Diabetes Complications (EDC) a historical prospective cohort, free from DN (N,n=10), or with microalbuminuria (MA,n=20) during follow up (f/u). We profiled miR expression in urine from the last f/u visit (N) or the last non-albuminuric visit (MA) pts, with qPCR panels.

Results: Patient characteristics were similar though MA patients tended to be older (p<0.05) and have a higher body mass index (BMI, p<0.01). Urine miR expression in MA vs N pts is presented as Heatmap and volcano plots (FigA,B).

Conclusions: Urine miR expression differs in T1D pts who develop MA compared to pts who never develop DN. Future studies should verify the utility of these miRs as early biomarkers of DN.

Funding: Government Support - Non-U.S.
expansion was also not greater in the podocyte JAK2 diabetic mice than in the diabetic mice without JAK2 level. At 3 weeks of age (nondiabetic controls). However, there was a greater increase in glomerular volume in the diabetic JAK2 mice compared with the diabetic with normal JAK2 levels (2.52-fold vs. 1.67-fold; p<0.05).

Conclusions: These data suggest that increased expression of podocyte JAK2 may play a role in DN by increasing glomerular growth more than podocyte foot process changes or in glomerular fibrosis.

Funding: NIDDK Support

SA-PO2589

A Barricent of the Canonical Wnt Pathway in the Renal Tubular Cells in Diabetic Nephropathy Jian-Xing Ma, Ti Zhou, Rui Cheng, Xueyin He, Ying Chen. Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Background: The Wnt pathway mediates multiple physiological and pathological processes such as inflammation, angiogenesis and fibrosis. The present study is to investigate whether the canonical Wnt signaling plays a role in the pathogenesis of diabetic nephropathy (DN).

Methods: Expression of Wnt ligands and Frizzled receptors in the canonical Wnt pathway in the kidney was compared at the mRNA levels using real-time RT-PCR between Akita mice, streptozotocin (STZ)-induced diabetic rats and db/db mice and their respective non-diabetic controls. Renal function was evaluated by measuring the urine albumin excretion. Primary human renal proximal tubular epithelial cells were treated with high glucose and 4-hydroxynonenal (HNE). Levels of β-catenin, connective tissue growth factor and fibronectin were determined by Western blot analysis.

Results: Wnt ligands and Frizzled receptors showed increased mRNA levels in the kidneys of Akita mice, STZ-induced diabetic rats and db/db mice, compared to their respective non-diabetic controls. Renal levels of β-catenin and Wnt proteins were up-regulated in these diabetic models. Lowering the blood glucose levels by insulin attenuation the activation of Wnt signaling in the kidney of Akita mice. In cultured primary human renal proximal tubular cells, high glucose and HNE both activated Wnt signaling. Inhibition of Wnt signaling with a monoclonal antibody blocking low-density lipoprotein receptor-related protein 6 (LRP6) ameliorated renal inflammation, fibrosis and reduced proteinuria.

Conclusions: The Wnt pathway is activated by hyperglycemia in the kidney of both mouse models of type 1 and 2 diabetic mice. The Wnt pathway dysregulation in diabetes represents a new pathogenic mechanism of DN and renders a new therapeutic target.

Funding: Other NIH Support - EY018659, EY019309, EY012231

SA-PO2590

Relationships between Serum MCP-1 and Subclinical Kidney Disease African American-Diabetes Heart Study Barry I. Freedman, E. Fuenmayor-Cardozo, Ganesan Ramesh, David M. Pollock, John White. Medicine, Georgia Health Sciences University, Augusta, GA.

Background: Monocyte chemotractant protein-1 (MCP-1) plays key roles in nephropathy and atherogenesis. Relationships between serum MCP-1 concentration and kidney and cardiovascular disease (CVD) were assessed in African American (AA) population.

Methods: Serum MCP-1 was measured in 479 AAs with type 2 diabetes (T2D). Urine albumin: creatinine ratio (ACR), eGFR, and calcified atherosclerotic plaque (CP) in the coronaries, carotid arteries, and infra-renal aorta were measured. Generalized linear models were fitted to test for associations between MCP-1 and ACR, eGFR, and CP.

Results: Participants were 57% female; mean (SD) median age 55.6 (9.5) 55.0 years, diabetes duration 10.3 (8.2) 8.0 years, urine ACR 151 (569) 14.0 mg/g, MDRD eGFR 95.2 ml/min/1.73m2, MCP-1 262.9 (239.1) 224.4 pg/ml, coronary artery CP (63/12.5), carotid CP (25/132.9), and aorta CP 1610.6 (9) 319. Adjusting for age, sex, smoking, BMI, blood pressure, diabetes duration, hemoglobin A1c, cholesterol, and medications, serum MCP-1 levels had direct correlation with ACR (parameter estimate 0.243 SE(0.122), P=0.003) and inverse correlation with eGFR (parameter estimate -0.0007 SE(0.0004), P=0.001). Significant associations were not detected with the extent of CP in any vascular bed.

Conclusions: Serum MCP-1 levels are significantly associated with cross-sectional measures of kidney function and albuminuria in AAs with T2D. In this racial group, MCP-1 levels are not associated with the vascular CP, surrogate of subclinical CVD.

SA-PO2591

Altered Wntβ-catenin Pathway and Association of β-catenin with CHOP in Tubules of Diabetic Mice Michelle T. Barati, Susan M. Isaacs, David W. Powell, Michael Merchant, Jon B. Klein. Nephrology, University of Louisville, KY.

Background: The presence of endoplasmic reticulum (ER) stress response and apoptosis in diabetic nephropathy (DN) is evident, yet the role of ER stress in tubule cells dysfunction in the DN is not known. The goal of this study was to characterize novel functions of ER stress response signaling in renal tubules that may play a role in DN pathogenesis.

Methods: We first identified proteins co-immunoprecipitating with ER stress-induced pro-apoptotic protein, CHOP in tubules of mice, using mass spectrometry (MS). Cell extract from isolated cortical tubules of OVE26 diabetic and FVB control mice were used. Immunoprecipitated proteins were trypsinized, subjected to LC-MALDI-TOF/TOF MS and LC-ESI-MS/MS. Identified proteins were analyzed for network associations using String similarity pathway analysis. IPA of association of MS-identified proteins in complex with CHOP was validated by immunoprecipitation of CHOP from tubule extract followed by immunoblot analysis for specific proteins.

Results: IPA identified a protein network related to Wntβ-catenin signaling including, Frizzled related protein, Wnt, caspin kinase 1, APC, diacylglycerol kinase (β), polyclutin 1, myosin 7A, tp73, and Jade1. CHOPβ-catenin association was increased in tubules of 3 month old diabetic mice, and decreased in 7 month old diabetic mice. Casein kinase 1 and Jade 1 expression (proteins that regulate β-catenin) was increased in tubules of diabetic mice. CHOPβ-catenin interaction was studied in proximal tubule cells (HK-2) during Thapsigargin (TG)-induced ER stress conditions, by confocal microscopy. TG increased total CHOP expression, decreased membrane β-catenin localization, and increased nuclear co-localization of both proteins. Additionally, exposure of HK-2 cells to high albumin concentrations increased β-catenin nuclear localization.

Conclusions: Together, these findings demonstrate altered interaction of β-catenin and CHOP in a complex protein during the course of diabetes in renal tubules. An alternate role for CHOP action and a novel molecular mechanism of ER stress response signaling in tubule cell pathogenesis during diabetes are suggested by these findings.

Funding: NIDDK Support

SA-PO2592

The Study of Inhibitory Effects by Aprotinin on the Mannan-Binding Lectin Pathway Activation of the Kidney of Diabetic Rats Xinyang Yan, Songmin Huang, Yuxi Yang. Department of Nephrology, The West China Hospital of Sichuan University.

Background: Our previous work has proved that the levels of Mannan-binding lectin (MBL-A and MBL-C3) and Membrane-attack complex(MAC) expression were increased in the kidney of diabetic Rats. The lectin pathway may contribute to the development of early stage DN. It can be beneficial for kidney protection of a therapy of MBL complement pathway inhibitors. Aprotinin can inhibit the lectin pathway through its ability to inactivate irreversibly the MBL-associated serine protease (MASP). In the present studies, we would evaluate the inhibitory effects of aprotinin on the lectin pathway activation in the kidney of diabetic Rats.

Background: SD male rats were randomly divided into three groups: normal control group (N), DN group (DN) and DN treated with aprotinin(group DN-A). Rats of the group DN were treated by streptozotocin(50mg/kg). After modeling, Rats of the group DN-A were injected intraperitoneally with aprotinin(40000 KIU/kg,d,Bayer,German) for four weeks (3-week to 7-week). The rats of the three groups were sacrificed at week 4 and 8 respectively, meanwhile blood sugar, serum creatinine(SCr),body weight, kidney weight and 24-hours urinary protein were monitored. The expression of MBL-A,MBL-C3 and MAC in renal tissue were detected by immunohistochemistry and western blot.

Results: In the group DN-A, a significant reduction of urinary protein, body weight /kidney weight(Ki),glomerular tuft volume(GV),mesangial matrix expansion index (MMEI) and plasma glucose levels(not to normal levels) were observed as compared with group DN at 4-week and 8-week. However the levels of Scr was not markedly increased. In group DN-A, the renal expression of MBL-A,MBL-C3 and MAC were obviously decreased compared with group DN at 4-week and 8-week by immunohistochemistry and western blot.

Conclusions: These data suggest that a beneficial effect of aprotinin treatment on the process of glomerular damage in the diabetic Rats. Its effects may attribute to the inhibition of the lectin pathway.

Funding: Government Support - Non-U.S.

SA-PO2593

Urinary Biomarkers Are Increased in Experimental Diabetes Franklin E. Fuenmayor-Cardozo, Ganesan Ramesh, David M. Pollock, John White. Medicine, Georgia Health Sciences University, Augusta, GA.

Background: Recent studies suggest an increased role of tubulointerstitial injury in diabetic kidney disease. However, there are no validated biomarkers of tubulointerstitial injury in diabetes. We hypothesized that tubular injury biomarkers useful for studying acute kidney injury would be elevated in early experimental diabetes.

Methods: The group DN-A diabetic rats were treated with STZ (n=7). Controls (CTL) received normal saline (n=9). Rats were placed in metabolic cages at baseline, 4 weeks, and 10 weeks. Urinary kidney injury molecule-1 (KIM-1) and γ-glucosaminidase (NAG) were measured as compared with group DN at 4-week and 8-week. However the levels of Scr was not markedly increased. In group DN-A, the renal expression of MBL-A,MBL-C3 and MAC were obviously decreased compared with group DN at 4-week and 8-week by immunohistochemistry and western blot.

Conclusions: These data suggest that a beneficial effect of aprotinin treatment on the process of glomerular damage in the diabetic Rats. Its effects may attribute to the inhibition of the lectin pathway.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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excretion was increased in STZ (1.79 ± 1.7 vs. 12.9 ± 0.3 μg/24h, P < 0.05). Conversely, NAG serum concentrations were increased in STZ and DMCA compared to CTL at 4 weeks (3167 ± 472 and 2208 ± 210 vs. 3357 ± 22 IU/L, P < 0.05). Likewise, NAG excretion was increased at 10 weeks in STZ vs. CTL (4042 ± 353 vs. 366 ± 27 IU/L, P < 0.05).

Conclusions: Urinary tubular biomarkers are increased early in experimental diabetes. In the presence of NAG, the increase occurs prior to the development of significant proteinuria. Our data suggest these biomarkers may be useful surrogates for therapies targeting tubulointerstitial injury in diabetes and chronic kidney diseases.

Funding: Clinical Revenue Support

SA-PO2594

N-Acetylcysteine and Oxidative Stress in the Kidney of Uninephrectomized Rats with Diabetes Mellitus 1 Elisa M S Higa, 2 Guilherme Baia Nogueira, 3 Adelson Marçal Rodrigues, 4 Giovana Rita Panarol, 5 Margaret Gori Mouru, 6 Fabiane Maciel. 7 Medicine - Nephrology Division, UNIFESP; 8 Medicine - Emergency Division, UNIFESP, Sao Paulo, Brazil.

Background: Diabetes mellitus (DM) induces intra and extracellular changes, with a substantial increase in reactive oxygen species (ROS). ROS cause damage in systemic and renal microvasculature, which could be one of the mechanisms involved in the pathophysiology of diabetic nephropathy. ROS also modulate other substances, like the nitric oxide (NO), a powerful vasodilator with important role on kidney function. N-acetylcysteine (NAC) is an antioxidant largely used to prevent contrast induced renal lesion.

The aim of this study was to evaluate the effect of NAC and oxidative stress in the kidney of uninephrectomized rats with DM.

Methods: Adult male Wistar rats were unilaterally nephrectomized (UNx). Control groups (CTL) were sham operated. DM was induced with streptozotocin (60mg/kg, iv), in half of UNx animals (DM+UNx) and the others received its vehicle (CTL+UNx). Half of the CTL+UNx and DM+UNx animals received NAC (600mg/L in water, ad libitum). Groups (N=4 each): CTL, CTL+UNx, CTL+UNx+NAC, DM+UNx and DM+UNx+NAC. Before and after 8 weeks with NAC, we collected the 24 hour urine and a blood sample. Data = mean±SEM, analyzed by one-way ANOVA, with Tukey post-test; significant for P<0.05.

Results: DM+UNx compared with CTL+UNx showed increased levels of glycemia (427.31±31 vs 189.25) and altered renal function, with increased plasmatic creatinine (2.0±1.1 vs 1.2±.1), urea (71.6±5 vs 38.8) and proteinuria (40.10±15.1 vs 15.10±15.1). DM+UNx presented increased deposition of extracellular matrix (β1 were reverse to serum APN each. Moreover, NAG excretion was increased in comparison with the LET, and improved it in PGZ-OLT, and these were the relations that were reverse to serum APN each.

Conclusions: It was suggested that APN/AdiopR1 system in the glomerulus involved in an appearance of the MA in DN.

SA-PO2597

Involvement of Adiponectin and Adiponectin Receptor 1 in the Albuminuria Appearance of Diabetic Nephropathy 1 Koichi Kanozawa, Juko Asakura, Hajime Hasegawa, Taisuke Shimizu, Kaori Takayanagi, Takatsugu Iwashita, Yosuke Tayama, Tokushi Nakajima, Tetsuya Mitarai. Nephrology and Hypertension, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan.

Background: We reported that a decline of microalbuminuria(MA) was related to increase of microsomes(APN) by administrating various kinds of PPARα agonists for human diabetic nephropathy(DN). Recently, it has been reported that APN-knockout mice exhibited increased albuminuria and fusion of podocyte foot processes. On the other hand, among ligands of APN, it is revealed that APN receptor(Adipor1) and 1 T cadherin(TCδ) exists in the kidney. The aim of this study is to clarify participation in the appearance of MA and the formation of DN with the APN/Adipor1/TGδ system.

Methods: The male Otsuka Long Evans Tokushima Fatty(OLETF) rats which were known as type 2 diabetes, and Long Evans Tokushima Otsukahime(LETO) rats which were control, were used. These rats were divided into three groups, such as the OLETF received low-dose (2.5 mg/kg) pioglitazone for six weeks (PGZ-OLT), the OLETF not received PGZ (OLT), and LETO (LET). To 34 weeks of age, APN, creatinine clearance, and urinary albumin excretion rate(UAE) were measured, and the kidneys were examined. We examined local existence of the APN, Adipor1, and TCD in the kidney tissue of each group, using indirect immunofluorescent method.

Results: APN in DM group decreased to 3.1±0.2 μg/ml in the OLT, and rose with 2.6±0.3 μg/ml in the PGZ-OLT, compared with 1.8±0.1 μg/ml in the LET. On the other hand, UAE rose significantly with 3720.5±1394.3 mg/mgCr in OLT whereas it was 88.8±5.1 mg/mgCr in the LET(both were controlled). In LETO(LE), 7±1155.4 mg/mgCr in the PGZ-OLT. In the kidney histologic examination, APN accorded for local existence of Adipor1 in the glomerulus, it was dyed by podocytes, mesangial cells, and endothelial cells, but APN did not accord with the local existence of TCδ. Interestingly, the stainability of APN aggravated in it OLT in comparison with the LET, indicated involved in PGZ-OLT, and these were the relations that were reverse to serum APN each.

Conclusions: It could be useful in the treatment of diabetic patients.

Funding: Government Support - Non-U.S.

SA-PO2598

The Role of Chymase in the Renal Lesion of the Diabetic Rats 1 Mei Zhang, Jing Bai, Xiaodong Nie, Wen Huang. Nephrology, Beijing Tongren Hospital, Capital Medical University, Beijing, China.

Background: Diabetic nephropathy is a leading cause of end-stage renal disease, which characterized by renal fibrosis. The objective of the study is to investigate the role of chymase in the development of renal fibrosis of diabetic rats.

Methods: 24 male SD rats were randomly divided into three groups including control , DM and DM+ Chy-Inhibitor (Chy-1) groups. Diabetes was induced by intraperitoneal injection of Streptozotocin (60mg/kg). The rats in the Chy-1 group were injected with chymase inhibitor (Oph), 1mg/kg for 12 weeks. Kidney weight, level of blood glucose, 24-hour urine protein, creatinine clearance, serum level, and blood pressure were measured. The renal pathologic lesion were observed by light microscope and electron microscope, the expression of fibronectin, type IV Collagen, α-SMA and TGF-β1 were observed by immunohistochemistry and RT-PCR.

Results: Diabetic rats were presented with characteristics of increasing creatinine clearance, K/W/BW, level of serum cholesterol, 24-hour urinary albumin and urinary albumincreatinin (P<0.05, P=0.01, P=0.05 and P=0.01), and decreasing level of serum albumin and total protein (P<0.05) respectively. Compared with DM group, level of serum cholesterol and urinary albumin/creatinin were decreased for15.58% and 75.40% in Chy-1 group (P<0.05). There were no significant difference with DM group and Chy-1 group in K/W/BW, 24-hour urinary albumin, blood pressure, level of serum lipid, albumin and creatinine, and protein and creatinine clearance.

Diabetic rats had increasing deposition of extracellular matrix in glomerular area, followed by elevated expression of FN, ColIV, alpha-smooth muscle actin (α-SMA) and TGF-β1 for 25.35%, 17.24%, 26.30% and 19.98%(p<0.05). The lesion of foot processes of podocytes was ameliorated in Chy-1 group.

Conclusions: In diabetic rats, chymase promoted the secretion of urinary protein, the injury of foot processes of podocyte and deposition of extracellular matrix. Inhibition of chymase could preserve the progression of renal lesion.
chemokines and growth factors, resulting in enhanced inflammatory and fibrotic responses. We aim to determine the role of SK1 on TGF-β1-induced EMT, inflammation and fibrosis in an in-vitro and in-vivo model of diabetic nephropathy.

**Methods:** HK2 cells were incubated with SKI-II (2μM) in the absence or presence of 1 μM TGF-β1 for 72 hours. The expression of SK1 was determined by western blotting and the mRNA expression of SK1 was determined by qRT-PCR. The effect of SK1 on TGF-β1-induced EMT was assessed by measuring the protein expression of αSMA and collagen type I. The effect of SK1 on the proliferation of HK2 cells was determined by MTT assay.

**Results:** The expression of SK1 was significantly increased in HK2 cells treated with TGF-β1 compared to the control group. The mRNA expression of SK1 was also significantly increased in HK2 cells treated with TGF-β1. The protein expression of αSMA and collagen type I was significantly increased in HK2 cells treated with TGF-β1. The proliferation of HK2 cells was significantly decreased in the presence of SK1.

**Conclusion:** SK1 plays an important role in TGF-β1-induced EMT and inflammatory responses in fibrosis and may direct future therapeutic strategies.

**Funding:** Government Support - Non-U.S.

**SA-PO2601**

**Identification of Novel Glomerular Molecules Implicated in Albuminuria**

**Background:** Albuminuria is a key risk factor for the progression of chronic kidney disease (CKD). However, the molecular mechanisms underlying albuminuria remain largely unknown.

**Methods:** Using a systems biology approach, we identified novel glomerular molecules implicated in albuminuria using a combination of proteomic and transcriptomic analyses.

**Results:** We identified several novel glomerular molecules, including proteins involved in the extracellular matrix, ion channels, and signaling pathways. These molecules were differentially expressed in control and albuminuric kidneys.

**Conclusion:** Our findings provide new insights into the molecular mechanisms underlying albuminuria and suggest potential therapeutic targets for the treatment of CKD.

**Funding:** National Institutes of Health (NIH)

**SA-PO2602**

**Heart Rate Variability Changes Induced by Reduction of Fluid Overload**

**Background:** Fluid overload is a common complication in patients with chronic kidney disease (CKD) and is associated with increased cardiovascular risk.

**Methods:** We studied 30 hemodialysis patients who were randomized to receive conventional fluid removal or fluid removal modified to achieve a lower heart rate variability (HRV).

**Results:** In the fluid overload group, there was a significant decrease in HRV compared to the control group. This decrease was associated with an increased incidence of cardiovascular events.

**Conclusion:** Reduction of fluid overload can improve HRV and reduce cardiovascular risk in patients with CKD.

**Funding:** National Institutes of Health (NIH)


Table 1. Median values (interquartile ranges) of HRV indices

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<th>HP</th>
<th>LF</th>
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<td>51(22.56)</td>
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<tr>
<td>1M</td>
<td>44(35.46)</td>
<td>40(35.48)</td>
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<td>1F</td>
<td>8(1.21)</td>
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Conclusions: In this pilot study, we demonstrate for the first time that reduction of FO has an effect on sympathetic ANS activity and HRV. Further studies in a larger population are needed to verify this finding and to determine whether these changes persist in the long-term.

SA-PO2603
Relationship between Visceral Fat Area, Percentage of Body Fat and Prevalence of Cardiovascular Disease in Chronic Dialysis Patients Yukie Kitajima,1 Yuzuru Sato,2 Tokyo Healthcare University, Tokyo, Japan; 3Sato Junkanki Hospital, Matsuyama, Ehime, Japan.

Background: The risk of cardiovascular disease is substantially high in hemodialysis patients. The risk factors for cardiovascular disease in hemodialysis patients include age, duration of hemodialysis, diabetes mellitus and hyperlipoproteinemia. However it is not clear the relationship as visceral fat area, percentage of body fat and percentage of body fat in hemodialysis patients. We investigated the relationship among visceral fat area, percentage of body fat, several clinical and biochemical parameters and anamnesis of cardiovascular complication in chronic hemodialysis patients.

Methods: Area of visceral fat was measured using computed tomographic scanning (CT) in 84 non-diabetic patients. Using a body composition analyzer, we measured total body fat, and percentage of body fat after completion of dialysis on the last day of the week. Patients were divided into two groups according to visceral fat area, those with visceral fat area < 100cm2 (Group A) and with > 100cm2 (Group B). Blood chemistry of lipid and complication of cardiovascular disease were evaluated in these patients. Body mass index (BMI) was calculated with body weight and height.

Results: Compared to Group A, Group B showed, significantly higher serum levels of triglyceride (p<0.001) and significantly lower, high-density lipoprotein-cholesterol levels (p=0.001). Group B also showed higher level of cardiovascular complication, especially ischemic heart disease (p=0.01). Visceral fat area correlated with BMI (R=0.5233), total body fat (R=0.544), percentage of body fat (R=0.283). BMI correlate with total body fat (R=0.792), percentage of body fat (R=0.491). Furthermore, Patients with ischemic heart disease showed higher percentage of body fat (p<0.05).

Conclusions: In this study, higher visceral fat area was found to have the high rate of cardiovascular disease. Visceral fat area can be a contributing risk factor to cardiovascular disease, especially ischemic heart disease in chronic dialysis patients. In addition, BMI and percentage of body fat can be measured more easily than CT. Those assessments will evade the risk of cardiovascular disease.

SA-PO2604
Osteoprotegerin as a Prognostic Marker of Mortality in Hemodialysis Patients with Cardiovascular Disease Simon Winther,1 Kaj A. Jørgensen,1 Erik Berg Schmidt,2 My Svensson,1 1Department of Nephrology, Aarhus University Hospital, Aarhus, Denmark; 2Department of Cardiology, Aalborg Hospital, Aalborg, Denmark.

Background: Patients with end-stage renal disease, treated with hemodialysis (HD) have an increased mortality, mainly caused by cardiovascular disease (CVD). Osteoprotegerin (OPG) is a glycoprotein involved in the regulation of the vascular calcification process. Previous studies have demonstrated that OPG is incorporated in atherosclerotic plaques and that elevated OPG in plasma is related both to severity and progression of vascular calcification. Furthermore, elevated OPG has been shown as a prognostic marker of mortality, in several high-risk populations. The aim of this study was to investigate if OPG was a prognostic marker of mortality in patients with end-stage renal disease in previously documented CVD.

Methods: We prospectively followed 206 HD patients with documented CVD. CVD was defined as previously documented myocardial infarction, angina pectoris, angographically documented coronary arteriosclerosis, stroke, transient ischemic attack, or percutaneous vascular disease. Plasma levels of OPG were measured at baseline and the patients were followed for 2 years or until reaching the primary endpoint, all-cause mortality.

Results: All-cause mortality during follow-up was 44% (90/206) and median follow-up to the primary endpoint was 314 days. Levels of OPG were divided into tertiles (first: 1.35-4.12, second: 4.12-6.04, third: 6.04-31.32 pg/ml). High OPG baseline levels were associated with increased mortality, using the first tertile as reference, with a HR of 1.83 (CI 1.05-3.20) for the second tertile and HR of 1.72 (CI 0.95-3.09) for the third tertile. In survival analysis this was not statistically significant with an adjusted p-value of 0.08 and an adjusted p-value for trend of 0.07. In multivariate Cox-regression analysis only age and OPG in the second tertile were associated with increased mortality.

Conclusions: In contrast to previous studies, we were not able to demonstrate that high levels of OPG, in a cohort of HD patients with documented CVD, were associated with an increased mortality.

Funding: Pharmaceutical Company Support, Private Foundation Support

SA-PO2605
Effect of Body Composition Monitor-Guided Volume Control on Inflammation and Adipokines in Hemodialysis Patients: A Prospective 16-Week Interventional Study Sejong Kim,1 Hayne C. Park,2 Hajeong Lee,2 Hong Jin Chin,2 Dong Ki Kim,3 Yon Suk Kim,3 Jin Suk Han,4 Kwon Wook Joo,2 1Internal Medicine, Gachon University of Medicine and Science, Incheon, Korea; 2Internal Medicine, Seoul National University College of Medicine, Seoul, Korea.

Background: Inappropriate volume control can be linked to an increased cardiovascular morbidity in hemodialysis (HD) patients, although there is no accurate method to monitor patients’ water contents. Also, inflammation and adipokines are related to cardiovascular outcome in HD patients. We evaluated the effect of body composition monitor (BCM)-guided volume control on inflammation and adipokines in hemodialysis patients.

Methods: We enrolled 120 patients who received more than 3-month hemodialysis in major 3 dialysis centers. According to the amount of fluid overload, which was provided by BCM (Fresenius Medical Care Korea), we divided into 3 groups: overhydrated group (OH; fluid overload ≥ 1.1L), nonhydrated group (NH; fluid overload < 1.1L), and dehydrated group (DH; fluid overload < -1.1L) and optimized body weight towards an objective target for normohydration for 16 weeks.

Results: The proportion of OH group was 36.6% (44/120), and that of DH group was 15% (18/120). Intervention failure rate was 16% (10/62). In OH group, serum levels of IL6 and MCP1 were significantly decreased after 8-week intervention period (IL6: 1.17 ± 1.34 at week 0 vs. 0.06 ± 1.18 log [pg/mL] at week 8, p<0.001; MCP1: 5.66 ± 0.77 at week 0 vs. 3.35 ± 0.39 log [pg/mL] at week 8, p<0.001). After 8-week intervention period, serum leptin levels were decreased, while serum adiponectin levels were increased in OH group. Those changes were persistent until another 8-week observation. In DH group, serum adiponectin levels were significantly increased every 8 weeks.

Conclusions: This study demonstrated that inflammation and adipokines improved after the correction of patients’ overhydrated status and adiponectin levels increased after the optimization of patients’ dehydrated status in hemodialysis patients.

Funding: Pharmaceutical Company Support

SA-PO2606
Vascular Calcification Inhibitors Are Promising Markers of Subclinical Cardiovascular Disease in Incident Hemodialysis Patients: The Predictors of Arrhythmic and Cardiovascular Risk in ESRD (PACE) Study Stephen M. Sozio,1 Rulan S. Parekh,2 Julia J. Scialla,3 Tarig Shafi,2 Bernard G. Jaar,1 Miguel Santaula-Tomas,4 Lucy A. Mooni,5 Wen Hong Linda Kao,6 Johns Hopkins University; 1University of Toronto.

Background: Vascular calcification is common in ESRD and likely contributes to clinical cardiovascular disease (CVD). The effect of circulating regulators of this dynamic process on subclinical CVD is unknown.

Methods: We investigated the association of the calcification inhibitors osteopontin (OPN), osteoprotegerin (OPG), bone morphogenetic protein-7 (BMP-7), and fetuin-A with simultaneously measured coronary artery calcium score (CAC), pulse pressure (PP), carotid-femoral pulse wave velocity (PWV), aortic augmentation index (AIX), and ankle-brachial index (ABI) in an incident hemodialysis cohort of 82 PACE participants. Laboratory and clinical tests were performed on a non-dialysis day at the baseline visit. Linear regression was used to assess the association of each inhibitor and subclinical CVD process.

Results: Mean age was 53 with 59% male, 67% African-American, 54% diabetic, and 50% with prior ASCVD. Mean±SD level of each calcification inhibitor was: CAC: 0.13±0.13 ng/mL, OPN: 9.73±3.4 pmol/L, BMP-7: 5.93±2.8 pg/mL, fetuin-A: 0.49±0.13 g/L. OPN, OPG, and fetuin-A were associated with several of the CVD processes.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.

718A
Coronary Artery Calcifications and Cardiovascular Mortality in Hemodialysis Patients Roberto Palumbo, Annalisa Node, Olga Durante, Simone Manca di Villahermosa, Michele Ferrarini, Efstathia Athanassopoulou, Sandro De Angelis, Mariarita Dessi, Pietro Sfregola, Giorgio Splendiani, Nicola Di Fabio, Department of Internal Medicine, Tor Vergata University, Rome, Italy; Department of Laboratory Medicine, Tor Vergata University, Rome, Italy; Regional Agency for Transplantations and Related Pathologies, Rome, Italy; Nephrology and Dialysis Service, Madonna delle Grazie, Velletri, Rome, Italy; Nephrology and Dialysis Unit, S.Eugenio Hospital, Rome, Italy.

Background: Coronary artery calcifications and long-term overall cardiovascular mortality were investigated in maintenance hemodialysis (mHD) patients.

Methods: Between June and December 2002 two-hundred and five patients with no history of major acute cardiovascular event (MACE), aged 59.85±12.77 years, on mHD mortality were investigated in maintenance hemodialysis (mHD) patients.

Results: One-hundred two patients (49.7%) died for cardiovascular disease during the follow up. Seven-year actuarial survival was more than 90% for patients of groups 1 and 2, but failed to about 50% in patients of group 3 and <10% in group 4. Patients with AS=400 thus showed a significantly higher cardiovascular mortality compared to patients of with AS=400 (p<0.0001).

Coronary Artery Calcifications and Cardiovascular Mortality in Hemodialysis Patients

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<td>4.46 (0.70, 8.21)</td>
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SA-PO2607

Coronary Artery Calcifications and Cardiovascular Mortality in Hemodialysis Patients

Background: Coronary artery calcifications and long-term overall cardiovascular mortality were investigated in maintenance hemodialysis (mHD) patients.

Methods: Between June and December 2002 two-hundred and five patients with no history of major acute cardiovascular event (MACE), aged 59.85±12.77 years, on mHD mortality were investigated in maintenance hemodialysis (mHD) patients.

Results: One-hundred two patients (49.7%) died for cardiovascular disease during the follow up. Seven-year actuarial survival was more than 90% for patients of groups 1 and 2, but failed to about 50% in patients of group 3 and <10% in group 4. Patients with AS=400 thus showed a significantly higher cardiovascular mortality compared to patients of with AS=400 (p<0.0001).

Conclusions: The risk/benefit ratio for warfarin use in AF among patients on HD is unclear. The objectives of this study are to: 1) measure Nephrologists’ certainty about warfarin use and 2) determine the patient variables influencing warfarin use in AF among Canadian Nephrologists.

Methods: Nephrologists at 57 hospitals in Canada were reviewed their patients with AF on HD. Using a 7 point scale, they documented their certainty regarding the use or nonuse of warfarin pre and post a 10 min presentation on risk and benefits of warfarin based on current literature. A survey was then developed consisting of 6 cases of patients with AF with a gradient of thromboembolic and bleeding risk and sent to a random sample of Nephrologists selected from the Canadian Society of Nephrology (CSN).

Results: Warfarin was used in 52.5 % of patients with AF. Nephrologists expressed uncertainty regarding both the use and nonuse of warfarin and this did not change with an update of the available literature. The survey response rate was 62.2 % (56/90).

Use of Warfarin for Atrial Fibrillation (AF) in Patients on Hemodialysis (HD) Salina Juma, Charmaine E. Lok, Catherine M. Clase, Peter G. Blake, Benjamin Ka Thomson, Louise M. Moist. University of Western Ontario, London, ON; University Health Network, Toronto, ON; McMaster University, Hamilton, ON.

SA-PO2609

Use of Warfarin for Atrial Fibrillation (AF) in Patients on Hemodialysis (HD)

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

719A
SA-PO2611
Statin Exposure over Time in Chronic Dialysis: An Observational, National Study of Dually Eligible Patients James B. Weismore, Theresa I. Shireman, Jonathan D. Malhnk, Qingjiang Hou, Puma Mukhopadhyay, Sally K. Rigler, Edward F. Ellerbeck. University of Kansas School of Medicine, Kansas City, KS.

Background: Although HMG CoA reductase inhibitors (statins) effectively lower cholesterol levels in patients on chronic dialysis, randomized trials have not consistently demonstrated a survival benefit in racial or ethnic groups that are still underrepresented in this population.

Methods: We examined factors associated with statin use over time in a large, national cohort of dually eligible, chronic dialysis patients from 2000-2005. Medication exposure was tracked through Medicaid prescription claims using the proportion of days covered (PDC) adjusted for inpatient and skilled nursing home stays. PDC was computed across the entire window of observation for each cohort member, reflecting medication exposure rather than adherence. Baseline characteristics and comorbidities were taken from linked USRDS Core Data.

Results: There were 18,757 dually-eligible, chronic dialysis patients taking statins during this period of observation (41.6% of the available cohort). The mean PDC for statin users was 0.57 (SD = 0.32). Statin PDCs increased with advancing age (10 year increments) but were lower among non-Caucasians (p < 0.0001). Persons with diabetes, CAD, or CVA at baseline also had higher PDCs (p < 0.0001). Cholesterol levels were not available for these study subjects, however, persons with higher BMIs had higher statin PDCs (p < 0.0001).

Conclusions: Even with their high potential for adverse cardiac outcomes, patients on chronic dialysis have inconsistent exposure to statins. In part, this may reflect the clinical equipoise during a period where clinical trials had not yet been completed. It may also reflect poor long-term adherence to statin therapy as has been demonstrated in other populations. Further examination of the effectiveness of statins in chronic dialysis is needed.

Funding: NIDDK Support

SA-PO2612
The Role of Statins and Cholesterol on Clinical Outcome of Continuous Ambulatory Peritoneal Dialysis Patients: A Retrospective Study Yong Kye Lee, Tae Ik Chang, Sug Kyun Shin. Nephrology Division, NHIC Ilsan hospital, Goyang, Gyeonggi, Korea.

Background: Patients who are on CAPD (Continuous Ambulatory Peritoneal Dialysis) show higher cholesterol levels and Triglyceride compared to patients who are on hemodialysis. But higher cholesterol level does not seem to effect on raising mortality or cardiovascular morbidity and CPAF failure. On the contrary, lower serum cholesterol level in CAPD patients tends to raise mortality and morbidity due to poor nutritional status.

Methods: This is a retrospective study designed to evaluate the effect of cholesterol level, statin on CAPD outcome and mortality. Patients who were on peritoneal dialysis for at least 6 months since March 1st, 2000 were included. A total of 467 patients were enrolled in this study. Patients’ biological parameter, biochemical parameter and morbidity/mortality during CAPD maintenance period were collected.

Results: Patients whose initial cholesterol level were above 240 mg/dL shows significantly low CAPD failure rate compared to patients whose initial cholesterol level were below 200 mg/dL (OR = 0.469, p=0.049). Patients whose average LDL-cholesterol during the period was above 100 mg/dL showed significantly higher mortality compared to patients whose initial LDL-cholesterol level were below 100mg/dL (OR = 1.848, p=0.024). Patients whose compliance to statin during CAPD period was over 80% showed significantly less low mortality compared to patients who did not take statin during CAPD period (OD = 0.556, p=0.020). Patients showed no significant difference in mortality due to total cholesterol, HDL cholesterol levels and patients showed no significant difference in CAPD failure due to HDL/LDL cholesterol, statin usage.

Conclusions: In CAPD patients, serum total cholesterol level should be targeted higher than HD or CKD patients. On the contrary, similar to HD or CKD patients, Statin should be administrated and LDL cholesterol should be lowered during CAPD period to lower mortality. To identify the difference in cholesterol mechanism of CAPD patients further, in depth study over adequate cholesterol level in CAPD patients needs to be proceeded.

SA-PO2613
SIRT1 Gene Polymorphisms Are Associated with Cholesterol Metabolism and Coronary Artery Calcification in Japanese Hemodialysis Patients Toshimitsu Niuwa,1 Yashuho Shimoyama,1 Hidehisa Shimizu,1 1Nagoya University Graduate School of Medicine; 2Meiyo Clinic.

Background: Sir2 is a longevity gene, protects cells against oxidative and genotoxic stress. This study aimed to investigate the association of SIRT1 gene single nucleotide polymorphisms (SNPs), rs7069102 and rs2273773, with various markers of Lipid metabolism in 219 Japanese patients with chronic kidney disease (HD) patients.

Methods: Genotyping of these polymorphisms was performed using polymerase chain reaction with confronting two-pair primers (PCR-CTPP) assay.

Results: The A allele frequency of rs7069102 and G allele frequency of rs7069102 were significantly higher in HD patients (0.228 and 0.131, respectively) than in 803 control subjects (0.289 and 0.181, respectively) (p=0.010 and p=0.012, respectively). However, the allele frequency of rs2273773 was not significantly different from that in the control subjects. Multivariate analysis adjusted for age and duration on HD demonstrated that the serum levels of total and low-density lipoprotein (LDL) cholesterol were significantly high in G allele carriers of rs7069102 compared with CC genotype in male HD patients. Coronary artery calcification score was significantly high in C allele carriers of rs2273773 in all and male HD patients.

Conclusions: This study first demonstrates that SIRT1 polymorphisms, rs7069102 and rs2273773, are associated with cholesterol metabolism and coronary artery calcification, respectively, in Japanese HD patients, especially in males.

SA-PO2614

Background: A consolidate evidence exists about the higher removal capabilities of HDF therapies with respect to conventional HD. It is also well known that postdilution HDF improves the removal rate of mid to high molecular weight uric acid toxins leading to some clinical benefits, like less dialysis related amyloidosis, better control of patient’s phosphatemia and likely better response to ESA. The aim of this study was to compare two post-dilution On-Line HDF in regards to exchanged volumes (EV) and middle molecules dialysis efficiency: standard On-Line (sOL) and ULTRAcontrol (UC).

Methods: Thirty ESRD patients (pts, 6/24 F/M) were enrolled in this prospective sequential study from 8 centres. The pts underwent a 3-month sOL (fixed exchanged volume) followed by 3-month UC. AK200 ULTRA has been employed in all treatments. In sOL, the EV were set according to a filtration fraction greater than or equal to 25%. In UC the EV was driven by a biofeedback system controlling in a double loop the transmembrane pressure (TMP) and its set point. In both HDF, pts maintained the treatment time, the dialyzer, the blood flow rate and anticoagulant regimen unchanged. Primary response variables were: EV, ß2-microglobulin (ß2m) clearance (Kß2m) measured at 60 and 120 min. Secondary response variables were: fß2m in pre and post dialysis and phosphate clearance (Kp).

Results: A greater EV was infused in UC than in sOL (20.8±3.7 l vs 16.9±4.4 litters; p=0.001). The average Kß2m values were higher in UC than in sOL (111.5±22 vs 123.3±24 ml/min, p=0.002), while the average Kp did not achieve a statistical significant difference (150.8±33.9 vs 156.2±38.9 ml/min, p>NS) despite they were higher in UC than sOL. The estimated fß2m concentrations at the beginning of dialysis were similar in both HDF (23.0±11.8 vs 22.1±15.0 ml/g, p>NS), while they significantly differed in the post dialysis (7.8±5.8 vs 5.8±4.2 ml/g, p=0.019).

Conclusions: This study shows that the biofeedback module, applied to the automatic control of TMP in On-Line HDF (ULTRAcontrol), allows higher EV and correspondingly higher Kß2m. These results were obtained by reducing both the technical complexity of HDF and staff workload.

SA-PO2615
Has the Introduction of Clinical Practice Guidelines Improved Dialysis Care? Héla K. Mogensen,1 Runolfur Pallsson,1 Ólafur S. Indridason,2 University of Iceland, Reykjavik, Iceland; 2Division of Nephrology, Landspítali - The National University Hospital of Iceland, Reykjavik, Iceland.

Background: Several clinical practice guidelines for dialysis care have been issued over the past decade. In this study we examined if the introduction of the KDQI and ERBP guidelines resulted in improvement of the quality of dialysis therapy at the University Hospital in Reykjavik.

Methods: This was a retrospective study of all patients receiving dialysis at the University Hospital’s Dialysis Unit for >3 months from 2003 to 2008. Data were obtained from electronic medical records. The study period was divided in two 3-year intervals, 2003-2005 and 2006-2008. Chi-square test was used to compare the proportion of patients reaching therapeutic targets in each interval and to compare hemodialysis and peritoneal dialysis patients.

Results: A total of 177 patients underwent dialysis treatment during the study period. The target level for URR (>65%) was achieved in 80.4% of hemodialysis patients during the first interval and 72.6% during the second interval (p=0.80). In the first interval, 65% of patients reached the target for hemoglobin (110-130 g/L) and 67.5% during the second interval (p=0.68). The target for serum calcium (2.0-2.6 mmol/L) was achieved in 72.1% of patients during the first and 70.7% during the second interval. Only 53.0% of patients reached the target for serum phosphate (1.1-3.178 mmol/L) during the first interval and 55.7% during the second interval (p=0.67). The target for PTH (150-300 ng/L) was achieved in only 23.5% of patients during the first and 33.0% during the second interval (p=0.30). The target for serum bicarbonate (>21 mmol/L) was achieved in 94.9% of peritoneal dialysis patients and 65.6% of hemodialysis patients (p>0.001); otherwise no significant difference existed between the two dialysis modalities.

Conclusions: Introduction of clinical guidelines has not been associated with substantial improvement in the care of dialysis patients in Iceland, as measured by selected quality indicators. The use of well defined clinical pathways may be needed to facilitate the implementation of clinical practice guidelines and to improve the quality of dialysis care.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

720A
Outcomes of Enhanced Systematic Screening for Transient Ischemic Attack in Hemodialysis

**Background:** Rapid recognition and management of transient ischemic attack (TIA) has been shown to reduce incident stroke in the general population but similar data are lacking in dialysis patients who form a high-risk group for cerebrovascular events. We hypothesized that systematic screening would reveal the true prevalence of TIA in hemodialysis (HD) and drive reductions in stroke incidence.

**Methods:** All patients on maintenance HD at one unit at our center were given verbal and written information about stroke symptoms ([UK Dept of Health], staff attended an educational session prior to data collection and patient areas prior to TIA. Prospective screening was performed once weekly using a dedicated questionnaire comprising all elements of the FAST test ([UK Dept of Health]), screening for sensory & visual symptoms and a record of any emergency attendances in an attempt to maximize yield.

**Results:** 209 patients were screened over 12 months from Nov 2009 [mean age 65.7±14.0yrs, 60% male, 53% Indo-Asian] with 2594 patient months follow-up. 44% were diabetic, 14% had prior cerebrovascular disease [stoke +/- TIA]. Mean HD length was 4.3±1.4yrs, single-pool Kt/V 2.0±0.3. Mean blood pressure was 140±78mmHg pre- and 135/75mmHg post-dialysis. Mean hemoglobin was 12.2±0.9g/dL, mean weekly darbepoietin dose 0.56±0.37mcg/kg. 9504 questionnaires [mean 32±16/patient] were administered. 6 strokes occurred, 5 ischemic- an incidence rate of 23.1/1000 pt yrs. No patients screened positive for TIA despite a predicted rate of 4.2/1000 pt yrs [95% CI 1.4-9.7/1000 pt yrs]. One ischemic stroke was preceded by symptoms compatible with a TIA ascertained in retrospect alone.

**Conclusions:** In the first study of its kind to date systematic screening for TIA in HD patients cannot be relied on alone as a method of identifying HD patients at higher risk of stroke events. The confounding presence of symptoms attributable to uremia, neuropathy, dyslipidemia, diabetes, hypertension and dysglycemia could reduce the sensitivity of establishing screened tests with significant implications for the detection and treatment of TIA in dialysis patients.

**SA-PO2617**

Improvement in Cardiac Strain Rate in Children on Chronic Hemodialysis after Carantine Supplementation

**Background:** Carnitine is essential for transport of fatty acids into mitochondria and plays a key role in energy production in the myocardium. Carnitine deficiency may occur in patients on chronic hemodialysis (HD) due to removal by dialysis and inadequate intake and may contribute to cardiomyopathy. We prospectively assessed cardiac response to IV carantine supplementation by standard echocardiographic (echo) and more sensitive parameters including left ventricular (LV) strain rate.

**Methods:** Carnitine levels (total,free,acyl,acyl:free ratio) and cardiac function of 9 children on chronic HD were assessed before and after carantine infusion (20mg/kg given 3 times a week for 6 months). Standard echo measures of LV size and systolic and diastolic function as well as echocardiographic and longitudinal strain rate analysis using speckle tracking were performed. Changes in echo parameters of a retrospective control group of 8 children on chronic HD not treated with carantine were assessed for comparison.

**Results:** The study group had mean age of 12.7±1.1 (range 9-16) years and dialysis vintage of 3.2±1.5 yrs. Carnitine deficiency did not differ from controls. After carantine supplementation, study total and free carantine levels increased (48.9±1.67 vs. 260.3±13.1 µmol/l and 29.0 ± 1.19 vs. 156.6 ± 8.44 µmol/l (p<0.001), whereas acyl:free ratio remained unchanged (0.73±0.04 vs. 0.69±0.05). There were no significant changes in standard echo measures of LV function including end diastolic dimension, mass index, ejection fraction, and shortening fraction after carantine supplementation. However, there was a significant improvement in longitudinal strain rate (−1.48±0.31 vs. −1.91±0.12, p=0.01) after supplementation. There were no changes in blood pressure, interdialytic weight gain, or hemoglobin of study patients pre vs post treatment. No improvements in LV strain rate occurred in control subjects.

**Conclusions:** Carantine supplementation improved total and free carantine levels in children on chronic HD without impacting acyl:free ratio. LV performance improved after carantine supplementation as assessed by strain rate analysis that was not obvious by standard echo measures.

**SA-PO2618**

Aspirin Resistance: Prevalence, Affecting Factors and Effects on Cardiovascular Complication and Vascular Access Failure in Hemodialysis Patients

**Background:** Even though aspirin has been effectively used for prevention of cardiovascular(CV) complication, some patients experience CV events during aspirin use. Association of aspirin resistance (AR) and CV events have been reported in many CV disease and kidney transplant patients. The prevalence and effects of AR on CV complication and vascular access failure of hemodialysis(HD) patients is not known.

**Methods:** 119 HD patients from two hospital who took 100-200mg of aspirin more than 1 month were enrolled. Patients who used NSAIDs or anti-platelet agents and thrombocytopenia were excluded. To define AR, measurement of anti-platelet effects was assessed by VerifyNow® assay device. AR was defined as aspirin resistance unit (ARU)≥550.

**Results:** Among 119 patients, female was 68(58.1%), mean age was 57, mean dialysis duration was 51.5 month and 68(57.5%) were diabetes. AR was observed in 19 of 119 patients (15.9%) and showed higher hsCRP(124.7±9.1 vs. 5.04±0.90, p=0.02) and higher prevalence of CV complication(43.8% vs. 30.6%, p=0.02), including cerebrovascular complication(53.8% vs. 19.5%, p=0.02). But vascular access failure prior to study did not different. Also it showed positive correlation with serum 25(OH) Vitamin D, total level(r=0.305, p=0.001), negative correlation with calcium(r=-0.271, p=0.003), phosphate(r=-0.177, p=0.05) and Kt/V urea(r=-0.196, p=0.036). Multivariability analysis showed vitamin D level OR 1.117, 95% CI 1.025-1.117, p=0.01, phosphate (OR 0.513, 95% CI 0.395-0.968, p=0.04), HDL (OR 1.065, 95% CI 1.010-1.124, p=0.02 ) as an independent factor. There were no relationship between AR with age, sex, dialysis duration, diabetes, BMI, smoking, aortic calcification.

**Conclusions:** AR was observed 15.9% in HD patients and was associated with higher incidence of CV disease but vascular access failure was not. Relative lower Kt/V urea and higher hsCRP suggest the possible influence of dialysis adequacy and inflammation. Correlation with some factors affecting vascular remodeling mechanisms such as Vitamin D, HDL, phosphate, and calcium need further investigations.
Magnesium: A Cardiovascular Risk Marker in Hemodialysis Patients

Patients - 24-Hours ABPM Monitoring and Cardiovascular Complications

Background: Hypomagnesemia seems to play a role in the pathogenesis of arterial hypertension, endothelial dysfunction and inflammation in the general population.

We studied 206 HD patients with mean age (± SD) of 63.6±14.3 years, 45% female, 26% diabetics, with mean HD time of 42.3±38.6 months. All patients were under pre dialution hemodiafiltration with a dialysate Mg concentration of 1 mmol/L. Univariate and multivariate analysis were performed and a p <0.05 was considered significant.

Results: Mean serum Mg was 1.36±0.18 mmol/L and none of the patients presented hypomagnesemia (Mg < 0.6 mmol/L). Mg levels were negatively correlated with age (r=-0.44, p=0.006), diabetes mellitus (r=-0.42, p=0.007), IPTII (r=-0.33; p=0.02), PP (r=-0.36; p=transplant), LVMI (r=-0.37; p=0.01) and VC (r=-0.40; p=0.008). Mg was positively correlated with albumin (r=0.57; p=0.001) and 25-hydroxvitamin D3 (r=0.45; p=0.04).

In multivariate analysis, lower Mg concentrations were predictors of an increased PP (> 65 mmHg) (p=0.002) and LVMI (> 140 g/m2) (p=0.03) and of a higher VC score (>3) (p=0.01).

Conclusions: Pre-dialysis Mg serum levels were associated with inflammation, bone disease and CV risk markers in HD patients.

Mechanical Circulatory Support in Dialysis Patients as a Bridge to Transplantation

Background: Advanced chronic kidney disease has generally been an exclusion criterion for cardiac transplant using standard listing criteria. Selected patients with end stage renal disease (ESRD) have undergone successful combined heart and kidney transplantation. However, the use of mechanical circulatory support as a bridge to transplantation has not yet been reported in patients with ESRD. We report the experience of a single transplant center where ESRD patients received continuous flow veno-arterial extracorporeal membrane oxygenation (VA-CO2M) as a bridge to transplant, and continued to receive dialysis in their usual outpatient setting.

Methods: 5 prevalent ESRD patients (3 in-center HD, 2 home PD) underwent VAD placement at our center between Nov 2009 and Oct 2010 as a bridge to transplantation. No ESRD patients were selected for destination therapy. Patients were observed from VAD placement until transplant or to 5/31/2011. Outcomes were length of stay (LOS) post-VAD placement, readmission, day, and duration of circulatory support.

Results: Following VAD implantation initial LOS was 22±8 days. 2 patients were successfully weaned from dialysis post-VAD and did not require renal transplantation. Patients remained on VAD support for 202±72 days. No dialysis modality changes were made pre-transplant. There were 4 readmissions in 3 patients with diagnoses of peritonitis, ventricular tachycardia, drive line infection, and weakness. Following initial discharge post-VAD implantation, patients remained as outpatients for 98% of observed days. 4 patients received transplants and 1 patient remains listed.

Conclusions: 1) ESRD patients on HD or PD can successfully receive mechanical circulatory support with VAD as a bridge to transplantation. 2) Outpatient HD and PD can be safely performed in patients with VAD. 3) Clinical outcomes, LOS and readmissions in ESRD patients are similar to VAD patients without ESRD. 4) Following VAD implantation, an average of 98% of follow-up days were spent as outpatients.

Characteristics of the Dipper and Non-Dipper Type of Hemodialysis Patients - 24-Hours ABPM Monitoring and Cardiovascular Complications over 10 Years

Background: 24-hour blood pressure monitoring (ABPM) was useful to determine whether the patient is a dipper or non-dipper. But characteristics of dipper and non-dipper on hemodialysis patients, the relationship between the two types, and the cardiovascular complications on each type are still unknown. In our study, ABPM was performed on 45 hemodialysis patients over 10 years.

Methods: 24-hour blood pressure monitoring (ABPM) was performed on 51 hemodialysis patients from 2000. Four patients were moved, two were transplanted and the other forty-five patients were followed for 10 years. ABPM started at the end of hemodialysis and BP was measured every 15 minutes at the daytime (defined from 6 AM to 9 PM) and every 1 hour at the nighttime (9 PM to 6 AM). Dipper status was defined on the change between the average SBP from daytime and nighttime. We considered two groups, dippers (BP nocturnal fall superior to 10mmHg) and non-dippers (BP nocturnal fall less than 10mmHg).

Results: The ABPM on 45 patients showed distinct groups, as 11 patients (24.4% of the whole group) were dipper type group A) and 34 patients (75.6%) were non-dipper type (group B). SBP of group A in the daytime was significantly higher than group B (163.7±16.8 vs. 150.2±19.4mmHg, p<0.05), but both group’s SBP were similar in the nighttime (138.3±17.0 vs. 146.8±22.8mmHg). The arterial calcification scores (ACS) were the same when computed tomography of the abdomen. Nine patients in group A and 13 in group B had cardiovascular complications for 10 years and the cumulative events-free rate of group B was significantly higher than group A’s one (Kaplan-Meier methods p=0.016, Log-rank test). ACS of the patients in group B with cardiovascular complications were significantly higher than without them (63.9±24.0 vs. 34.6±23.4, p<0.01).

Conclusions: In this study, two types of hemodialysis patients were characterized over 10 years. Higher SBP in the daytime for dipper type patients and pre-existing severe arterial calcification for non-dipper type patients suggested several risk factors of cardiovascular complications in hemodialysis patients.

SA-PO2624

Early Interdiastolic Fluid Retention Is Associated with Cardiovascular Outcomes in Incident Hemodialysis Patients

Background: Fluid retention is a major clinical problem in patients undergoing hemodialysis (HD) and is associated with cardiovascular outcomes. Patient with end stage renal disease have similarities to heart failure. Volume overload in heart failure is associated with worse outcome. Removal of fluid during the HD is the cornerstone of volume management in this population. Therefore, we hypothesized that greater inter-diastolic fluid retention (IDFR) is associated with poor cardiovascular event and survival in incident HD patients.

Methods: We analyzed the 174 patients who newly started and maintained the HD over 6-month in Gachon University Gil Hospital between January 1, 2003 and December 31, 2008. We did not take the first 3 month IDFR into account by reason of stabilized period in incident HD patients. According to the average IDFR of 4~6 month, we divided into 2 groups by the median value: Lower IDFR (< 2.17 kg), Higher IDFR (Fluid retention ≥ 2.17 kg). The associations of IDFR with cardiovascular outcomes were evaluated with the use of Cox proportional regression analysis.

Results: Higher IDFR showed higher prevalence of diabetes, better nutritional status (higher phosphorus and nPNA level). In univariate analysis, higher IDFR, hemoglobin (Hb), total cholesterol and LDL cholesterol level were associated with cardiovascular events. After multivariate adjustment, higher IDFR, low Hb and high LDL cholesterol were associated with increased risk of cardiovascular events. The odds ratio (95% confidence interval) of cardiovascular events 2.213 (1.011 – 4.846, P = 0.047) in the higher IDFR, 0.557 (0.355-0.876, P = 0.011) in Hb level, and 1.106 (1.003 – 1.028, P = 0.014) in LDL cholesterol level, respectively. However, all-cause and cardiovascular mortalities were not significantly different between two groups.

Conclusions: In incident HD patients, early greater IDFR is associated with higher risk of cardiovascular events. Further research with large subjects needed to elucidate the pathophysiological mechanisms that link fluid retention to increased cardiovascular events.

SA-PO2625

Pre-Procedural Serum Albumin and C-Reactive Protein Levels Predict Clinical Outcome after Endovascular Therapy in Hemodialysis Patients with Peripheral Artery Disease

Background: Although endovascular therapy (EVT) has become widely performed for peripheral artery disease (PAD), adverse events such as high restenosis rate or premature death after EVT remain major clinical problems in patients on hemodialysis (HD). On the other hand, malnutrition and chronic inflammation status are frequently observed, and linked to poor cardiovascular outcome in such population. We evaluated the possible prognostic values of serum albumin and C-reactive protein (CRP) levels on clinical outcomes after EVT in patients on HD.

Methods: A total of 450 HD patients successfully undergoing EVT for PAD were enrolled and were followed-up for up to 8 years. Serum albumin and CRP levels were measured prior to EVT. They were divided into tertiles according to serum albumin and CRP levels; the lowest tertile (T1), the middle tertile (T2) and the highest tertile (T3), respectively. We analyzed the incidence of major adverse cardiovascular events (MACE) as a composite endpoint including target lesion revascularization (TLR), amputation and all-cause death.

Results: During follow-up period (mean 36.1±31.9 months), 206 MACE (46%) including 69 TLR, 26 amputation and 71 death were observed. T1 and T2 patients were older (p<0.0001) and 41.2%.
36.3% and 18.9% in T1, T2 and T3 of CRP (p<0.0004), respectively. After adjustment, lower albumin (HR 2.00, 95%CI 1.29-3.10, p=0.0071 for T1 vs T3) and elevated CRP (HR 1.93, 95%CI 1.29-2.90, p=0.0061 for T3 vs T1) were independent predictors for MACE, respectively. In the combined setting of albumin and CRP, the risk of MACE was 5.22-fold (95%CI 2.34-11.64, p<0.0011) higher in the T1 of albumin with T3 of CRP compared to the T3 of albumin with T1 of CRP even after adjustment.

Conclusions: Lower albumin and elevated CRP levels could predict MACE after ETV in patients on HD. However, the combination of these variables is more markedly related to increased MACE than either variable alone.

SA-PO2626
Risk Factors of Progression of Aortic Arch Calcification in Patients with Maintenance Hemodialysis and Peritoneal Dialysis Byun Guvim Kim, Young Ok Kim. Department of Internal Medicine, Seul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea.

Background: Vascular calcification is accelerated during the dialysis and known as an important risk factor for cardiovascular disease. Progression of AoAC can be simply estimated with an AoACS score (AoACS) on chest radiography. The objective of this study was to evaluate the risk factors of the progression of AoAC.

Methods: The enrolled subjects were 125 hemodialysis and 59 peritoneal dialysis patients, newly treated at the dialysis unit. In the patients who had undergone chest radiographies before initial dialysis therapy and every year, we estimated AoACS and then divided into two groups by the presence or absence of the AoAC progression. We also compared the baseline clinical and biochemical profiles in two groups.

Results: Eighty-five (46.2%) were men and the mean age was 58.6 ± 12.7 years. 76 patients (41.3%) had AoAC before initial dialysis, and the mean AoACS of 13.0 ± 20.4%. The mean duration of follow-up of AoACS was 2.7 ± 1.0 years. The half of the patients (50%) had the progression and the others non-progression of AoAC. Old age more than 65 years (p=0.003), diabetes duration (p=0.004), diabetes (p=0.015) and the presence of AoAC at baseline (p=0.001) were related to the progression of AoAC. No significant association was detected between the AoAC progression and baseline clinical parameters including gender, obesity, hypertension and dialysis modality. In multivariate analysis, the duration of dialysis (p=0.004) and the presence of AoAC at baseline (p=0.001) were independent risk factors of the progression of AoAC in dialysis patients.

Conclusions: The duration of dialysis and the presence of AoAC before initial dialysis were significantly related to the progression of AoAC in dialysis patients. We suggest that we should focus on the through management from pre-dialysis stage to prevent the progression of AoAC and reduce cardiovascular morbidity in chronic dialysis patients.

SA-PO2627
Reduction of Nurse Intervention Using Blood Volume Tracking (BVT) System Maria Doria,1 Simonetta Genovesi,2 Antonio Santoro.3 Policlinico S. Donato I.R.C.C.S., San Donato, Italy; 1San Gerardo Hospital, Monza, Italy; 2San Orsola Malpighi Hospital, Bologna, Italy.

Background: Intradialytic hypertension (IDH) still represents the most common acute complication of hemodialysis (HD) therapy accounting for up to 30% of HD sessions. BVT system is a tool capable of reducing the occurrence of IDH by avoiding sudden drop of BV below hazard values during HD by adjusting both UF rate and the dialysate sodium content. The present study was aimed to understand if BVT system is able to reduce the occurrence of IDH by avoiding sudden drop of BV below hazard values during HD by adjusting both UF rate and the dialysate sodium content. The present study was aimed to understand if BVT system is able to reduce the occurrence of IDH by avoiding sudden drop of BV below hazard values during HD by adjusting both UF rate and the dialysate sodium content.

Methods: Ten HD patients (M/F: 5:5; age: 76.7±8.3 yrs) prone to IDH were selected for the study. The presence of cancer, mental illness, pregnant, residual diuresis greater than 600 ml/day were excluded. The variables monitored were: number of session requiring nurse interventions (NI), effective treatment time (ETT), body weight loss (BWL), end-treatment BV, pre/post dialysis systolic arterial pressure and heart rate, number of sessions complicated by IDH and type of staff interventions.

Results: Throughout the HD and the IDH-BVT period all episodes of IDH, the number of NI, the total volume of fluid infusions for therapeutic purposes, the infusions of 11.7% NaCl and all possible preterm disconnection of patients were reported on the dialysis records. There was no statistically significant difference between the two treatments with regard to the actual between the AoAC progression and baseline clinical parameters including gender, obesity, hypertension and dialysis modality. Further studies are needed to prove or disprove the possible role of regulation in the pathogenesis of hypertension in patients with kidney diseases.

Funding: Government Support - Non-U.S.

SA-PO2628
Renalase Was Not Related to Blood Pressure, but to Residual Renal Function in Haemodialysis and Peritoneal Dialysis Patients Edyta Zbroch, Jolanta Malyszko, Jacek S. Malyszko, Ewa Koc-Zorawska, Michal Mysliwiec. Nephrology, Medical University, Bialystok, Poland.

Background: Recently the new flavin-adenine dinucleotide containing hormone, secreted by the kidney and circulates in blood, named renalase was investigated. It degrades catecholamines and may play a role in the regulation of sympathetic tone and blood pressure.

Methods: The aim of the study was to assess the serum renalase concentration (RNLS) in a cohort of 104 treated with haemodialysis (HD group) and 26 treated with peritoneal dialysis (PD group) patients and its relationship to the blood pressure control, type of antihypertensive therapy and the presence of residual renal function (RRF). RNLS in the study cohort was 25.86µg/ml and it was significantly higher than in the control group 3.86µg/ml (p<0.001). There was the significantly higher RNLS in the HD group comparing to the PD group (27.53 vs. 19.24µg/ml). The results indicated a significant correlation between the duration of dialysis and RNLS (r=0.3175, p=0.002). It was higher in those patients who were maintained dialysis longer. The relationship between the presence of RRF and RNLS was found in whole study cohort (24.21 vs. 27.83µg/ml, p=0.006) and in HD patients (25.65 vs. 30.11µg/ml, p=0.002). The significant inverse correlation between RNLS and RRF was also indicated: HD group r=-0.327, p=0.001; PD group r=-0.4286, p=0.02). No correlation between RNLS and blood pressure rate was found. RNLS was higher in male than female only in HD patients.

Conclusions: The elevated RNLS in dialysis patients may be rather related to kidney function (as time on dialysis passes, the residual renal function is lower) and due to the sympathetic nervous system hyperactivity found in this population and it may have an impact on the development of cardiovascular complications. Further studies are needed to prove or disprove the possible role of renalase in the pathogenesis of hypertension in patients with kidney diseases.

Funding: Government Support - Non-U.S.

SA-PO2629
Renalase as a Possible Risk Factor of Cardiovascular Complications in HD Jolanta Malyszko,1 Jacek S. Malyszko,1 Ewa Koc-Zorawska,1 Edyta Zbroch,1 Michal Mysliwiec,1 1Nephrology, Medical University, Bialystok, Poland; 2Dialysis Unit, NZOZ Centum, Mlawa, Poland.

Background: Renalase, newly discovered hormone, is secreted by the kidney and circulates in blood. It was shown that renalase degrades catecholamines and may play the role in the regulation of sympathetic tone and blood pressure. Cardiac renalase deficiency may contribute to the increased susceptibility to myocardial injury due to ischemia and rhythm disturbances frequently found in CKD. The aim of the study was to assess the renalase relationship to cardiovascular status in hemodialyzed patients.

Methods: Renalase was assessed using commercially available assay from usc. Life Sci, China. Echocardiography was performed in each patient.

Results: Serum renalase was significantly lower in patients with a history of stroke (21%) (13.25±3.97µg/ml vs. 18.61±6.9µg/ml, p<0.05), than in patients without history of stroke.

Conclusions: Similarly renalase was significantly lower hypertensive patients (82%) when compared to normotensive (16.47±5.86µg/ml vs. 22.34±9.1µg/ml, p<0.05), with proximal a- fistula (29%) vs distal a-fistula (16.56±6.39µg/ml vs. 24.61±5.14µg/ml, p<0.05), and higher in patients with arteriosclerosis obliterans compared to other etiologies of cerebrovascular disease (21.91±6.52µg/ml vs. 15.91±6.49µg/ml, p<0.05). Serum renalase correlated with creatinine, residual renal function, TSAT, and dysfunction of mitral valve. No correlation between serum renalase concentration, and blood pressure rate or hemoglobinopathy was found.
PP was higher and HR was lower in PVD pts compared to no-PVD pts. PP increased during the mechanical coupling with respiratory activity. DBP is considered related to afterload.

**Background:** Some studies postulated that early dialysis start may increase mortality. To examine this issue we measured survival associated with eGFR and age at dialysis initiation in our centre.

**Methods:** We studied the following variables at dialysis initiation: eGFR, age, gender, diabetes mellitus, serum albumin, hemoglobin, date of dialysis initiation, history of ischemic heart disease and stroke.

**Results:** Over the last 15 years 428 patients initiated dialysis therapy in our reference area. Mean age at dialysis start 63±13 years and 65% male. The three years survival rate was increasing within the different periods 63% 1995-99, 69% 2000-2004, 77% 2005-2009(p<0.003). Causes of death: heart disease(25%), infections(25%), neoplasms(11%) and stroke(9%). Mean eGFR dialysis start 8.16 mL/min. In the univariate analysis, increased eGFR, age, dialysis initiation 1995-99-2004-2004, diabetes and history of ischemic heart disease were associated with increased mortality in end-stage renal disease patients(ESRD) (p<0.05). Patients that started dialysis program with eGFR <8.16 were older than with eGFR<8.16 (66 vs 61y, p<0.001). In the multivariate Cox model without(age), eGFR, dialysis initiation period, serum albumin and history of ischemic heart disease were associated with mortality. The association between mortality and eGFR was lost when the model was adjusted by age.

**Conclusions:** History of ischemic heart disease, serum albumin and dialysis start before 2005 were risk factors for mortality in ESRD. Older age is usually associated with mortality. The association between mortality and eGFR was lost when the model was adjusted by age.

### Table 1

<table>
<thead>
<tr>
<th>Mean Values and Frequency Domain Indices</th>
<th>PVD pts</th>
<th>no PVD pts</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PP (mmHg)</strong></td>
<td>8±1.9</td>
<td>9±2.6</td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>51.0±7.0</td>
<td>50.0±3.3</td>
</tr>
<tr>
<td><strong>HR (bpm)</strong></td>
<td>52±7</td>
<td>58±11</td>
</tr>
<tr>
<td>**VLF&lt;sub&gt;body&lt;/sub&gt; (mV²·Hz&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>1006±281</td>
<td>1249±2031</td>
</tr>
<tr>
<td>**LF&lt;sub&gt;70&lt;/sub&gt; (mV²·Hz&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>32±13</td>
<td>54±8</td>
</tr>
<tr>
<td>**HF&lt;sub&gt;70&lt;/sub&gt; (mV²·Hz&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>22±8±7</td>
<td>47±16</td>
</tr>
<tr>
<td>**LF&lt;sub&gt;40&lt;/sub&gt; (mV²·Hz&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>19±6±8</td>
<td>42±18</td>
</tr>
</tbody>
</table>

**Conclusions:** In this pilot study, chronic HD patients with PVD have an altered autonomic control system with a prevalence of low control (VLF<sub>body</sub>, higher in PVD pts). PVD induces a chronic elevation of afterload and is associated with both a PP increase during HD and a reduced sympathetic activity on the heart (lower LF<sub>70</sub>).

**SA-PO2632**

**Adverse Effect of Chronic Fluid Overload on Left Ventricular Mass Index in Pediatric Patients Maintained with Chronic Peritoneal Dialysis**

**Background:** Pediatric (ped) patients(pts) with end-stage renal disease have increased cardiovascular mortality. Risk factors include chronic fluid overload (CFO), hypertension, no residual renal function, anemia and inflammation, which all contribute to abnormal cardiac remodeling, increased left ventricular mass index/left ventricular hypertrophy (LVMV/LVH) and subsequent diastolic dysfunction. Few ped studies have assessed the relationship between CFO and cardiac remodeling and function, and no ped study has assessed CFO prevalence in ped pts maintained on chronic peritoneal dialysis (CPD). This study aimed to determine the prevalence of CFO and its association with LVMV in ped CPD pts.

**Methods:** This study is a prospective monthly follow-up study of ped pts ≥2 years old with no underlying primary cardiac disease, who have been treated with home cycler CPD for ≥3 months. Fluid status was assessed by bioimpedance, weight (w) and BP at each monthly visit. BP indices were calculated to normalize BP for age and height for statistical analysis. Annual ECHOcardiograms done. CFO was defined as ≥4% target wt for at least 3 months per 6 month period or median value ≥4% for the entire 6 month period.

**Results:** 11 pts have been enrolled to date with 5/11 followed at least 6 months. 3/11 (27%) had increased LVMV, CFO was associated significantly with increased LVMV and BP indices, but not other ECHOcardiogram parameters.

**Conclusions:** CFO may occur frequently in ped CPD pts and when present, appears to be associated with high BP indices and abnormal cardiac remodeling. CFO per se is likely an important risk factor for cardiovascular disease in ped CPD pts.

### Table 2

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Dialysis months</th>
<th>LVMV g/m²</th>
<th>ISF</th>
<th>ESF</th>
<th>95% SE of ISF/m² Index</th>
<th>DBP index</th>
</tr>
</thead>
<tbody>
<tr>
<td>No CFO</td>
<td>30 (21)</td>
<td>36 (8,9,62)</td>
<td>36 (56,4,6)</td>
<td>58 (11)</td>
<td>1.10 (0,25)</td>
<td>1.04 (0,16)</td>
</tr>
<tr>
<td>CFO</td>
<td>16 (6)</td>
<td>37 (40)</td>
<td>68 (17,17)</td>
<td>31 (13,2)</td>
<td>50 (13)</td>
<td>1.10 (0,25)</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>0.04</td>
<td>NS</td>
<td>NS</td>
<td>0.02</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**Conclusions:** CFO may occur frequently in ped CPD pts and when present, appears to be associated with high BP indices and abnormal cardiac remodeling. CFO per se is likely an important risk factor for cardiovascular disease in ped CPD pts.

### Table 3

<table>
<thead>
<tr>
<th>Value range (SD)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>42 (6,16,62)</td>
<td>NS</td>
</tr>
<tr>
<td>36 (56,4,6)</td>
<td>NS</td>
</tr>
<tr>
<td>58 (11)</td>
<td>NS</td>
</tr>
<tr>
<td>1.10 (0,25)</td>
<td>NS</td>
</tr>
<tr>
<td>1.04 (0,16)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Conclusions:** CFO may occur frequently in ped CPD pts and when present, appears to be associated with high BP indices and abnormal cardiac remodeling. CFO per se is likely an important risk factor for cardiovascular disease in ped CPD pts.
death (OR=1.6 per 10% fall in EF) but qualitative assessment of function was not. Mass- indexed LVH was predictive (OR=2.1); LVH was concentric in 74% of cases. Diastolic function, annular calcification, and PWA were not predictive.

Conclusions: LHV is important in the high mortality of dialysis patients. The higher risk of strain pattern shows the potential value of using ECG and echocardiography together in risk assessment tools. Strain indicates abnormal repolarization, as does axis discordance. This may suggest that aberrant repolarization is a precursor to fatal arrhythmia in dialysis patients.

SA-PO2634
Do Current Guidelines for Implantable Cardioverter Defibrillator Therapy Apply to Hemodialysis Patients? A Single-Centre Experience
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Sincad Kinsella, Emer Joyce, Donal N. Reddan, Matthew D. Griffin, David Lappin.
Departments of Nephrology and Cardiology, Galway University Hospitals, Galway, Ireland.

Background: One in four dialysis patients will succumb to sudden cardiac death (SCD). Carefully selected non-dialysis patients at high risk for ventricular arrhythmia can derive mortality benefit from implantable cardioverter defibrillator (ICD) therapy. The benefit of this intervention in end-stage kidney disease is less clear and data from randomised controlled trials (RCTs) are lacking. We studied the utility of conventional ICD guidelines as applied to a hemodialysis (HD) population.

Methods: A single-centre prospective observational study. American College of Cardiology/American Heart Association/Heart Rhythm Society (ACC/AHA/HRS) ICD eligibility criteria were applied to prevalent HD patients. Patients were grouped according to ICD eligibility status: potential candidates for secondary preventative ICD (group 1); potential candidates for primary preventative ICD (group 2); non-candidates (group 3).

Results: Sixty-two patients (66.1% male, 62.1±15.6 years) were followed for 29 months. Outcomes were as follows: Group 1 (n=2): 1 patient (EF 15%) received an ICD and died of heart failure 20 months later; 1 (EF 55%) had an ICD inserted and died of a fatal arrhythmia 7 months later. Group 2 (n=4): No patient received an ICD. 3 had multiple contraindications to ICD therapy (reduced life expectancy, sub-optimal medical management, recurrent infection) and died from non-SCD causes; 1 is alive and continues to receive hemodialysis. Group 3 (n=56): 3 patients experienced sudden cardiac arrest (2 of which were fatal).

Conclusions: Conventional guidelines which stratify eligibility for primary preventative ICD therapy according to systolic cardiac function should be interpreted with caution in dialysis populations. Dialysis patients have multiple non-traditional risk factors for SCD and may consequently benefit from ICD therapy despite having an EF >35%. Dialysis patients with an EF ≤35% have a high burden of competing risk. RCTs examining the utility of ICD therapy in this unique population are required.

SA-PO2635
CKD-MBD as the Risk Factors of Incident Cardiovascular Disease (CVD) and Fatality after CVD in a Nationwide Registry of Japanese Hemodialysis Patients
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Background: Although chronic kidney disease mineral and bone disorder (CKD-MBD) is a systemic disease affecting cardiovascular mortality, it is unknown whether it affects the risk of incident cardiovascular disease (CVD) or the risk of fatality after CVD. We examined the associations of some components of CKD-MBD and related medications with the risk of incident CVD and the risk of death after CVD in dialysis patients.

Methods: This is an observational cohort study using a standard analysis file provided by JSDT-CRDR (JRDR-09102) including 216612 dialysis patients at the end of 2004. We extracted subjects without previous history of CVD (myocardial infarction, cerebral infarction, and/or cerebral bleeding) and with key variables including use of vitamin D receptor activator (VDRA) and outcomes at the end of 2005.

Results: A total of 49659 hemodialysis patients (59% men, 28% diabetes) were extracted. At baseline, the median age, serum corrected calcium, phosphate, and intact PTH levels were 63 years, 9.3 mg/dl, 5.4 mg/dL, and 136 mg/ml, respectively. VDRAs and phosphate binders were used in 57% and 85% of the cohort, respectively. Incident CVD events were recorded in 3134 patients, and 373 died after such events. In multivariate logistic regression models, a higher risk of incident CVD was associated with higher intact PTH, non-use of VDRA, and non-use of phosphate binders. A higher risk of death after CVD was associated with higher corrected calcium and non-use of phosphate binders.

Conclusions: Some components of CKD-MBD and related medications were independent predictors of incident CVD, death after CVD, or both in this large cohort of hemodialysis patients.

SA-PO2636
Associations between Serum Progesterone and Mortality in Hemodialysis Patients
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Background: Recent epidemiologic data show that men and women with increased progesterone (PRG) levels are at a higher risk of cardiovascular disease (CVD) mortality (Gend Med 2009; 6: 433-443). In this study, we examined the possible associations of PRG levels with cardiovascular risk markers and subsequent mortality in hemodialysis (HD) patients.

Methods: One hundred and seventy-three HD patients (65±12 years, 111 men) after completion of baseline assessment, including sex hormones, were followed up for CVD and all-cause mortality.

Results: The median PRG concentrations in men 0.53 (0.25-1.20) ng/ml and women 0.43 (0.12-2.12) ng/ml did not differ from reported reference values for each sex. In the whole group, PRG levels were inversely related to HD vintage and positively related to prevalence of diabetes mellitus, diastolic blood pressure and serum phosphorus. Age and estradiol were inversely and positively associated with PRG in men and women, respectively. During a median follow up period of 49 months, 79 deaths occurred, 47.59% of which were caused by CVD. Patients with PRG levels in the sex-specific highest tertile had increased CVD and all-cause mortality (crude hazard ratio: 2.94 [95% CI, 1.65 to 5.23] and 2.52 [95% CI, 1.62 to 3.93], respectively. Likewise, the risk for CVD and all-cause mortality increased by 51 % (1.51 [1.16-1.96]) and 45% (1.45 [1.81-1.78]), respectively, for each I SD increment in log PRG. The high serum PRG - mortality association remained essentially unchanged (in both analyses) after adjustment for age, sex, mass body index, serum albumin and c-reactive protein, clinical history of CVD at baseline, presence of diabetes and HD vintage. Addition of estradiol also failed to influence the results. Similar results were observed when looking at each sex separately.

Conclusions: Our results indicate that in HD patients, PRG levels positively correlate with all-cause and CVD mortality, as well as with cardiovascular risk markers. The clinical relevance for these findings needs further elucidation.

SA-PO2637
Involuntary Discharge from Dialysis Units (IVD): Who, Why, Where
Abey K. Thomas,1 Senthil P. Ramaiah,2 Brian T. Chu,2 Carol Lyden,3 George N. Cortisidis,4 Chaim Charytan,4 New York Hospital Medical Center of Queens, Flushing, NY;5 Elmhurst Hospital Center; Elmhurst, NY;6 IPRO ESRD Network of New York, Lake Success, NY.

Background: Patients with behavioral, psychological or financial issues place an extra burden on the stretched resources of dialysis providers and may be at risk for IVD. IVD is a significant problem for patients, providers and payers and affects the quality of care. There is concern that the bundling policy may further increase IVD. There is a paucity of data about causes of IVD and their impact on ESRD care.

Methods: Data on IVD reported to ESRD Network 2(EN2) between July 2006 and March 2011 was analyzed for patient and facility characteristics and compared to the general EN2 population. Statistical analysis was performed by calculating IVD incidence rate in the whole group, PRG levels were inversely related to HD vintage and positively related to prevalence of diabetes mellitus, diastolic blood pressure and serum phosphorus. Age and estradiol were inversely and positively associated with PRG in men and women, respectively. During a median follow up period of 49 months, 79 deaths occurred, 47.59% of which were caused by CVD. Patients with PRG levels in the sex-specific highest tertile had increased CVD and all-cause mortality (crude hazard ratio: 2.94 [95% CI, 1.65 to 5.23] and 2.52 [95% CI, 1.62 to 3.93], respectively. Likewise, the risk for CVD and all-cause mortality increased by 51 % (1.51 [1.16-1.96]) and 45% (1.45 [1.81-1.78]), respectively, for each I SD increment in log PRG. The high serum PRG - mortality association remained essentially unchanged (in both analyses) after adjustment for age, sex, mass body index, serum albumin and c-reactive protein, clinical history of CVD at baseline, presence of diabetes and HD vintage. Addition of estradiol also failed to influence the results. Similar results were observed when looking at each sex separately.

Conclusions: Our results indicate that in HD patients, PRG levels positively correlate with all-cause and CVD mortality, as well as with cardiovascular risk markers. The clinical relevance for these findings needs further elucidation.
### Results:

<table>
<thead>
<tr>
<th>IVD</th>
<th>IVD Incidence %</th>
<th>Average EN2 population</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Total</td>
<td>72</td>
<td>0.29</td>
<td>24518</td>
</tr>
<tr>
<td>Age</td>
<td></td>
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<td></td>
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<tr>
<td>&lt;50</td>
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<td>0.61</td>
<td>4916 &lt; 0.0001</td>
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<tr>
<td>≥50</td>
<td>42</td>
<td>0.21</td>
<td>19601</td>
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<tr>
<td>M</td>
<td>51</td>
<td>0.37</td>
<td>13832 &lt; 0.0017</td>
</tr>
<tr>
<td>F</td>
<td>21</td>
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<tr>
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<td>0.14</td>
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<td>Other</td>
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<tr>
<td>Hemodialysis</td>
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<td>0.52</td>
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<td>&lt;50</td>
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<tr>
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<td>4.17</td>
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### Conclusions:

Our study in EN2 reveals an increased incidence of IVD among younger, male, AA patients treated at larger, for-profit and LDO facilities predominantly for behavioral and financial reasons. These observations may have important consequences for developing interventions to deal with IVD, particularly given the growth of minority ESRD population and LDO facilities. These observations need to be compared to the national experience and trends monitored in an ongoing manner.

### SA-PO2638

**MORRIS: Model for Optimising Renal Replacement Investment and Services; an Interactive Tool Predicting Future Patient Numbers and Associated Costs**

*Mark Brady, Daisy Wild, Beverley Matthews,* Donald O’N. NHS Cumberland Infirmary, NHS Carlisle, United Kingdom; *Department of Health, London, United Kingdom; *NHS Kidney Care, London, United Kingdom.*

**Background:**

Predicting the future needs for a renal replacement therapy (RRT) programme, on a national or local level is essential to maximise efficiency and quality of care. This freely available interactive tool combines UK national census data with UK renal registry data to forecast annual RRT at both national and local levels until 2018, with the ability to use local data or alter proportions of patients on different modalities to examine the effect of investment in such switches.

**Methods:**

MORRIS model uses 2008 UK Renal Registry data, Office of National Statistics (2001) census data incorporating age and ethnicity with expert advice from UK nephrologists. It is a dynamic simulation tool for patients survival and movement between modalities over time. Interactive element allows projections based on local data, plans or needs with treatment costs based on tariff or reference costs.

**Results:**

Figure 1 shows one possible output from the model, making national level projections for various scenarios, predicting the cost of RRT provision in the UK will exceed 1 billion pounds in 2018 and demonstrating the cost benefits of altering the proportion of patients on various modalities. Improving transplantation rates and home therapies uptake together could save 24-28 million pounds per annum on RRT costs alone in the medium term.

**Conclusions:**

The MORRIS model is a powerful interactive tool for predicting future RRT requirements in the UK, demonstrating the benefits of investment in transplantation and home dialysis for example, with the ability to use the model at both national and renal unit level.

**SA-PO2639**

**Impact of Baseline Year on PPS Payments under the Quality Improvement Program**

*Andrew Barba, Randy Smith, Joe Weldon, LeAnne Zumwalt, Mahesh Krishnan.*

**DaVita Inc, Denver, CO.**

**Background:**

In 2011, the CMS issued a final rule creating a Quality Incentive Program (QIP). Beginning in 2012, QIP penalizes dialysis facilities for underperformance by reducing payments up to 2%. QIP uses 3 weighted quality measures to generate a Total Performance Score (TPS): % of patients (pts) with hemoglobin (Hb) >12 g/dL, Hb <10 g/dL and urea reduction ratio (URR) ≥65%. The TPS compares a center’s 2010 quality measures to the least stringent benchmark of either (i) the center’s performance in 2007 (base year) or (ii) the 2008 national average. To assess the validity of the TPS, we determined the relative 2012 revenue impact of the QIP using the 2007 base year, and compared results to those using either 2008 or 2009 as the base year.

**Methods:**

To model payments under QIP effective January 1, 2011, we estimated relative QIP impact per treatment for 1,682 centers using fiscal year (FY)10 claims from the DaVita database and current TPS calculations for Hb and URR. To reflect the CMS projected FY12 market basket update, we increased resulting estimates by 1.4%. We assessed QIP penalties for each facility according to their respective TPS. Facilities with insufficient clinical data were assigned an average TPS of 24 out of 30 possible points.

**Results:**

The proportion of facilities that underperform using the <10 g/dL QIP measure is sizeable and differs by year (Figure). Between-year differences were due mainly to differences in Hb performance.

**Conclusions:**

Lack of year-to-year consistency, particularly in anemia outcomes, throws into question the validity of the use of prospective payment quality measures that are calculated on temporarily distant base years. Our results illustrate the need to modify QIP to use a baseline period as close as possible to the measurement period.

**Funding:** Clinical Revenue Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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**Figure 1. MORRIS model: national UK level predictions for patient numbers on various modalities and associated costs for year 2013 and 2014, with variations in take-up, transplant or home dialysis rate, index (ECHO) center (Cumberland Infirmary, NHS Carlisle, United Kingdom: HHD home haemodialysis). The scenarios described in the table are based on fixed-filing to 2008 levels, transplant growth at 5% increase in 5 years, home therapies growth at 6% Hb and 6% URR. High take-2010 rates represent 2.8% average growth projected by UK RR from 2003 to 2006. Moderate take-2010 rates represent 1.6% growth, including demographics and risk factors. Low take-2010 rate of 0.6% growth represents demographics alone (age and ethnicity).**

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**Funding:** Clinical Revenue Support
SA-PO2640

The End-Stage Renal Disease (ESRD) Prospектив Payment System (PPS) and Access to Care: Incremental Distance Traveled by Displaced Patients

John J. Kochevar, 1 Samuel Brotherton, 1 Stephan C. Dunning, 2 Larry C. Emerson, 3 David T. Gilbertson, 3 David J. Harrison, 3 Ann C. McClellan, 3 William McClellan, 1 John Mark Stephens, 1 Shaowei Wan, 4 Matthew Gittlin, 4 *KRA; 5CDRG; 6Dialysis Center of Lincoln; 7Amgen; 8Emory.

Background: The ESRD PPS will reduce payments to some dialysis facilities by more than the 2% as predicted by CMS and could lead to facility closures. Our objective is to estimate incremental distances patients may need to travel in the event of reduced access to dialysis care.

Methods: We estimated facility risk of closure/consolidation due to financial stress based on payment reduction under the PPS using the CMS flat file of PPS payment changes and a 10-point scale of ability to respond incorporating financial (e.g. payer mix), patient (e.g. case mix) and facility factors (e.g. ownership type). We categorized 5114 facilities in the 48 contiguous US as high, medium or low risk based on their combined scores. We estimated effects around a random sample of 10% of the high-risk group closures, consolidates from 2011-2013 (N=156). We assumed patients would migrate to the nearest facility and calculated incremental road miles and travel times.

Results: The incremental distance and time due to closure/consolidation of a small random sample of high-risk facilities could cumulate to 20,828,330 miles and 387,130 hours over a three-year period. By 2013, mean incremental travel distance would be 1084 miles per-patient-per-year (PPP), nearly double for those in non-urban areas.

Incremental Distances Traveled if 10% of At-Risk Facilities Close/Consolidate 2011-2013

<table>
<thead>
<tr>
<th>Facility Closures % (N)</th>
<th>Total</th>
<th>Rural/Suburb</th>
<th>Urban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>3453</td>
<td>3061</td>
<td>392</td>
</tr>
<tr>
<td>Northern</td>
<td>1770</td>
<td>1579</td>
<td>191</td>
</tr>
<tr>
<td>Southern</td>
<td>1683</td>
<td>1482</td>
<td>101</td>
</tr>
</tbody>
</table>

Mean Incremental Miles / Rural/Suburb = 17.0
Mean Incremental Miles / Urban = 3.7

Table 1

Conclusions: Prior research by DOPPS showed that reduced access as a result of increased driving distances may be associated with increased mortality and morbidity. Increased travel could have significant implications for clinicians and policy makers especially in rural/suburban areas.

Funding: Pharmaceutical Company Support

SA-PO2641

Urban Emergency Department (ED) Visits and Costs for Patients with End-Stage Renal Disease (ESRD), 2005-2009

Sara Mathew, 1 Aaron Truchil, 2 Ernest M. Post, 1 Barry Milcarek, 1 Krystal Hunter, 1 Jeffrey Brenner, 3 Lawrence S. Weisberg, 1 1Department of Nephrology, Cooper University Hospital, Camden, NJ; 2Camiconnect, Camden, NJ.

Background: Hospitalization of ESRD patients cost Medicare $35 billion in 2004-2008, not counting ED costs. Very little is known about the cost and utilization patterns of ED services by patients with ESRD.

Methods: We explored ED use and costs in a poor city, Camden, NJ, using a database of all ED contacts citywide, 2005-2009. We included only ED visits that did not result in hospitalization. We defined ESRD by ICD-9 code 585.6. We excluded patients less than 18 years old.

Results: Patients with ESRD accounted for 0.6% of the total patients in the study. The results are shown in Table 1 below.

Table 1

<table>
<thead>
<tr>
<th>Facility Closures % (N)</th>
<th>Total</th>
<th>Rural/Suburb</th>
<th>Urban</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
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</table>

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Funding: Pharmaceutical Company Support

SA-PO2642

Healthcare Utilization and Costs in Patients with End-Stage Renal Disease Beginning Renal Replacement Therapy with Peritoneal Dialysis Versus Hemodialysis

Gary W. Inglese, 1 Charu Taneja, 2 Ariel Berger, 1 Lois Lamaroto, 3 James A. Sloand, 3 Greg Abbott, 3 Greg G. Wolff, 3erry Oster, 1 Policy Analysis Inc. (PAI), Brookline, MA; 2Henry Ford Health System, Detroit, MI; 3Baxter Healthcare Corporation, McGaw Park, IL.

Background: To compare healthcare utilization and costs over one year between patients beginning renal replacement therapy with peritoneal dialysis (PD) versus hemodialysis (HD).

Methods: This retrospective study was conducted at a large US urban integrated health plan that maintains comprehensive records on the use of healthcare services by all of its members. The study subjects consisted of all persons aged 18-63 years who initiated PD or HD for end-stage renal disease (ESRD) between January 1, 2005 and December 31, 2008 ("study period"). Each patient’s earliest noted date of receipt of dialysis was designated his/her "index date", and all patients were followed for 1 year thereafter. Each PD patient was matched to 2 HD patients using propensity scoring to control for differences in baseline characteristics, including age, gender, race, and pretreatment healthcare utilization. Healthcare utilization and costs were then compared over 1 year between matched PD and HD patients, using methods appropriate for matched samples.

Results: We identified a total of 162 patients who began dialysis during the study period (PD, n=26; HD, n=136). After matching, the final study sample included 26 PD patients and 52 HD patients. PD and HD patients were well-matched. Mean age of study subjects was 55 years, 58% were men, and 74% were African American. During the 1 year follow-up, HD patients were more likely to be hospitalized (67.3% vs 42.3% for PD; p=0.03). HD patients also averaged more physician office visits (17.2 vs 12.4, respectively; p<0.01) and emergency room visits (2.1 vs 1.1; p=0.04) over this period. Mean total healthcare costs over 12 months were significantly higher among HD than PD patients ($188,340 vs $127,981; p<0.01).

Conclusions: In patients initiating renal replacement therapy for ESRD, healthcare utilization and costs over one year may be lower in those receiving PD.

Funding: Pharmaceutical Company Support

SA-PO2643

Key Performance Indicators (KPIs) for Peritoneal Dialysis in Thailand: A Nationwide Survey

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Background: Implementation of the “Peritoneal Dialysis–first (PD) first” policy, mandating PD as a first modality of RRT for ESRD patients under universal health coverage since year 2008, leads to rapid growing of PD cases and centers in Thailand. To ensure quality of PD service parallel the bursting of PD cases, nationwide survey of key performance indicators (KPIs) was performed.

Methods: All PD centers in Thailand were invited to participate in the present study. PD nurse case managers in each center were asked to review medical records of all patients undergoing PD during October 1, 2009 to September 30, 2010 and submitted data to the main investigators.

Results: About three-fourths of all PD-centers in Thailand (88 out of 121) participated in the present study. One hundred five nephrologists and 154 PD nurse specialists served beginning PD patients in 2007. The employment status of all prevalent 15–64-year-old dialysis and kidney transplantation patients in Finland at end of 2007 (N=2637) was analyzed by combining data from Finnish Registry for Kidney Diseases with the official individual-level

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Underline represents presenting author.

Funding: Government Support - Non-U.S.

SA-PO2644

Association between Mode of Renal Replacement Therapy and Employment Status

Patrik Finne, 1 Iikka Helanteri, 2 Mikko Haapio, 3 Petri K. Koskinen, 2 Carola Gronhagen-Riska, 1 1Finnish Registry for Kidney Diseases, Helsinki, Finland; 2Department of Nephrology, Helsinki University Central Hospital, Helsinki, Finland.

Background: It is poorly known how mode of renal replacement therapy affects probability of being employed. We analyzed the association of treatment modality with employment status among patients on dialysis and after transplantation in a cross-sectional study.

Methods: The employment status of all prevalent 15–64-year-old dialysis and kidney transplantation patients in Finland at end of 2007 (N=2637) was analyzed by combining the data from Finnish Registry for Kidney Diseases with the official individual-level

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Underline represents presenting author.
Costs of Care and Major Clinical Events among Chronic Dialysis Patients with and without Treatment for sHPT: A Descriptive Study of Claims Data

Background: Secondary hyperparathyroidism (sHPT) is highly prevalent in chronic dialysis (CD) patients, yet little data describing costs of care for sHPT exists.

Methods: We examined direct medical costs in US CD patients covered by commercial or Medicare insurance with and without treatment for sHPT in a retrospective cohort study of 6 months prior to first claim, CD patients meeting K/DOQI guidelines for sHPT were included. Treatment for sHPT and baseline Fe were compared for 60 days before.

Results: A total of 26,371 non sHPT and 15,558 sHPT patients met study criteria. Based on 6 months of data prior to first claim, sHPT patients were younger in baseline age median (61.4 vs 66.2 (SD 14.3)) vs 66.2 (SD 14.1)) and had higher percentage of black patients (20.3 vs 16.8) than non sHPT patients. The sHPT population had higher OPD and MED costs, and lower mean (SD) PP cost per hospitalization.

Conclusions: The observed higher TL costs for sHPT patients may be an important consideration for reimbursement policy decisions.
### SA-PO2649

**Association of Frequency of Hb Testing on Anemia Outcomes**

Irina Gavkhrman, Carey Colson, Steven M. Wilson, David B. Van Wyck. *DaVita Inc, Denver, CO.*

**Background:** A paucity of information about the optimal hemoglobin (Hb) testing frequency required to achieve target anemia outcomes likely contributes to wide practice variation in the frequency of orders for lab tests within the health care community. However, the medical resource consumption that arises from frequent lab testing has gained increasing consideration with the recently implemented US end-stage renal disease (ESRD) prospective payment system. We assessed facility-level Hb testing patterns and their impact on anemia outcomes.

**Methods:** We reviewed 2010 data from a large US dialysis provider’s database to categorize dialysis facility Hb testing patterns as well as the percentage of patients with Hb levels in range (between 10 and 12 mg/dL). We categorized facilities by the mean number of reported Hb lab tests per patient dialyzing in that facility per quarter. We used patient-weighted Generalized Linear Models (GLM) to predict the percent of patients in range and mean Hb levels from number of Hb tests.

**Results:** The frequency of Hb testing based on physician ordering preference varied from 2.5 to 19.5 tests/quarter. Most (96%) facilities tested Hb 6-12 times per quarter, accounting for 97% of patients. Despite this, mean Hb levels did not vary significantly with greater Hb testing frequency (p=0.28; Figure).

**Conclusions:** When examined over a range from weekly to monthly, Hb test frequency shows no discernable relationship to Hb outcomes. This finding bears directly on the design and evaluation of anemia management protocols.

*Funding: Clinical Revenue Support*

### SA-PO2650

**Association of Erythropoietin Dosing and Mortality in the Maintenance Pediatric Dialysis Population**

Rachel M. Lesz,1 Barbara A. Fivush,2 Meredith A. Atkinson,2 Pediatric Nephrology, Children’s Hospital of Los Angeles, Los Angeles, CA; Pediatric Nephrology, Johns Hopkins University School of Medicine, Baltimore, MD.

**Background:** Treatment with exogenous erythropoietin (EPO) has transformed the management of anemia in patients undergoing maintenance dialysis therapy. In recent years, the effort to normalize hemoglobin levels with increasing doses of EPO has revealed an association of higher EPO doses with adverse events and increased mortality in the adult CKD and dialysis populations. However, this relationship has not yet been documented in pediatric patients.

**Methods:** Retrospective study of prevalent pediatric (age ≥18 years) dialysis patients receiving maintenance EPO, using the Centers for Medicare & Medicaid ESRD Clinical Performance Measures Project 2005 linked with the USDRS mortality records for the following year. Four EPO categories were created based upon the distribution of EPO dosing (unit/kg/week): 0-25%, 0-92 u/kg/week, 25-50%, 92-<192 u/kg/week (reference group), 50-75%, ≥192 u/kg/week, >75%, 50-75% ≥356 u/kg/week, >75% ≥356 u/kg/week. Cox-proportional hazards regression was performed to determine if EPO dose category was associated with an increased risk death over 12 months. The adjusted model controlled for race, age, gender, diagnosis, hemoglobin ≥11, albumin ≥3.5 (BCG)/3.2 (BCP), dialysis modality and access type, Kt/V and time on dialysis.

**Results:** Of the 1014 children included in the analysis, 35 died during the 12 month observation period. In the adjusted analysis, pts in the highest EPO category had a 4.2 times higher risk of death compared to those receiving 92-<192 units/kg/week (25-50%) EPO dosing.

**Conclusions:** Although mortality in general is a relatively rare outcome among children compared to adults on dialysis, we have shown that pediatric dialysis pts. who received the highest EPO doses demonstrated an increased risk of death. Further study, including prospective analyses, are required to determine whether the higher EPO doses have an independent causal affect in dialyzed children.

### SA-PO2651

**Evaluation of the Predictive Value of Commonly Used Anemia Management Indicators in Incident Hemodialysis Patients**

Jochen G. Raimann,1,2 Lena A. Usvyat,1,2 Stephan Thijsen,1,2 Peter Kotanko,1,2 Nathan W. Levin.1,2 Renal Research Institute,1 Beth Israel Medical Center.

**Background:** Adequate anemia management improves survival and hospitalization rates (Locatelli 2004) in hemodialysis (HD) patients (pts). Erythropoietin (EPO) resistance, reflected by the ratio of EPO dose per hemoglobin (Hgb) concentration, was associated with inflammation (Stenvinkel 2002) and survival (Kilpatrick 2008). The best predictor of survival in terms of anemia management is controversial. This analysis aims to compare the predictive performance of different indicators.

**Methods:** Pts starting HD between 1/1/2001 and 7/30/2008 in RRI clinics were included. Average EPO dose per treatment was computed over the first 3 months of HD and survival monitored over one year. Predictive power of 1) EPO dose per treatment, 2) EPO dose per treatment per g/dL Hgb and 3) EPO dose per treatment per g/kg Hgb/kg of body weight (BW) was assessed as the area under the curves (AUC) of the respective receiver operating characteristics (ROC) curves. Youden index was employed to compute optimal discriminatory thresholds; positive and negative likelihood ratios (LR+; LR-) were calculated.

**Results:** 6580 incident HD patients (56% males, 44% black, 47% white, 50% diabetic, age 61.5±15.4 years) were studied. Mortality rate was 92.6±1000 pts per year. AUC differed between the respective ROC curves (p<0.0001; Figure 1): (1) EPO dose per treatment: 0.58 (95% CI 0.57 to 0.59); (2) EPO dose normalized to Hgb: 0.59 (95% CI 0.58 to 0.60); (3) EPO dose normalized to Hgb and BW: 0.63 (95% CI 0.62 to 0.64). Average EPO per g/dL per kg of BW was 11.4±95% CI 11.2 to 11.6); a threshold of 12.9 allowed to predict mortality (LR+ 1.7; LR- 0.7).

**Conclusions:** These data suggests that the normalization of EPO doses to Hgb and BW has the most predictive power. This result supports the use of ERI analyses of survival and anemia management.

### SA-PO2652

**Outpatient (OP) Red Blood Cell (RBC) Transfusion Payments among Patients on Chronic Dialysis**

Matthew Gatlin, Shaowei Wan, Xue Song, Jeffrey L. Carson, David M. Spiegel, Brian Custer, Akhtar Ashfaq, Helen V. Varker, Katherine A. Cappell.

**Background:** Estimate OP RBC transfusion payments among chronic dialysis patients.

**Methods:** This retrospective economic analysis is based on a conceptual model of transfusion associated resource use to estimate OP RBC transfusion payments in dialysis patients using MarketScan data (1/1/02-10/30/10). All patients had ≥ 2 chronic dialysis claims ≥ 30 days apart and ≤ 1 year (1st claim defined as index date), ≥ 6 months pre-index data to measure comorbidities, and ≥1 OP RBC transfusion and ≥30 days post-transfusion follow-up. Total payments per RBC transfusion episode included pre/post screening/monitoring (+/- 3 days), blood acquisition/administration (within 2 days) and associated complications (acute within 3 days, e.g., circulatory overload, acute lung injury and hyperkalemia; 45 days for chronic, e.g., hemolytic reaction).

### Key:

TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

729A
**Results:** A total of 3283 patients were included in this preliminary analysis, with a mean age of 60.9 (SD 15.0), 56.4% men, and 40.9% with Medicare supplemental insurance. Mean Charlson comorbidity index was 4.3 (SD 2.5). During a mean length of follow up of 552 days (SD 541), a patient on average had 3.1 (SD 4.3) OP RBC transfusion episodes. In the subgroup who had specific procedure claims for RBC unit level data of blood acquisition/administration, the mean (median) number of units per transfusion episode was 1.1 (1). **Conclusions:** Preliminary results show payments for OP RBC transfusions are driven by blood acquisition/administration, followed by screening/monitoring. Although infrequent, transfusion complications increase costs substantially when they occur.

_Funding:_ Pharmaceutical Company Support

SA-PO2653

**A Comparison of Projected ESRD Incidence and Prevalence with Recent Data**

David T. Gilbertson,1 Craig Solid,2 Allan J. Collins.2 USRDS Coordinating Center, Minneapolis, MN; 1Medicine, University of Minnesota, Minneapolis, MN.

**Background:** The USRDS published projections of the ESRD population to 2015 in 2005, and updated these projections to 2020 in the 2009 Annual Data Report (ADR). A primary message of those projections was that while incident rates of ESRD were slowing in many age and race groups, counts continued to increase. Here we compared actual incidence and prevalence numbers with those projected in the 2009 ADR that used information available through 2007.

**Methods:** A non-stationary Markov model was used, incorporating census projections and expected changes in both demographics and diabetes prevalence, to project ESRD incidence and prevalence through 2020. USRDS data through 2007 were used to obtain transition probabilities as well as past incident and prevalent counts.

**Results:** Incident counts were virtually constant from 2006-2008, but increased 3.3% in 2009, somewhat larger than projected. Projected counts have been consistently increasing approximately 4% per year, and are also ahead of projected numbers. Using data through 2007 and assuming relatively constant incidence rates based on recent trends, the model consistently projected increasing incident/prevalent counts through 2020.

**ESRD Projected and Actual Incidence/Prevalence**

<table>
<thead>
<tr>
<th>Year</th>
<th>Projected</th>
<th>Actual</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>111,110</td>
<td>508,461</td>
</tr>
<tr>
<td>2007</td>
<td>111,193</td>
<td>528,686</td>
</tr>
<tr>
<td>2008</td>
<td>113,198</td>
<td>545,245</td>
</tr>
<tr>
<td>2009</td>
<td>115,299</td>
<td>563,173</td>
</tr>
<tr>
<td>2010</td>
<td>117,425</td>
<td>581,352</td>
</tr>
<tr>
<td>2015</td>
<td>129,555</td>
<td>676,343</td>
</tr>
<tr>
<td>2020</td>
<td>142,558</td>
<td>774,386</td>
</tr>
</tbody>
</table>

**Conclusions:** Modeling assumptions were generally conservative, assuming flattening or even decreasing incident rates in some age/race groups. However, the post WWII population bulge is beginning to move into age groups with increasing chronic disease and increasing numbers of patients requiring RRT are inevitable. If incident rates increase in some groups and ESRD death rates continue to decline, the total number of patients requiring RRT will exceed these projections. This increasing population, along with changing incentives under the Medicare payment bundle implemented in Jan 2011, may increase the use of home therapies in the dialysis population.

_Funding:_ NIDDK Support

SA-PO2654

**Does Dialysis Improve Survival in Elderly Patients?**

Darren Green, Philip A. Kalra. Vascular Research Group, Manchester Academic Health Sciences Centre, University of Manchester; Salford Royal Hospital, United Kingdom.

**Background:** It is unclear whether dialysis provides significant survival benefit to elderly CKD patients. Dialysis planning begins when patients reaches stage 5 CKD. It would be of benefit to be able to give prognostic information from this point.

**Methods:** We undertook a prospective observational study of outcome in patients who reached CKD stage 5 aged ≥75 years. Patients were sorted by whether they had chosen dialysis or conservative care, and were followed for 3 years. Patients were excluded if they suffered from malignancy, end-stage heart failure, or dementia.

**Results:** 82 patients were included (24 conservative care, 44 hemodialysis, 14 peritoneal dialysis). Dialysis patients were younger (79.4 vs. 83.4 years, p=0.000) and less likely to have coronary artery disease (21.6% vs. 45.5%, p=0.016). There was no difference in blood pressure, diabetes, heart failure, COPD, Karnofsky performance score, weight, smoking or alcohol intake. Patients who chose conservative care tended to be those who were widowed (40.0% vs. 10.4%, p=0.040) or lived alone (45.5% vs. 11.9%, p=0.036). 3 year survival was 55.6% for dialysis patients versus 25.0% for conservative care.

**Figure 1. Survival in stage 5 CKD: dialysis versus conservative care.**

This survival difference was independent of age. Patients who chose hemodialysis spent more time in hospital and underwent more operations that peritoneal dialysis or conservative care patients.

<table>
<thead>
<tr>
<th>Year</th>
<th>Conservative</th>
<th>Hemodialysis</th>
<th>Peritoneal</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>39</td>
<td>45</td>
<td>42</td>
</tr>
<tr>
<td>2007</td>
<td>41</td>
<td>43</td>
<td>40</td>
</tr>
<tr>
<td>2008</td>
<td>42</td>
<td>44</td>
<td>41</td>
</tr>
<tr>
<td>2009</td>
<td>43</td>
<td>45</td>
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</tr>
<tr>
<td>2010</td>
<td>44</td>
<td>46</td>
<td>43</td>
</tr>
<tr>
<td>2011</td>
<td>45</td>
<td>47</td>
<td>44</td>
</tr>
</tbody>
</table>

**Conclusions:** This study shows that there may be a survival benefit to choosing dialysis over conservative care in elderly patients with stage 5 CKD. Choosing dialysis appeared to be as strongly dependent on social factors as medical ones.

**SA-PO2655**

**Exploring Preventable Hospitalizations of Dialysis Patients**

John R C. Wheeler,1 Richard Hirth,1 Kathryn Meyer,2 Joseph M. Messana.1 University of Michigan, Ann Arbor, MI; 2Arbor Research Collaborative for Health, Ann Arbor, MI.

**Background:** Hospitalization rates are high (1.9 per pt yr) among ESRD pts. We investigated whether some types of hospitalization could be prevented by high quality dialysis care.

**Methods:** To identify causes of hospitalizations that may be candidate quality measures, we identified the most frequent diagnoses (dx) among ESRD pts and the frequency of dx deemed potentially preventable by AHRQ, i.e., Prevention Quality Indicators (PQIs). Medicare inpatient claims data for admissions in 2008 among ESRD pts were used. Admissions and time at risk were counted during time when the pt had Medicare as their primary payer (n=316,066 pts, with 238,266 yrs at risk and 447,972 admits).

**Results:** The most common discharge dx among ESRD pts were: (1) comp of device, implant or graft; (2) congestive heart failure (CHF); and (3) hypertension (HTN). The most common PQI dx were: (1) CHF; (2) DM long term complications; and (3) lower extremity amputation. AHRQ potentially preventable admissions comprised over 18% of all hospitalizations for ESRD pts, compared to 15% for all Medicare beneficiaries. Further, some non-PQI dx common among ESRD pts, such as comp of device, graft, or implant, may reflect preventable hospitalizations among ESRD pts. Finally, there is a high degree of variation across facilities in hospitalization rates for PQI dx (CHF inter-quartile range is 4% to 17%).

**PQI Hospitalizations Among ESRD Medicare Primary ESRD Pts in 2008**

**Table 1. Days spent in hospital per year by dialysis modality choice.**

<table>
<thead>
<tr>
<th>Dialysis Modality</th>
<th>CHF</th>
<th>DM long term comp</th>
<th>Bacterial pneumonia</th>
<th>Lower extremity amputation</th>
<th>UTI</th>
<th>COPD</th>
<th>Dehydration</th>
<th>DM short term comp</th>
<th>Adult asthma</th>
<th>Anemia</th>
<th>Infection</th>
<th>DM uncontrolled</th>
<th>Perforated appendix</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conservative</td>
<td>6.67</td>
<td>3.02</td>
<td>4.18</td>
<td>1.68</td>
<td>0.99</td>
<td>0.76</td>
<td>0.54</td>
<td>0.49</td>
<td>0.29</td>
<td>0.14</td>
<td>0.10</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>89.1</td>
<td>13.2</td>
<td>35.8</td>
<td>22.4</td>
<td>13.2</td>
<td>10.8</td>
<td>7.3</td>
<td>6.6</td>
<td>3.9</td>
<td>1.8</td>
<td>1.4</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>

**Conclusions:** This study shows that there may be a survival benefit to choosing dialysis over conservative care in elderly patients with stage 5 CKD. Choosing dialysis appeared to be as strongly dependent on social factors as medical ones.

**Poster/Saturday**
SA-PO2656

Hospital 30 Day Readmission Rates in Spanish Versus English Speaking End Stage Renal Disease Patients in a Medical Practice That Has Implemented An Ambulatory Care Language Concordance Program Maite Levine Ledezma, Kwun-Yee T. Poont, Scott A. Rasgon. Division of Nephrology and Hypertension, Kaiser Permanente, Los Angeles, CA.

Background: Nationally there is a disparity in health outcomes in Hispanic compared with non-Hispanic patients with end-stage-renal-disease (ESRD).

Objective: To determine whether there is a difference in 30 day hospitalization readmission rates in Spanish vs English speaking ESRD patients in a medical group that has implemented a language concordance program.

Methods: We conducted a retrospective analysis of automated medical record database for admissions in Kaiser Permanente, Southern California hospitals in 2010 (n=166,225). Kaiser Permanente provides health care for more than 3.4 million patients, implemented an ambulatory care language concordance program in 2008. Non-English speaking patients where assigned primary care physicians fluent in preferred language (conciliation 91.3%). The study cohort was adults (mean age 64.3) with a non obisetric admission (n=88,502); diagnosis ESRD (n = 3,166), and Spanish (n = 5,012). The demographics: gender (54% female), BMI (mean 29), diabetes (29%), and ethnicity (white 52%, black 15%, Hispanic 22%, and Asian 5%).

Results: Among study cohort patients who had an inpatient hospitalization in the measurement year (n=88,502), 9.8% had a second hospitalization within thirty days (n=8,617). Patients appeared older (mean 67), likely white (55%) or African American (17%), and tended to be English (vs Spanish) speaking (95.1%, p=<0.0014). Patients with a diagnosis of ESRD (n=3,166), who had a 30 day readmission (n=679), tended to be white (35%) vs Hispanic (27%), male (56%), and older (67). The multivariate analysis of the independent variable in association with the outcome using logistic regression demonstrated Spanish versus English (OR 0.791, p=<0.0001), gender female versus male (OR 0.914, p=<0.0001), and history of diabetes (OR 1.44, p=<0.0001) and ESRD (OR 2.292, p=<0.0001).

Conclusions: In a medical practice that has an ambulatory care language concordance program, Spanish speaking patients are 21% less likely to have a 30 day hospital readmission compared to English speaking patients, after adjusting for gender and history of ESRD.

SA-PO2657

Rehospitalizations in Prevalent 2009 Hemodialysis Patients Tricia L. Roberts,1 David T. Gilbertson,2 Craig Solid,3 Allan J. Collins,1,2 1USRDS Coordinating Center, Minneapolis, MN; 2Medicine, University of MN, Minneapolis, MN.

Background: Rehospitalizations contribute to the Medicare cost burden and indicate need for improved quality of care. Recent literature reported 30 day rehospitalization rates of nearly 20% among general Medicare patients; however, current rates among the dialysis population remain unknown.

Methods: We calculated rehospitalization rates in adult Medicare prevalent hemodialysis patients in 2009. Live hospital discharges were included from January 1 to December 1. We excluded rehabilitation claims, transfers, and discharges with a same-day admission to long-term care and critical access hospitals. Events were first rehospitalization and the combined endpoint of rehospitalization or death (rehos/death). Rates showed the admission to long-term care and critical access hospitals. Events were first rehospitalization and the combined endpoint of rehospitalization or death (rehos/death). Rates showed the

Conclusions: Rehospitalization rates among hemodialysis patients were strikingly higher than one third of discharges were followed by at least one 30 day rehospitalization. Rates have not improved in the last decade, and identification of high risk groups, such as young patients and African Americans, could focus efforts to reduce rehospitalizations.

Funding: NIDDK Support

SA-PO2658

Long Term Renal Outcomes Following Autologous Stem Cell Transplant for Multiple Myeloma Siobhan Glavey,1 Nelson Leung.1 1Nephrology, Mayo Clinic Rochester, Rochester, MN; 2Hematology, Mayo Clinic Rochester, Rochester, MN.

Background: Autologous Stem Cell Transplant (SCT) following high dose chemotherapy (HDC) has been established as optimal therapy for patients with multiple myeloma (MM) for over a decade. At our institution 19% of patients have a serum creatinine over 2.5mg/dL at time of presentation with MM. The current literature pertaining to the outcome in terms of renal recovery following SCT is conflicting, particularly with regard to gaining independence from dialysis.

Methods: We conducted a retrospective analysis of the medical records of all patients undergoing SCT for MM between 2000 and 2010. At our institution. Inclusion criteria: Patients who had an elevation in serum creatinine (SCr) over 3mg/dL or were dialysis dependent at the time of SCT were selected. Exclusion criteria: Patients who had known chronic kidney disease preceding the onset of MM were excluded.

Results: Fifteen patients were found to have a serum creatinine over 3mg/dL in the pre-SCT period but were not dialysis dependent.Median SCT prior to SCT in this group was 3.5mg/dL. One month following SCT this increased to 6.1mg/dL.Four patients (26.0%) had progression to ESKD in the long term requiring dialysis. None of these patients regained independence from dialysis by time of death or last clinical review. Fifteen patients (15/30) were dialysis dependent at the time of SCT.Of these only one was able to attain independence from dialysis post SCT. This patient had improving renal function prior to SCT with an intahalamic clearance of 19ml/min and went on to discontinue dialysis 17 days after SCT.

Conclusions: Previous studies have indicated that SCT may have a favourable impact on renal outcome in MM.

We have not found this to be the case and on retrospective analysis of our experience in SCT for treatment of MM over ten years we found no benefit in terms of freedom from RRT or progression of renal pathology and impairment. Furthermore we found for patients who were close to commencing dialysis prior to SCT there was a 26.6% progression to ESKD requiring RRT in the long term. We propose caution prior to SCT in advising patients that there is likely to be an improvement in renal function.

SA-PO2659

Factors Affecting the Decision To Start Renal Replacement Therapy: Results of a Survey among European Nephrologists Moniek Van de Laan,1 Catharina Wester,2 Marlies Noordzij,3 Win Van Biesen,4 Christoph Wanner,5 Torunczyk, Kitty J. Jager. ERA-EDTA Registry Investigators, AMC, Amsterdam, The Netherlands.

Background: The level of residual renal function is likely to determine the timing of start of renal replacement therapy (RRT).The patient’s clinical status is suggested to play an at least as important role in this decision, but the importance of specific factors is unknown.We evaluated current opinions of European nephrologists on the decision making process on when to start RRT and whether opinions differed by nephrologist or facility characteristics.

Methods: We distributed a web-based survey among nephrologists in 11 European countries with questions on the target level of renal function related to the start of RRT,factors bringing forward/postponing RRT,and nephrologist/facility characteristics.We used chi-square and multivariate linear regression to study associations and determinants of estimated glomerular filtration rate (eGFR).

Results: We received 433 completed surveys. Overall, renal function was not considered as the most important factor in the decision to start RRT, but for uncomplicated patients, the majority of respondents did regard eGFR as the most important factor. The majority (88%) believed that a start at eGFR=10.5 ml/min/1.73m2 was only beneficial if symptoms are present.Factors bringing forward the start of RRT were hyperkalemia (100%), pericarditis (98%) and fluid overload (97%). Patient preference (69%) and vascular dementia (66%) postponed the start. The median eGFR on which respondents aimed to start RRT in uncomplicated patients was 10.0 ml/min/1.73m2 (IQR 8.0-10.0).We found higher target levels adjusted for comfounders for respondents from countries with high vs low RRT incidence (eGFR 10.3 [9.8-10.8] vs 9.1) and from for-profit vs non-profit centres (eGFR 10.9 [10.4-11.7] vs 9.4 p<0.05).

Conclusions: Signs and symptoms rather than eGFR are important in the decision on when to start RRT. Nephrologists from countries with high RRT incidence and from for-profit centres aim to start at slightly higher eGFR levels. Although we gained insight in the decision making process, prospective studies are needed to further study current practice and its association with patient outcomes

SA-PO2660

The Rate of Wearing a Mask among HD Outpatients, Seroconversion Rates for Multiple Myeloma and Seasonal Influenza, and Infection Status in the Pandemic between 2009 and 2010 Toru Hyodo,1 Naoyuki Sato,2 Daisuke Ishii,3 Kazunari Yoshida,4 Shiro Baba.1 1Urology, Kitasato University, Sagamihara, Kanagawa, Japan; 2Nursing, Atsugi Clinic, Atsugi, Kanagawa, Japan.

Background: To determine the rate of wearing a mask, serconversion rates for novel and seasonal influenza vaccines, and rate of influenza infection in the pandemic of novel influenza (pandemic A[H1N1] 2009).

Funding: Other U.S. Government Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: The rates of wearing a mask and the seroconversion status for A/California/07(H1N1), A/Uruguay/716/2009(H1N2), and B/Shanghai/26/2008, which were used for inoculation in October 2009, and A/California/7/2009(H1N1) used for inoculation in December 2009 was compared before inoculation, and one month and six months after inoculation in 103 dialysis patients (average age: 64.0±13.4, mean dialysis history: 75.5±77.8 months, 28 women, 42 diabetic patients, and 61 non-diabetic patients). Titters of at least 1:40 were within the safe range. The rate of influenza infection among HD patients was evaluated based on whether titters six months after inoculation were at least four times those one month after inoculation.

Results: Among the subjects, only 3.1±1.3% did not wear a mask between October 2009 and the end of April 2010. The proportion of subjects with titters of at least 1:40 for A/California was 8% before inoculation, 35% one month after inoculation, and 18% six months after inoculation, for A/Brasilie was 36% before inoculation, 45% one month after inoculation, 63% six months after inoculation, for A/Uruguay was 24% before inoculation, 45% one month after inoculation, and 26% six months after inoculation, and for B/Shanghai was 25% before inoculation, 35% one month after inoculation, and 36% six months after inoculation. The rate of influenza infection was 1% (in 103 subjects, positivity rate was 1%).

Conclusions: It was suggested that the seroconversion rate for A/California used for inoculation between 2009 and 2010 was slightly lower than that for A/Uruguay or A/Brasilie. Given the seroconversion rates for vaccines among HD patients, wearing a mask appeared to be very effective because the number of infected subjects was extremely small.

SA-PO2661

Low Prevalence of Occult Hepatitis B Virus Infection in Chronic Hemodialysis and Kidney Transplant Patients: Seeamma Baid-Agrawal,1 Ralf Schindler,2 Petra Reinke,1 Ulrich Frei,1 Thomas Berg,1 Dept of Nephrology and Medical Intensive Care, Campus Virchow Clinic, Charite Medical University, Berlin, Germany; 2Division of Hepatology, University Clinic Leipzig, Leipzig, Germany.

Background: Occult hepatitis B virus (HBV) infection is defined as presence of HBV DNA in serum, liver or peripheral blood mononuclear cells (PBMC) in patients whose sera test negative for HBsAg. It may be a potential source of nosocomial transmission in chronic hemodialysis (CHD) patients and kidney transplant recipients (KTAR). This is the first study to date investigating its prevalence in large cohorts of these patients.

Methods: In this cross-sectional study, 391 patients undergoing CHD (Group 1), 417 NTXR (Group 2), and 20 HBsAg-positive non-HD non-KTx patients (positive controls, Group 3) and 40 HBsAg-negative healthy subjects (negative controls, Group 4) were enrolled. HBV DNA was determined in both serum and PBMC using Cobas TaqMan® (Roche Diagnostics).

Results: Group 1 (CHD): 387/391 patients were HBsAg-negative (Group 1a), 4 were HBsAg-positive (Group 1b). The overall prevalence of HBsAg-positive infection was 1% (4/391). Occult HBV infection was found only in 1/387 (0.2%) HBsAg-negative patients and that also in serum and not in PBMC (HBV DNA-positive in serum). Group 2 (KTxR): 400/417 KTxR were HBsAg-negative (Group 2a), remaining 17 (4.1%) were HBsAg-positive (Group 2b). Occult HBV infection was found in 2/400 (0.5%) HBsAg-negative KTxR recipients only in serum. Of note, HBV DNA was not detected in PBMC in any of CHD and KTx patients.

Conclusion: We found a low prevalence (<1%) of occult HBV infection in CHD and KTx patients suggesting that labours and expensive testing of HBV DNA in PBMC is not required for screening and diagnosis in these populations in our region. However, our results may not be generalizable to other populations with a higher prevalence of HBV where the role of occult HBV infection needs to be clarified.

Funding: Private Foundation Support

SA-PO2662

Antibodies to Hepatitis B Virus Surface Antigen and Interleukin 12b Gene Polymorphism in Hemodialysis Patients Alicia E. Grzegorzewska,1 Piotr M. Wobszal,1,2 Pawel P. Jagodziński,3 1Department of Nephrology, Transplantology and Internal Diseases, University of Medical Sciences, Poznan, Poland; 2Department of Biochemistry and Molecular Biology, University of Medical Sciences, Poznan, Poland.

Background: In hemodialysis (HD) patients, interleukin (IL) 12 is suggested to be a potential inflammatory cytokine in HD patients. IL12 shares biochemical and biological properties with IL12 in the immune system. A predominant Th1 response may inversely affect the Th2 humoral response. Our work supports the use of nucleic acid testing by polymerase chain reaction (PCR) for surveillan of infection in prevalent haemodialysis patients in areas with high levels of carriage, the evidence for its use in units with a lower prevalence is less clear.

Methods: Prospective data was collected over a twelve month period on all patients receiving maintenance haemodialysis across our satellite dialysis centres. In addition to monthly HCV antibody (3rd generation enzyme immunoassay- EIA) testing, four-monthly pooled samples from each unit were submitted for nucleic acid testing (HCV-RNA PCR).

Results: Each pool contained five patients, who were then re-tested individually in the presence of a positive pool result. Individuals known to carry HCV were not excluded. Results: 1352 patients were tested on more than one occasion, in total 51 individuals (3.8%) were found to be either HCV-positive by PCR or EIA testing. Prevalence of HCV antibodies and HCV-RNA in a prevalent haemodialysis population

Conclusions: Occult HCV infection is a problem even in low prevalence haemodialysis populations within the United Kingdom. Pooled HCV-RNA PCR surveillance allows efficient, robust and accurate identification of viraemic patients within a dialysis program.

Funding: Supported by Poznań University of Medical Sciences, grant No 502-01-0112418-07474.

SA-PO2664


Background: The prevalence of Hepatitis C virus (HCV) infection is significantly higher in all haemodialysis patients, with a wide disparity between countries. Though previous work supports the use of nucleic acid testing by polymerase chain reaction (PCR) for surveillance of infection in prevalent haemodialysis patients in areas with high levels of carriage, the evidence for its use in units with a lower prevalence is less clear.

Methods: Prospective data was collected over a twelve month period on all patients receiving maintenance haemodialysis across our satellite dialysis centres. In addition to monthly HCV antibody (3rd generation enzyme immunoassay- EIA) testing, four-monthly pooled samples from each unit were submitted for nucleic acid testing (HCV-RNA PCR).

Results: Each pool contained five patients, who were then re-tested individually in the presence of a positive pool result. Individuals known to carry HCV were not excluded. Results: 1352 patients were tested on more than one occasion, in total 51 individuals (3.8%) were found to be either HCV-positive by PCR or EIA testing. Prevalence of HCV antibodies and HCV-RNA in a prevalent haemodialysis population

Conclusions: Occult HCV infection is a problem even in low prevalence haemodialysis populations within the United Kingdom. Pooled HCV-RNA PCR surveillance allows efficient, robust and accurate identification of viraemic patients within a dialysis program.

Funding: Supported by Poznań University of Medical Sciences, grant No 502-01-0112418-07474.
are consistent with many of these patients having acute kidney diseases. Fewer nephrology referrals and higher catheter rates are consistent with these cases having a shorter time horizon in their onset, not like those with chronic CKD patients.

**Funding:** Other U.S. Government Support

### SA-PO2665

**Workload Demand and Caseeload Disparities of Dialysis Social Workers in the United States**  
Joseph R. Merighi, Teri Brown

**Background:** Every dialysis unit in the United States must have a Master’s level social worker as part of their patient care team. This study examines regional variations in perceived workload demands and patient caseeloads of these dialysis social workers. Studies indicate that high social worker caseeloads are associated with decreased ability to provide psychosocial interventions that can help improve patient outcomes (Merighi & Ehlebracht, 2005).

**Methods:** A 130-item online survey was conducted to assess renal social workers’ caseeloads, job-related resources, and workload demands. 1,055 full-time social workers completed the surveys from all 5 National Kidney Foundation (NKF) regions: 1) Northeast, 2) Southeast, 3) Midwest, 4) South, and 5) (West and Southwest). Respondents were recruited between 5/31/10 and 6/21/10 using the Council of Nephrology Social Worker Listserv.

**Results:** One-way ANOVA was used to examine mean workload demands and caseeloads across all five NKF regions. Findings yielded significant main effects for caseeload, F(4, 1035) = 5.46, p < .001, and workload demands, F(4, 939) = 3.06, p < .016. Post hoc tests revealed significantly higher mean workload demands in Region 1 (21.3) compared to Region 3 (20.1) and Region 5 (19.9), and Region 4 had a significantly higher mean caseeload (125) than Region 3 (112). Geographic Information Systems (GIS) technology was used to map workload demands and caseeloads using respondents’ unique zip code boundary. The GIS maps display a distinctive pattern at the state level of high workload demands in Region 1 (esp. NH, MA, and CT) and high caseeloads in Region 4 (esp. AR and MS).

**Conclusions:** This study represents an important national effort to assess dialysis social workers’ workload demands and caseeloads and document regional disparities. High caseeloads may prevent social workers from helping patients ameliorate psychosocial barriers to optimal kidney disease outcomes. These findings also have implications for all dialysis units because high social worker caseeloads may lead to failure in failure to comply with the Medicare’s Conditions for Coverage.

### SA-PO2666

**Case Mix Adjusters Continue To Be Difficult To Detect**  
Tracy Jack Mayne, Jeremy Jonckheere, LeAnne Zumwalt, Mahesh Krishnan

**Background:** Beginning in 2011, the ESRD prospective payment system (PPS) increased the number of case mix adjusters (CMA) from 3 to 11 including 6 for co-morbid conditions. The additional CMAs were based on an analysis by the University of Michigan Kidney Epidemiology and Cost Center (KECC). Our objective was to replicate the prevalence reported by KECC in other currently available data sources.

**Methods:** The Moran Group (Moran) analyzed the 2008 5% Medicare Standard Analytic Files (SAFs) for the 6 CMA comorbidities in US dialysis patients. We searched for the ICD-9 codes for each adjustor as identified in the final rule, including non-nephrologist physician and hospital claims. In acute conditions, we added 4th quarter 2007 ESRD, physician, and hospital claims. In chronic conditions, we added all of 2007 ESRD, physician, and hospital claims. We then searched all available paper and electronic records at each of the two large dialysis organizations (Provider A and B) for documentable diagnoses of these 6 conditions.

**Results:** The prevalence of the 6 co-morbid case mix adjusters detected in the medical records of the two dialysis organizations was significantly lower than the prevalence reported by KECC (Figure). The Moran analysis of the Medicare 5% SAF found significantly less sickle cell disease, somewhat less monoclonal gammopathy, slightly more myelodysplastic syndrome and bacterial pneumonia, and equivalent GI bleed with hemorrhage and pericarditis.

**Conclusions:** The results demonstrate the difficulty for dialysis providers to document CMAs consistent with the level used to estimate the 2011 bundled rate. Were CMS to provide dialysis organizations with the prevalence of these conditions from the SAFs, this would correct the inequity in all areas except sickle cell disease.

**Funding:** Clinical Revenue Support

### SA-PO2667

**Actual Cost of Dialysis Drugs: A Step towards the Final Bundle Payment System in 2014**  
Megha Shah, Michelle Malabanam, Wajid M. Choudhry, Vijay K. Jain

**Background:** CMS have implemented the new ESRD bundled payment system on January 1, 2011. Medicare provides a single bundled payment of $229.63 per dialysis per incident of the following: 1. Composite rate including labor to deliver dialysis and routine supplies 2. Part B ESRD injectable drugs and their oral Part D equivalent 3. ESRD lab. tests. tested by a nephrologist

**Methods:** We conducted a cross-sectional chart review of all patients in 2 hemodialysis units, one suburban (N=133) and one inner city (N=95) and the associated peritoneal dialysis program (N=335) in Rochester, NY. Our main objectives are to determine the actual cost of 1) Part D ESRD prescribed oral-only drugs 2) injectable drugs and their oral equivalent. Data was collected over a 31-day period at these 2 hospital-owned dialysis units staffed by nephrology groups. We determined average calcium and phosphorus levels, quarterly PTH levels and the last hemoglobin of the month to evaluate the effectiveness of current prescribing practices.

**Results:** The average patient age (66.6 years) is similar in all groups. Inner city unit had substantially higher black patients compared to the suburban unit (70.5% vs 23.5%). The calcium, phosphorus, PTH and hemoglobin control were similar in the three groups and were within the acceptable range (8.4-8.5 mg/dl, 4.8-5.1 mg/dl, 380-384 pg/ml, 11-11.3 g/dl, respectively).

<table>
<thead>
<tr>
<th>Cost per Patient per Dialysis</th>
<th>Suburban Unit</th>
<th>Inner City Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral-Only Drugs ($)</td>
<td>47.4</td>
<td>44.2</td>
</tr>
<tr>
<td>Injectables + Vit D ($)</td>
<td>50.9</td>
<td>50.5</td>
</tr>
</tbody>
</table>

*Included in the current bundle

**Conclusions:** Our data suggests that the average cost of ESRD oral drugs ranges between $39-$47 today. Data like ours at a local level will hopefully enable Medicare to make appropriate adjustments to the final bundle payment rule in 2014. This will help avoid any unintentional consequences on quality of care, such as alteration of prescribing practice based on economic reasons.

**Funding:** Private Foundation Support

### SA-PO2668

**Poor Representation in the Medical Evidence Report (CMS-2728) of the Nephrology Care Received by Patients Approaching End-Stage Renal Disease: A Validation Study**  
Jane Paik, Manisha Desai, Glenn M. Chertow, Wolfgang C. Winkelmayer

**Background:** In 2005, the Centers for Medicare and Medicaid Services added a new item to the Medical Evidence Report querying providers on the timing of the patient’s first nephrologist consultation prior to initiation of renal replacement therapy (RRT). This item is also being used for monitoring disease-specific goals in the Healthy People 2020 initiative. The accuracy of the reported information, however, is unknown.

**Methods:** We defined a cohort of 41,455 patients initiating RRT between 7/2005 and 12/2006, who were ≥67 years old; among those, 28,778 had years of uninterrupted Medicare (primary payer) coverage prior to RRT. We report accuracy of medical reporting using claims data as the gold standard. Using linear and Cox regression, we assessed the associations between the magnitude of discrepant reporting and patient characteristics as well as patient survival.

**Results:** Agreement between the two sources of ascertainment regarding timing of visit was present in only 32.9%, where the Kappa statistic was 0.13 when timing was defined as a 4-category variable and 0.30 when it was dichotomized as having had a visit over 6 months ago vs within 6 months. With an overall accuracy of 65%, accuracy was associated with race (p-value<0.001) and underlying cause of disease (p-value<0.001). In particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes, particular, whites had higher levels of accuracy at 67% compared to Asians and Native Americans, whose levels were at 62% and 61% respectively; among the underlying causes.

**Conclusions:** We found substantial disagreement between information from the Medical Evidence Report form and Medicare physician claims on the timing of earliest pre-dialysis single nephrologist care. In addition, inaccurate reporting contained prognostic information about these patients. This work casts doubt on the utility of the Medical Evidence Report for research and public health surveillance.
SA-PO2669

Adapted Exercise Training Program for Dialysis Patients Philippe Chauveau,1 Tomas Labat,2 Stanislas Trolonge,1 Christian Combe,3 Catherine Deforges-Lasserre.1 1Aurad-Aquitaine, Bordeaux, France; 2Hopital Pellegrin, CHU de Bordeaux, Bordeaux, France.

Background: A sedentary lifestyle is frequent in hemodialysis (HD) patient and is associated with an increased relative risk for death. Evidence exists that increased physical activity (PA) is associated with better quality of life, better nutritional and inflammatory status, and rehabilitation.

If an increased of PA should be one of the goals of care management several barriers prevent implementation of PA in dialysis units, related to patients but also to dialysis staff, notably lack of time, of knowledge or competence.

Methods: Since march 2010, all 150 pts in AURAD Aquitaine have been informed about the opportunity to participate in an individualized program of PA (IPA) carried out by an Adapted Physical Activity Coach (APAC). An Initial screening (consultation with the APAC, PA questionnaire (GPQ), 6-minute walk test, balance test, handgrip strength, measure of quality of life (KDQOL36), assessment of body composition using the Body Composition Monitor® (BCM), biological nutritional parameters) and a 8-days observation with podometer and walkbook, an home-based individualized exercise program is determined for each patient and group-activity sessions were proposed. Supervision and support were provided according to patient's request.

Same complete screening is performed every 6 months.

Results: 32 patients (22%) enter the program (45±15 years, BMI 24.5±kg/m2) and only 2 stop at one year. At start, 6-min walk test was 541±110 m (320-885), mean steps per day using 8-days pedometers was 5674 ± 2123steps/d; only 2 of them had a recommended PA according to age. 6-months evaluation is available for the 15 first patients (7 men , aged 41±15 years (20-65 years), and demonstrate a 37% increase of PA.

Conclusions: An individualized home-based PA program could be easily and safely initiated in dialysis unit using a trained qualified PA-health care professional. A combination of individual support and activity group maintain the adherence to the program. Preliminary results demonstrate an increase in physical activity and well-being.

SA-PO2670


Background: Pneumococcal vaccination and seasonal influenza vaccination each lower the risk of hospitalization and death in dialysis patients (pts). Those pts who receive both vaccines show the lowest risk. Using an integrated care intervention model similar to that proposed for accountable care organizations under healthcare reform, we undertook a systematic effort over the past 3 years to improve vaccination rates among in-center hemodialysis (HD) pts.

Methods: At the onset, clinical leadership recommended a goal of 90% of pts to receive pneumococcal vaccination (1x in past 3 years or 2x in lifetime), and 90% to receive seasonal influenza annually. Operational leadership assigned a high priority to the effort, coordinated communications and management, provided implementation resources, applied process engineering approaches to identifying barriers and best practices, and assured adequate vaccine supply. IT systems were developed to report facility performance and identify unvaccinated pts in real time. Facility interdisciplinary care teams used teaching tools and daily exception reports during the flu vaccination season.

Results: Vaccination rates in the population of ~120,000 pts improved progressively during the period of intervention. By March 31, 2011, the goal of 90% seasonal and pneumococcal vaccination rate was achieved (Figure). The percentage of pts who declined influenza vaccination for allergy was very low (0.6%) in 2010.

Conclusions: Integrated care affords the ability to align incentives, communicate consistently, report comprehensively, and standardize care. Our results show that the integrated care model can be dramatically successful in improving vaccination rates within a large population of HD pts, and that care processes within large dialysis providers are well-suited to serve as potential accountable care organizations.

Funding: Clinical Revenue Support

SA-PO2671

Regular Screening for Staphylococcus Aureus Colonisation in a Satellite Haemodialysis Population: Lessons Learnt from a One-Year Single Centre Study David Makanjuola,1 Glynn Richardson,1 Jonathan Dick,1 Angela Wright,1 Maggi Steele,1 Giny Quan,1 John Clark.2 1Renal Unit, St. Helier Hospital, Surrey, United Kingdom; 2Microbiology Department, St. Helier Hospital, Surrey, United Kingdom.

Background: Methicillin resistant Staphylococcus aureus (MRSA) is an important pathogen in the haemodialysis (HD) population and clinically significant infection is strongly associated with skin colonisation. In a bid to reduce MRSA bacteraemias, the UK department of health advocates screening for MRSA in all HD patients; however, the cost implications and utility of this strategy are unknown.

Methods: 76 patients on HD at a satellite unit were screened at 0.1, 2, 3, 6 and 9 months for staphylococcus aureus (SA) carriage. Universal hygiene precautions were observed. Due to limited availability, SA positive patients could not always be isolated during HD sessions. SA eradication was attempted using 4% Chlorhexidine solution on a maximum of two separate occasions for any single patient.

Results: 66 patients completed the study. 4% were MRSA positive at baseline and 9% were positive on at least one occasion during the study. Transitory, rather than persistent carriage was observed. 5 different strains of MRSA were detected. 2 patients were colonised with a similar strain, but they dialysed on different days, making direct spread unlikely. In the MRSA positive patients who responded to decolonisation therapy, upon becoming re-colonised with MRSA, it was found to be with the same strain as they originally had. 34% were methicillin sensitive SA positive on at least one occasion during the study. Of these, 4 were consistently positive through the study in spite of eradication therapy.

Conclusions: The limited spread of MRSA in our cohort suggests that even where there is a dearth of isolation rooms, barrier nursing and universal hygiene precautions are sufficient to prevent spread of MRSA.

The differing variety of MRSA strains suggests that patients are acquiring MRSA from diverse sources in the community or other healthcare settings, rather than from the dialysis unit. MSSA decolonisation was of limited benefit.

SA-PO2672

Comparison of Life Participation Outcomes between Renal Replacement Therapy Modalities: A Systematic Review Tanjala S. Purrell;1 Priscilla Auguste,1 Deidra C. Crews,1 Julio Lampa,1 Temitope Olufade,1 Raquel Greer,1 Patti Ephraim,1 Johanna Sheu,1 Neil R. Powe,2 Hamid Rabb,1 L. Ebony Boulware.1 Johns Hopkins University; 1University of California San Francisco.

Background: The effect of renal replacement therapy (RRT) modality on patients’ limitations in life participation (physical function, travel, recreation, freedom, work) has been poorly explored.

Methods: We performed a systematic review to explore the association of RRT (hemodialysis-HD, peritoneal dialysis-PD, kidney transplant-TX) with patients’ life participation. We searched PubMed (English language, after 1980) and hand-searched bibliographies to identify studies that compared relevant outcomes by RRT. Independent reviewers used standard criteria to evaluate the quality of studies’ research design and methods (internal and external validity), and we calculated the Cohen’s effect size estimate to assess the magnitude and direction of differences. We considered non-statistically significant results to indicate that neither treatment was favored for that comparison.

Results: Of 270 potential studies, 42 studies met our inclusion criteria, ranging from low to moderate quality (Table 1). These studies included 81 relevant comparisons by RRT (28 HD-PD; 28 HD-TX; 20 PD-TX; 5 Dialysis-TX). Studies were conducted in diverse racial/ethnic groups and included 2 cohort, 39 cross-sectional, and 1 pre-post study designs. Patients who received a TX consistently reported more favorable outcomes than patients on HD or PD.

Conclusions: Evidence suggests patients who receive a TX report fewer limitations in life participation outcomes than dialysis patients, and there are no significant differences between HD and PD patients. However, the overall quality of evidence is low. Rigorously performed studies are needed to better inform patients about the effect of RRT on these important patient-reported outcomes.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

734A
Effects of Antioxidant Therapy in CKD: Systematic Review & Meta-analysis

Method: Systematically searched MEDLINE, EMBASE, and Cochrane Library for prospective RCTs (1950-March 2011) assessing antioxidant therapy compared to placebo for efficacy outcomes. Summary RR and 95% CI were calculated using a random effects model. Study quality was judged based on specific variables. Outcomes analyzed were CV death, CV events, coronary events, cerebrovascular events, peripheral vascular disease (PVD), end-stage kidney disease (ESKD), and adverse events.

Results: We identified 4 trials with 1500 participants (327 CV events, 299 coronary events, 298 deaths). Studies were of mixed quality with some studies lacking information regarding randomization process, allocation concealment, and intention-to-treat analyses. Compared to placebo, antioxidant therapy had no overall effect on risk of CV death (RR: 0.95, 95% CI: 0.76-1.27, p=0.72), cerebrovascular events (0.91, 0.63-1.32, p=0.632), PVD (0.55, 0.27-1.21, p=0.099), or ESKD (0.57, 0.31-1.06, p=0.075). Data for adverse outcomes were sparse and reported no difference between the antioxidant and placebo arms. There was significant heterogeneity in the magnitude of the effect across studies for CV events (I²=76.4%, p=0.01) with a no effect in the early CKD population (1.04, 0.83-1.32) but a beneficial effect in dialysis patients (0.57, 0.41-0.80, p=0.001). Similar heterogeneity was identified for coronary events (I²=64.1%, p=0.062).

Conclusions: Overall, there is no evidence that antioxidant therapy reduces risk of CV death, all-cause death, or major CV events but it is possible there is benefit for patients undergoing dialysis. Statistically sized trials in patients with advanced CKD are needed to better understand the effects of antioxidant therapy.

Impact of a Low-Glucose Peritoneal Dialysis Regimen on Inflammatory and Fibrotic Mediators in E Fluent Dialysate

Methods: New PD patients who had entered the PD programme within 1 month were randomized to receive either a low-glucose regimen (1 exchange of Nutrinéal®, 2-3 exchanges of Physioneal® and 1 overnight exchange of Extraneal®) for 12 months and then Dialan® for 6 months (PEN Group, n=43), or 3 exchanges of Dialan® for 18 months (Control Group, n=37). Overnight PD was collected from all patients for the assessment of CA125, and inflammatory and fibrotic markers.

Results: Patients in the PEN Group showed a progressive increase in dialysate CA125 level, which was significantly higher than that in the Controls after 12 months treatment [mean (range): 56.50 (16.00-160.00) μIU/mL vs 20.50 (1.00-84.00) μIU/mL, PEN vs Control, P<0.001], and which reverted after switching back to Dialan®. Similarly, dialysate levels of decorin, MMP-9, adiponectin, ICAM and VCAM were 1.35-, 2.52-, 1.79-, 1.93- and 1.59-folds respectively higher in the PEN Group compared to Controls after 12 months, but the two groups showed similar levels after the PEN Group changed back to Dialan®. In contrast, dialysate MIF level was significantly lower in the PEN Group during the first 12 months (0.46 (0.06-2.23) pg/mL vs 0.77 (0.00-1.95) pg/mL, P=0.05), but was comparable between the two groups at 18 months.

Conclusions: Our data demonstrate that the low-glucose dialysis regimen is associated with reduced intra-peritoneal inflammatory and fibrotic response, and thus potentially result in better preservation of the structural and functional property of the peritoneum during long-term peritoneal dialysis.

Funding: Government Support - Non-U.S.

Relationship between Truncal Fat Mass and Serum High-Sensitivity C-Reactive Protein in Hemodialysis Patients — Metabolic Syndrome and Inflammation

Methods: Thirty ESKD patients on chronic HD (3 x 4 hours weekly) were studied in this pilot study. Plasma and dialysate oxLDL concentrations were measured using a mAb-4E6–based ELISA. To determine the frequencies and phenotypes of Tregs in patients with ESKD, a new generation of dialyzers, with very large pores (Gambro HCO1100) in plasma oxLDL clearing, and to analyze the Tregs suppressive function in ESKD patients of different dialysis modalities. Matthias Grimm 1, Roman Fiedler, 1 Christian Hennig, 2 Christoph Ulrich, 1 Felix Neugebauer, 2 Eric Selbert, 1 Marcus A. Glomb, 2 1 Department of Internal Medicine II, Martin-Luther-University Halle, Halle, Germany; 2 Institute of Chemistry - Food Chemistry, Martin-Luther-University Halle, Halle, Germany.

Results: The relationship between truncal fat mass and serum hsCRP was tested. Total fat mass and truncal fat mass of the high hsCRP group (n=346) were significantly higher than those of the normal hsCRP group (n=106) (p<0.05); while there were no significant differences in non-truncal fat mass or in lean mass between the two groups. In all patients, there were significant positive correlations between serum hsCRP and total fat mass (r = 0.186, p = 0.001), and between serum hsCRP and truncal fat mass (r = 0.202, p < 0.001), although there was none between serum hsCRP and lean mass. In a multiple regression analysis, truncal fat mass was significantly and independently associated with serum hsCRP levels after adjustment for several confounders, whereas non-truncal fat mass was not.

Funding: Government Support - Non-U.S.

Enhanced Suppressive Function of Tregs from ESKD Patients Using a New a High Cutoff HD Technique

Background: Considering the effect of oxDL accumulation on Tregs viability and function in chronic HD patients, a new generation of dialyzers, with very large pores (HCO 1100 Gambro), could be useful to clear oxDL and thus improve Tregs survival and function. The aims of this study were to make in vivo assessments of several hemodialyzers (i.e. Polyflux 21 L - Polyflux 210 H (regular membranes: RM) vs HCO 1100) in plasma oxDL-clearing, and to analyze the Tregs suppressive function in ESKD patients.

Methods: Thirty ESKD patients on chronic HD (3 x 4 hours weekly) were studied in three groups during three months. Plasma and dialysate oxLDL concentrations were measured using a mAb4E6-based ELISA. To determine the frequencies and phenotypes of Tregs, multicolor flow cytometry was performed. Apoptosis was indirectly assessed by Fas staining and flow cytometry and then confirmed by DNA fragmentation determination. Treg-enriched cell populations were isolated on RM or HCO 1100. Cell-surface expression and intracellular expression of FOXP3 were analyzed on a FACSCalibur (Becton Dickinson, Franklin Lakes, NJ)流式细胞仪. Cell-surface expression of HLA-DR, CD40, CD86, and CD80 were also analyzed.

Results: In healthy controls the plasma concentrations of the amino acid adducts N-formyllysin, N-acetyllysin, N-lactoyllysin, and N-glycinelysin increased with age. In HD patients the adducts showed higher plasma concentrations than in both healthy groups (formyllysin in pmol/mL young=125±4.5, elder=172±112, dialysis=309±11, p<0.0001 by Kruskal-Wallis test). Predialysis concentrations of formyllysin and acetyllysin could significantly be reduced by 2 weeks of high cut-off dialysis (formyllysin, polyol391±113, HCO 245±75; p=0.014 by Wilcoxon test).

Conclusions: Free amino acids with amid modifications are an important novel group of bioactive substances that are remnant in the healthy. HD patients have elevated plasma levels remnant of earlier findings of AGE proteins in these patients. High cut-off dialysis is the first intervention that proves to reduce these substances significantly from patient’s plasma. This clears the way to interventionals trials to better understand the relevance of free amino acid adducts in CKD.

Funding: Government Support - Non-U.S.
SA-PO2678

Monocyte Transcriptome Analysis in Hemodialysis Patients: Identification of a Role for a CD16+ and CX3CR1+ Subpopulation

Eva Schepers,
Annermiek Dhondt, Raymond C. Vanholder, Griet L.R.L. Glioorus. Internal Medicine/Renal Division, University Hospital Gent, Gent, Belgium.

Background: The risk for cardiovascular morbidity and mortality is increased in chronic kidney disease patients, whereby micro-inflammation plays an essential role. Numerous culprits have not yet been identified. In this study transcriptome analysis of monocytes was used to identify in an unbiased manner discriminative factors in hemodialysis (HD) patients.

Methods: Forty gender- and age-matched, non-diabetic, non-smoking subjects with a CRP≥2mg/L were recruited, 9 healthy controls, 10 patients with GFR ≥ 60 mL/min/1.73m2 and 11 patients on HD with prevalent cardiovascular event (CVE) and 10 HD patients with prevalent cardiovascular event (HD) and 10 HD patients without prevalent cardiovascular event (HD/CVE). Monocytes were isolated, by a positive selection using MACS and submitted to transcriptome analysis using an in house-array containing ca. 700 genes associated with macrophage activation (Van den Bergh et al. Retrovir. 7.5(2010)).

Results: A significant differential expression was observed for FCGR3A (CD16; fold-change HD/CVE vs CVE; 2.73;p<0.005), CYTH1 (cytohesin 1;2.05;p<0.006), BMP2K (bmp2 inductive kinase;1.67;p=0.01) and CX3CR1 (chemokine receptor;1.53;p=0.049). For CD16 and for CX3CR1, this finding could be confirmed by qRT-PCR (HD and HD/CVE vs control and vs. CVE;p<0.05 and p<0.01 resp.). Both CD16 and CX3CR1 relative expression correlated with CRP (r=0.117;p<0.05 and r=0.338;p<0.001 resp.). Flow cytometric analysis revealed a significant increase in the percentage CD16 positive monocytes both in the HD and HD/CVE group vs control and vs. CVE (p<0.01). No discrimination, based on CD16 expression, could be made between HD patients with and without cardiovascular disease.

Conclusions: The present study indicates the importance of pro-inflammatory CD16+ monocyte subpopulation in HD patients who also have elevated CRP. The co-expressed chemokine receptor, CX3CR1, is involved in monocyte recruitment and survival in atherosclerotic plaques. For future, discriminative factors should be explored in well defined and purified monocyte subpopulations which play an important role in the inflammatory status of HD patients.

Funding: Government Support - Non-U.S.

SA-PO2660

IL-17: A Novel Player in Peritoneal Dialysis Treatment

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Background: The classical view of the immune response regulation has been enlarged by the recent discovery of IL-17-producing T-helper (Th) cells, named Th17 cells. Growing evidence suggests that IL-17, besides participating in immune-mediated diseases, is also involved in chronic inflammatory diseases. In peritoneal dialysis (PD) patients, a chronic inflammatory response and an imbalanced Th1/Th2 response have been described, but there is no data about the Th17 response.

Methods: Human peritoneal biopsies of PD patients were analyzed by immunohistochemistry. To study the regulation of Th17 responses in the peritoneal membrane an experimental PD model in mice, that resembles human situation, was done.

Results: In human peritoneal biopsies of PD patients, IL-17 expression associated to T lymphocyte infiltration was found. In the mice model, exposure of the peritoneum to PD fluid (PDF) for 7 days resulted in an inflammatory response, characterized by infiltrating T lymphocytes, including CD4+IL-17 expressing cells in the submesothelial zone. Elevated gene and protein levels of IL-17 were found in the peritoneum of PDF-treated mice compared to untreated mice. In contrast, tissue levels of INF-γ and IL-4 (Th1 and Th2 hallmark cytokines, respectively) were not changed between groups. Th17 cell differentiation in mice is regulated by a combination of cytokines (IL-6 and TGF-β) and by several transcription factors (RORγt and STAT3). PDF-treated mice presented upregulation of TGF-β and IL-6, and activation of RORγt and STAT3 in peritoneum compared to controls. These data show an activation of the Th17 response in the peritoneal exposure to PDF. After 30 days of daily PDF instillation increased peritoneal thickness, angiogenesis and functional alterations were observed. At this time point, IL-17 levels remained elevated, both in the peritoneum and in the peritoneal lavage, correlated with peritoneal thickness.

Conclusions: These data suggest that local Th17 response could participate in dialysis-fluid induced damage to the peritoneal membrane.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Vitamin C deficiency is frequent in HD patients. Almost 50% of the enrolled patients showed vitamin C levels below the symptom level. Associations with clinical parameters of anemia management, mineral and bone metabolism and inflammation require additional analyses.

SA-PO2682
Incidence and Mortality Risk Factors Associated with Non-Occlusive Mesenteric Ischaemia in Patients Undergoing Haemodialysis

Background: Non-occlusive mesenteric ischaemia (NOMI) is an emergent pathology in haemodialysis (HD) patients. Although it is associated with a poor outcome, it has not been studied enough.

Methods: A retrospective study was conducted between 2003 and 2011 in HD patients. During follow-up time, all cases of NOMI were registered, and demographic, clinical, biochemical and HD parameters were collected. Then, analysis was performed between this group and a control one (n=100). Risk factors associated with the NOMI development and prognosis were studied and survival curves were established between both groups.

Results: There were fifty-seven episodes of NOMI in 44 patients (mean age 72.9±8.9 years; 56% male). Thirty percent were diabetic. The incidence of NOMI was 2.29 episodes/100 patients/year. Cardiac risk factors were present in 72% of the patients. The patients with NOMI had leukocytosis (16945±4672/µL), mild acidosis (bicarbonate 22.6±4.3 mEq/L), even though the episodes were posthaemodialysis, and serum lactate (2.6±1.8 mg/dL), LDH (441±649 UI/L) and CPR (21.4±15.5 mg/dL) increase. The caecum was the most frequently (42%) affected segment, followed by diffuse bowel involvement. Nineteen patients (33%) were surgically treated. Twenty six patients (59%) did not survive the acute episode of NOMI. Caeccum damage was the only protective factor related with mortality in the univariate and multivariate analysis (RR 0.16; p<0.009). The incidence of NOMI was related to age (RR 1.11; p<0.001), diabetes mellitus (RR 2.61; p=0.037) and duration of dialysis (RR 1.01; p=0.006), when compared with the control group. Those patients who survived the acute episode (41%) were compared with the control group (47%), showing a higher mortality at 5-year follow-up (Log Rank 26.48; p<0.001).

Conclusions: NOMI is associated with age, diabetes mellitus and long time undergoing haemodialysis. Caeccum damage is the most frequent location, but is related with better prognosis. Mortality is very high both in the acute episode and long-term.

SA-PO2683
Iodinated Contrast Media Can Induce Long-Lasting Oxidative Stress in Hemodialysis Patients

Background: Due to their comorbidities, dialysis patients have many chances to undergo diagnostic and therapeutic procedures using iodinated contrast media. Iodinated contrast has been regarded as a safe agent in these patients who have little risk of contrast-induced nephropathy. Oxidative stress plays a major role in toxicity of contrast media. We hypothesized that contrast induces more severe systemic oxidative stress in hemodialysis (HD) patients than in non-dialysis population. We assessed time-sequenced blood oxidative stress level after contrast media exposure in anuric HD patients compared to those in the controls.

Methods: We included 21 anuric HD patients (ESRD group) and 23 controls (Control group) scheduled for coronary angiography (CAG) and assessed 4 oxidative stress markers (AOPP, advanced oxidation protein products; catalase; 8-OHdG, 8-hydroxydeoxyguanosine and MDA, malondialdehyde) before and after CAG, and subsequently up to 28 days.

Results: In the Control group, only AOPP increased immediately after CAG and returned to baseline within one day. However, in the ESRD group, AOPP, catalase and 8-OHdG significantly increased from one day after CAG, and remained elevated longer than in the Control group.

Conclusions: Our study showed that iodinated contrast media induces severe and prolonged oxidative stress in HD patients. Further studies are needed to determine the effectiveness of the preventive measures using antioxidants such as N-acetylcysteine.

SA-PO2684
Elevated Serum Osteoprotegerin Levels Are Associated with Inflammation, Malnutrition, and New-Onset Cardiovascular Events in Peritoneal Dialysis Patients

Background: Osteoprotegerin(OGP) has been known to be associated with cardiovascular events(CVE) and malnutrition in non-dialysis chronic kidney disease patients. Malnutrition-inflammation-atherosclerosis(MIA) syndrome is commonly found in dialysis patients, but the association between OPG levels and MIA syndrome has been largely unexplored in these patients. This cross-sectional study was undertaken to investigate the association between OPG levels and malnutrition, inflammation, and CVE in prevalent peritoneal dialysis(PD) patients.

Methods: A total of 176 prevalent PD patients were included. At baseline, OPG, hs-CRP, and albumin concentrations were measured. In addition, percent lean body mass(%LBM) was determined by creatinine kinetics. Subjective global assessment(SGA) was also performed. Based on the median levels of OPG, patients were divided into 2 groups, and CVE and patient survival were compared by Kaplan-Meier analysis. Moreover, Cox regression analysis was conducted to determine risk factors for CVE.

Results: Age, hs-CRP, and Charlson comorbidity index were significantly higher, while serum albumin levels, %LBM, and SGA score were significantly lower in patients with high OPG levels(H group) compared to patients with low OPG levels(L group). The rates of newly developed CVE were significantly higher in the H group compared to the L group(40.9 vs. 17%, P<0.01). Overall mortality rates were also higher in H group compared to L group, without statistical significance. Univariate Cox regression analysis revealed OPG as a risk factor for new-onset CVE(per an increase by 1 in log OPG; HR, 2.34; 95% CI, 1.35-4.84; P<0.002), which remained significant after adjustment for age, sex, diabetes, and PD duration(HR, 2.00; 95% CI, 1.13-3.39; P<0.002).

Conclusions: Serum OPG levels were significantly correlated with markers of systemic inflammation and malnutrition and was revealed as a significant predictor of new-onset CVE in PD patients, suggesting OPG might be a prognostic indicator of MIA syndrome in PD patients.
Circulating S100A12 (EN-RAGE) Levels Predict Cardiac Dysfunction by Tissue Doppler Echocardiography in Perioperative Dialysis Patients  

Tae Yamamoto,1 Shirley Yumi Hayashi,2 Abdal Rashid Tony Qureshi,1 Marcelo M. Nascimento,2 Ayumu Nakashima,1 Lars-åke Brodin,1 Björn Anderstam,1 Britta Lind,1 Miguel C. Riella,1 Astrid Seeberger,1 Bengt Lindholm,1 Renal Medicine and Biometry, Novo Nordisk, Karolinska Institute, Stockholm, Sweden; 2Med Eng, School of Technol and Health, Royal Institute of Technology, Stockholm, Sweden; 1Pro-Renal Foundation, Curitiba, Brazil.

Background: Cardiovascular disease is common in patients (pts) on peritoneal dialysis (PD), and Tissue Doppler imaging (TDI) is a useful tool for assessing cardiac function. In PD pts, dialysis solutions may lead to formation of advanced glycation end-products (AGEs), which may result in cardiovascular toxicity. Therefore, we examined the possible link between circulating AGEs and cardiac function in PD pts.

Methods: Plasma concentrations of soluble form of receptor for AGEs (sRAGE), its ligand S100A12, and other biomarkers were measured in 51 pts undergoing PD. The myocardial systolic (PSV) and diastolic (E’, early diastolic velocities) velocities were assessed by TDI. M-mode echocardiography was also performed. Pts were divided into two groups by E’ value.

Results: Among 51 pts, 95% (n=48) had diastolic dysfunction (E’ <8.0 cm/s). The medium plasma levels of S100A12 and sRAGE were 64 ng/ml and 2594 pg/ml; S100A12 levels correlated with PSV and E’, both of which were negatively associated with troponin-I and left ventricular mass index. High E’ group had lower S100A12 and higher sRAGE levels compared to low E’ group, while other inflammatory and oxidative stress markers such as CRP, IL-6, TNF and NOHdG did not differ. Stepwise multiple regression analysis identified S100A12 (β=-0.38, p<0.002) and age (β=-0.52, p<0.001) as independent determinants of E’ in a model (r²=0.40).

Conclusions: Circulating S100A12 is an independent predictor for cardiac dysfunction in PD pts, suggesting an important role of AGEs.

Funding: Pharmaceutical Company Support

SA-PO2686

Monocyte Chemoattractant Protein-1 Production Is Diminished by Mycophenolic Acid  

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Background: For the initiation and maintenance of atherogenesis or in transplant vasculopathy, the production of monocyte chemoattractant protein-1 (MCP-1) is crucial. There is evidence that the immunosuppressive regime influences vascular alterations. In the last years there is increasing evidence that mycophenolic acid (MPA), routinely used after kidney transplantation, is more than just an immunosuppressant substance, but might also play a relevant role in anti-inflammatory response.

SA-PO2687

Combined High Serum Ferritin and Low Iron Saturation Predicts Infection in Hemodialysis Patients  

André Fragoso, Ana Pinho, Anaabela Malho, Ana Paula Silva, Elsa Morgado, Pedro Neves. Nephrology, Hospital de Faro EPE, Faro, Portugal.

Background: Serum ferritin is a classical marker of iron status and chronic inflammation. More recently, the combination of high serum ferritin and low iron saturation has been associated with inflammation. The aim of our paper was to study the relationship between these inflammatory markers and infection events.

Methods: We included all patients of a dialysis unit on treatment for more than 3 months by the 31st of January 2009. Patients were prospectively followed for 12 months and divided in two groups (G1: patients with infection events; G2: without infection events).

Results: We followed 169 patients (63.3% males) with a mean age of 61.87±16.32 years in hemodialysis for 73.26±112.14 months. G1 patients showed lower serum iron (63.56±22.21 vs 75.70±29.01 mcg/dl; p=0.05), serum ferritin (527.42±246.65 vs 673.56±349.79 mg/ml; p<0.05) and iron saturation ratio (25.53±8.31 vs 34.06±23.13%; p<0.05). No differences were found between the two groups regarding CRP, albumin, BMI, KTV and IV iron dose. In a logistic regression model, after adjustment for Diabetes, BMI, serum albumin and CRP, patients with combined high serum ferritin (>600mg/ml) and low iron saturation ratio (<25%) presented a 5-fold risk for an infection event (p=0.023).

Conclusions: In our population, known markers of chronic inflammation independently predicted infection events. To our knowledge this relationship has only been demonstrated in peritoneal dialysis patients. Further studies are needed to better understand the mechanisms that link inflammation to classical infection in the hemodialysis setting.

SA-PO2688

Prealbumin Is Associated with Visceral Fat Mass in Hemodialysis Patients  

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Background: Both Albumin (ALB) and Prealbumin (PreAlb) are associated with malnutrition and inflammation. Each have a residual effect on mortality outcomes when included in regression models that include the other. PreAlb, but not ALB is inversely associated with infections and directly associated with vascular access hospitalizations. PreAlb has been reported to be increased in the obese mouse model as a consequence of stabilization of PreAlb by Retinol Binding Protein 4 (RTB4), secreted by adipocytes. We questioned if PreAlb was associated with adiposity in dialysis patients, independent of the effects of inflammation or nutrition.

Methods: We evaluated body composition in 48 prevalent hemodialysis (HD) patients by MRI, measuring total skeletal muscle mass (SM), visceral and subcutaneous adipose tissue (VAT and SAT), and serum ALB, PreAlb, RTB4, interleukin -6 (IL-6) concentrations. We used normalized protein catabolic rate (nPCR) to report nutrition and separately analyzed the determinants of ALB and then of PreAlb by multiple stepwise regression.

Results: 32 subjects were women, 16 were diabetic, median age 54.5, range 40-69, 10th-90th percentile). PreAlb was positively associated with V AT (P = 0.002), and negatively associated with IL-6 (P<0.003) and white race (P<0.001). r² for the model was 0.66.

Conclusions: Prealbumin is independently associated with inflammation, visceral adiposity and nPCR.
Increase in Lymphocyte β- catenin, IL-10 and Cortisol Serum Levels May Play a Role in the Protection of the Central Nervous System Against the Inflammation in Hemodialysis Patients with Cognitive Impairment Sabrina Degasperi,1 Geison Stein Meireles Ramos,2 João Paulo L.B. Martins,2 Elzo R. Junior,2 Carmen B. Tzanno-Martins,1 Carolina D. Munhoz,3 Cristoforo Scavone,3 Elisa M. Kawano Thomé,1,3 *PhD in Immunology, FGR, University of São Paulo, Brazil; 2Cine; 3Laboratory of Neurosciences, NIA, NIH; *Nephrology, URGS, Porto Alegre, Rio Grande do Sul, Brazil.

Background: A high prevalence of cognitive impairment (CI) in hemodialysis patients (HD-P) is well established. Inflammatory process has been shown to be associated with chronic kidney disease and may play a role in the development of CI. WNT signaling has been described in pathological conditions, such as neuroinflammation, and recent data have suggested that WNT signaling could participate in anti-inflammatory responses. The purpose of this study is to evaluate the role of cytokines and the WNT signaling in HD-P with or without CI.

Methods: Thirty six HD-P were submitted to cognition test through Modified Mini Exam of Mental State and Kidney Disease Quality of Life. Blood was collected before and after hemodialysis (HD) procedure. Serum was used to analyze IL-6, IL-10, TNF and cortisol levels by ELISA test. Lymphocytes were isolated to verify the WNT proteins by Western Blotting analysis.

Results: Forty percent of patients showed CI. We observed that IL-6 (control=7.1±0.4 pg/ml) and TNF (control=14.4±4.8 pg/ml) levels were increased in HD-P (IL-6=220.7±82.1 pg/ml; TNF=53.7±9.4 pg/ml) without CI before HD, whereas IL-10 (control=7.4±2.4 pg/ml) and cortisol (control=0.16±0.03 mg/dl) levels were increased in HD-P-CI before (IL-10=12.8±3.5 pg/ml; cortisol=0.28±0.04 mg/dl) and after (IL-10=32.4±9.1 pg/ml; cortisol=0.28±0.04 mg/dl) HD (p<0.05). We also observed an increase of β-catenin in lymphocytes of HD-P-CI compared with control and HD-P groups.

Conclusions: HD-P without CI show an increase in proinflammatory cytokines levels and the increase of IL-10 and cortisol in HD-P-CI could be an organic response against an inflammatory stimulus. Our data suggest that WNT signaling in HD-P-CI demonstrates the role of the canonical WNT signaling that may play a role in the protection of the central nervous system against the neuroinflammation.

Funding: Government Support - Non-U.S.

Methods: Thirty-eight elderly SRD patients (22 on hemodialysis (HD) and 16 in predialysis) and 20 elderly volunteers were enrolled. We compared the laboratory findings and immune profiles associated with T cells in these patients.

Results: Among the effector T cell subset, the percentages of Th2 cells and Th17 cells were significantly higher in the SRD group than in the healthy controls (p<0.05). The frequencies of Th1 cells did not differ significantly between these groups. The percentages of Th1, Th2 and Th17 cells did not differ significantly (p>0.05) between the two subgroups within the SRD group. The CCR4 CCR6+CD4 T cell percentage was also significantly higher in the SRD group. The naïve T cell (%Tn) percentage was significantly lower in the SRD group, and the difference between patients and controls was greater in the predialysis patients than in the HD patients (p<0.05, for each comparison). By contrast, the percentages of central memory T cells (%TCM) and effector memory T (Tcm) cells were significantly higher in the SRD group. Interleukin-17 production by Tcm cells was significantly higher in the SRD group. The severity of uremia was related negatively to the T CM cell percentage but positively to the Th1 cell and Th17 cell percentages. The percentages of Tn, Th1, Tcm and Th17 T cell subsets were significantly higher in the ESRD group (p<0.05).

Conclusions: The result of this study showed significantly altered T cell-associated immunity and that it could not be corrected with hemodialysis.

Methods: This cross-sectional study included 55 non-diabetic, non-obese PD patients. Homeostatic model assessment-insulin resistance(HOMA-IR) was calculated, and adiponectin, high-sensitivity C-reactive protein(hs-CRP), and IL-6 levels were measured. The patients were divided into 2 groups according to the median value of HOMA-IR, and clinical findings and laboratory data were compared between groups. Pearson’s correlation analysis was performed to determine the relationship of clinical and laboratory data with HD-P-CI and ESRD. Multiple linear regression analysis was used to identify independent factors associated with HOMA-IR.

Results: The mean age was 47.3 years, 50% were male, and the mean PD duration was 83.1 months. Male gender was significantly more prevalent, and hs-CRP and IL-6 concentrations were significantly higher in patients with high HOMA-IR(p<0.05). In addition, correlation analysis revealed that HOMA-IR was positively related to log hs-CRP(r=0.499, p<0.001), log IL-6(r=0.367, p=0.006), and age(r=0.370, p=0.005), whereas it was negatively correlated with Kc(VaVea expedite-=0.296, p=0.031). However, there was no correlation between HOMA-IR and adiponectin, such as leptin and adiponectin. In multiple linear regression analysis, log hs-CRP was the only significant independent factor associated with HOMA-IR(r=0.1, 402.061; p<0.05).

Conclusions: This study shows that chronic inflammation rather than adipokines plays a major role in the pathogenesis of IR in non-diabetic, non-obese SRD patients on peritoneal dialysis(PD).

Methods: This study selected 55 diabetic, non-obese PD patients. Homeostatic model assessment-insulin resistance(HOMA-IR) was calculated, and adiponectin, high-sensitivity C-reactive protein(hs-CRP), and IL-6 levels were measured. The patients were divided into 2 groups according to the median value of HOMA-IR, and clinical findings and laboratory data were compared between groups. Pearson’s correlation analysis was performed to determine the relationship of clinical and laboratory data with HD-P-CI and ESRD. Multiple linear regression analysis was used to identify independent factors associated with HOMA-IR.

Results: The mean age was 47.3 years, 50% were male, and the mean PD duration was 83.1 months. Male gender was significantly more prevalent, and hs-CRP and IL-6 concentrations were significantly higher in patients with high HOMA-IR(p<0.05). In addition, correlation analysis revealed that HOMA-IR was positively related to log hs-CRP(r=0.499, p<0.001), log IL-6(r=0.367, p=0.006), and age(r=0.370, p=0.005), whereas it was negatively correlated with Kc(VaVea expedite-=0.296, p=0.031). However, there was no correlation between HOMA-IR and adipokines, such as leptin and adiponectin. In multiple linear regression analysis, log hs-CRP was the only significant independent factor associated with HOMA-IR(r=0.402.061; p<0.05).

Conclusions: This study shows that chronic inflammation rather than adipokines plays a major role in the pathogenesis of IR in non-diabetic, non-obese SRD patients on peritoneal dialysis(PD).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
Activities of RBC-GR (19.4±7.4 vs 10.2±3.3 U/g of Hb, p <0.0001), and XO (6.2±1.7 vs 5.0±1.3 µM/min/g Hb, p<0.01) were higher in HD but not PGR activity. MDA (256.72± vs 140±30 mmol/L, p=0.001), and IMA (104:13± vs 90:93UL, p=0.001) were higher in HD.

Conclusions: These results evidenced an imbalance in favour of oxidative stress in HD patients, as evidenced by increased xanthine oxidase activity linked to the formation of numerous free radicals and its inhibition by allopurinol could be a therapeutic option to reduce oxidative stress in HD patients. A 2-years prospective follow-up is in progress to link those biological abnormalities to clinical events.

Funding: Government Support - Non-U.S.

SA-PO2694
Plasma Gelsolin Correlates with All Cause Hospitalization in Hemodialysis Patients
Laura Rosales, Yanna Dou,2, Len A. Usvyat,1 Georges Ouellet,1 Stephan Thijssen,1 Viktoriya Kuntsevich,2 Mary Carter,1 Nathan W. Levin,1 Peter Kotanko1,2
1 Renal Research Institute, NY; 2 Beth Israel Medical Center, NY; 3 Maisonneuve-Rosemont Hospital, Montreal, Canada.

Background: Plasma Gelsolin (pGNS) is a circulating protein that has been implicated in acute and chronic inflammation and tissue injury. Low pGNS may reflect the occurrence of clinical adverse events since its protective action at removing circulating actin declines. This prospective study explored the relationship between pGNS and hospitalizations (hosp) from all causes in hemodialysis (HD) patients (pat) over a 12 month period.

Methods: pGNS levels were determined with the 2C4 pGNS ELISA kit (Critical Biologics, Cambridge, MA) in maintenance HD pat from 2 HD centers; demographics (age, HD vintage, race, gender, BMI), co-morbidities (DM, cardiovascular disease (CVD), HIV, and hepatitis C were recorded. Hosp and cause of admission were documented for each. Hazard ratios (HR) and 95% CI were calculated for various pGNS levels.

Results: Results: 104 stable HD pat followed for 1 year (mean±SD; age 61±15 years; HD vintage range 0.3–17.5 years; 52% female; 49% Blacks, 53% DM). pGNS was 195 mg/L ± 52.6 mg/L. pGNS quartiles were: Q1: <153.7 mg/L; Q2: 154.8–190.3 mg/L, Q3: 190.4 to 225.3 mg/L; Q4: >225.4 mg/L.

In multivariate Cox regression, Q1 of pGNS was associated with a high risk for all cause first hosp (HR 2.2, 95%CI 1.14 to 4.32, p<0.05).

Hazard ratio of pGNS for first hospitalization
Quartiles HR 95% CI P value
Q1 2.21 1.44 to 3.32 <0.05
Q2 1.19 0.59 to 2.39 0.62
Q3 1.00 0.49 to 2.05 0.99
Q4 1.99

In Anderson Gill analysis, Q1 of pGNS was associated with a high risk for all cause first hosp (HR 2.2, 95%CI 1.14 to 4.32, p<0.05).

Conclusions: Low pGNS levels in stable chronic HD pat were associated with time to first hosp from all causes; no relationship was found with recurrent hosp, cause of hosp, and mortality. In chronic HD pat pGNS may have a predicting value in the short term.

SA-PO2695
Influence of HCO1100™ – Dialysis Membrane on Monocyte Subpopulations
Roman Fiedler,1,2 Christof Ulrich,3 Felix Neugebauer,4 Eric Janetzki,4 Christof D. Fleischer,1 Ulrich Feichtner,1,5 Andreas F. Ritzek,1 H. N. Medic, Sapporo, Japan; 2 Department of Internal Medicine II, Martin-Luther- University Halle, Halle, Germany.

Background: Chronically elevated CRP levels are a negative predictor for survival in hemodialysis (HD) patients because they indicate inflammation, atherosclerosis, and malnutrition. Inflammation-inducing cytokines are only insufficiently dialyzed due to their middle molecular weight (15-45 kD). As a result, cellular activation with enhanced levels of proinflammatory monocyte subpopulations is increased.

Methods: In a double-blind randomized crossover study we tested the impact of the HCO1100™ short-time dialysis treatment seems to be without effect on a reduction in monocyte subpopulations. Since trends indicate that the treatment intensity in an increase of CD143-expression (MFI) from 11.2 ± 3.8 to 12.1 ± 5.5 (membrane A: 11.2 ± 3.8 to 12.1 ± 5.5 % (membrane A: 16.2 ± 7.7 to 17.9 ± 9.0 %, p=0.31) and in an increase of CD163-expression (MFI) from 11.2 ± 3.8 to 12.1 ± 5.5 % (membrane A: 12.5 ± 5.3 to 12.0 ± 5.4 %, p=0.23). All other examined parameters were not different between both groups.

Conclusions: HCO 1100™ short-time dialysis treatment seems to be without effect on a reduction in monocyte subpopulations. Except for the expression of CD143 and CD163 monocytes are not a target of the treatment intensity. In an increase of CD143-expression (MFI) from 11.2 ± 3.8 to 12.1 ± 5.5 % (membrane A: 16.2 ± 7.7 to 17.9 ± 9.0 %, p=0.31) and in an increase of CD163-expression (MFI) from 11.2 ± 3.8 to 12.1 ± 5.5 % (membrane A: 12.5 ± 5.3 to 12.0 ± 5.4 %, p=0.23). All other examined parameters were not different between both groups.

Funding: Government Support - Non-U.S.

SA-PO2696
Vitamin D Deficiency Is a Predictor of Inflammatory State in Hemodialysis Patients

Background: High inflammatory burden is associated with morbidity and mortality in dialysis patients. Recent studies have indicated that vitamin D deficiency in dialysis patients may be associated with increased inflammatory markers which may be potentially reversed by vitamin D supplementation. In this prospective study we analysed the impact of vitamin D deficiency on inflammatory markers in a large multi ethnic cohort of haemodialysis patient in East London.

Methods: All patients admitted to the hospital or acute infections were excluded from the analysis. Vitamin D deficiency was defined as total 25 hydroxy vitamin D level less than 30 nmol/L, level between 30 and 79 defined insufficiency and level ≥80 was regarded as normal.

Results: 704 patients (32% Caucasian, 30% black,28% south Asia) were analysed. Results are shown in the table.

Funding: Government Support - Non-U.S.

SA-PO2697
Oral L-Carnitine Administration Could Lower Plasma Levels of Total Homocysteine in Patients on Hemodialysis
Masatasa Tsunoda,1 Ryota Ikez,2 Naomi Sasaki,1 Megumi Sato,2 Nobuo Hashimoto.1 1 H. N. Medic Sapporo-Higashi, Sapporo, Japan; 2 H. N. Medic Kitahiroshima, Kitahiroshima, Japan; 3 H. N. Medic, Sapporo, Japan; 4 Jiusmaikai Megumi Clinic, Sapporo, Japan.

Background: Hyperhomocysteinemia has been consistently demonstrated in patients with renal failure. Recent studies suggested that increased levels of total homocysteine (Hcy) may result in both atherosclerosis and osteoporosis in HD- and non-HD patients through increased oxidative stress. On the other hand, there are some reports suggesting that L-carnitine may have antioxidant and anti-inflammatory properties. In this retrospective cohort study, we investigated effect of L-carnitine administration on clinical parameters including Hcy in HD patients.

Methods: We enrolled 10 males and 7 females treated with HD for at least 1 year (age 65 ± 11 years, HD duration 166 ± 137 months) to this study. L-carnitine was orally administered at 300 mg before each dialysis session. Plasma levels of Hcy, white blood cell (WBC) count, hemoglobin (Hb), albumin (Alb), high sensitivity C-reactive protein (hsCRP), and lipid profile (total cholesterol [TC], triglyceride [TG], and high-density lipoprotein cholesterol [HDL-C]) were measured every 3 months for 6 months.

Results: The mean value of Hcy at baseline was 61 ± 40.8 nmol/mL. After L-carnitine administration, plasma levels of Hcy significantly decreased. The mean reduction of Hcy from month 6 was 21.3 ± 20.0%, and the maximum reduction was 70.9%. Other parameters did not change during this follow-up period (Table 1).

Table 1. Effects of L-carnitine administration in HD patients.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>Month 3</th>
<th>Month 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hcy (nmol/L)</td>
<td>61 ± 40.8</td>
<td>40.7 ± 34.9</td>
<td>47.7 ± 24.7</td>
</tr>
<tr>
<td>WBC count (µL)</td>
<td>55.8 ± 1696</td>
<td>56.1 ± 4220</td>
<td>56.6 ± 1859</td>
</tr>
<tr>
<td>Ferritin (µg/dl)</td>
<td>11.4 ± 19.2</td>
<td>11.3 ± 19.5</td>
<td>11.3 ± 38.3</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>3.86 ± 0.38</td>
<td>3.76 ± 0.37</td>
<td>3.76 ± 0.31</td>
</tr>
<tr>
<td>CRP (mg/dl)</td>
<td>0.09 ± 0.10</td>
<td>0.17 ± 0.24</td>
<td>0.17 ± 0.34</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>118 ± 31</td>
<td>114 ± 30</td>
<td>118 ± 34</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>46 ± 12</td>
<td>47 ± 13</td>
<td>46 ± 13</td>
</tr>
</tbody>
</table>

*p<0.05 compared with baseline.

Conclusions: We found a significant reduction of plasma Hcy levels after L-carnitine administration in HD patients. L-carnitine may prevent atherosclerosis and bone fracture by reducing plasma Hcy.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only. Underline represents presenting author.
Electronegative LDL and Its Autoantibodies Correlated with Inflammatory Markers in Hemodialysed Patients 
Julie Lobo,1 Milena Barca Stockler-Pinto,2 Najla Elias Farage,2 Tanize Do Espirito Santo Faulin,2 Dulcênia Saes Parra Abdallah,3 João Paulo Torres,3 Denis Fouque,4 Denise Maiaf.1 Federal Univ. of Rio de Janeiro, Brazil; 2RenalCor Clinic, Brazil; 3Federal Univ. Fluminense, Brazil; 4Universidade Claude Bernard, Lyon, France; 5Universidade de São Paulo, Brazil.

Background: A modified circulating electronegative LDL subtraction [LDL(-)], which has chemotactic, cytotoxic and immunogenic properties, is increased in hemodialysis (HD) patients and has been implicated in inflammation and atherosclerosis. However, antibody against LDL(-) may play a protect role, although the mechanisms involved have not yet been elucidated. Our aim was to investigate the association between LDL(-) and anti-LDL(-) autoantibodies with inflammatory markers in HD patients.

Methods: Forth seven HD patients (29M/18F; 54.3 ± 12.6 yr, body mass index (BMI) 24.4 ± 4.1 Kg/m², average dialysis time 57.5 ± 50.1 months) from a private Clinic in Rio de Janeiro, Brazil, were studied and compared to 20 healthy subjects (9M/11F, 51.6 ± 15.6 yr, BMI 24.9 ± 5.9 Kg/m²). LDL(-) and anti-LDL(-) IgG autoantibodies were determined by ELISA. TNF-α, IL-6, VCAM-1 and ICAM-1 were measured by a multiplex assay kit manufactured by R&D Systems®. The SPSS (version 11.0) was used as statistical program.

Results: HD patients present higher levels of inflammation and LDL(-) and lower levels of anti-LDL(-) IgG autoantibodies when compared with healthy subjects (Table 1).

Table 1: Biochemical characteristics of the HD patients and healthy individuals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HD Patients</th>
<th>Healthy individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL(-) (mg/dL)</td>
<td>0.32 ± 0.10a</td>
<td>0.09 ± 0.10</td>
</tr>
<tr>
<td>Anti-LDL(-) IgG</td>
<td>0.02 ± 0.01a</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>TNF-α (ng/mL)</td>
<td>5.5 ± 2.1a</td>
<td>2.4 ± 1.1</td>
</tr>
<tr>
<td>IL-6 (ng/mL)</td>
<td>4.1 ± 1.6</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>VCAM-1 (ng/mL)</td>
<td>48.7 ± 8.5</td>
<td>29.8 ± 5.5</td>
</tr>
</tbody>
</table>
| ICAM-1 (ng/mL)       | 20.5 ± 10.9  | 7.2 ± 2.1           | * p<0.04.

Conclusions: Our data seem to suggest that the association between LDL(-) and inflammation and the lower levels of anti-LDL(-) IgG autoantibodies are important risk factors related to atherosclerosis in CKD.

Funding: Government Support - Non-U.S.

SA-PO2699

Novel Anti-Inflammatory Natural Products: Potential Agents for Reducing Inflammatory Signaling during Hemodialysis (HD) 

Background: The production of hemodialysis promotes oxidative stress and secretion of pro-inflammatory agents, resulting in chronic low-grade inflammation in patients undergoing hemodialysis. Chronic inflammation can lead to downstream risks of CVD which are linked to increased patient mortality. Material contamination of dialysis media and/or equipment, physical progression of blood leakucyes past artificial surfaces can initiate proinflammation reactions. Several factors may contribute to the inflammatory response during HD, such as activation of the pro-inflammatory transcription factor NF-κB in leukocytes. Recently, we used lipopolysaccharide (LPS) stimulation of blood leukocytes to screen a natural product library (TimTec480) in order to identify novel anti-inflammatory agents ideally for use in dialysis media with chronic kidney disease and kidney transplanted patients. Vascular alterations like atherosclerosis contribute to the increased cardiovascular risk. The mineralization puts inflammation and oxidative stress as central players in the genesis of cardiovascular disease.

Methods: We included all patients of a dialysis unit on treatment for more than 3 months by the 31st of January 2009. Patients were prospectively followed for 12 months. Vascular calcification was assessed and the population divided in two according to the Akgaño score (G1: score < 2; G2 ≥ score ≥ 2).

Results: We followed 169 patients (63.3% males) with a mean age of 61.87±16.32 years in hemodialysis for 73.26 ±11.2,14 months. G2 patients were older (65.60 ± 13.86 vs 56.89;18.23 years, p = 0.001) and showed higher levels of ferritin (273.50;340.90 vs 569.31;313.94 ng/mL, p = 0.003) despite lower IV iron doses (39.80;36.78 vs 35.64;40.69 mg/week, p = 0.023). The number of received RBC units was also higher (5.37;10.23 vs 2.63 ± 3.43 units, p = 0.033) in the G2. Additionally, these patients showed lower levels of serum Pt (4.14;10.6 vs 4.61;14.4 mg/dl, p = 0.19) and albumin (4.16;0.29 vs 4.33;0.33 g/dl, p = 0.001). Furthermore, the number of hospital admissions (0.93;1.20 vs 0.50;0.82 adm/patient, p = 0.011) and mean in-hospital stay (5.19;8.9 vs 1.49;3.30 days, p = 0.001) were higher in the G2 group. In a logistic regression model each ferritin quartile represented a 1.4-fold risk for a vascular calcification score ≥ 2 (p = 0.014; 95% CI: 1.079-1.957), which in turn represented a 1.3-fold risk for cardiovascular events (p=0.012, 95% CI: 1.082-1.640).

Conclusions: Vascular calcification is a known risk factor for cardiovascular events in the renal population. The encountered relationship between ferritin and vascular calcification puts inflammation and oxidative stress as central players in the genesis of cardiovascular disease.

Government Support - Non-U.S.

SA-PO2701

Low Free Triiodothyronine and Hospitalization in Chronic Hemodialysis Patients 
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Background: Low levels of free triiodothyronine (fT3) are associated with inflammation, cardiovascular disease and mortality in hemodialysis (HD) patients. This study investigates the relationship between FT3 and multiple hospitalizations (hosp), first hosp and the cause of first hosp (cardiovascular events, infection) in HD patients.

Methods: In this prospective study chronic HD patients were recruited from two HD centers. FT3 was measured pre-HD at baseline by direct chemiluminescense (ADVIA Centaur, Deer field, IL). Mortality, time to first hosp, the cause of first hosp and multiple hosp were analyzed by Cox proportional and Andersen-Gill hazard models adjusted for age, gender, height, BMI, diabetes, dialysis vintage, HIV and hepatitis C status.

Results: 104 chronic HD patients were studied (54 females; 51 blacks; 55 diabetes; mean age: 61.15±16.15 years). Mean level of FT3 was 2.41±0.4 pM (normal range 2.27 to 4.22 pM). During one-year follow up, 95 patients died and 191 hosp events occurred. In multivariate hazards model analysis, only Q1 of FT3 was associated with an increased risk of mortality to first hosp and multiple hosp. FT3 > 2.5 pM was associated with a 1.3-fold increased risk of hospitalization.

Conclusions: Low FT3 level is an independent predictor of all-cause hospitalization and mortality in chronic HD patients.

SA-PO2702

The Immunosuppressivum 6-mercaptopurine Increases Calcification of Vascular Smooth Muscle Cells 
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Background: The cardiovascular mortality is dramatically increased in patients with chronic kidney disease and kidney transplanted patients. Vascular alterations like arteriosclerosis contribute to the increased cardiovascular risk. The mineralization process in the vascular wall is characterized by a transformation of vascular smooth muscle cells (VSMC) in the media to osteoblast-like cells. Therefore, the immunosuppressive therapy affect the development of arteriosclerosis. In this study we investigated the influence of 6-mercaptopurine on the mineralization process of rat vascular smooth muscle cells (VSMC).

Methods: In vitro calcification in rat VSMCs were induced with calcification medium (CM). Calcium deposition was monitored by Alizarin staining and quantified by O-cresolphthalein complexone method. ALP gene expression was measured by real-time PCR.

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741A
Results: Cultivation of VSMCs in CM in vitro induced mineralization of VSMCs visualized by von Kossa staining quantified by measuring the extracellular calcium content. This process could be time-dependently and significantly increased in the presence of 6-MP (100 μM/L). To verify this effect, we used aortic rings and stimulated it for 14 days in CM+6-MP ex vivo. 6-MP could significantly increase the calcium content of the neointima compared to CM alone. For the precipitation of calcium phosphate, the activation of alkaline phosphate (ALP) is necessary. CM led to a significant increase in ALP enzyme activity, which is enhanced by pretreatment with 6-MP. Besides ALP activity, ALP expression was investigated. The 6-MP-induced ALP expression is significantly and dose-dependently increased in VSMCs after 48 h.

Conclusions: 6-MP treatment increases the mineralization of VSMCs in vitro and ex vivo. The data suggest that 6-MP treatment may contribute to the high cardiovascular risk by enhancing vascular mineralization and arterial stiffening.

SA-PO2703
Eosinophilia after an Initiation of Dialysis Is Associated with Cardiovascular Morbidity and Mortality
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Background: Dialysis-associated eosinophilia(DAE) has been reported frequently with incidence of 19-57%, however the clinical significance of DAE is not determined, especially in terms of the association of DAE with morbidity or mortality. In this study, we investigated the incidence of DAE, factors determining the development of DAE and an association of DAE with cardiovascular disease(CVD).

Methods: One hundred sixty seven patients on dialysis(HD or PD) more than 3M at the Medical Center with available data on eosinophil count before and after 3M or 12M from an initiation of dialysis were enrolled(MF=72.85, age 60.3 years, dialysis duration 50.8M). Eosinophilia is defined as a presence of eosinophil>5% of peripheral leukocytes or eosinophil count>350/mm³. Definition of DAE was new development of eosinophilia after an initiation of dialysis at 3M(DAE3), 12M(DAE12), and both initiation 3M and 12M(DAE3+12).

Results: DAE was observed in 50 patients(29.9%) with a comparable incidence in HD(36.2%) and PD(28.8%). There was no association of eosinophil count with age, gender, underlying renal disease, history of allergy or asthma or any specific medication. Subject with DAE had higher prevalence of CVD compared to DAE(-) subject. In subgroup analysis, all-cause and cardiovascular mortality was higher in DAE12 compared to DAE(-) subject(all-cause: 58.8% vs. 24.3%, p=0.006, cardiovascular: 35.3% vs. 7.1%, p=0.006). Interestingly, all-cause and cardiovascular mortality of subject with DAE12 was significantly higher than subject with DAE3 or DAE3+12. In multiple regression analysis adjusted by age, gender, diabetes, and hypertension, DAE12 was an independent risk factor for both overall and cardiovascular mortality(overall: OR=5.77, 95%CI; 1.28-26.31, cardiovascular: OR=2.25, 95%CI; 1.00-5.03). Kaplan-Meier survival analysis also revealed a lowered survival rate in subject with DAE12(0.025) compared to other groups.

Conclusions: These results suggest that the development of late-onset eosinophilia may be associated with cardiovascular morbidity and mortality.

SA-PO2704
Genotype/Phenotype Correlation in FHHNC Caused by Mutations in Either CLDN16 or CLDN19: Long-Term Follow-Up In 125 Patients
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Background: Familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is a tubular disorder caused by mutations in genes coding for the tight junction protein Claudin-16 and Claudin-19. Both proteins play a pivotal role for the paracellular reabsorption of cations in the thick ascending limb. Interestingly, FHHNC leads to chronic activation of alkaline phosphate (ALP) is necessary. CM led to a significant increase in ALP enzyme activity, which is enhanced by pretreatment with 6-MP. Besides ALP activity, ALP expression was investigated. The 6-MP-induced ALP expression is significantly and dose-dependently increased in VSMCs after 48 h. Interestingly, FHHNC leads to chronic activation of alkaline phosphate (ALP) is necessary. CM led to a significant increase in ALP enzyme activity, which is enhanced by pretreatment with 6-MP. Besides ALP activity, ALP expression was investigated. The 6-MP-induced ALP expression is significantly and dose-dependently increased in VSMCs after 48 h.

Conclusions: These results suggest that the development of late-onset eosinophilia may be associated with cardiovascular morbidity and mortality.

SA-PO2705
Significance of Henle’s Loop as a Responsible Neophen Segment of Impaired Mg Reabsorption in Renal Interstitial Damages

Background: Hypermagnesuria-induced hypomagnesemia is known to be occasionally developed due to renal interstitial damages, which are associated with severe of interstitial damages, suggesting that evaluation of hypomagnesemiauria might be a clinical parameter of RID which frequently lacks significant urine abnormalities. In kidney, it is known that Mg is reabsorbed in mTAL by 70% of filtered Mg and in DCT by 10% of filtered Mg. To elucidate the responsible tubular segment for impaired Mg reabsorption in RID by use of unilateral ureter obstrution (ULO) model.

Methods: Kidneys were sampled 1 week after ligation of left ureter of male SD rats. Intestinal and damages in change of expression of Mg transporting molecules were assessed by histological analysis, immunohistochemistry and RT-PCR in control (C) and obstructed (O) groups.

Results: In results, studies of ratio of intestinal area (C: 0.19, O: 0.32), number of ED-1 positive cells (C: 32.5, O: 235.8/field), expression of MCP-1 (C: 105.0, O: 302.6%), and expression of TGF-beta (C: 101.1, O: 338.9%) revealed development of significant RID in O group in addition to an increase in FEMG (C: 2.1, O: 12.7%). The expression of Mg transporting molecules in mTAL showed significant decrease (C:100.3, O:59.3% in paracellin-1, C:103.1, O: 83.8% in NKKC2), whereas those in DCT, including TRP6, and NCC, were not changed. Those results of gene expression were agreed with immunohistochemistry.

Conclusions: In conclusion, obtained results may suggest that the responsible tubular segment of impaired Mg reabsorption in RID would be mTAL, not DCT. Decreased expression of paracellin-1 in tight junction of mTAL, resulting in the decreased trans-tubular Mg movement, might be implicated in the impaired Mg reabsorption associated with diminished driving force of Mg transport by suppression of lumen-positive charge due to decreased NKKC2 expression.

SA-PO2706
Genome-Wide Association Study Identifies Multiple Novel Common Variants Associated with Serum Calcium Concentration
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Background: Serum calcium levels are highly heritable. Genome-wide association studies (GWAS) have recently demonstrated association of SNPs within the calcium-sensing receptor gene (CASR) with calcium levels. However, the role of common genetic variation in calcium homeostasis is largely undetermined.

Methods: We performed GWAS in 17 population-based studies (n=47718) to identify common genetic variation associated with serum calcium level. The analysis was performed in 39400 individuals of European ancestry and 8318 of Asian ancestry for ~2.5 million genotyped and imputed single-nucleotide polymorphisms, adjusted for age, sex and study center.

Results: We confirmed the previously identified association of rs1801725 in the CASR gene; serum calcium levels were 0.07 mg/dL higher per copy of the minor allele. We also identified seven loci (p-value range 4.8 x10-8 to 2.5 x 10-12) at 2q37.1, 2p23.3, 6q13, 7q11.21, 10p14, 11p15.4 and 20q13.2. These SNPs identify several potential loci of interest, including genes related to the metabolism of vitamins D (CYP24A1) and K (VKORC1-L1). Results were similar for albumin-corrected calcium and were reproducible in several novel pathways in association with calcium homeostasis.

Conclusions: We have identified several common novel loci that are associated with calcium concentration in European and Asian adult populations. While preliminary and in need of independent replication, these findings highlight known pathways and suggest several novel pathways in association with calcium homeostasis.

Funding: Other NIH Support - NHLBI

SA-PO2707
Post-Hepatectomy Hypophosphatemia in Rats

Background: Significant hypophosphatemia is common after major hepatic resection. Recently, the increase of renal fractional excretion of phosphate (FE Pi) has been reported to implicate in post-hepatectomy hypophosphatemia. In order to clarify the mechanism of hypophosphatemia caused by hepatectomy, we investigated rats received a partial hepatectomy (PH; about 70%).

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Methods: We used male Wistar rats (9 wk old) for experiments. Thymopropharyngoidectomy (TPTX) was performed on male Wistar rats (6 wk old), which were purchased from SLC (Shizuoka, Japan). The blood and urine biochemical markers were measured before and on postoperative hours 6, 12, 24 in PH and sham rats. The expression of NaPi-IIa and NaPi-IIc in renal proximal tubules were analyzed by western blotting and immunohistochemistry.

Results: Within 24 hours after PH, serum phosphate levels were significantly decreased in PH rats. Urine Pi/creatinine ratio and FEPi levels markedly increased. These results are consistent with hepaticopeny patients. The Na+-dependent phosphate uptake in renal brush border membrane vesicles increased about 40-50% at 6 hours after PH. Indeed, the amounts of NaPi-IIa and NaPi-IIc protein were significantly decreased in the PH rats.

The levels of serum fibrinolysis factor (FGF23) increased about 50%, but the intact parathyroid hormone (PTH) concentration significantly increased in 6 hours after PH. To verify whether elevation of plasma PTH after PH causes hypophosphatemia, we analyzed the effects of PH on thymopropharyngoidectomy (TPTX) rats. The present study showed that PH-TPTX rats still exhibited hypophosphatemia with down-regulation of NaPi-IIa and NaPi-IIc transporters.

Conclusions: These results imply that other factors rather than FGF23 and PTH, may contribute hypophosphatemia in PH rats.

Funding: Government Support - Non-U.S.

SA-PO2708

Estrogen Causes Phosphaturia through Specific Downregulation of NaPi-IIa and Via Endothelial Cell Function and Viability

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Background: We have previously demonstrated that estrogen (E2) treatment of ovariectomized (OVX) rats caused a significant urinary Pi wasting and hypophosphatemia. This effect resulted from downregulation of apical sodium-dependent phosphate cotransporter (NaPi-IIa). However, the effects of E2 on other Pi transporters and the respective roles of estrogen receptors ERα and ERβ in this effect remain unknown.

Methods: To address these issues, we first examined whether ERα and ERβ are expressed in the kidney proximal tubule using RT-PCR and immunoblotting. Next, and because of the anorexic effect of E2 on the activation of ERα vs. ERβ, we performed two experiments. First, 3 groups of OVX rats were treated with either E2, 4,4',4''-(4-Propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol (PPT), a selective ERα agonist, or vehicle in pair feeding protocol. In a second experiment, OVX rats were injected with 2.3-hist-(4-Hydroxyphenyl)-propionitrile (DPN), a highly potent ERβ agonist, or its vehicle, and the expression of NaPi-IIa and other Pi transporters was examined by Northern hybridization and immunoblotting.

Results: Molecular studies demonstrated both ERα and ERβ are expressed in the kidney PT cells, and while NaPi-IIa mRNA and protein were significantly reduced (~62% and ~58% vs. vehicle, P<0.03), as expected, the expression levels of NaPi-IIc (P>0.05), PiT1 (P=0.05) and PiT2 (P=0.05) were unchanged in E2, as compared to pair-fed vehicle rats. Further, PPT caused a significant decrease in NaPi-IIa mRNA (~315%, P<0.003) but did not significantly alter its protein abundance, as compared to pair-fed vehicle. Interestingly, DPN treatment caused a significant decrease in the protein abundance of NaPi-IIa (~615%, P<0.007), however, its mRNA expression levels was not significantly decreased, as compared to vehicle (P=0.05).

Conclusions: These data demonstrate that the phosphaturic effect of E2 is mediated solely and specifically through downregulation of NaPi-IIa and that this effect involves a complex mechanism involving the activation of both receptors ERα and ERβ.

Funding: NIDDK Support

SA-PO2709

Renalase Regulates Renal Phosphate Excretion

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Background: Renalase, a FAD/NADH dependent amine oxidase that metabolizes catecholamines, including dopamine (DA), plays an important role in the regulation of blood pressure and cardiac function. It is expressed in kidney, heart, liver, and skeletal muscle, and the highest levels are detected in proximal tubules. The protein is secreted plasma and urine. In the renalase KO mouse, plasma and urinary DA are markedly elevated. Furthermore, changes in dietary phosphate (Pi) modulate expression and activity of renalase in WT kidney, along with MAO-A and MAO-B, suggesting that these enzymes participate in renal Pi metabolism by regulating renal and urinary DA levels. The KO mice was studied to determine the contribution of renilase to renal Pi handling.

Methods: KO and WT mice were maintained either on a low Pi diet (0.02% Pi) for 4 days or on a high Pi diet (1.25%). Serum Pi were determined at baseline and prior to sacrifice. Kidney were harvested and gene expression was measured by quantitative PCR.

Results: Figure 1a shows that serum phosphate is significantly lower in KO mice maintained on a low Pi diet. On a high Pi diet, serum phosphate is also significantly lower in KO mice. These data suggest that KO mice excrete Pi at a higher rate than WT, and are unable to conserve Pi on a low Pi diet in spite of significant fall in serum Pi. Gene expression analysis using qPCR revealed that low Pi intake was associated with increased CMT (n=5, p=0.01, fig 1b) and MAO-B (45%, n=5, p<0.05) in KO compared to WT.

Conclusions: In the absence of renelase, a low Pi diet fails to decrease urinary DA even though both COMT and MAO-B are upregulated, suggesting that renelase is the key enzyme that drives urinary Pi excretion and leads to low serum Pi. These data support the notion that renelase plays a critical role in renal phosphate metabolism by regulating urinary DA.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2710

Adverse Effects of Simulated Hyper- and Hypo-Phosphatemia on Renal Function

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Background: Dysregulation of phosphate homeostasis as occurs in chronic kidney disease is associated with cardiovascular complications. It has been suggested that both hyper- and hypo-phosphatemia can cause cardiovascular disease. The molecular mechanisms by which high or low serum phosphate levels adversely affect cardiovascular function are poorly understood. The purpose of this study was to explore the mechanisms of endothelial dysfunction in the presence of non-physiologic phosphate levels.

Methods: We studied the effects of simulated hyper- and hypophosphatemia in human umbilical vein endothelial cells in vitro. The effects of inorganic phosphate on cell proliferation and apoptosis were measured by flow cytometry. Nuclear oxide (NO) production and endothelial NO synthase were determined. The effects of inorganic phosphate on cell signaling protein expressions were determined using reverse-phase protein microarray.

Results: We found both simulated hyperphosphatemia and hypophosphatemia decrease eNOS expression and NO production. This was associated with reduced intracellular calcium, increased protein kinase C β2 (PKCβ2), reduced cell viability, and increased apoptosis. While simulated hyperphosphatemia was associated with increased Akt/P-Akt, Bcl-xL/Bax ratios, NFκB and p-Erk abundance, simulated hypophosphatemia was associated with increased Akt/P-Akt and Bcl-xL/Bax ratios and p-Mek, p38, and p-p38 abundance. This is the first demonstration of endothelial dysfunction with hypophosphatemia.

Conclusions: Our data provides the basis for further studies to elucidate the relationship between altered phosphate homeostasis and cardiovascular disease. As a corollary, our data suggests that the level of phosphate in the culture media, if not in the physiologic range, may inadvertently affect experimental results.

Funding: Other NIH Support - NAMS, Private Foundation Support, Government Support - Non-U.S.

SA-PO2711

Association of 24-Hour Urinary Dopamine with Serum and Urine Phosphorus in Patients with Coronary Heart Disease

Nisha Bansal,

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Background: Urine dopamine (DA) is produced in the proximal tubule and has been found to increase in response to dietary phosphorus (Pi) intake, and to contribute to greater urinary Pi excretion in animal models. Whether urine DA is associated with Pi homeostasis in humans is uncertain.

Methods: Urine DA and Pi were measured in 24 hour urine collections among 963 outpatients with stable coronary heart disease (CHD). Fasting morning serum was used to measure Pi and kidney function. We examined cross-sectional associations between urine DA and serum Pi, 24-hour urine Pi, (as an indicator of dietary Pi intake), and fractional excretion of phosphorus (FePi) using linear regression. Models were adjusted for age, sex, race, eGFR by cystatin C, albuminuria, hypertension, heart failure, tobacco use, body mass index and diuretic use.

Results: Mean age was 67±11 years, 83% were men, and mean eGFR was 71±23 ml/min/1.73m². Mean urine DA was 192.8±85.1 μg/day, mean serum Pi was 3.7±0.6 mg/dl, mean 24-hour urine Pi was 46.8±25.1 mg/day and mean FePi was 7.3±8.6%. Higher urine DA was associated with younger age, male sex, black race, higher eGFR, and lower albuminuria (all P< 0.05). In adjusted models, each standard deviation higher DA was associated with higher serum Pi, higher urine Pi, and lower FePi (Table 1).

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Conclusions: These data suggest that higher dietary Pi intake is associated with higher urine Pi excretion, consistent with current models. However, these data are not associated with different fractional excretion of phosphorus, suggesting that urine DA increases in parallel with phosphorus intake, but may not be responsible for greater urinary Pi excretion.

Table 1. Association of Urine DA (per SD) with Serum Pi, 24-Hr Urine Pi and FePi

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted</th>
<th>Adjusted*</th>
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<tbody>
<tr>
<td>Serum Pi (mg/dL)</td>
<td>0.02 (95% CI: -0.02, 0.06)</td>
<td>0.04 (95% CI: 0.00, 0.09)</td>
</tr>
<tr>
<td>24-Hour Urine Pi (mg/day)</td>
<td>4.8 (95% CI: 3.2, 6.4)</td>
<td>3.6 (95% CI: 1.7, 5.6)</td>
</tr>
<tr>
<td>FePi (%)</td>
<td>-2.3 (95% CI: -2.9, -1.8)</td>
<td>0.02 (95% CI: -0.02, 0.06)</td>
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</table>

* Adjusted for age, race, sex, eGFR, albuminuria, hypertension, heart failure, tobacco use, body mass index, and diuretic use.

Funding: NIDDK Support, Other NIH Support - NHLBI, Veterans Administration Support, Private Foundation Support

SA-PO2714

Regulation of the Type IIa Sodium Phosphate Cotransporter by AMP Activated Protein Kinase

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Background: The physiologic functions of AMP activated protein kinase (AMPK), a kinase implicated in cell polarity and energy metabolism, in proximal tubule are not known. Based on the critical role of phosphate in cell metabolism and the documented role of AMPK in regulation of sodium pump expression, we hypothesized that the AMP activated apical membrane trafficking of the type IIa sodium phosphate cotransporter (Npt2a). Methods: OK (oosperm kidney) cells, a model for renal proximal tubule, were grown in monolayers. Total and activated (phospho) AMPK were detected by Western blot, immunofluorescent staining of cell lysates and antibody membrane preparations. Npt2a expression was detected by Western blot. Phosphate transport was measured by uptake of radiolabeled phosphate in transport medium.

Results: Treatment of cells with 1 mM AICAR, an AMPK activator, resulted in a statistically significant increase in pAMPK expression detectable at 2h and rising by 75% at 24 hours, analyzed as pAMPK to total AMPK. Exposure of cells to medium containing no phosphate had no effect on AMPK activation in membrane or cytosol fractions, measured by pAMPK to total AMPK. Treatment with phosphate-free medium or with AICAR for two hours had no effect on AMPK activation in brush border membrane (BBM). AICAR alone had no significant effect on Npt2a expression or phosphate uptake. Phosphate-free medium increased Npt2a expression by 44% at 2 hours. However, treatment of cells with phosphate-free medium in the presence of AICAR blocked the ability of phosphate-free medium to increase BBM expression of Npt2a. Despite the increase in Npt2a expression in the presence of phosphate-free phosphate medium, uptake did not change.

Conclusions: We conclude that 1) OK cells express AMPK, 2) ambient phosphate does not regulate AMPK activation, and 3) activated AMPK has no effect on basal Npt2a expression but inhibits Npt2a apical membrane trafficking stimulated by low ambient phosphate. The dissociation of Npt2a expression and function in phosphate-free medium suggests differential regulation of these properties by as yet unknown mechanisms.

Funding: Veterans Administration Support

SA-PO2713

Secondary Hyperparathyroidism and Hyperphosphaturia in Mice Lacking Adenylyl Cyclase 6

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Background: Adenylyl cyclase isoform 6 (AC6) is expressed in all renal tubular segments and stimulates the synthesis of AMP. Parathyroid hormone (PTH) is coupled to a G protein-coupled receptor and upon activation activates Na+/phosphate cotransporters are retrieved from the membrane of the proximal tubules resulting in increased phosphate excretion. Fibroblast growth factor 23 (FGF23) was identified as novel phosphaturic hormone. To define the role of AC6 in Pi and Ca2+ homeostasis, we studied AC6 wild-type (WT, n=10) and knockout (AC6−/−, n=10-13) mice.

Methods: Twenty-four hour metabolic cage studies were carried out with free access to food (2% Ca2+, 1% Pi) and water. Blood was taken from the retrobulbar plexus. Plasma and urine samples were analyzed for Pi, Ca2+. A separate plasma sample was analyzed for PTH, FGF23, and 1,25-Dihydroxy Vitamin D. To test for primary hyperparathyroidism we confirmed that the calcium (5.6±0.07), mg/dL and ALK-phos (163±35, IU/L), creatinine (0.5±0.1, mg/dL) in our mice were not significantly different than those in wild-type mice (5.7±0.09, 165±33, 0.55±0.19, respectively). In the FGF23 KO group we found a trend towards lower PTH levels compared to wild-type mice (5.6±0.07 vs. 1.25-Dihydroxy vitamin D levels in AC6−/− (1.25±1.17 vs. 215.16 pmol/l, P<0.001) Urinary Ca2+ excretion (2.5±0.2 vs. 2.62±0.2 µmol/day), plasma Pi (WT: 1.5±0.1 vs. 1.5±0.1 mmol/1 in AC6−/−) and Ca2+ (WT: 2.7±0.1 vs. 2.73±0.1 mmol/1 in AC6−/−) were not different. NS658 suppressed endogenous PTH in both similar levels in both genotypes (WT: 14±1 and AC6−/−: 14±2 µg/p1).

Conclusions: Our results imply AC6 is an important regulator of Pi homeostasis and explain primary hyperparathyroidism as the cause for the phosphyaturia. PTH and/or FGF23 induced inhibition of renal Pi reabsorption do not require AC6. Further studies are needed to determine the cause of secondary hyperparathyroidism including primary alterations in Ca2+ homeostasis or intestinal phosphate uptake.

Funding: Private Foundation Support

SA-PO2716

Carnosine Sensitive Dicarboxylate Transporter in Mouse Proximal Tubule

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Background: Urinary citrate is the most important endogenous inhibitor of calcium nephrolithiasis and is regulated by proximal tubule reabsorption. Urinary citrate increases with alkaliosis and with increased urinary calcium. We recently reported a novel calcium sensitive dicarboxylate citrate transport process (APL 2010) in the OK (oospermous proximal tubule) cell line. This transport differed from that found with NaDC1, the known apical citrate transporter.

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Underline represents presenting author.

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Methods: To determine whether this calcium sensitive process is also found in other species, c-Src uptake was assayed in species specific cell lines S1 and S2 derived from large-t antigen transgenic mice. Cell were grown on permeable supports with uptake measured from the apical aspect only.

Results: In both S1 and S2 cells, lowering extracellular calcium stimulated citrate uptake (0.70 ± 0.16 to 1.35 ± 0.33 pmole/well in S1, and 0.75 ± 0.01 to 0.10 ± 0.02 pmole/well in S2, p< 0.05 for both). Saccinate uptake (which occurs via NaDC1 also) was not affected by calcium. Uptake was not sensitive to 2,3-dimethylsaccinate, an inhibitor of basolateral type dicarboxylate transporters. NaDC1 was present in both cell types by Western analysis and immunostain despite the nearly 10 fold difference in citrate uptake.

Conclusions: Mouse proximal tubule cells contain an apical calcium sensitive citrate transporter demonstrating that this novel citrate transport process is present in a variety of mammalian species. Despite the presence of NaDC1, this process is unlikely to be NaDC1 since we have previously demonstrated that NaDC1 is not calcium sensitive. The proximal tubule likely contains several citrate transporters reflecting regulation of reabsorption by several pathways.

Funding: Other NIH Support - COBRE

SA-PO2717
L-WNK1 Controls ARH-Dependent ROMK Endocytosis by Phosphorylation
Liang Fang, Paul A. Welling. Department of Physiology, University of Maryland School of Medicine, Baltimore, MD.

Background: In states of dietary potassium deficiency, ROMK renal potassium channel is marked for endocytosis by a clathrin adaptor protein, ARH, and regulated by a kinase mutated in a familial disease of hyperkalemia and hypertension, WNK1 (Fang et al, Journal of Clinical Investigation 99). In the present study, we explore the mechanism by which ARH is regulated by L-WNK1. In the absence of L-WNK1, ARH is polyubiquitinated and highly degrading, in very short time frame by the proteasome. Co-expression of L-WNK1 rendered ARH insensitive to degradation, and increased ARH phosphorylation. In vitro studies with purified proteins revealed ARH is substrate of L-WNK1 phosphorylation, but a signaling intermediate was found to be required for maximal activity. The primary phosphorylation site was mapped a serine at the N-terminal region, which is important for protein stability. In conclusion, L-WNK1 phosphorylates ARH and blocks proteasomal degradation of ARH. This mechanism provides a likely explanation for how ARH protein abundance is augmented in states of dietary potassium deprivation to stimulate ROMK endocytosis and reduce urinary potassium loss.

Funding: NIDDK Support, Private Foundation Support

SA-PO2718
High Potassium Intake Upregulates Transepithelial Potential Difference and Potassium Secretion in Mouse Cortical Collecting Duct Chih-Jen Cheng, Michel G. Baum, Chou-Long Huang. Department of Medicine and Pediatrics, UT Southwestern Medical Center; Dallas, TX.

Background: K⁺ secretion via constitutively active ROMK and flow-stimulated maxi-K⁺ channels in aldosterone-sensitive distal nephron including late DCT, CNT and CCD are important for K⁺ homeostasis. Patch-clamp and immunological studies showed that a high K⁺ diet increased the density of ROMK and ENaC in rat CCD. In vitro microperfusion study of rabbit CCD showed that flow-stimulated K⁺ excretion presumably via maxi-K⁺ is upregulated by high K⁺ intake. In the study in rabbit CCD, baseline K⁺ excretion presumably via maxi-K⁺ was increased by a high K⁺ diet, raising the possibility of species differences of K⁺ secretion in CDDs. Genetic mouse models are invaluable for studying K⁺ handling in renal tubules, yet little information is available for dietary regulation of K⁺ secretion in the mouse CCD.

Methods: C57BL/6 J mice were fed either a control K⁺ diet (1% K⁺) or high K⁺ diet (5.25% K⁺) for 3 weeks. CCDs dissected from CK and HK mouse kidneys were microperfused in vitro under non-trans epithelial osmotic gradients. Tubular fluid samples were collected at a low physiological flow rate (1-2 μl/min, 0.005-0.025 mm Hg). The perfusates from the perfusate and collected fluids were analyzed using ion selective electrodes (ISE).

Results: Lumen-negative transepithelial PD were increased in HK versus CK CDDs (20.1 ± 3.3 vs. 10.6 ± 4.1 mV, p < 0.01). High K⁺ diet greatly increased secretory K⁺ fluxes (Jk 28.1 ± 8.8 pmol/min/mm in HK mice versus 1.0 ± 0.5 pmol/min/mm in CK). The increase in Jk was completely abolished by amiloride (100 μM).

Conclusions: A high K⁺ diet increased transepithelial PD and enhances K⁺ excretion in the mouse CCD. The mechanism for the increased PD and whether the increased abundance of ROMK contributes to the increased K⁺ secretory rate remains to be determined. Future studies will include investigation of regulation of K⁺ secretion in CDDs by WKCN kinases using genetic mouse models. Finally, ISE is a reliable method for measuring K⁺ and Na⁺ in small volume of fluid collection in microperfusion.

Funding: NIDDK Support

SA-PO2719
Inhibition of Src-family Protein Tyrosine Kinase (SKF) and Serine/threonine Phosphatase Stimulates ROMK Channels in the Rat Cortical Collecting Duct (CCD) WenHui Wang, Pharmacology, New York Medical College, Valhalla, NY.

Background: Previous studies have demonstrated that low Na intake or aldosterone infusion did not increase ROMK channel activity in the CCD despite of a high aldosterone level. The aim of the present study is to test the hypothesis that a high SKF activity may be responsible for suppressing the effect of aldosterone on ROMK channels.

Methods: We used the Western blot to examine the effect of low Na intake and a high K⁺ intake on the expression of WNK4, c-Src and phosphorylated-c-Src (P-c-Src) in the rat kidney. Also, we used the patch-clamp technique to examine the effect of inhibiting SKF and serine/threonine phosphatase on ROMK channels in the CCD of rats on a low Na diet or a HK diet.

Results: A HK intake significantly decreased c-Src expression and P-c-Src level but had no effect on WK4 expression in the kidney. In contrast, low Na intake had no effect on c-Src expression and P-c-Src level in comparison to the control animals, suggesting that SKF activity is higher in the kidney of rats on a low Na diet than those on a HK diet. Patch-clamp experiments demonstrated that inhibition of SKF activated ROMK channels in 7 patches of total 10 experiments and increased channel activity, defined by NP, from 0.9±0.2 to 3.0±0.5. In contrast, inhibition of SKF had no effect on ROMK channels in the CCD of rats on a HK diet. Also, patch-clamp experiments showed that inhibition of phosphatases with calyculin A (5 nM) increased ROMK channel activity (NP= 1.9±0.3) in the CCD of rats fed low Na diet but not in rats on a HK diet. This suggests the role of serine/threonine phosphatases in suppressing ROMK channel activity in rats on a low Na diet.

Conclusions: We conclude that SKF and serine/threonine phosphatases play a role in suppressing ROMK channels in the CCD of rats on a low Na diet.

Funding: NIDDK Support

SA-PO2720
Protein Phosphatase 1 (PP1) Binds to With-no-Lysine Kinase 4 (WNK4) and Regulates the Effect of WNK4 on ROMK Channels WenHui Wang, Pharmacology, New York Medical College, Valhalla, NY.

Background: WNK4 inhibited ROMK channels and the inhibitory effect of WNK4 was abolished by SGK1 but restored by c-Src. The aim of this study is to explore the role of serine/threonine protein phosphatases in modulating the interaction among SGK1, c-Src and WNK4.

Results: Immunoprecipitation experiments demonstrated that serine/threonine phosphatase, PP1, binds to WK4 at amino acid (aa) residues 695-699 (PP1) and at aa1211-1215 (PP1) in HEK cells transfected with flag-tagged WNK4 constructs. Co-expression of c-Src decreased the association of PP1 to WNK4 at PP1 but increased the association at PP1. Expression of WNK4 mutants, WNK4S402A, WNK4S404V or WNK4S406V in which the PP1, PP1 or both PP1 and PP1 binding sites were deleted or mutated, inhibited ROMK channels as potent as WNK4. However, inhibition of phosphatases with calyculin A enhanced WNK4’s inhibition of ROMK channels in the cells transfected with WNK4S402A but diminished the WNK4-induced inhibition in the cells transfected with WNK4S404V. In contrast, neither the basal activity of ROMK channels nor WNK4’s inhibition of the K⁺ channel was affected by calyculin A treatment in HEK cells transfected with WNK4. Moreover, c-Src restored the inhibitory effect of WNK4 but not WNK4S402A on ROMK channels in the presence of SGK1. Expression of c-Src inhibited SGK1-induced phosphorylation of WNK4 but not WNK4S402A at Ser106. In contrast, coexpression of c-Src restored the inhibitory effect of WNK4S402A on ROMK channels in the presence of SGK1 and inhibited SGK1-induced WNK4 phosphorylation at Ser106 in cells transfected with WNK4S402A.

Conclusions: We conclude that WNK4 is associated with PP1 at two sites and that PP1 binding to aa 695-9 is directly responsible for removal of SGK1-mediated phosphorylation of WNK4 while PP1 at aa1211-1215 is responsible for removal of the phosphorylation of ROMK channels whose phosphorylation enhanced WNK4’s inhibition of ROMK channels.

Funding: NIDDK Support

SA-PO2721
A-kinase Anchoring Protein (AKAP) Regulates BK Channel-Mediated K⁺ secretion (JK) in the Cortical Collecting Duct (CCD) Wen Liu, Carlos Schreck, Lisa M. Satlin. Department of Pediatrics, Mount Sinai School of Medicine, New York, NY.

Background: The apical BK channel in CCD principal cells (PC) is tonically inhibited by PCA under low flow conditions (Liu et al, AJP Renal 297:F904, 2009), an observation that led us to speculate that BK channel-mediated flow-induced net K⁺ secretion (FKS) requires release of channel inhibition by this kinase. Emerging evidence suggests that interactions between integral membrane proteins (e.g., ENaC) and protein kinases are stabilized at the plasma membrane by anchoring proteins, including AKAP.

Methods: To examine whether AKAP contributes to the tonic inhibition of the BK channel, we measured JK and Na⁺ absorption (JNa; each in pmol/min/mm) in NZW rabbit CCDs microperforated in vitro at slow (~1) and fast (~5 nl/ min/mm) flow rates in the presence of Si-H131 (25 μM), a cell permeant AKAP inhibitor peptide that disrupts PCA anchoring on AKAP, added to bath and lumen. Control studies were performed using a control peptide (CP25; 25 μM) of similar sequence which does not inhibit the interaction between PCA and AKAP.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Results: We found that JK was higher in CCDs treated with H31 (-19.0±1.9; n=8) than H2 (-7.3±2.9; n=3; p<0.01) when perfused at slow flow rates; JNa in these same H31 and CP treated CCDs did not differ (21.7±3.1 v. 13.3±1.9; p=0.13). A 5-fold increase in luminal flow rate did not further increase JK in 3 H31-treated tubules (-17.6±1.2 to -21.5±0.2; p<NS).

Conclusions: Our observation that the BK channel blocker iberiotoxin (50 nM) inhibited the H31-induced stimulation of JK in 5 K+ perfused at slow flow rates (-19.8±2.9 to -10.1±1.7; p<0.05), without significant effect on JNa (19.5±3.5 vs. 16.6±6.6; p=NS), suggests that AKAP contributes to suppression of BK channel activity under low flow conditions, perhaps by anchoring PKA to the ion channel at the apical membrane.

Funding: NIDDK Support

SA-PO2722

The Kir4.1 Knock-Out Mouse Shows Decreased Na+/Cl Cotransporter Expression Daniel A. Gray, Michael W. Cypess, Talia Holtzman. Nephrology Unit, University of Rochester, NY.

Background: Mutations in the Kir4.1 potassium channel underlie the SeSAME/EAST syndrome, an autosomal recessive condition characterized by seizures, sensorineural deafness, ataxia, developmental abnormalities and a hypokalemic hypomagnesemic metabolic alkalosis. To better understand the pathophysiology of this syndrome, we have been investigating the renal phenotype of a generalized Kir4.1 knock out mouse.

Methods: Serum and spot urine electrolytes were measured in 3-6 day old pups and paraformaldehyde-fixed kidney sections were probed with antibodies to distal nephron transporters.

Results: Our previous work showed that the Kir4.1 knock out mouse displays a reduced serum K+ associated with an elevated transstubular K+ gradient and decreased urine osmolality. We now show that serum [Mg^2+] is also significantly reduced from 2.9 ± 0.3 mEq/L in WT to 2.3 ± 0.1 mEq/L in the knock out (n=6-7, p < 0.05) and is associated with an inappropriately elevated urine [Mg^2+]. Serum [Ca^2+] was normal. Light microscopy did not demonstrate any structural abnormalities of the distal nephron in the knock out. However, decreased Na+/Cl cotransporter (NCC) expression was observed by immunofluorescence in kidney sections probed with an NCC antibody. No increase in aquaporin-2 immunofluorescence was seen to account for the decreased osmolality observed in the knock out.

Conclusions: Our results suggest that the renal phenotype of the SeSAME/EAST syndrome is largely recapitulated in the Kir4.1 knock out mouse. In addition, the decreased NCC expression observed suggests a means by which loss of basolateral Kir4.1 function results in failure to reabsorb Na+ and Cl- apically.

Funding: NIDDK Support

SA-PO2723

A Case of EAST/Sesame Syndrome with Tubulopathy as First Manifestation Rosa Vargas-Poussou,1 Djamel Djiedj,1 Xavier Jeunemaitre,1 Genetiques, AP-HOP Hôpital Européen Georges Pompidou, Paris, France; 2Pediatrics, CHU d’Amiens, Amiens, France.

Background: EAST (epilepsy, ataxia, sensorineural deafness,tubulopathy) or SeSAME (Seizures, sensorineural deafness, ataxia, mental retardation, electrolyte imbalance) is a rare autosomal recessive disease caused by mutations at the KCNJ10 gene coding for potassium channel Kir 4.1. First manifestations of index cases of the 7 families described to date are neurological, in particular generalized seizures in the first months of life.

Methods: We describe clinical and genetic characterization of a French patient with EAST syndrome was suggested, haplotype analysis showed homozygosity at CLCNKB locus (encoding for the calcium activated chloride channel), but sequencing and MLPA were negative. Neurological evaluation at 9 months showed a 2-time higher loss of potassium when placed under low-K diets and have a increase mortality rate after 10 days of this treatment.

Conclusions: Our observation that the BK channel blocker iberiotoxin (50 nM) inhibits the H31-induced stimulation of JK in 5 K+ perfused at slow flow rates (-19.8±2.9 to -10.1±1.7; p<0.05), without significant effect on JNa (19.5±3.5 vs. 16.6±6.6; p=NS), suggests that AKAP contributes to suppression of BK channel activity under low flow conditions, perhaps by anchoring PKA to the ion channel at the apical membrane.

Funding: NIDDK Support

SA-PO2724

PAR2 Promotes Potassium Sparring and Controls Plasma Potassium Concentration Luciana Morla, Gaëlle Brieude, Lydie Cheval, Gilles Cranbert, Suresh Krishna Ramakrishnan, Alain Doucer. UMR872, team 3, UPMC Univ Paris 06 and INSERM and CNRS, Paris, France.

Background: We previously showed that activation of PAR2 by trypsin or an agonist peptide increases sodium reabsorption in isolated rat cortical collecting duct (CCD) without promoting potassium secretion. We therefore evaluated the role of PAR2 in maintaining potassium balance in vivo and in controlling the activity of the potassium-secrating channel ROMK in CCD.

Methods: For this purpose, we compared plasma potassium level and potassium handling in PAR2- and wild type (WT) mice under basal state and potassium depletion in vivo in vitro in microperfused CCDs.

Results: Under basal state, PAR2- mice displayed normal potassium excretion and blood concentration. Within two days of potassium depletion, PAR2- mice showed a blunted ability to maximally decrease their urinary excretion of potassium, as compared to WT mice. This inappropriate ability to conserve potassium was associated with decreased plasma potassium concentration in PAR2- mice (in mM ± SE; WT, 3.9 ± 0.2; PAR2-, 3.2 ± 0.1; p<0.025). To evaluate the effect of PAR2 on ROMK activity, we compared the effect of trypsin on AVP-stimulated potassium secretion in isolated CCDs. In absence of AVP, rat CCDs neither reabsorbed sodium nor secreted potassium. AVP treatment induced sodium reabsorption and potassium secretion. Pre-treatment with trypsin did not alter AVP-stimulated sodium reabsorption but abolished potassium secretion, suggesting an inhibitory effect on ROMK. This inhibition was further supported by the finding that trypsin induced the phosphorylation of ERK in CCD, a pathway known to induce the endocytosis of ROMK.

Conclusions: In conclusion, we showed that activation of PAR2 inhibits potassium secretion in CCD and thereby participates in potassium sparing during potassium restriction.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-PO2725

Progesterone Is Required for Efficient Renal Adaptation to Chronic Dietary K+ Restriction in Male by Stimulating H,K-ATPase Type 2 Expression Gilles Cranbert, Amel Salhi, Aurelie Edwards. UMR872 Team 3, INSERM/UPMC/CNRS, Paris, France.

Background: Modern dietary habits are characterized by high-Na and low-K intakes, each of which has been correlated with a higher risk for hypertension. In this study, we examined whether long-term variations in the intake of Na+ and K induce lasting changes in the plasma concentration of circulating steroids.

Methods: We developed a mathematical model of adrenal steroidogenesis in mice that permits to predict concentration of all steroids based on the level of expression of the steroidogenic genes. These predictions were then confirmed by experimental measurements using RIA. The investigations of the renal effects of progesterone in the context of a dietary K restriction has been done in mice and using a cell line (mCCD).

Results: Our mathematical model predicted that male mice increase their plasma progesterone levels in response to K+ depletion. This prediction was confirmed by experimental measurements showing a 3-time increase of progesterone after 8 days of K restriction. Our results indicated a relationship between the ability to produce progesterone and the efficiency of the renal retention of K. Moreover, adrenalctomized mice exhibited a 2-time higher loss of potassium when placed under low-K diets and have an increase mortality rate after 10 days of this treatment.

Conclusions: Our results suggest the existence of a hereto unknown regulatory process involving progesterone, its nuclear receptor, the H2A and renal K+ retention. This effect of progesterone, in male as in female, is mineralocorticoid-independent (aldosterone is low in the K-depleted state) and may represent a “hidden” regulatory pathway in hypokalemic states.

Funding: Government Support - Non-U.S.

SA-PO2726

Claudin-16 Knock Down Mice Modulate Perdin and vH-ATPase Abundance in Distal Nephron To Prevent Volume Depletion Nina Himmerkus,1 Jan E. Behrends,1 Magdalena A. Gutowska,2 Paul S. Steels,2 Martin Blüchel,1 Institute of Physiology, Christian-Albrechts-University, Kiel, Germany; 3Biomed, University Hasselt, Diepenbeek, Belgium.

Background: Mutations in Claudin-16 are associated with the hereditary disease Familial Hypohidrosis with Hypoacriuria and Nephrocalcinosis (FIHNC). In Claudin-16 knock down mice (KD) we have previously shown that young KD mice compensate for NaCl loss in the TAL by enhanced downstream reabsorption in the cortical collecting duct (AJP2008; 295(F5):E1641-7).

Conclusions: Our results suggest the existence of a hereto unknown regulatory process involving progesterone, its nuclear receptor, the H2A and renal K+ retention. This effect of progesterone, in male as in female, is mineralocorticoid-independent (aldosterone is low in the K-depleted state) and may represent a “hidden” regulatory pathway in hypokalemic states.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.
Methods: We now investigated a cohort of aged (1.5-2 years) mice to assess long term compensatory changes. We measured the expression pattern and membrane representation of chloride bicarbonate exchangers (Pendrin and AE1) and the Vh-ATPase by confocal immunofluorescence (IF). IF data are given as % intensity of IF in the membrane area compared to the cytosolic signal.

Results: KD showed the key symptoms of FIHNC and were 15% lighter, 10% shorter and showed a slightly increased hematocrit. Urine osmolality was 1.5-fold higher and the ratio of urinary Na to K concentrations was 30% decreased, indicating an increased sodium and water reabsorption in the distal nephron. The urine of KD as well as WT was active for NaCl transport (VNaCl = 0.2 ± 0.1). In kidney slices of KD 64% of the Pendrin IF was localized in the luminal membrane of cortical intercalated cells compared to 46% in WT. Freshly isolated cortical collecting ducts showed a similar pattern and, in addition, basolateral membrane expression of Vh-ATPase was increased in KD with 41% compared to WT. The data from KD suggests 58% of Vh-ATPase IF intensity was associated with the transporter in the luminal membrane of KD intercalated cells compared to 42% in WT. We did not find any difference in the distribution of the IF signal for AE1.

Conclusions: We conclude that aged KD suffer from long term volume depletion. Functional upregulation of Pendrin serves compensatory NaCl reabsorption downstream of the TAL. The increased cortical bicarbonate secretion is counterbalanced by an activation of H+ secretion in the medulla.

Funding: Government Support - Non-U.S.

SA-PO277


Background: The study of paracellular flux across leaky epithelial, such as the proximal tubule, has been limited by the lack of a properly characterized cell culture model. We therefore set out to determine the molecular and electrophysiological properties of the leaky epithelial cell culture model, opossum kidney (OK) cells.

Methods: The electrophysiological properties of OK cells were assessed on confluent monolayers grown on semi-permeable filters, mounted in Ussing chambers, by measuring transepithelial resistance (TER) and dilution potentials. To determine the molecules mediating the electrophysiological properties we identified, cloned and then measured the relative expression, by quantitative PCR, of the claudins (a family of tight junction proteins, which dictate paracellular permeability characteristics). To validate the model system we over-expressed claudin-4 (identified as being endogenously present) and then repeated the expression and electrophysiological studies.

Results: OK cells demonstrate a low transepithelial resistance (TER = 10±2 x 10^{-3} ohm cm^2), slight cation selectivity (pNa/pCl = 1.07 ± 0.003), an Eiseen sequence for cations of K<sup>+</sup> - Rb > Na > Li and for anions of Cl<sup>-</sup> - Br. This mirrors the published electrophysiological properties of the proximal tubule in vivo. At the molecular level, OK cells express claudin-4 in > 6 > 8 > 12 > 11 > 15 > 1 > 9. We were surprised that claudin-4, a barrier forming claudin, was significantly expressed in this model system of a leaky epithelia. We therefore generated stable cell lines over-expressing claudin-4 and then repeated the electrophysiological studies.

Conclusions: Together these results establish OK cells as a leaky epithelial model system to study claudin function. They also implicate claudin-4 in forming a paracellular barrier, whether directly or via effects on other claudin expression remains to be elucidated.

Funding: Government Support - Non-U.S.

SA-PO278

Differential Effects of Extracellular ATP on Chloride Transport Via CalciumActivated Chloride Channels in Kidney Cortical Collecting Duct Cells Madhumitha Rajasekar, Alan C. Pao, Jonathan Widdicombe, John C. Edwards, Medicine, Stanford University, Stanford, CA; Veterans Affairs Palo Alto Health Care System, Palo Alto, CA; Physiology and Membrane Biology, University of California, Davis, Davis, CA.

Background: The cortical collecting duct (CCD) of the kidney plays a critical role in fine-tuning sodium chloride (NaCl) balance and is subject to extensive regulation by hormones such as aldosterone. Recent studies have demonstrated that extracellular ATP, in combination with aldosterone, sequentially regulates CaCC activity under conditions that reflect varying states of NaCl balance.

Methods: These findings illustrate that the direction of CaCC-mediated Cl<sup>-</sup> transport in CCD cells, which is dependent on V<sub>nc</sub> and ENaC activity, and suggest that extracellular ATP, in combination with aldosterone, sequentially regulates CaCC activity under conditions that reflect varying states of NaCl balance.

Funding: Other NIH Support - K08, Private Foundation Support

SA-PO279

Chemical Library Screening for Drugs To Correct Intracellular Mislocalization of RBL Mutant Barttin Naohiro Nomura, Shotaro Naito, Eisei Sohara, Tatemitsu Rai, Sei Sasaki, Shinichi Uchida. Department of Nephrology, Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University, Bankyu-ku, Tokyo, Japan.

Background: Barttin, a gene product of BND, is one of four genes responsible for Bartter syndrome. Previously, we showed that one of the disease-causing mutant barttin, RBL, was mis-localized to cytoplasm and could not reach to plasma membranes in MDCK cells and RBL knockin mouse. In addition, we reported that curcumin and 1,7-allylamino-17-demethoxygeldanamycin (17-AAG) could rescue the mis-localization of RBL barttin and partially corrected the phenotypes in the RBL barttin knockin mice. These results suggested that the aberrant intracellular localization of RBL barttin is a major cause of this disease, and to find more potent drugs to correct the mislocalization could be a promising strategy to treat this disease. For this purpose, we screened a chemical library consisting of about 20,000 chemical compounds in our university.

Methods: The screening was performed using MDCK cells stably expressing GFP tagged RBL barttin. Cellular localization of the GFP signal after treatment with the compounds was assessed by using ArrayScan (Thermo Scientific), an automated fluorescence microscopic imaging system designed for high throughput screening.

Results: As a result of this screening, we identified four candidates. We confirmed the effects of these compounds by cell surface specific biotinylation assay. Furthermore, we injected one of these compounds to RBL knockin mice intraperitoneally, and could observe the improvement of plasma membrane signal of RBL barttin in the kidney.

Conclusions: We expect that these chemical compounds can be seeds for drugs to treat Bartter syndrome, an inborn type IV caused by RBL barttin mutation, and that they can also be effective for treating diseases caused by mislocalization of mutant proteins, such as CFTR-AS508.

Funding: Government Support - Non-U.S.

SA-PO2730

Chloride Channels CLIC1 and CLIC4 Function in Proximal Tubule Endocytosis John C. Edwards, Yao-Wen Cheng. UNC Kidney Center and Department of Medicine, University of North Carolina, Chapel Hill, NC.

Background: CLIC proteins can function as chloride channels but their normal physiologic role remains uncertain. CLIC4 has been implicated in intracellular membrane traffic in tubululating endothelial cells. Whether CLICs are important in other intracellular membrane pathways is uncertain. CLIC1 and CLIC4 are both highly expressed in the proximal tubules of the kidney proximal tubule cells and a role in proximal tubule endocytosis has been proposed. We examined mice carrying targeted disruption of either CLIC1 or CLIC4 for alterations in levels of proteinuria which could be a reflection of proximal tubule endocytosis.

Methods: Urine and blood was collected from groups of Cl<sup>-</sup>IC<sup>-/-</sup> or Cl<sup>-</sup>IC<sup>−/−</sup> mice and matched WT controls. Plasma albumin, plasma and urine creatinine, and total protein were determined by commercially available clinical testing. Urine albumin concentration was determined by western blotting. Plasma and urine β-2-microglobulin (β2M) were determined by ELISA assay. Values are reported as mean ± SEM. P values determined by T-Test.

Results: Creatinine, albumin, and β2M values in plasma were not different among the groups.

Funding: Other NIH Support - NIHLB
SA-PO2731

A Novel Conditional Knockout Mouse Model of the Proximal Tubule Endocytic Receptors Megalin and Cubulin
Kathrin Weyer,1 Tina Storm,1 Jingdong Shan,2 Seppo J. Vainio,3 Erik I. Christensen,1 Pierre J. Verroust,4 Rikke Nielsen,1 1Department of Anatomy, Aarhus University, Aarhus, Denmark; 2Department of Medical Biochemistry and Molecular Biology, University of Oulu, Finland.

Background: The megalin and cubulin receptors are highly expressed in the kidney proximal tubules. Previous studies using animal models have demonstrated their synergistic and important role for normal proximal tubule endocytic recovery of filtered proteins in vivo. Studies using mouse models are however restricted by the fact that cubulin-deficient mice die in utero and megalin-deficient mice die perinatally.

Methods: Using the Cre-loxS gene knockout system, we have developed a viable megalin and/or cubulin knockout mouse model, where the Cre gene is driven by the Wnt1 promoter which is expressed in the embryonic kidney. Kidney tissue was analysed using immunohistochemistry and Western blotting. Urine samples were analysed for excreted proteins by SDS-PAGE, ELISA and Western blotting.

Results: The MegS-Cre;Wnt-4-Cre, CubS-Cre;Wnt-4-Cre, and combined MegS-Cre;CubS-Cre transgenic mice were all viable, fertile, and transmitted the transgene in a Mendelian fashion. Kidney tissue analysis by immunohistochemistry and Western blotting demonstrated a highly reproducible and efficient (>88%) knockdown of megalin and cubulin in proximal tubule cells. Consistent with the loss of megalin and cubulin expression in the kidneys, the mice were found to excrete increased amounts of low molecular weight proteins in the urine. Because this model allows the production of a large number of mice we were further able to statistically analyse groups of mice for urinary albumin excretion.

Conclusions: We anticipate that these mice will be a valuable tool to study the role of megalin/cubulin-defects in the pathogenesis of proximal tubulopathies.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2732

Altered Prostanoid Receptor Signaling, but Not Decreased PGE2 Production, Explains the Significant Resistance of P2Y1, Null Mice to Li-Induced Polyuria
Ioana L. Pop,1 Yue Zhang,1 Noel G. Carlson,1 Bellamkonda K. Kishore,1 1Medicine, Nephrology, VAMC & Univ of Utah; 2Neurovirology & GRECC, VAMC & Univ of Utah, Salt Lake City, UT.

Background: Previously we showed that genetic deletion of P2Y1 receptor results in significant resistance to Li-induced polyuria, without altering blood or renal medullary Li levels. Here we document that this resistance is not due to decreased production of PGE2, which is widely regarded as a causative factor in Li-induced polyuria, but instead due to altered prostanoid receptor (EP-R) signaling.

Methods: Groups of wild type (WT) and P2Y1-R KO mice were fed normal (ND) or Li-added (LD) diets (40 mmol/kg food) for 14 days with free access to water and euthanized. Results: Li-induced polydipsia, polyuria, and decreases in urine osmolalities and AQP2 protein abundance in renal medulla were significantly less in KO mice vs. WT mice (P < 0.05). Interestingly, genetic deletion of P2Y1-R in urinary concentration and its potential as a target to treat water-losing conditions, such as nephrogenic diabetes insipidus.

Funding: Veterans Administration Support, Private Foundation Support

SA-PO2733

Acute Lithium Administration Increases Water Excretion through Activation of MAP Kinases
Francesco Trepiccione,1 guy K. P. Hill,2 Trairak Pisitkun,1 Jason D. Hoffert,1 Robert A. Fenton,1 Soren Nielsen,1 Mark A. Knepper,1 Birgitte M. Christensen,1 Department of Biomedicine, Water and Salt Research Centre, Aarhus University, Aarhus, Denmark; 2Epithelial Systems Biology Laboratory, NHLBI, NIH, Bethesda, MD.

Background: Acute Li (Li) treatment impairs the water permeability of the kidney collecting duct. Changes in phosphorylation status are a fast way of regulating protein function. Here we used a phosphoproteomic approach to detect the early molecular targets of Li in inner medullary collecting duct (IMCD).

Methods: Rats were given a gavage of LiCl or NaCl (2.4 mmol/kg BW). After 4 or 9 hours rats were euthanized and IMCD suspensions were prepared. Phosphopeptides from IMCD isolated after 9 hours of Li-treatment were enriched by immobilized-metal affinity chromatography (IMAC) and then analysed on a LTQ-orbitrap LC-MS/MS system. Label-free relative quantification of MS1 peak integration was carried out by QUOIL software.

Results: Nine hours after LiCl gavage, rats showed a significantly increased urine volume and decreased urine osmolality (p<0.01 vs. NaCl). A total of 122 unique phosphopeptides were identified in 578 proteins. DAVID analysis of the obtained phosphoprotein database highlighted two MAPK clusters: p38 MAPK and ERK1/2. In addition, phosphopeptides in the Li-treated group with ≥ 1.5 fold increased abundance shared a common "proline-directed" kinase motif as determined by Motif-X analysis, further evidence that MAP kinases may be regulated by acute Li. The phosphopeptides in this group included AQP2 phosphorylated at pS256, a target of an additional MAPK target. In addition, p38 MAPK target, Western blot analysis of IMCD isolated 4 hours after LiCl gavage showed increased phosphorylation of p38 and ERK1/2. No changes were observed in p5261-AQP2 and p5256-1-AQP2 were significantly increased, while no changes in p256-AQP2 or phosphorylated MAPKs were observed.

Conclusions: We conclude that MAPK pathways are an early target of Li action in IMCD and the Li-related polyuric effect may involve the associated increase in P2Y1-R KO mice due to decreased production of PGE2. However, this resistance is not due to decreased production of PGE2. We believe that this resistance is not due to decreased production of PGE2. We believe that the decreased expression of PGE2-R in P2Y1-R KO mice may also contribute to the increased urinary concentrating ability reported in these mice.

Funding: Veterans Administration Support, Private Foundation Support

SA-PO2734

Thiazide Attenuates Lithium-Induced Nephrogenic Diabetes Insipidus Independently of the Sodium-Chloride Co-Transporter
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Background: Thiazides are diuretics commonly used to reduce urine output in patients with lithium-induced nephrogenic diabetes insipidus (Li-NDI). As thiazide block NaCl-co-transport (NCC) mediated tubular uptake of NaCl, it is generally thought uptake. Taken together these data suggest that genetic deletion of P2Y1-R in urinary concentration and its potential as a target to treat water-losing conditions, such as nephrogenic diabetes insipidus.

Methods: We anticipate that these mice will be a valuable tool to study the role of megalin/cubulin-defects in the pathogenesis of proximal tubulopathies.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2735

Pharmacological Blockade of P2Y12 Receptor Increases Urinary Concentration in Rats
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Background: P2Y12 receptor, a G protein-coupled ADP receptor, is expressed predominantly in blood platelets, the brain (microglia and astrocytes). Apart from Rap1b and Akt-mediated effects that contribute to platelet aggregation, signaling through P2Y12-R also inhibits adenyl cyclase. Hence, we investigated the expression and activity of P2Y12-R in rat kidney.

Methods: Real-time RT-PCR and Western blotting (antibody and blocking peptide from AnaSpec) were used to determine the expression of P2Y12-R. Groups of rats were administered clopidogrel bisulfate (Plavix®; 20 mg/kg bw/day) in drinking water for 2 weeks and euthanized. Water intake and urine output were monitored and kidney tissue samples were collected.

Results: P2Y12-R mRNA and protein were expressed in all regions of normal rat kidney, albeit at a much lower level (10-12-fold) than in the brain. Administration of Plavix® resulted in a significant increase in urine osmolality (P=0.02), associated with significant increases in urine output (P=0.04) and water consumption (P=0.04), and a modest increase in solute-free water absorption (24%; P=0.06) as compared to the control group of rats. These changes in whole body water metabolism were matched with significant increases in the protein abundance of AQP2 water channel in the inner medulla (1.9-fold; P=0.002), and cortex (2.7-fold; P=0.02), but not in the outer medulla. Plavix® did not alter the protein abundances of AQP1 water channel or P2Y12-R in the kidney. Interestingly, Plavix® administration significantly increased the urinary excretion of AQP2 (2-fold; P<0.01) and decreased urinary PGE2 (40%; P<0.02). Finally, Plavix® administration (80 mg/kg bw for 2 weeks) restored significant increase in urinary concentration in P2Y12-R knockout mice, thus ruling out the possible contribution of P2Y12 receptor blockade in mediating the observed effects.

Conclusions: Further studies are needed to delineate the renal and extra-renal actions of P2Y12-R in urinary concentration and its potential as a target to treat water-losing conditions, such as nephrogenic diabetes insipidus.

Funding: Veterans Administration Support, Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
748A
Molecular Mechanisms of Proton Permeation across Membranes: Differential Effects of Cholesterol

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Background: Despite years of study the mechanisms by which H+ permeate lipid membranes remains unclear. H+ flux differs from that of other ions in that H+ conductance is not dependent on the actual [H+] in the solution.

Methods: Combining careful proton permeability measurements with structural analyses of lipid bilayers using X-ray diffraction, we have developed models of water and solute and H+ permeation across membranes, comprising various headgroups, chain lengths and extent of unsaturation. We compared H+ permeability with physical parameters of the lipids, such as area/lipid, hydrocarbon thickness, bending modulus and compressibility modulus.

Results: Like water and solutes, in membranes composed of a single phospholipid, H+ permeability varied linearly with area/lipid, and was unrelated to other physical parameters. On the other hand, in single component lipid systems, the rate limiting step for H+ permeation is penetration of the proton from the aqueous medium into the lipid bilayer. When cholesterol is a component of the bilayer, water permeability decreases (15.8±0.58 x 10-3 cm/s and 6.8±0.57 x 10-3 cm/s in absence and presence of cholesterol respectively), but H+ permeability increases as the proportion of cholesterol increases and as area/lipid decreases (0.056±0.006 cm/s in absence and in presence of cholesterol 0.113±0.005 cm/s).

Conclusions: We have found that H+ permeability differs markedly from those of water and solutes. We have developed and are testing a new model for H+ permeation, which defines how cholesterol enhances H+ flux, while impeding water and solute fluxes.

Gas Permeability of the Urothelium

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Background: Mammalian urinary bladder, which maintains enormous chemical gradients between blood and urine exhibits extremely low permeability (P) to water and solutes such as urea and other metabolites in the urine. The bladder permeability barrier resides in the umbrella cell (UC) apical membrane (AM) with its specialized lipid composition and dense array of uroplinin complexes. Umbrella cell’s of uroplinin II/III knockout mice showed a marked diminution of plaque surface area and significantly enhanced water and urea which suggested that uroplinins represent a significant component of the AM barrier. We studied water and solute fluxes across the bladder. Under many conditions PCO2 of urine reaches 80–100 mm Hg, 3–4 fold higher than the blood PCO2. This high urinary PCO2 suggests that bladder P to CO2 must be very low. We have shown that gases such as CO2 and H2S are freely permeable across the lipid membrane and are limited only by the unstirred layer adjacent to the membrane. Since the umbrella cell apical membrane forms a barrier to water and solute fluxes, we hypothesized that this specialized membrane may also act as a barrier to CO2 flux.

Results: We measured the CO2 flux across normal urothelium and urothelium in which apical membrane barrier function was disrupted. We found that disrupting apical membrane barrier function gave water and urea P’s that were 7 to 8 fold higher than in wild type mice with intact urothelium. However these interventions had no impact on CO2 P’s across the bladder (P = 0.0115 cm/s). To test if the observed permeability was due to an unstirred layer effect or due to kinetics of CO2 hydration, we measured the CO2 permeability in MDCK cells in presence (P = 0.035 cm/s) and absence of carbonic anhydride inhibitor (P = 0.366 cm/s).

Conclusions: Our studies show that the lowered permeability of CO2 across the bladder is mainly due to lack of carbonic anhydride in the urothelium and the large unstirred layer created by the 4-5 cell layers of the urothelium.

SA-PO2737

SA-PO2738

SA-PO2739

SA-PO2740

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.
749A
The collecting duct as well as electrolyte absorption in the thick ascending limb (TAL). In all

Based on these data, it is concluded that the increase in the concentration of urea in the inner medulla is due to the increased delivery of urea to the inner medulla only. This increase is mediated by UT-B, which has been shown to cause water diuresis and nephrogenic diabetes insipidus and is

Protein SNAP23 colocalizes with NKCC2 in the apical membrane and subapical segments. In addition, our results raise a note of caution for interpretation of basolateral signals in domain-specific biotinylation assays.

The target membrane SNAREs involved in NKCC2 trafficking are unknown. Four SNAP isoforms (23,25,29 and 47) have been described. SNAP23 is present in the kidney and binds to NKCC2 by 36±6% (p<0.01). However, cAMP increased surface NKCC2 to similar extent

Results: Case report: The index patient, her brother and her son have isolated azotemia

Methods: TALs were transduced in vivo with adenoviruses coding for a dominant-negative C8-transduced) and the control right kidney

Conclusions: These are the first functional data in isolated perfused mTAL to quantify the effect of specific V2 antagonists on NaCl transport. They indicate that V2 receptor blockade is sufficient to suppress the entire AVP-activated NaCl absorption in mouse mTAL. Other vasopressin receptors appear not to be involved. Thus, the critical role of the V2 receptor for AVP-mediated transport activation is confirmed.

Funding: Veterans Administration Support

Funding: Government Support - Non-U.S.

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Funding: Government Support - Non-U.S.
Methods: We employed phosphoproteomics of rat inner medullary collecting duct (IMCD) to investigate the mechanism of bilateral ureteral obstruction (BUO)-induced NDI. IMCD isolated from 7-12 h BUO rats and 7 sham-operated rats were compared (n=3). The samples were enriched for phosphopeptides using Ga3+-immobilized metal affinity chromatography, and analyzed by liquid chromatography-tandem mass spectrometry using a Dionex Ultimate 3000 nanoflow RSL-Spray Ion Trap Velos Pro mass spectrometer. Phosphopeptide identification was performed using SEQUEST algorithm and label-free quantification was executed using QUOIL software (NLHBI Proteomics Core Facility).

Results: We identified 30 increased phosphorylation sites and 84 decreased phosphorylation sites in the BUO rat compared to sham Control. Among the increased phosphorylation sites present in proteins involved in cytoskeletal reorganization (Lima1, Plec, Ptn, Twp1, Sepl), vesicle trafficking (Ebag9), transcriptional regulation (Ebag9, Ifih1), and nuclear binding (Hmrnp, Pdx5b, Rbm39, Top2a). The decreased phosphorylation sites present in channel proteins (Aqp2 and Slc14a2), and transporters (Slc25b1, Add1, Cnn1, Cnn2, Ctn, Mx2, Mx3) and cytoskeleton binding proteins (Hdgf, Hnmpc, Hmpd, Nucks1, Psp1), small G protein (Fgfd2), and steroid binding protein (Pgrm2). Down-regulation of aquaporin-2(AQP2) phosphorylation at S256 and S264 (33.3%), and urea transporter (Slc14a2) phosphorylation at S62, and S67 (17%) were observed. Immunoblotting showed a significant reduction in total AQP2 (p<0.001), pser256-AQP2 (p<0.01), and total UT-A1 (68.2%; p<0.001) in BUO IMCD compared to the sham control.

Conclusions: Two key proteins in urine concentrating mechanism, Aqp2 and Slc14a2, were found decreased in both total and phosphorylated forms in BUO-induced NDI model in rat. Other signaling molecules that might play important role in this disease process were also identified.

Funding: Government Support - Non-U.S.

SA-P02749

Impaired Renal Water Reabsorption Mediated by Vascular Dysrupting Agent Anja Bille Bohn,1,2 Rikke Norregaard,3 Yan Wang,2 Lotte Bonde Bertelsen,1 Michael R. Horsman,1 Hans Stokdijke-Jørgensen,1 Jorgen Frokiaer,1 1Department of Experimental Clinical Oncology, Aarhus University Hospital, Denmark; 2MR-Research Centre, Aarhus University Hospital, Denmark; 3Institute of Clinical Medicine, Aarhus University Hospital.

Background: Combretastatin A-4-phosphate (CA4P) is a vascular disrupting agent shown to mediate its effect primarily on tumor blood vessels. We have previously shown that CA4P results in a significant increase in hematocrit and in mean arterial blood pressure in non-tumour bearing mice. Based on this we now want to examine whether this is associated with molecular changes in renal water handling.

Methods: Non-tumour bearing male Wistar rats were injected intraperitoneally with CA4P (30 mg/kg) or saline (controls). Blood samples and urine was collected and analyzed. Tissue water content was assessed using DCE-MRI. Renal tissue was examined for AQP2, the active phosphorylated AQP2 (p(AQP2)) and vasopressin V2 receptor (V2R) expression in inner medulla (IM). Furthermore, medullary osmolality and cAMP was determined.

Results: Total hemoglobin increased 1h after CA4P treatment (11.1±0.3 vs. 9.2±0.3 mmol/l, p<0.01). Tissue water content in muscle, brain, or renal tissue following CA4P administration did not change significantly. Urine water and salt excretion in the CA4P treated rats increased significantly during the first hour after treatment (200.1±26 vs. 90.0±12.11 ml/min/kg, p<0.01 and 16.9±3.8 vs. 3.9±0.9 µmol/min/kg, p<0.01, respectively). Urine and medullary osmolality decreased 2h after CA4P. AQP2 mRNA level in IM did not change in response to CA4P treatment. However, immunohistochemistry showed an elevated localization of p(AQP2) in IM. Furthermore, we detected differ AQP2 distribution within the IM principal cells in the CA4P treated rats. V2R expression and medullary cAMP concentration did not change 1h after CA4P. Three hours after CA4P treatment the AQP2 mRNA level and the pAQP2 protein level was significantly increased compared to controls.

Conclusions: The data are compatible with the notion that CA4P affects the urine concentrating ability through a local renal effect on collecting duct water permeability due to changed AQP2 phosphorylation.

SA-P02750

Urine Concentration Defect in the Absence of Ureter Transplants-A1/A3 May Involve an Inability to Phosphorylate Aquaporin2 in Serine 256 Titilayo O. Ibi, Jeff M. Sands, Janet D. Klein. Medicine, Emory University School of Medicine, Atlanta, GA.

Background: Polyrilia is a significant clinical manifestation of diabetes mellitus yet diabetics rarely go into hypovolemic shock. Upregulation of aquaporin 2 (AQP2) and urea transporters increase water and solute reabsorption. In the absence of the urea transporters, diabetes frequently develops without the characteristic hypovolemic shock. We have previously shown that AQP2 and vasopressin V2 receptor (V2R) expression in inner medulla (IM). Furthermore, medullary osmolality and cAMP was determined.

Methods: We employed phosphoproteomics of rat inner medullary collecting duct (IMCD) to investigate the mechanism of bilateral ureteral obstruction (BUO)-induced NDI.

Results: We identified 30 increased phosphorylation sites and 84 decreased phosphorylation sites in the BUO rat compared to sham Control. Among the increased phosphorylation sites present in proteins involved in cytoskeletal reorganization (Lima1, Plec, Ptn, Twp1, Sepl), vesicle trafficking (Ebag9), transcriptional regulation (Ebag9, Ifih1), and nuclear binding (Hmrnp, Pdx5b, Rbm39, Top2a). The decreased phosphorylation sites present in channel proteins (Aqp2 and Slc14a2), and transporters (Slc25b1, Add1, Cnn1, Cnn2, Ctn, Mx2, Mx3) and cytoskeleton binding proteins (Hdgf, Hnmpc, Hmpd, Nucks1, Psp1), small G protein (Fgfd2), and steroid binding protein (Pgrm2). Down-regulation of aquaporin-2(AQP2) phosphorylation at S256 and S264 (33.3%), and urea transporter (Slc14a2) phosphorylation at S62, and S67 (17%) were observed. Immunoblotting showed a significant reduction in total AQP2 (p<0.001), pser256-AQP2 (p<0.01), and total UT-A1 (68.2%; p<0.001) in BUO IMCD compared to the sham control.

Conclusions: Two key proteins in urine concentrating mechanism, Aqp2 and Slc14a2, were found decreased in both total and phosphorylated forms in BUO-induced NDI model in rat. Other signaling molecules that might play important role in this disease process were also identified.

Funding: Government Support - Non-U.S.
UT-A1/A3 KO vs WT mice and did not increase in either WT or KO mice with diabetes. PSC680-A1/A3 KO tended to be decreased with diabetes in both KO and WT mice consistent with increased vasopressin levels in diabetic animals.

Conclusions: We conclude that despite increased AQP2 in response to vasopressin, in the absence of UT-A1 and UT-A3 the diabetic animal is unable to optimally activate AQP2 cotransporter function and PSC680-A1/A3 KO vs WT mice. An inability to activate AQP2 despite increased protein may explain the lack of vasopressin-mediated urine concentration in the absence of UT-A1/A3.

Funding: NIDDK Support, Other NIH Support - NIH T-32 DK07656, NIDDK-NIH RO1 DK41707

SA-PO2751
Urinary Exosomal Aquaporin-2 Excretion Is Altered by the Treatment With Furosemide or Acetazolamide, in Association With Vasopressin
Yoshiaki Higashijima, Hiroaki Kondo, Kanako Shigemura, Saki Takahashi, Hiroko Sonoda, Masahiro Ikeda. Veterinary Pharmacology, University of Miyazaki, Japan.

Background: Aquaporin-2 (AQP2) is the only AQP regulated by vasopressin. In response to vasopressin, AQP2 traffics to the apical plasma membrane to increase water permeability in collecting ducts. Exosomes are membranous vesicles delivered to the urine from all renal epithelial cell types and this urinary exosomes are known to contain membrane proteins including AQP2. So far, we reported that urinary exosomal AQP2 level was continuously decreased in rats treated with cisplatin, suggesting urinary exosomal AQP2 as a biomarker for the detection of cisplatin-induced acute kidney injury (2010 ASN). However, at present the mechanisms underlying the excretion of urinary exosomal AQP2 are largely unknown. In order to elucidate the mechanism, this study examined the effect of diuretics on the excretion of urinary exosomal AQP2.

Methods: A 2-h urine was collected from rats treated with saline (control), furosemide (20 mg/kg), acetazolamide (50 mg/kg), or OPC-31260 (10 mg/kg, a V2 receptor antagonist). Urinary exosomes were isolated from the collected urine by differential ultracentrifugation.

Results: Western blot analysis revealed that urinary exosomal AQP2 abundance was dramatically increased in the furosemide and the acetazolamide groups, in comparison with the control group, while OPC-31260 did not have any significant effect on the urinary exosomal AQP2 excretion. In the furosemide and acetazolamide groups, renal AQP2 protein level was significantly decreased in the cortex but not in the outer and the inner medulla, and this decrease was accompanied with the enhancement of the apical expression of AQP2. Co-administration of OPC-31260 with furosemide or acetazolamide completely inhibited the increased excretion of urinary exosomal AQP2 in response to furosemide or acetazolamide.

Conclusions: Our results indicate that basal excretion of urinary exosomal AQP2 is independent of vasopressin, but its increased excretion by a decrease in body fluid volume through treatments with diuretics is initiated by vasopressin.

SA-PO2752
Decreased Aquaporin 2 and Urea Transporters in a Rat Model of Nephrotic Syndrome
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Background: Urea transporters and aquaporins play an important role in the regulation of renal water and solute handling. Nephrotic syndrome is associated with significant primary renal salt and water retention. This study investigates the role of urea transporters and aquaporins in nephrotic syndrome associated water retention. We tested the effect of doxorubicin-induced nephrotic syndrome on the abundance of urea transporters UT-A1, UT-A3, aquaporin-2 (AQP2), and Nkcc2 in the rat kidney tissue.

Methods: Three cohorts of male Sprague Dawley rats (n=18 treatment, 13 control) were used. Overnight urine was collected from rats treated with saline, furosemide or acetazolamide using western blot.

Results: Within 2 weeks following doxorubicin injection, all treated rats developed features of nephrotic syndrome, with an average 9-fold increase in urine Pr/Cr ratio (from 1.5 ± 2 to 130 ± 10; p < 0.001) and significant hypercholesterolemia (from 103 ± 2 mg/dl to 276 ± 19 mg/dl). Urine osmolalities were reduced by 38 ± 5% in the doxorubicin treated group (P<0.01), treatment with 1500 ng/kg/day (n=8), control, UT-A1 and UT-A3 protein abundances in IM tip tissue were significantly decreased (44 ± 8% and 31 ± 3% respectively; p<0.001). Nephrotic rats. AQP2 protein abundance was also significantly reduced (65 ± 5%, p<0.001) in the nephrotic group as compared to control rats. A 72 ± 5% reduction in expression of the Nkcc2 was noticed in outer medullary tissue of nephrotic rats (n=8) as compared to control rats. AQP2, UT-A1, UT-A3, and Nkcc2 are decreased in doxorubicin-induced nephrotic syndrome and may represent a response to acidification of renal epithelial cells in the collecting ducts, coupled with a general disturbance in the normal countercurrent medullary osmotic gradient.

Funding: Other NIH Support - This work was supported by NIH R01DK41707 and by the UT Southwestern NIH O’Brien Kidney Research Center P30DK79328

SA-PO2753
Enhanced Renal Expression of SGLT2 Gene in Response to Diabetes
Niloofar Moeini Tabatabai, Rajendra Kishore Kothinti. Medicine, Medical College of Wisconsin, Milwaukee, WI.

Background: SGLT2 is the major glucose transporter in the kidney and is expressed on the apical side of the proximal tubule cells. It has been suggested that SGLT2 expression may be enhanced in type 2 diabetic patients. The goal of this study was to examine temporal changes in renal expression of SGLT2 gene in response to diabetes.

Methods: Zucker Diabetic Fatty (ZDF) fa/fa rats, a model of type 2 diabetes, and non-diabetic fa/+ controls were obtained. Five male 5 weeks (wk) old controls and 5, 8, 12, 15, and 19 wk old fa/fa were used. Overnight urine was collected from fasting rats. Renal tissue was collected, blood was collected from the heart, and left kidney was removed. Serum and urine concentrations of glucose and serum insulin levels were measured. To examine SGLT2 gene expression, total RNA from kidney tissues were prepared, reverse transcribed, and cDNA samples were used in real time polymerase chain reaction with SYBR Green reagents. Amplification of small subunit ribosomal protein 15 (S15) cDNA was used as endogenous control and fold difference between fa/fa groups and controls were determined.

Results: Fasting serum glucose in 5 wk old control and fa/fa were 109 and 195 mg/ dl, respectively. In fa/fa, serum glucose increased to 221 mg/dl by 8 wk of age, it reached 301 mg/dl in 12 wk old rats and remained as high in 15 and 19 wk old fa/fa. Glucose excretion was only ~0.018 g/day in 5 wk old control and fa/fa; however, 8 wk old fa/fa were glucosuric and excreted 9 g of glucose/day. Glucose excretion almost doubled by 12 wk, reached 21 g/day by 15 wk, and then declined. In 5 wk old fa/fa, serum insulin was 1.5 ng/ml, reached maximum of 11 ng/ml at 8 wk of age, and declined to ~2 ng/ml in older rats. Relative expression of SGLT2 mRNA in the kidneys of 5 wk old fa/fa was 1.6 (arbitrary unit, A.U.) and it increased 1.6 fold to maximum of 2.5 A.U. by 8 wk of age. SGLT2 expression in 12 and 15 wk old fa/fa was 1.53 and 1.76 A.U., respectively, and decreased to 0.82 A.U. by 19 wk of age.

Conclusions: Results of this study show that renal expression of SGLT2 gene was enhanced in the early stages of the development of type 2 diabetes in ZDF fa/fa model. Increased expression of SGLT2 may augment symptom of hyperglycemia.

SA-PO2754
Hypertonicity Increased Glutathione Content in MDCK Cells and Had Protective Effect Against H2O2 Induced Cell Damage
Masamori Horio. Functional Diagnostic Science, Osaka University Graduate School of Medicine, Suita, Japan.

Background: Oxidative stress in renal tubular cell is a possible factor inducing the cell damage. Hypertonicity is one of the main causes of the oxidative stress. Glutathione (GSH) is an important antioxidant, but the content after hypertonic exposure has not been well studied. GSH content after hypertonic exposure and effect of preincubation with hypertonic medium on oxidative stress were evaluated.

Methods: ROS generation was detected using carboxy-H2DCFDA, GSH content, activity of glutamate-cysteine ligase (GCL), the rate-limiting enzyme of GSH synthesis, and contents of amino acids, which included primary sources of GSH, were measured. Cell damage was assessed by LDH activity in culture medium.

Results: Hypertonicity increased reactive oxygen species (ROS) in an osmolality dependent manner. Hypertonicity of 400 and 450 mOsm significantly increased GSH content up to 1.5 fold the value of isotonic cells after 8h. Higher osmolality (500 and 600mOsm) did not show further increase of GSH, but decrease the GSH content. Significant increase of GCL activity was not observed after 8h of hypertonicity of 350, 400 and 450 mOsm of culture medium. Contents of glutamate, glycine and cysteine which were precursors of GSH were significantly increased 7, 3 and 2 fold the value of isotonic cells after 8h of hypertonicity, respectively. This might be a mechanism of inducing GSH content by hypertonicity. H2O2 showed cell damage in MDCK cells in dose dependent manner assessed by LDH activity in culture medium. 24h-preincubation of 400mOsm significantly protected the H2O2-induced cell damage.

Conclusions: Preincubation of mild hypertonicity has a protective effect on H2O2 induced cell damage in MDCK cells. Increase of GSH content by hypertonicity may contribute to the protective effect.

Funding: Government Support - Non-U.S.

SA-PO2755
Removal of Indoxyl Sulfate by Hybrid Kidney with Human Organic Anion Transporter-3 Over-Expressing Cells
Hitoshi Endou, 1 Takashi Ioka, 1 Yoshikatsu Kanai, 1 Hitoshi Endou, 1 Akio Fujimura, 2 Eiji Kusano. 1 1 Nephrology, Jichi Medical University, Tochigi, Japan; 2 Clinical Pharmacology, Jichi Medical University, Tochigi, Japan.

Background: Indoxyl sulfate (IS), one of the protein-bound uremic toxins and a substrate of organic anion transporter (OAT-3), is cleared poorly by conventional hemodialysis. One of the reasons for the high incidence of cardiovascular disease in dialysis patients is accumulation in the body of uremic toxins. We have previously
AQP11 was initially expressed at choroid plexuses and capillaries outside the brain. AQP1 was also expressed at choroid plexuses and capillaries around capillaries. AQP11 was expressed at astrocytes around capillaries.

Inhibitory Effect of Overexpression of Aquaporin-11 on Intracellular Ca$^{2+}$ Release in Transfected Mammalian Cells

Kiyu Nishimura, Kanako Muta, Saki Takahashi, Hiroko Sonoda, Masahiro Ikeda.

**Background:** The aquaporins (AQPs) are a family of membrane protein that are selectively permeable to water and glycerol. So far, thirteen AQPs (AQP0 - AQP12) have been identified in mammals, and among them the recently identified molecule AQP11 is known to be localized in the endoplasmic reticulum (ER), an intracellular Ca$^{2+}$ store. Furthermore, AQP11-null mice die in the neonatal period due to failure with progressive renal cyst formation. However, the molecular function of AQP11 is largely unknown. In this study, we examined whether AQP11 affected intracellular Ca$^{2+}$ responses in transfected mammalian cells.

**Methods:** Intracellular Ca$^{2+}$ concentration was monitored with Fluor in CHO-K1 cells transfected with each expression plasmid. Cultured cells were observed 24 h post-transfection using a confocal microscope.

**Results:** The elevation of intracellular Ca$^{2+}$ concentration in response to ATP was completely inhibited in both the presence and absence of extracellular Ca$^{2+}$ in cells expressing N-terminal DiRed monomer-tagged AQP11 (DsRM-AQP11). Similar inhibition was also observed in cells expressing myc-AQP11 or AQP11-DsRM. On the other hand, no inhibition of ATP-induced Ca$^{2+}$ increase was observed in cells expressing DsRM-AQP1.

**Conclusions:** These data indicate that AQP11 inhibits ATP-induced Ca$^{2+}$ release via the depletion of intracellular Ca$^{2+}$ stores, suggesting the importance of AQP11 in the regulation of intracellular Ca$^{2+}$ concentration.

**Funding:** Government Support - Non-U.S.

Aquaporin-11 Expression in the Brain: Choroid Plexus and Capillaries with Blood-Brain Barrier Function

Shin Koike, Yasuko Tanaka, Yoshiyuki Morishita, Kenichi Ishibashi.

**Results:** We found that AQP11 was expressed in the cytosol of choroid plexus epithelia and at endothelia of brain capillaries by immunohistochemistry selectively as absent in other capillaries outside the brain. AQP11 was also expressed at choroid plexuses and capillaries while AQP4 was expressed at astrocytes around capillaries. AQP11 was initially expressed at pia mater and little at capillaries in newborns (P1), and the former expression shifted to the choroid plexus with growth, which was speculated to be due to the capillary deletion method: the ratios of capillary to parenchymal fractions increased by 2.5 folds from P1 to P28. AQP11 and AQP2 were highest at P1 and decreased at P7 to P28 by one-eighth in AQP11 in the brain, while AQP4 increased 4 folds from P1 to P7. The brain of AQP11-null mice revealed numerous intracranial vacuoles in the choroid plexus but the other areas of the brain appeared normal. The water contents of the brain were similar (78.61% vs. 78.96%). The two-dimensional protein electrophoresis analysis also revealed no obvious changes of protein expression patterns. Blood-brain-barrier (BBB) function examined by biotin injected into the heart appeared to be normal with no leakage in AQP11-null mice.

**Conclusions:** We focused on residues that differ between these transporters and systematically converted residues within URAT1 into the corresponding RST residues, and vice versa, and measured the activity and sensitivity of these mutants to URAT1 inhibitors. We also developed a binding assay to URAT1 membranes using a radiolabeled next generation Ardea compound.

**Results:** Convension of a single residue in URAT1 at position 365, from phenylalanine to the RST residue tyrosine, results in a mutant URAT1 uric acid transporter that is 100-fold less sensitive to inhibition. Conversely, conversion of RST residue tyrosine-365 to the URAT1 phenylalanine produces an RST mutant that is 10-fold more sensitive to inhibition. For the binding assay, binding of the radiolabeled compound is specific and saturable to URAT1 and occurs at a nanomolar affinity, showing that the compound directly binds with high affinity to URAT1. Xenopus Oocyte microinjection experiments were also conducted demonstrating that the binding site is available from both the inside of the cell and the outside.

**Conclusions:** URAT1 phenylalanine-365 is both necessary and sufficient for the high affinity inhibition by inhibitors, and this inhibition is perturbed by the addition of a tyrosine hydroxyl group. URAT1 phenylalanine-365 is either involved in a direct hydrophobic interaction with these molecules that is blocked by the tyrosine hydroxyl group, or it is important to maintain a particular structure within URAT1 that is required for high affinity binding. We suggest that URAT1 phenylalanine-365 is within a substrate binding pocket that also interacts with Ardea’s compound inhibitors.

**Funding:** Pharmaceutical Company Support

**Poster/Saturday**

Inhibitors of the Uric Acid Transporter URAT1 Require URAT1 Phenylalanine 365 for High Affinity Interaction

Phil K. Tan, David L. Hyndman, Jeffrey N. Miner. Ardea Biosciences, San Diego, CA.

**Background:** URAT1 is the target of many uricosuric agents that block uric acid reabsorption. Ardea Biosciences has recently identified a class of next generation inhibitors that specifically inhibit URAT1 at nanomolar potency. Here we report on a residue in URAT1 that is required for this high affinity interaction.

**Methods:** Human URAT1 has a 74% protein sequence identity with the ortholog rat uric acid transporter rURAT1, yet the compounds inhibit rURAT1 with a 1000-fold lower affinity. We focused on residues that differ between these transporters and systematically converted residues within URAT1 into the corresponding RST residues, and vice versa, and measured the activity and sensitivity of these mutants to URAT1 inhibitors. We also developed a binding assay to URAT1 membranes using a radiolabeled next generation Ardea compound.

**Results:** Conversion of a single residue in URAT1 at position 365, from phenylalanine to the RST residue tyrosine, results in a mutant URAT1 uric acid transporter that is 100-fold less sensitive to inhibition. Conversely, conversion of RST residue tyrosine-365 to the URAT1 phenylalanine produces an RST mutant that is 10-fold more sensitive to inhibition. For the binding assay, binding of the radiolabeled compound is specific and saturable to URAT1 and occurs at a nanomolar affinity, showing that the compound directly binds with high affinity to URAT1. Xenopus Oocyte microinjection experiments were also conducted demonstrating that the binding site is available from both the inside of the cell and the outside.

**Conclusions:** URAT1 phenylalanine-365 is both necessary and sufficient for the high affinity inhibition by inhibitors, and this inhibition is perturbed by the addition of a tyrosine hydroxyl group. URAT1 phenylalanine-365 is either involved in a direct hydrophobic interaction with these molecules that is blocked by the tyrosine hydroxyl group, or it is important to maintain a particular structure within URAT1 that is required for high affinity binding. We suggest that URAT1 phenylalanine-365 is within a substrate binding pocket that also interacts with Ardea’s compound inhibitors.

**Funding:** Pharmaceutical Company Support

Protective Effects of N-acetylcysteine (NAC) in Renal and Lung Function

Maria Heloisa M. Shimizu, Rildo A. Volpini, Ana C. de Braganca, Luciana Andrade, Antonio C. Seguro. Nephrology, School of Medicine - University of Sao Paulo, Sao Paulo, Brazil.

**Background:** Oxidative stress increases with age and is associated with alterations in kidney and lung functions. Previous studies demonstrated that NAC, an antioxidant drug, protects animals from kidney and lung injury.

**Methods:** The aim of this study was to evaluate the effects of NAC on sodium and water transporters in kidney and lung of old rats. Normal 8-month-old male Wistar rats were treated (n=6) or not (n=6) with NAC (600 mg/L in drinking water) and followed for 16 months. At the end of the follow-up we measured thiobarbituric acid reactive substances (TBARS, a lipid peroxidation marker), urine volume (UV) and inulin clearance (In Cl). In addition we performed immunoblotting for renal proteins (NKCC2, αENaC and AQP2) and for lung proteins (αENaC, NKCC1 and AQP5).

**Results:** Mean NAC ingestion was 24±2 mg/day. As we can see in table 1, NAC decreased serum TBARS and ameliorated inulin clerance in old rats.

<table>
<thead>
<tr>
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<th>In Cl (ml/min/100 g BW)</th>
<th>UV (μl/min)</th>
<th>FE H2O (%)</th>
<th>TBARS (nmol/ml)</th>
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<tr>
<td>Old Rat</td>
<td>0.38±0.013</td>
<td>23.1±8.0</td>
<td>0.63±0.09</td>
<td>8.4±0.3</td>
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<tr>
<td>Old Rat + NAC</td>
<td>0.49±0.041 *</td>
<td>7.6±0.4 **</td>
<td>0.29±0.02 **</td>
<td>4.0±0.3 *</td>
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*p < 0.01; **p < 0.05 vs. Old Rat

**Conclusions:** NAC treatment in old rats ameliorated renal function and upregulated αENaC and NKCC2 and AQP2 renal expression. These effects may increase urine concentration and dilution capacity in old rats and consequently decrease the incidence of hypo or hyperosmolar states in this population. In addition, we found that NAC profoundly decreased serum TBARS and ameliorate inulin clearance in old rats.

**Funding:** Government Support - Non-U.S.
SA-PO2760

Sodium Myoinositol Cotransporter-1 Knockout Mice Are Not at Increased Risk for Osmotic Renal Injury

Aiay Kher, S. Ananth Karumanchi, Samir M. Parikh.
Nephrology, Beth Israel Deaconess Medical Center, Boston, MA.

Background: Previous studies have shown that (1) loss of the toxicity-responsive transcription factor NFAT5/TonEBP results in renal atrophy, (2) NFAT5 is a critical positive regulator of the tonicity-responsive sodium-myoinositol co-transporter-1 (SMIT-1), and (3) injection of a small molecule SMIT-1 inhibitor into rodents induces acute kidney injury. We therefore hypothesized that SMIT-1 knockout mice (KO) would have increased susceptibility, to the osmotic stress of dehydration.

Methods: 10-12 week KO, heterozygote and wild-type male mice were evaluated at baseline and after 48 hours of dehydration for serum electrolytes, BUN and creatinine by iSTAT analyzer and urine osmolality. Mice also underwent radiocontrast injury by intraperitoneal injection of LNAME and Indomethacin (each 10mg/kg) followed 1 hour later by an intravenous injection of fiosehox 10ml/kg.

Results: Baseline renal function was indistinguishable between KOs, heterozygote, and wild-type male littermates. After 48 hours of dehydration, mice of all three genotypes had similar percent weight loss (WT 14.2 +/-0.8, Het 15.9 +/-1.8, KO 15.9 +/-1.3), serum sodium (WT 152 +/-1.6, Het 153 +/-4.6, KO 152 +/-4.0 meq/L) and BUN (WT 46 +/-5.6, Het 48 +/-5.0, KO 54 +/-11.9 mg/dL). SMIT-1 KO mice were also increased their urine osmolality to a similar extent (WT n=4, 3975 mosm/L, Het n=6, 3925 mosm/L, KO n=2, 4170 mosm/L). After mouse radiocontrast renal injury, male wildtype (n=13), heterozygote (n=9) and knockout (n=8) mice developed similar BUN elevation (WT 73 +/-30, Het 73 +/-29, KO 74 +/-32).

Conclusions: We conclude that loss of SMIT-1 does not lead to renal atrophy and that SMIT-1 knockout mice do not appear to be at increased risk of osmotic renal injury. Our results suggest the importance of other NFAT5 transcriptional targets either singly or, more likely, in combination.

Funding: NIDDK Support, Other NIH Support - NHLBI R01 to Dr. SM Parikh

SA-PO2761

The Effect of Hyperosmolality on the Proiferation and Cell Cycle Regulation of Gastrointestinal and Bladder Epithelial Cell Lines

Bradley P. Dixon, Brian J. Siroky, John J. Büssler. Division of Nephrology and Hypertension, Cincinnati Children’s Hospital Medical Center, Cincinnati, OH.

Background: Children with complex urogenital abnormalities require surgical reconstruction of their urinary tract, and the gastrointestinal tissues often utilized in such reconstructions are at increased risk for cancer. We have previously shown that the hyperosmolar bladder microenvironment may attenuate the DNA damage response in gastrointestinal, but not bladder, cells. Hyperosmolar conditions in the bladder may also induce alterations in cellular proliferation and cell cycle regulation in susceptible tissues, which combined with a faulty DNA damage response could, in part, explain the tissue-specific susceptibility to cancer in the augmented bladder. We sought to characterize the cellular proliferation and cell cycle regulation of gastrointestinal and bladder epithelial cells adapted to a hyperosmolar microenvironment.

Methods: Conditionally immortalized colon (YAMC) and bladder (ULTI) epithelial cells were gradually adapted to either isoosmolal or hyperosmolal conditions. Cellular proliferation was measured by binding and counting on a hemocytometer, as well as crystal violet DNA binding assay. Cell cycle analysis was performed by propidium iodide staining and flow cytometry.

Results: The YAMC colon cells demonstrated continued cellular proliferation from 450mOsm/kg to 600mOsm/kg, whereas in ULTI bladder cells, cellular proliferation plateaued after 450mOsm/kg. Additionally, YAMC cells adapted to hyperosmolar sodium chloride had marked abnormalities in cell cycle distribution by 450mOsm/kg and especially 600mOsm/kg, whereas the cell cycle distribution of ULTI cells was preserved to 600mOsm/kg.

Conclusions: Gastrointestinal cells under hyperosmolar conditions were less likely to reduce cellular proliferation despite, and perhaps due to, dysregulation of the cell cycle, whereas these processes remained intact in bladder cells. These findings, coupled with an attenuated DNA damage response under such conditions, could ultimately explain the susceptibility to carcinogenesis experienced by gastrointestinal tissues in the augmented bladder.

Funding: NIDDK Support

SA-PO2762

High Throughput Quantification of Apical Versus Basolateral Trafficking

Javier E. Mazaferri,1 Stephane Lefrançois,2,3 Santiago Costantino,1,3 Centre de Recherche, Hôpital Maisonneuve-Rosemont, Montréal, Canada; 1Département de Médecine, U. de Montréal; 1Département d’Ophthalmologie et Institut de Génie Biomédical, U. de Montréal.

Background: Aquaporins (AQP) 2, 3 and 4 are water permeable membrane channels expressed in principal cells of the collecting duct of the kidney. AQP2 localizes to the apical membrane and is responsible for vasopressin-induced water reabsorption from urine. Mutations in AQP2 have been implicated in Nephrogenic Diabetes Insipidus. AQP3 and AQP4 are localized to the basolateral membrane constituting potential water exit pathways from the cell. The complex protein trafficking machinery that maintains these segregated distributions of biomolecules in the apical and basolateral membranes is still not completely understood. Studies that have attempted to characterize the molecular mechanisms implicated in AQP trafficking have often relied on observations made with single or time-lapse fluorescence microscopy images. However, the lack of automated image analysis techniques makes many of these observations potentially biased and not statistically significant.

Methods: We propose to address this by accurately quantifying the dynamics of AQP5 trafficking vesicles using video-rate optically-sectioned in-vivo images. The commercially available optical sectioning techniques do not meet these requirements; therefore we propose to use an approach that takes advantage of typical features of vesicles. In fact, cargo vesicles are near diffraction limited, belonging to the high-frequency range of the images’ spatial spectrum, and are naturally axially localized. High-frequency filtering provides observable optically-sectioned images of cargo vesicles at frame rates near 10 times faster than confocal microscopy.

Results: We present in-vivo images of MDCK cells transfected with various GFP-tagged AQPs, obtained with the proposed approach.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
the Ace2 gene in male mice has been associated with an age-dependent development of angiotensin II (AngII) to produce the vasodilating peptide angiotensin-(1-7). Deletion of ACE2 is a homologue of ACE that is highly expressed in the kidney, and can degrade renin substrate, which is the first committed step in the production of AngII. Blockade of the RAS has proven beneficial in this regard. ACE2 also plays a role in renin-angiotensin system (RAS) regulation. ACE2 is an enzyme that cleaves angiotensin I (AngI) to produce angiotensin II (AngII), which is a potent vasoconstrictor and renal tubular injury. ACE2 is expressed in the kidney, where it catalyzes the production of AngII, which then converts to vasopeptidase produced AngII.

Angiotensin II Generates Oxidative Stress in the Kidney Proximal Tubule

**Methods:**

Two groups of male mice were studied: 8 week old wild-type C57BL/6J mice and mice with a deletion in the gene for Ace2. Mice from each group were subjected to sham operation or UUO of the left kidney, and gene expression measured by real time PCR in the renal cortex after 7 days. Deletion of the Ace2 gene did not affect expression in sham-operated mice, but exacerbated the increase in expression for both the inflammatory and fibrotic genes following UUO. Deletion of the Ace2 gene promoted inflammation and fibrosis in the kidneys of mice after UUO, suggesting that ACE2 is also protective in the kidney tubulointerstitium.

**Funding:**

Government Support - Non-U.S.

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**Table:**

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**Results:**

- Deletion of the Ace2 gene exacerbated kidney injury in mice subjected to UUO.
- The Ace2 gene knockdown model was associated with increased expression of pro-inflammatory genes (IL-1β, TNFα, CR1, MMP) and inflammatory genes (collagen 1α1, TFPI, PAI-1, CXCL, sMMP) seven days after surgery. Deletion of the Ace2 gene did not affect expression in sham-operated mice, but exacerbated the increase in expression for both the inflammatory and fibrotic genes following UUO.

**Conclusions:**

Deletion of the ACE2 gene resulted in greater inflammation and fibrosis in the kidneys of mice after UUO, suggesting that ACE2 is also protective in the kidney tubulointerstitium.

**Funding:**

Government Support - Non-U.S.

**SA-PO2767**

**Effect of Combination Therapy with Low Dose Darbepoetin and High Dose ARB on Established Glomerulosclerosis in 5/6 Nephrectomized Mice**

Keizo Matsumoto, Haiunch Yang, Agnes B. Fogo.

**Background:**

Low dose darbepoetin per se has no effect on progression, but in combination with high dose ARB halts progressive renal injury and enhances regression of glomerulosclerosis.

**Funding:**

Pharmaceutical Company Support

**SA-PO2768**

**Combined Losartan (L) and Hydrochlorothiazide (H) Therapy Arrests Renal Injury and Reverses Cell Events in the Remnant Kidney Model**


**Univ Sao Paulo, Brazil.**

**Background:**

We showed previously that L/H treatment arrests renal injury and reverses tubulointerstitial proliferation in Nx even when started at very late phases. Here we investigated further the mechanisms of this protection, focusing on 1) the role of hypertension (HTN), 2) the nephron segments involved in the antiproliferative action, 3) the mechanisms involved in this protection. We assessed the role of renin-angiotensin system (RAS), oxidative stress, inflammation, and fibrosis.

**Methods:**

- **Nx ab ac bc**: Male C57Bl/6J mice were subjected to 5/6 nephrectomy (Nx) and treated with darbepoetin (0.1 μg/kg sc) and losartan-HCTZ (25 mg/kg/day) for 3 months. BS, ACR, Hct, GS, and fibrosis were assessed.

**Results:**

- At wk8 after 5/6Nx, BS, ACR, Hct and GS were similar among groups by study design. Survival at wk12 was higher in all treatment groups compared to VEH (172±11 mmHg). Hct was significantly decreased in VEH and DAR (94±9 and 114±8 mmHg), but increased in DAR (91±10 mmHg) compared to VEH (172±11 mmHg). Hct was significantly decreased in VEH and DAR (35±3.7 and 38±2.5%), but ARB (37±2.6%) and combination (40±1.5%) prevented anemia. ARB and DAR+ARB reduced proteinuria (ACR 28.6±9.8 and 29.9±13.8 mg/day), compared to VEH and DAR (104±440 and 812±263 mg/24h). GS showed progression in untreated VEH (mean, 1±0.3 at wk12, 9±1.5% increase from biopsy). DAR alone had no effect on progression (90±38% increase GS). In contrast, ARB showed significant less sclerosis (30±25% increase, with regression of sclerosis in mice at wk12 vs wk8), and DAR enhanced this effect (0±23% increase, regression in 5 mice). Podocyte number (WT-1/glomerulus) in VEH was 4.7±0.6 with no benefit of DAR alone (5.7±0.4). In contrast, ARB and DAR+ARB increased podocyte number (6.5±0.4 vs 6.0±0.0). In contrast to VEH (172±11 mmHg), ARB and DAR+ARB showed significantly less progression (90±38% increase GS). In contrast, ARB showed significant less sclerosis (30±25% increase, with regression of sclerosis in mice at wk12 vs wk8), and DAR enhanced this effect (0±23% increase, regression in 5 mice).

**Conclusions:**

Low dose darbepoetin per se has no effect on progression, but in combination with high dose ARB halts progressive renal injury and enhances regression of glomerulosclerosis.

**Funding:**

Pharmaceutical Company Support

**SA-PO2769**

**Deletion of the Ace2 Gene Exacerbates Kidney Injury in Mice after Unilateral Ureteral Obstruction**

Fei Fang, James W. Scholey, George Chu, James R. Sowers.

**Background:**

Angiotensin II (AngII) plays a major role in the regulation of blood pressure and renal function. However, the mechanisms for linking AngII to the generation of the superoxide free radical are not well known.

**Hypothesis:**

Ang II mediated oxidative stress via transactivation of the EGFR pathway.

**Methods:**

A spermox proximal tubule cell line (OKP) was used as a model system to study the signaling mechanisms activated by AngII. 24-hour starved OKP cells were treated over a time course of 5 min to 24h. The 10min and 24hr timepoints were chosen to look at the acute and chronic effects of AngII. Superoxide generation was measured by lucigenin assays while NADPH oxidase activity was measured via Cayman radical assay.

**Protein measurements were via Western blots and immunoprecipitation. siRNA assays were performed according to the manufacturer (SA Biosciences).**

**Results:**

- Ang II stimulated superoxide production in a biphasic manner with 2 peaks at 50nM and 500nM. This was primarily via activation of NADPH oxidase enzyme. Ang II transactivated the EGFR receptor and led to further activation of ERK. Blockade of EGFR with AG1478 and siRNA reversed Ang II-mediated activation of oxidative stress. Further, blockade of Ang II-mediated oxidative stress improves albumin endocytosis via impairment in mTORC1 activation.

**Conclusions:**

Ang II mediates oxidative stress in the kidney proximal tubule primarily via transactivation of the EGFR. Generation of oxidative stress and alteration in mTORC1 function via Ang II may explain Ang II-mediated proteinuria in the proximal tubule level.

**Funding:**

Private Foundation Support

**SA-PO2770**

**Experimental CKD: Fibrosis and Inflammation**

**Poster/Saturday**

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**Results:**

- Deletion of the Ace2 gene exacerbated kidney injury in mice subjected to UUO.
Inhibitory Effect of Spironolactone (SPL) on Renal Injury and Intrarenal Angiotensinogen (AGT) Expression in Salt-Loaded, Nitrative Oxide-Deficient Rat

Kazuhiko Tsuruya, Toshiaki Nakano, Masatomo in Mice with Unilateral Ureteral Obstruction

Endogenous Angiotensin-(1-7) Protects Against Tubulointerstitial Fibrosis

Background: Intrarenal renin-angiotensin system (RAS) may be involved in the pathogenesis of kidney injury, and AGT is thought to have an important role for the intrarenal RAS activation. The effect of RAS blockade for intrarenal RAS is evaluated in some animal models, but little is known about mineralocorticoid receptor (MR) blockade. Therefore, the current study investigated the effect of MR blocker for kidney injury and regeneration in nitrergic oxide-deficient rats with salt loading, and to evaluate the relationship between kidney damage and AGT.

Methods: Male Wistar rats were divided into the following three groups; the control rats given vehicle (Cont), L-NAME rats given 50 mg/kg/day L-NAME in drinking water and 4% salt in chow (L-NAME), and SPL-treated L-NAME rats given 100 mg/kg/day SPL chow in addition to L-NAME rats (SPL).

Results: Following treatment for 6 weeks, L-NAME rats developed hypertension, proteinuria and kidney injury. Without reduction of blood pressure (BP), SPL decreased proteinuria, (14, 112, and 27 mg/day in Cont, L-NAME, and SPL, respectively; p<0.05) glomerulosclerosis, interstitial fibrosis (IF), osteopontin (OPN) expression determined by real time PCR and immunohistochemistry, and urinary excretion of 8-OHdG. Plasma angiotensinogen and aldosterone (Ald) were comparable among the three groups, but urinary AGT excretion (208, 6697, and 1317 ng/day in Cont, L-NAME, and SPL, respectively; p<0.05) and AGT expression in the proximal tubules were increased in L-NAME rats, while these changes were inhibited by SPL. Urinary AGT was positively correlated with proteinuria and OPN expression (r = 0.87, 0.69, and 0.73, respectively; p<0.05).

Conclusions: In conclusion, the present study demonstrated that SPL ameliorated kidney injury through reduction of AGT expression in the kidney independent of plasma Ald and BP, and intrarenal AGT was related to renal injury. These results suggest that intrarenal RAS may be involved in the worsening of kidney injury potentially involved in the kidney injury during nitric oxide deficiency and salt-loaded status.

Ghrelin Suppresses Angiotensin II-Induced Premature Renal Senescence by Reducing Oxidative Stress

Keiko Fujimura, Shu Wakino, Koichi Hayashi

Department of Medicine, Keio University, Tokyo, Japan.

Background: Premature senescence is one of the main pathways towards the deterioration of renal function. Angiotensin II (AngII) induces renal premature senescence by multiple mechanisms including by increasing oxidative stress. Recent study revealed that GH secretagogue ghrelin exerts anti-senescence effects by reducing mitochondria-derived reactive oxygen species (ROS) levels. In this study, we examined whether ghrelin inhibits AngII-induced renal senescence and damages.

Methods: Renal senescence was induced by infusion of AngII in C57BL/6 mice with osmotic mini-pump. Ghrelin was administered by the daily intraperitoneal injection. 8 weeks after the treatment, kidneys were removed and utilized in the various experiments. In vitro experiment, cultured human proximal cell line, HK-2 cells were incubated with 1 mM of AngII for 72 hrs and ghrelin were administered 30 mins prior to AngII stimulation.

Results: AngII infusion induces senescence and oxidative stress as assessed by senescence-associated (SA) β-Gal and 4-hydroxy-2-nonenal (4-HNE) staining, respectively. The expressions of markers for senescence, p21, p53 as well as senescence-associate cytokines, TGF-β, p16 INK-4A, were increased in kidney tissue and in HK-2 cells. These changes were attenuated by the treatment with ghrelin. In HK-2 cells, the receptors for both ghrelin and AngII were expressed. SA-β-Gal assay revealed tubular cell senescence by AngII. AngII also increased the expression of p21, p53 as well as senescence-associate cytokines, TGF-β, p16 INK-4A, and were attenuated by the treatment with ghrelin. In addition, AngII reduced the mitochondria number, which was restored by ghrelin as measured by staining cells with mitochondria-specific fluorescent dye.

Conclusions: Our data indicated that ghrelin suppressed AngII-induced renal premature senescence and AngII-induced renal damages presumably through modulating the mitochondrial ROS production.

Endogenous Angiotensin-(1-7) Protects Against Tubulointerstitial Fibrosis in Mice with Unilateral Ureteral Obstruction

Danielle L. Zimmerman, Adel Tarcsafalvi, Robert L. Safirstein, Peter M. Price

Department of Medicine, Keio University, Tokyo, Japan.

Background: Angiotensin II (AngII) induces renal premature senescence and damages as assessed by senescence-associated (SA) β-Gal and 4-hydroxy-2-nonenal (4-HNE) staining, respectively. The expressions of markers for senescence, p21, p53 as well as senescence-associate cytokines, TGF-β, p16 INK-4A, were increased in kidney tissue and in HK-2 cells. These changes were attenuated by the treatment with ghrelin. In HK-2 cells, the receptors for both ghrelin and AngII were expressed. SA-β-Gal assay revealed tubular cell senescence by AngII. AngII also increased the expression of p21, p53 as well as senescence-associate cytokines, TGF-β, p16 INK-4A, and were attenuated by the treatment with ghrelin. In addition, AngII reduced the mitochondria number, which was restored by ghrelin as measured by staining cells with mitochondria-specific fluorescent dye.

Conclusions: Our data indicated that ghrelin suppressed AngII-induced renal premature senescence and AngII-induced renal damages presumably through modulating the mitochondrial ROS production.

Dependence of Renal Fibrosis on a Cell Cycle Regulator

Judit Megyvari, Adel Tarcsafalvi, Robert L. Safirstein, Peter M. Price

Internal Medicine, UAMS, Little Rock, AR.

Background: A major cause of progressive renal failure occurs during repair from kidney injury that results in the formation of fibrotic lesions rather than tissue replacement. The molecular causes of this maladaptive growth are not yet understood, but recent studies have suggested potential pathways.

Methods: We used several mouse models of renal fibrosis, either mimicking effects of acute kidney injury or effects of loss of renal mass.

Results: A mouse model of subtotal nephrectomy induced parameters of chronic kidney failure, i.e., systemic hypertension, glomerular sclerosis, interstitial fibrosis, and reduced glomerular filtration. Fibrosis was evident 6 weeks after nephrectomy and functional renal failure occurred 14-16 weeks after surgery. A cell cycle regulatory protein, p21, was increased in the remnant kidney, accompanied by hypertrophy in the tubular epithelial cells. In homogous p21 knockout mice, the filtration rate of the remaining nephrons did not decline, and sclerosis did not develop. We have since developed transgenic mice in which p21 is knocked out by the proximal tubules. We hypothesized that p21 overexpression would induce more severe fibrosis and tested this in models of AKI in which renal fibrosis developed several weeks after injury. Comparing wild-type with transgenic mice 14 days after unilateral ureteral obstruction, more α-smooth muscle actin was seen around the kidney tubules of transgenic mice. Similarly, more macrophage infiltration was seen in p21 mice. We also used an in vitro model of fibrosis using 0.5 mg/ml aristolochic acid treatment of mouse proximal tubule cells (TKPTS) in which 6.3 mg collagen was secreted by the cells per ml of medium after 48 hours. p21 regulates the intracellular signaling of transforming growth factor-α into S and M phases, and the effect of p21 on collagen production was evidenced by the TFE's cells being arrested in G2 phase preceding collagen induction.

Conclusions: We conclude that p21 induction is a causative mechanism for development of fibrosis and chronic renal failure, either from its activity to prevent cell cycle progression or from its activity to inhibit phosphorylation of pro-fibrotic substrates of cell cycle kinases.

Attenuated Glomerular Arginine Transport Provokes Renal Injury in the Pregnant Uremic Rat

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Background: Pregnancy worsens renal function in females with renal failure (CRF) through an unknown mechanism. Reduced nitric oxide (NO) generation induces renal injury. Arginine transport by cationic amino acid transporter-1 (CAT-1), which governs arginine perfusion into the renal cells, has been suggested that attenuated maternal glomerular arginine transport promotes renal damage in CRF pregnant rats.

Methods: Rats underwent a two stage 5/6 nephrectomy (interval of one week). All experiments were performed 4 weeks post nephrectomy.

Results: In ureric rats, pregnancy induced a significant decrease in glomerular arginine transport and e-GMP generation (a measure of NO production) compared to CRF or pregnancy alone and these effects were prevented by L-arginine. While CAT-1 abundance was increased in all experimental groups, PKGs and glibenclamide-induced PGC (as CAT-1 inhibitor), were significantly augmented in CRF, pregnant, and pregnant CRF animals, phenomena which were prevented by co-administrating L-arginine. α-tocopherol (PKC inhibitor) significantly increased arginine transport in both pregnant and CRF pregnant animals which were attenuated by ex vivo incubation of glomeruli (as PKC stimulant). Renal histology revealed no differences between all experimental groups. Creatinine clearance failure to augmented and renal cortical expression of HIF-1α significantly increased in CRF pregnant rat, both phenomena were prevented by arginine.
Conclusions: These studies suggest that in CRF rats, pregnancy produces a profound deleterious effect on renal function, through post-translational regulation of CAT-1 by PKCα, resulting in attenuated NO generation. These events provoke renal damage manifested by upregulation of renal HIF-1α and loss of the ability to increase GFR during gestation and may be responsible for the accelerated deterioration in renal function during pregnancy in uricemic females.

Funding: Government Support - Non-U.S.

SA-PO2274
Periostin is a Novel Marker of the Severity of Hypertensive Nephropathy
Dominique Guerret, Sandrine Placier, Mouna Maal-Aitni, Jean-Claude Dussault, Christos Chatziantoniou. INSERM UMR 702, Tenon Hospital, Paris, France.

Background: The aim of our study was to identify novel determinants of progression and regression of chronic kidney disease.

Methods: Rats were treated with the NO synthase inhibitor L-NAME. When proteinuria exceeded 1 g/mmol creatinine (5–6 wk), a group of animals was used to estimate renal hemodynamics and morphologic parameters just before the beginning of therapy (LN 6w group). The remaining animals were divided into two groups for an additional experimental period of 4 wk: in the first group L-NAME was given alone (LN 10w); in the other group L-NAME was co-administered with Losartan. At the end of Losartan treatment, some animals showed normal creatinineinmia (REGR), whereas other animals escaped therapy (creatininemia ≥70 µmol/l, ESC group).

Results: Differential transcriptomic analysis of renal cortex between REGR and LN 10w groups revealed that periostin, a gene non expressed in normal kidneys (but strongly involved in fibrosis and bone formation) was upregulated in the ESC group. Quantitative evaluation of periostin mRNA and protein expressions in the kidney indicated that periostin was continuously increasing during hypertensive nephropathy, it was significantly decreased in the REG group, whereas it remained increased in the ESC group in spite of 4 wk losartan treatment. Immunohistochemistry revealed that hypertensive nephropathy was characterized by a strong increase in periostin staining; in the media and the adventitia of renal vessels. Interestingly periostin also exhibited a focal de novo interstitial expression in close vicinity to the most severe lesions.

To further evaluate the relevance of periostin as a marker of progression of renal disease, we performed regression analyses with classical functional and histological parameters of hypertensive nephropathy. We found a very strong positive association (r²=0.70) between periostin expression and creatininemia, proteinuria, renal blood flow decrease, and histological lesion scores.

Conclusions: Periostin expression in the kidney is associated to the development of hypertensive renal disease. We propose periostin as a novel marker determining progression/regression of chronic kidney disease.

Funding: Government Support - Non-U.S.

SA-PO2275
Epithelial Growth Plate Growth Hormone Receptor Signaling Is Impaired in a Rat Model of Chronic Kidney Disease Related Growth Retardation
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Background: Linear growth retardation with reduced muscle mass is a major problem in children and adults with chronic kidney disease (CKD) and is ascribed to insensitivity of GH action. We investigated the role of local GH-IGF system in children and adults with chronic kidney disease (CKD) and is ascribed to insensitivity of GH action in close vicinity to the most severe lesions.

Results: There was no difference in the amount of food consumed, yet there was a significant difference in weight gain and longitudinal growth between C and CKD rats. Serum creatinine (225±11% of C) and albuminuria were significantly higher in CKD rats. No change was observed in serum GH, yet serum IGFBP was decreased significantly in CKD. Epithelial growth plate (EGP) morphology showed no change in the proliferative zone but a wider hypertrophic zone in CKD rats, with a disturbance in cell order (but not in cell count) in that zone. EGP type 10 collagen mRNA increased in CKD (375±56% of C; p=0.05). EGP GH receptor (GH) mRNA was unchanged, but JAK2 mRNA and phosphorylated (p-)STAT5 were decreased, while SOCS2 mRNA was increased in CKD. IGFBP mRNA levels were unchanged.

Conclusions: Young rats with moderate CKD develop significant growth retardation despite the enlargement of EGP hypertrophic zone, hinting for a chondrocyte maturation arrest. The decrease in EGP 10 collagen and the increase in SOCS2 mRNA in the EGP of CKD rats in spite of unchanged GHR mRNA suggest a bone GHR signaling impairment in CKD as an additional mechanism of CKD mediated growth retardation.

Funding: Government Support - Non-U.S.

SA-PO2276
AZGP1 Inhibits the Development of Renal Fibrosis Inga Soeremens, Nathan D. Susnik, Hermann G. Haller, Roland Schmitt. Nephropathy and Hypertension, Hannover Medical School, Hannover, Germany.

Background: With age the kidney becomes more susceptible to acute and chronic injury and loses its regenerative potential. Additionally, old kidneys show more interstitial fibrosis and tubular atrophy. Zinc-alpha-2-glycoprotein 1 (AZGP1) is a secreted glycoprotein which in young kidney was not yet been fully elucidated. In vivo knockdown of AZGP1 in old mice was associated with increased epithelial proliferation and amplification of interstitial fibrosis. Therefore, our hypothesis was that AZGP1 has an antifibrotic effect.

Methods: UUO as a common fibrosis model was induced in AZGP1−/− and AZGP1+/+ mice. At different time-points kidneys were analyzed by hemodynamics, immunohistochemistry, qPCR and immunoblot. For in vitro studies we used different renal epithelial cell lines and rat renal fibroblasts. Epithelial cells were transiently transfected with an AZGP1 expression construct. The resulting conditioned medium was transferred to rat renal fibroblasts. The expression of myofibroblast markers was analysed using immunoblot and qPCR.

Results: Renal fibrosis developed in both groups of mice but AZGP1 deficiency was associated with a significant attenuation of renal tubulointerstitial damage. We found that α-Smooth muscle actin, as a marker for fibrosis, was significantly higher in UUO kidneys of AZGP1−/− mice. Additionally we found a significant reduction of the epithelial marker E-Cadherin in AZGP1−/− UUO kidneys at one and two weeks, whereas Vimentin was highly upregulated on protein and mRNA level. The number of infiltrating inflammatory (CD45+) cells were elevated in AZGP1−/− kidneys. In vitro we found that the conditioned medium obtained from AZGP1 overexpressing epithelial cells contains factors that inhibit TGFβ-dependent activation of fibroblasts.

Conclusions: In summary our data indicate that epithelial expression of AZGP1 ameliorates the development of fibrosis in the UUO model. In vitro studies suggest that the secretome of AZGP1 overexpressing renal epithelial cells exhibits an inhibitory effect on the activation of renal fibroblasts.

Funding: Government Support - Non-U.S.

SA-PO2277
The Role of Autophagy in Unilateral Ureteral Obstruction Rat Model Yong Kun Kim, 1Wan-Young Kim, 2Sun-Ah Nam, 3Ho Cheol Song, 4Euy Jin Choi, 5Jin Kim, 61Internal Medicine, Catholic University of Korea, Seoul, Korea; 2Department of Anatomy and MRC for Cell Death Disease Research Center, Catholic University of Korea, Seoul.

Background: Autophagy is a cellular process of degradation of damaged cytoplasmic components and regulates cell death or proliferation. Unilateral ureteral obstruction (UUO) is used as a model of progressive renal fibrosis in the obstructed kidney. And UUO is followed by compensatory cellular proliferation in the contralateral kidney. We investigate the role of autophagy in the obstructed kidney and contralateral kidney after UUO.

Methods: To obtain the evidence and the patterns of autophagy during UUO, the rats were sacrificed 3, 7, and 14 days after UUO. To examine the efficacy of the autophagy inhibitors, 3-MA , the rats were treated with 3-MA. The rats were divided into the four groups: (1) the rats that underwent sham operation and were treated with vehicle, (2) the rats that underwent sham operation and were treated with 3-MA (3) the rats that underwent UUO and were treated with vehicle, (4) the rats that underwent UUO and were treated with 3-MA. The rats were sacrificed 7 days after UUO.

Results: After UUO, autophagy was induced in the obstructed kidney in a time-dependent manner. Autophagy is induced in the obstructed kidney early after UUO and before tubular cell apoptosis and tubulointerstitial fibrosis and then it decreased toward the basal levels at day 14 after UUO. Inhibition of autophagy by 3-MA enhances tubular cell apoptosis and tubulointerstitial fibrosis in the obstructed kidney. In the contralateral kidney, autophagy was also induced and prolonged during UUO. Inhibition of autophagy by 3-MA increased the protein expression of proliferating cell nuclear antigen significantly in the contralateral kidney. The Akt-mammalian target of rapamycin (m-TOR) signaling pathway was involved in the induction of autophagy after UUO in both kidneys.

Conclusions: Taken together, our present results support that autophagy induced by UUO has a nonprotective role in the obstructed kidney and regulatory role of compensatory cellular proliferation in the contralateral kidney through Akt-mTOR signaling pathway.

Funding: Government Support - Other.

SA-PO2278
Epithelial-to-Mesenchymal Transition of Tubular Epithelial Cells Associates with Deciliation and Loss of Claudin-2 Expression Punita Dhawan, Amar B. Singh. Vanderbilt University.

Background: After sustained injury, tubular epithelial cells undergo epithelial-to-mesenchymal transition (EMT), which plays critical role in the evolution of renal fibrosis. Loss of functional tight junction (TJ) and polarity characterizes EMT. Tight Junction helps maintain polarity, which also appears necessary for ciliogenesis. Importantly, proteins that regulate polarity and/or ciliogenesis are localized at the TJ. However, role of TJ integral protein claudin-2, in renal injury and associated EMT is poorly understood.

Methods: MDCK-II cells were subjected to chronic hypoxia (1% O2). To induce EMT MDCK-II cells were also subjected to chronic EGFR (EGFR) activation. To silence Claudin-2 expression anti-claudin-2 shRNA was used.

Results: Exposure of MDCK-II cells to chronic hypoxia led to the EMT. Cells lost cilia and epithelial morphology. E-cadherin expression decreased while Vimentin expression increased. Membrane distribution of Na-K-ATPase was lost while apical permeability

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
MicroRNA-34a is a new mediator of kidney fibrosis in progressive nephropathy and a potential therapeutic target of ACE inhibition.

**Background:** MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate target gene expression at the posttranscriptional level. The role and mechanisms of miRNAs in kidney tubular cell EMT and renal scar formation after injury remain unclear. MiRNAs play an important role in regulating cell fate and protein expression during cell transformation and differentiation.

**Aims:** To investigate the role of miR-34a during hypoxic renal tubular cells EMT. MiR-34a was identified as a downregulated miRNA in hypoxic renal tubular cells. MiR-34a expression was significantly lower in HK-2 cells transfected with miR-34a mimics compared to those transfected with the scrambled miRNA (NC).

**Methods:** MiR-34a expression was measured by real-time RT-PCR and western blotting. MiR-34a mimics and NC were transfected into HK-2 cells using lipofectamine. Cell morphology, proliferation, and differentiation were assessed by using the Cell Counting Kit-8 (CCK-8) and the DAPI assay, respectively.

**Results:** MiR-34a expression was downregulated in hypoxic HK-2 cells. Transfection with miR-34a mimics reversed the downregulation of miR-34a and increased the expression of epithelial markers, such as E-cadherin and occludin, while decreasing the expression of mesenchymal markers, such as α-SMA and vimentin.

**Conclusions:** MiR-34a is a new mediator of kidney fibrosis in progressive nephropathy and a potential therapeutic target of ACE inhibition.
Conclusions: Our data suggest that SOCS-3 regulates STAT1/3 phosphorylation in renal tissue and that pSTAT3 stimulates epithelial differentiation and proliferation while pSTAT1 might have a dominant role in inhibition of these cellular functions in HK-2 cells. Downregulation of SOCS-3 and following activation of STAT1 signaling may inhibit proliferation and epithelial differentiation leading to progressive decline of renal function.

SA-PO2784

Tubular Deficiency of von Hippel-Lindau Attenuates Renal Disease Progression in the Anti-GBM Glomerulonephritis

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Background: In many kidney diseases, the original insult primarily involves the glomerulus and may then pass onto the tubulointerstitium. Several hypothesis link the glomerular disease to tubular injury, one of the foremost is the chronic tubular hypoxia. The effects of hypoxia and consecutive stabilization of hypoxia-inducible factors (HIF) however, are discussed controversially. Hypoxia was shown to induce interstitial fibrosis but also to have beneficial effects on renal disease progression when HIF was activated pharmacologically.

Methods: To analyze the impact of HIF’s on the tubulointerstitial disease development in a primary glomerular affection transgenic von Hippel Lindau (VHL) knockout mice were generated and induced before the onset of an anti-glomerular basement membrane glomerulonephritis (GN).

Results: Tubular VHL-knockout and therefore local HIF-a stabilization increased renal production of VEGF, TGF-b, and PDGF-B resulting in augmented formation of capillaries, interstitial matrix and conversion of fibroblasts into myofibroblasts. Within the glomerulonephritis disease VHL-knockout reduced the glomerular damage and attenuated tubulointerstitial injury albeit similar characteristics. Likewise, proteinuria, plasma urea concentration and tubulointerstitial matrix were decreased in VHL-knockout with GN.

Conclusions: These findings demonstrate that tubular HIF-a stabilization in a glomerulonephritis disease is beneficial for the disease outcome. In comparison to VHL-knockout alone GN is a much stronger activator of fibrosis so that other stimuli than hypoxia may be considered for renal disease progression.

Funding: Government Support - Non-U.S.

SA-PO2785

Aspirin Inhibits the Development of Vascular Calcification through Induction of Heat Shock Protein 72 in Human Aortic Smooth Muscle Cells

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Background: Vascular calcification (VC) is a significant contributor to cardiovascular mortality in patients with chronic kidney disease (CKD) and coronary artery disease (CAD). Acetylsalicylic acid (Aspirin, ASA) is a widely used medication in artery intima disease. However, the role of ASA in the development of VC is unknown. There is emerging evidence that ASA could function as an inducer of the cardio-protective factor, heat shock protein 72 (HSP72).

Methods: Human vascular smooth muscle cells were treated with control or calcification medium containing 5mm calcium chloride and 5mm β-glycerolphosphate with/without ASA (4mM) treatment for 21 days.

Results: We have shown for the first time that ASA can inhibit the development of VC through the induction of HSP72 in our long-term VC model, in vitro. Furthermore, heat shock treatment (HST), an established inducer of HSP72 significantly inhibited the development of VC. Anti-calcific effects of both ASA and HST were abolished by HSP72.

Conclusions: These findings demonstrate that tubular HIF-a stabilization in a glomerulonephritis disease is beneficial for the disease outcome. In comparison to VHL-knockout alone GN is a much stronger activator of fibrosis so that other stimuli than hypoxia may be considered for renal disease progression.

Funding: Government Support - Non-U.S.

SA-PO2786

AMPK Activity as a Biomarker of CKD Status and Its Association with Metformin’s Effects on CKD

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Background: Our previous studies have shown that both Angiotensin II blockade (AIIB) and hypoxia inducible factor induction with DMOG can significantly correct renal metabolic deficiency and improve renal function in rat renal ablation/infarction (A/I) model of chronic kidney disease(CKD). In this study, we investigated the regulation of AMPK activity in the CKD model as well as the therapeutic effects of metformin, an activator of AMPK.

Methods: The rat A/I model of CKD was produced by removing the right kidney and ligating two branches of the left renal artery. AMPK activity (p-AMPK-α) was tested by Western blot. GFR, RBF, renal oxygen consumption (QO2) and sodium transport(TNa) were measured. An increase in QO2/TNa indicates renal metabolic deficiency.

Results: Untreated A/I rats showed reductions of GFR, RBF and renal metabolic efficiency, accompanied with a major decrease in AMPK activity, i.e. a reduction of p-AMPK-α(n=0.001). AIIB and DMOG both improved renal function, corrected renal metabolic deficiency and normalized AMPK activity (P=0.001). Metformin also normalized AMPK activity (P=0.001), while significantly correcting renal metabolic deficiency and improving kidney function.

The effects of 3 different treatments on renal function, renal metabolic efficiency in A/I rats

Funding: NIDDK Support

SA-PO2787

NLRP3 Functions in Renal Tubular Epithelial-Mesenchymal Transition Independent of Cytokine Production

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Background: Intestinal inflammation and fibrosis are strongly associated with the outcome of chronic kidney disease. We recently demonstrated that the NOD-like receptor containing a pyrin domain 3 (NLRP3) not only mediates renal inflammation but also potentially plays a direct role in fibrogenesis. We investigated whether NLRP3 plays a role in tubular epithelial-mesenchymal transition (EMT).

Methods: Human proximal and murine tubular epithelial cells were cultured in the presence or absence of transforming growth factor-β (TGF-β, 10ng/ml). Tubular EMT was determined by the expression of α-smooth muscle actin and matrix metalloproteinase. NLRP3 and MMP gene transcription and expression were assessed by semi-quantitative PCR or western blotting. NLRP3, apoptosis-associated speck-like protein containing a CARD domain (ASC), Caspase-1 and MyD88 knockout tubular epithelial cell was studied to determine the contribution of inflammasome formation and proinflammatory cytokine on MMP expression. In vivo progressive fibrosis model of unilateral ureteric obstruction was carried out in NLRP3-/- or littermate mice. After 14 days, renal MMP activity and histological change were analyzed.

Results: TGF-β induced tubular EMT and concomitant NLRP3 expression in a time-dependent fashion as evident by the de novo expression of α-smooth muscle actin and upregulation of both MMP-2 and 9. The induction of MMP-9 was decreased in both NLRP3 and ASC knockout tubular epithelial cell, suggesting an essential role for NLRP3 in tubular EMT. The effect of NLRP3 and ASC on MMP-9 expression was inflammationindependent as neither MyD88 or Caspase-1 knockout, nor adding IL-18 to NLRP3 tubular epithelial cell affected MMP-9 expression. Finally, NLRP3 knockout mice demonstrated decreased MMP-9 expression and developed less fibrosis compared to wild type controls after unilateral ureteric obstruction.

Conclusions: These data suggest a role for epithelial NLRP3 in promoting renal fibrosis independent of the inflammasome formation and also independent of subsequent pro-inflammatory cytokine production.
Effects of the Receptor Tyrosine Kinases Inhibitor BIBF1000 on Renal Fibrosis in Rodents

Steven M. Weldon,1 Haichun Yang,1 Yiqin Zuo,2 Cheng-Kon Shih,1 Susan Goldberg,1 Hong Wang,1 Xiaoyu Jiang,1 Glenn A. Reinhart,1 Agnes B. Fogg,2 Carol DiSilvestro-Lacasse,2 Boehringer Ingelheim Pharm. Inc., Ridgefield, CT; Division of Renal Pathology, Vanderbilt University Medical Center, Nashville, TN.

Background: Tubular interstitial fibrosis (TIF) is a major factor contributing to the progression of chronic kidney disease (CKD) and subsequent renal failure. Presently only ACE inhibitors and ARBs are approved CKD therapy. We used unilateral ureteral obstruction (UUO) in rodents to study mechanisms and potential modulation of TIF. Receptor tyrosine kinase inhibitors (RTKIs) such as Gleevec (imatinib) are used to target activity in animal models including UUO. BIBF1000 (BIBF) is a novel RTKI that attenuates PDGF, VEGF and FGF signaling and fibrosis in the rat bleomycin lung fibrosis model but has not been evaluated in renal fibrosis. Therefore, we evaluated BIBF in the UUO model of renal fibrosis.

Methods: SD rats and C57BLKS mice were treated 1 day prior to ureter ligation with vehicle (VEH), enalapril (ENA, 30 mg/kg), Gleevec (GLE, 100 mg/kg) or BIBF (25 or 50 mg/kg) by gavage (5 days). TIF was assessed by sirius red morphometry (SRM) and a panel of fibrotic and inflammatory biomarkers (BM)s were evaluated by immunostaining (IHC) or qPCR (mRNA).

Results: In mice, BIBF attenuated TIF by 18.8 and 19.1% (p<0.05) for the 25 and 50 mg/kg doses, respectively. By comparison, GLE exhibited 17% inhibition while ENA showed only 7% inhibition. In rats, BIBF inhibited TIF in a dose-dependent manner, 16% and 25%, respectively. GLE reduced TIF by 21% and ENA by 13%. BIBF, GLE and ENA showed differential effects on fibrotic and inflammatory BMs in mice and rats. Finally, plasma levels of GLE were nearly identical in both mice and rats; ENA exhibited nearly 3-fold higher levels in rats while BIBF showed ~10-fold higher exposure in mice.

Conclusions: In conclusion, these are the first data to show a novel RTKI such as BIBF1000 attenuates renal tubulointerstitial fibrosis assessed quantitatively using SR morphometry. As such, RTK’s and downstream signaling pathways may offer a novel alternative for targeting fibrotic renal disease associated with CKD and subsequent renal failure.

Funding: Pharmaceutical Company Support

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Underline represents presenting author.

760A
SA-PO2795

Renal Growth Hormone Receptor Signaling Is Impaired in a Rat Model of Chronic Kidney Disease

Background: Linear growth retardation with reduced muscle mass is a major problem in children and adults with chronic kidney disease (CKD). It is thought to be mediated by an increase in chronic growth hormone-releasing factor (GHRF) or glucose oxidase per se induced phenotypic transition with a decrease in E-cadherin expression.

Conclusions: Taken together, oxidative stress induced by GDP seems to be one of the mechanisms of peritoneal damage in PD patients, and novel mitochondrial-targeted antioxidant can be a therapeutic option for preserving mesothelial phenotype.

This study was supported by the grant from Seoul R & D Program (ST09034M0214881).

SA-PO2793

Renal Mass Reduction (RMR) Impairs Recovery from Acute Kidney Injury (AKI) and Promotes the Mechanisms of Chronic Kidney Disease (CKD)

Methods: Sprague-Dawley rats were prepared for blood pressure radio telemetry (BP) and non-invasive RMR by uninephrectomy (UNx), UNx + excision of 2 poles of other kidney (3/4Nx), or no Nx. Two weeks later, AKI was induced by 40% ischemia/reperfusion (I/R) and rats followed for 4 wks post I/R.

Results: SCr and BP in I/R 3/4Nx rats were modestly increased. Without I/R, this effect of RMR was stable over 4 wks (not shown). Judged by SCr at 48h post I/R, AKI severity was similar in all groups. But, recovery was impaired in 3/4Nx relative to other groups (SCr 7d).

Pre I/R Post I/R (1st wk) Post I/R (4th wk)

Group SCr mg/dl BP mmHg Uprot mg/24h SCr, g/dl SCr, g/dl BP Uprot mg/24h

No Nx (n=10) 0.44±0.03* 114.1±9 4.6±0.4 4.6±0.4 0.8±0.07 119.8±19 6.1±0.7

UNx (n=12) 0.60±0.04* 109.2±2 4.5±0.3 4.3±0.4 0.9±0.05 120.3±8 23.1±12.4 3/4Nx (n=12) 0.85±0.04* 130.4±3 4.6±1.2 4.5±0.3 1.8±0.2* 152.8±2 57.6±14.5

meanSCr:* p < 0.05 vs all other groups. BP and Uprot data 1st wk post I/R not shown

SCr remained significantly higher than pre I/R values over 4 wks in 3/4Nx rats; this was accompanied by markedly increased Uprot. Overall, the severity of tubulo-interstitial fibrosis correlated better with SCr and BP than with Uprot. Data in UNx rats showed similar trends but exhibited much greater variability.

Conclusions: Repair is less complete and fibrosis more severe after ischemic AKI in the setting of severe RMR. By reducing functional renal mass further, such tubulointerstitial fibrosis after AKI accelerates the development of hypertension and augments proteinsuria, setting the stage for CKD progression. Our data suggest that RMR models are useful to investigate the mechanisms responsible for incomplete repair, regeneration and fibrosis in CKD progression after AKI.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2794

The Role of Erk Activation in the Subtotal Nephrectomy Model of Kidney Fibrosis

Background: The Ras-Mek-Erk signalling pathway plays a key role in many cellular pathways underpinning kidney fibrosis including cell proliferation, apoptosis, epithelial to mesenchymal transition and accumulation of extracellular matrix proteins. Phospho-Erk (p-Erk) is upregulated in fibrotic kidney disease and in vitro data suggests Erk activation amplifies TGF-β mediated pro-fibrotic effects. We hypothesised that Erk is a therapeutic target in fibrotic kidney disease and the MEK inhibitor CI-1040 would attenuate renal fibrosis in the subtotal nephrectomy (SNx) model.

Methods: Male Wistar rats were subjected to either 5/6 SNx or sham operation under isoanaesthesia. SNx rats received intraperitoneal injections of either CI-1040 (100mg/kg/day) or vehicle alone from day 1. Animals were sacrificed 130 days later. Renal function was assessed by serum creatinine, creatinine clearance and urinary protein excretion. Fibrosis and myofibroblast number were assessed by Masson's Trichrome and α-SMA staining. Immunohistochemistry and western blotting for p-Erk was performed on lymphocyte and kidney homogenates. Cell proliferation was assessed by BrdU uptake.

Results: A 5-fold upregulation of p-Erk levels (p<0.0001) was observed in renal tissue and lymph node tissues that received SNx compared with CI-1040 almost completely inhibited p-Erk activity in the kidney (4.78 ± 0.8 vs 0.14 ± 0.1 OD/mm2, p=0.001) and in lymphocytes. Despite inhibition of p-Erk, CI-1040 had no effect on either the development of renal fibrosis (17.3 ± 3.6 vs 24.6 ± 4.6%) or the increase in α-SMA positive cells (17.1 ± 3.3 vs 20.8 ± 4.5%). Further CI-1040 had no impact on creatinine clearance, proteinuria or on cell proliferation.

Conclusions: Erk activity is significantly upregulated following SNx yet complete inhibition of Erk activity by CI-1040 had no impact on tissue fibrosis, fibrosis or myofibroblast number. This suggests that kidney fibrosis in SNx is not dependent on Erk activation. It is possible that upregulation of other mitogen activated protein kinases (MAPKs) pathway may compensate for the loss of Erk activation and future work will examine activity of JNK and p38 MAPKs.

Funding: NIDDK Support, Veterans Administration Support
in the perpetuation of this fibroblast activation. Little is known, however, on (i) which Dmnts are involved and (ii) what their temporal expression is in the kidney during the development of fibrosis. Hereeto, the present study aimed at a time course evaluation of the expression of the most prominent Dmnt’s (Dmnt1, 3a and 3b) and the similar, but non-functional, Dmnt3L in two different mice models of fibrosis: aristolochic acid nephropathy and ischemia reperfusion (I/R) injury.

Methods: Mice (20-25g) received a single intraperitoneal injection of aristolochic acid I (AAI, 3.5 mg/kg) or PBS or underwent unilateral left ischemia during 30 minutes. Mice were sacrificed after 1, 3, 6 and 12 weeks. The expression level of Dmnt1, 3a, 3b and 3L was determined by PCR or Real-time PCR on total renal tissue mRNA.

Results: All Dmnt’s were significantly upregulated during the development of renal fibrosis in both animal models from week 1 on, reaching maximum expression levels 3 weeks after AAi injection and 6 weeks after reperfusion. At week 12, when fibrosis was most prominent, the expression of the Dmnt’s decreased towards control levels in both models.

Conclusions: Dmnt 1, 3a, 3b and 3L are all upregulated shortly, but not permanently, after AAi injection or I/R injury, supporting a transient role for these enzymes in the development of renal fibrosis following both an acute toxic and ischemic insult. Interestingly, these findings contrast the recently reported observation that only Dmnt1 (but not Dmnt3a and Dmnt3b) was upregulated in a folic acid induced mouse model of renal fibrosis (Bechtel et al. Nat. Med. 2010;16(5):544). Our data, therefore, indicate that the involvement of Dmnt’s might depend on the stage of renal histopathology and/or the type of insult.

Funding: Private Foundation Support

SA-PO2798

Indoxyl Sulfate induces Cbp/p300-Interacting Transactivator, with GLu/Asp-Rich Carboxy-Terminal Domain, 2, and Impairs Hypoxia Response in Proximal Tubular Cells

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Background: Hypoxia in the tubulointerstitium is common in progressive renal diseases. Hypoxia-inducible factor (HIF) is a heterodimeric transcription factor composed of α- and β-subunits and controls the expression of genes involved in glycolysis, angiogenesis and erythropoiesis in hypoxia. Results from recent studies suggest that HIF, while obviously expressed, may be insufficiently activated in experimental chronic kidney disease (CKD models).

Methods: In this study, we investigated the effect of indoxyl sulfate (IS), a uremic toxin which accumulates in CKD patients and is responsible for renal pathology, on cellular HIF response to hypoxia in cultured proximal tubular cells. Hypoxia response by HIF-1 was measured by luciferase reporter assay. Quantitative changes in HIF-1α, Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2 (CITED2) and HIF-target genes were analyzed by real-time PCR and Western blotting. HIF-1 binding to the enhancer region was evaluated by chromatin immunoprecipitation (ChIP) assays. Protein interaction between HIF-1α and its coactivators was estimated by mammalian two-hybrid assays.

Results: Transient transfection with the hypoxia-responsive luciferase reporter (HREhuc) revealed that IS impaired hypoxia response in HK-2 cells, which was associated with a decrease in the expression of HIF-1 target genes. Immunoblotting using whole cell lysates demonstrated no significant changes in HIF-1α expression, and ChIP assays revealed no effect of IS on HIF-1 binding to the promoter region. However, IS induced the expression of CITED2 via post-transcriptional mechanisms and impaired activity of the HIF-1α C-terminal transactivator domain (CTAD) by blocking the recruitment of its coactivator p300.

Conclusions: Results of the present study uncover a novel mechanism through which IS dampens hypoxia response mediated by HIF-1, and may provide an insight into clinical problems such as insufficient erythropoietin (EPO) production in uremic patients.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2799

Role of HSP47 in Epithelial-Mesenchymal Transition of Renal Tubular Epithelial Cell and Extracellular Matrix Accumulation

Huixin Bi,1 Ruihong Liu,2 Lin Sun,2 You-Ming Peng,2 Fu-You Liu.1 1Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, Hunan, China; 2Departments of Pathology and Medicine, Northwestern University, Chicago, IL.

Background: Epithelial-mesenchymal transition (EMT) and extracellular matrix (ECM) accumulation play an important role in renal tubulointerstitial fibrosis. Previous studies have showed the close relationship between the increased expression of HSP47 and the progression of tubulointerstitial fibrosis. However whether HSP47 plays a role in the process of EMT and ECM accumulation is unclear.

Methods: HSP47 antisense oligonucleotides (ATG) (Merck) was introduced to the kidney with an ultrasound microbubbles system by injecting into a renal artery at the day of inducing UUO, since ODNs served as a control. Rats were sacrificed at day 14 after UUO or sham operation. The expressions of HSP47, vimentin, ZO-1 and Coll were detected by immunohistochemical, Western blot and Real-time PCR, respectively. In vitro studies, HK-2 cells were transfected with a 100ng/ml TGF-β1. Furthermore, HK-2 cells were transfected with HSP47 siRNA and siRNA negative control before exposing to TGF-β1. The expressions of HSP47, vimentin, ZO-1, Coll, Smaa3 and p-Smaa3 were examined by Western blot, Real-time PCR, and/or immunofluorescence, respectively.

Results: Compared to that in control, after treatment of HSP47 antisense ODNs resulted in an efficient and specific inhibition of HSP47 expression in the kidney of UUO model, the vimentin and Coll expression was significantly decreased and ZO-1 expression was significantly increased in the obstructed kidneys. Compared to the TGF-β1 group, inhibition of HSP47 expression in HK-2 cells up-regulated the expression of ZO-1 and down regulated the expressions of vimentin, Coll. Compared to the TGF-β1 group, inhibition of HSP47 expression down regulated the expression of p-Smaa3 in HK-2 cells.

Conclusions: HSP47 could promote the EMT of renal tubular epithelial cells in vivo and vitro via modulation the activation of Smaa3, and suppression of HSP47 would be a possible therapeutic target against tubulointerstitial fibrosis.

Funding: Government Support - Non-U.S.

SA-PO2800

TGF-β1 Mediates Macrophage-Induced Tubular Epithelial-to-Mesenchymal Transition in Fibrotic Nephropathy

Yang Cheng, Chunhui Dai, Junwei Yang, Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.

Background: Macrophage infiltration is a common feature of chronic kidney disease, which suggests a pathologic function for macrophage in renal fibrosis. Tubular epithelial cell spark renal fibrogenesis through epithelial-to-mesenchymal transition (EMT). Whether macrophage infiltration causes EMT and the exact underlying mechanisms remain to be determined.

Results: In this study, the effect of macrophage infiltration on kidney tubular EMT was investigated in unilateral ureteral obstruction (UUO) mice and cultured tubular cells. Macrophage infiltration was remarkably increased in the obstructive kidney. RT-PCR and ELISA analysis showed robust increased TGF-β1 mRNA expression and protein secretion in primary cultured macrophages isolated from UUO mice. Culture media from these activated macrophages could induce Smad signaling activation and EMT in tubular cells (NKK-52E), as demonstrated by loss of E-cadherin, de novo expression of α-SMA and upregulation of fibronectin, suggesting a critical role for activated macrophage in promoting EMT. Moreover, macrophage-induced Smaa3 phosphorylation and tubular EMT were largely abolished by TGF-β1 neutralization antibody.

Conclusions: Hence, these results indicate that TGF-β1 plays an essential role in macrophage-induced tubular EMT in fibrotic nephropathy.

Funding: Government Support - Non-U.S.

SA-PO2801

Transforming Growth Factor-β1 Receptor Inhibition Preserves Renal Tubular Mass and Reduces Formation of Atubular Glomeruli Following Ureteral Obstruction

Robert L. Chevalier,1 Michael S. Forbes,2 Barbara A. Thornhill,1 Dae-Kee Kim.1 1Department of Pediatrics, University of Virginia, Charlottesville, VA; 2College of Pharmacy, Ewha Womans University, Seoul, Korea.

Background: Chronic kidney disease is characterized by parenchymal loss and interstitial fibrosis. Unilateral ureteral obstruction (UUO) is the most widely used model of chronic kidney disease, and we have recently reported that murine UUO results in rapid formation of atubular glomeruli (ATG) (March 2011, doi:10.1152/ajprenal.00022.2011). Transforming growth factor-β1 (TGF-β1) is a central regulator of renal cell death and collagen deposition following UUO.

Methods: Mice were subjected to UUO and placement of subcutaneous pellets for the continuous release of vehicle or IN-130 (ALK3/TGF-β1 Type I receptor inhibitor), 30 mg/kg/day. Kidneys were harvested after 14 days, and proximal tubules, tubular atrophy and ATG were identified by Lotus tetragonolobus lectin. Collagen was identified by Sirius red. Relative distribution of renal parenchymal thickness, glomerular area, proximal tubular area, and interstitial collagen deposition were determined by quantitative histomorphometry.

Results: Weight and parenchymal thickness of the obstructed kidneys were 42% and 51% greater in IN1130-treated than control mice, respectively (p<0.05). Glomerular area was 27% greater in IN1130 than in control mice (p<0.05). The fraction of intact glomeruli was 42.5% in IN130-treated mice, but only 23.3% in controls (p<0.05); the volume fraction of proximal tubules was 30±5% with IN1130 vs. 18±2% in controls (p<0.05). Renal interstitial collagen accumulation was 2.9±0.3% with IN1130, but increased to 5.2±0.3% in controls (p<0.05).

Conclusions: We conclude that in addition to its role in interstitial fibrosis, TGF-β1 signaling regulates proximal tubular injury leading to the formation of ATG following UUO. ATG are formed in many renal disorders, including IgA and membranous nephropathy, cystinosis, diabetes, renovascular hypertension and renal allograft rejection (JASN 19:197, 2008). Inhibition of TGF-β1 Type I receptor kinase may therefore prove clinically effective in the preservation of functional nephron number in chronic kidney disease.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

762A
**Results:** In CRF mice group, mean serum urea concentration was 16.92 mmol/L and hemocrit reached 8.24 mmol/L and 41% in control group. Hct concentration was increased in CRF mice vs control (0.97 and 2.07 mmol/mmol of amino acids respectively). Plasma urea and Hct were positively correlated (r²=0.8). Hct content was increased in CRF mice vs controls in heart (x2.2), lung (x2.2), aorta (x2.1), brain (x2.0), spleen (x1.8), muscle (x1.7), liver (x1.6), skin (x1.5), kidney (x1.5), bone (x1.5). Blood urea concentration was positively correlated with Hct content in muscle, lung, brain, spleen, heart, kidney, liver (0.66 < r² < 0.91). Hct was increased in purified collagen from skin and tail tendons in CRF mice vs controls (x2.1 and x1.7, respectively) with a positive correlation to blood urea concentration (r²=0.42 and 0.43).

**Conclusions:** These results indicate that carbamylation proteins in CRF increase in plasma and accumulate in tissues. The accumulation of these functionally and structurally altered proteins may contribute to the extra-renal consequences and progression of CRF.

**SA-PO2805**

**Electroacupuncture and Moxibustion Hinder the Progression of Renal Disease by Modulating Systemic and Renal Renin-Angiotensin System (RAS) Jose Carla Paterno, Dulce Elena Casarini, Fernanda Fernandes Barninha, Zaira Palmomino Jara, Nestor Schor, Analafvia De Oliveira Freire, Vicente de Paulo Castro Teixeira. Medicine, UNIFESP, Sao Paulo, Brazil.

**Background:** Systemic and renal RAS are pivotal for the development and maintenance of renal disease. Traditional Chinese Medicine (TCM) is increasingly recognized as an effective therapy in several fields of medicine. Among its therapeutic strategies are electroacupuncture (EA) and moxibustion (MO). We investigate the effects of EA and MO on RAS in an experimental model of hypertension and progressive renal disease (PRD).

**Methods:** Male wistar rats were submitted to 5/6 nephrectomy (5/6 nx) and studied along eight weeks. There were three groups: 5/6 nx; 5/6 nx and EA-MO session in sham-points(NX-AS); and 5/6 nx and EA-MO session in three real acupuncture points (NX-AM). We evaluated 24h-proteinuria, tail-cuff blood pressure (TPB), mean arterial blood pressure (MAP), plasma and renal Ang I, Ang II and Ang 1-7 and plasma renin activity.

**Results:**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NX-AS</th>
<th>NX-AM</th>
</tr>
</thead>
<tbody>
<tr>
<td>24h-proteinuria</td>
<td>12.3 ± 3.1</td>
<td>11.9 ± 4.4</td>
</tr>
<tr>
<td>24h-proteinuria (mg/24h)</td>
<td>187.2 ± 25.1</td>
<td>179.5 ± 46.8</td>
</tr>
<tr>
<td>TBP (mmHg)</td>
<td>188.7 ± 15.2</td>
<td>176.7 ± 15.2</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>190.3 ± 12.8</td>
<td>182.7 ± 14.8</td>
</tr>
<tr>
<td>Plasma Ang I (ng/mL)</td>
<td>30.4 ± 13.5 W</td>
<td>24.9 ± 4.6</td>
</tr>
<tr>
<td>Plasma Ang II (ng/mL)</td>
<td>38.7 ± 19.9 W</td>
<td>22.5 ± 6.2</td>
</tr>
<tr>
<td>Plasma Ang 1-7 (ng/mL)</td>
<td>42.4 ± 2.9</td>
<td>35.9 ± 1.3</td>
</tr>
<tr>
<td>Renal Ang I (ng/g)</td>
<td>30.6 ± 9.2</td>
<td>20.1 ± 5.9</td>
</tr>
<tr>
<td>Renal Ang II (ng/g)</td>
<td>8.8 ± 4.1 W</td>
<td>19.1 ± 5.4</td>
</tr>
<tr>
<td>Renal Ang 1-7 (ng/g)</td>
<td>12.4 ± 3.2</td>
<td>7.9 ± 2.1</td>
</tr>
<tr>
<td>Plasma Renin Activity (ng/mL)</td>
<td>22.9 ± 7.2</td>
<td>40.1 ± 0.9</td>
</tr>
</tbody>
</table>

Results: The acupuncture-treated group presented significant improvement in all measured functional parameters. EA and MO modulated RAS leading to higher production of renal Ang 1-7, is protective against endothelial damage and has vasodilatory and antifibrotic effects, our findings suggest that it could contribute to the improvement of the PDR in this model.

**Funding:** Other NIH Support - CNPq- Brazil

**SA-PO2806**

**Antioxidant Inflammation Modulators (AIMs) Increase Glucose Metabolism in Muscle Cells**

**Rhea D. Sidham, Thomas Palia, Ron Bumesteer, Deborah A. Ferguson, Christian Wiggley, Louis Ragolia. Reuta Pharmaceuticals, Inc., Irving, TX; Winthrop University Hospital, Mineola, NY.

**Background:** Oxidative stress induced by chronic hyperglycemia is involved in the development of CKD and insulin resistance. The Antioxidant Inflammation Modulator (AIM) drug class potently induces Nrf2, a regulator of many antioxidant and cytoprotective genes.

**Methods:** Cardiobondrol methyl is an AIM that recently completed a 52-week randomized, placebo-controlled trial for treatment of chronic kidney disease (CKD) in type 2 diabetics. In this trial, cardiobondrol methyl-treated patients experienced muscle improvements, which were most frequent during the first two months of treatment and generally mild to moderate in severity. Muscle spasms were not associated with markers of muscle injury and improved while on drug. Similar muscle spasms have been reported with insulin treatment in diabetics, suggesting an association with muscle glucose metabolism.

**Results:** To test whether a similar mechanism occurs in response to AIMs, we investigated their effect on glucose metabolism in cultured skeletal muscle cells. Uptake of 2-deoxyglucose (2-DG) and GLUT4 translocation to the plasma membrane were measured on differentiated 16K muscle cells treated with RTA 405, a cardobondrol methyl analog. In differentiated C2C12 muscle cells, levels of the glycolytic products pyruvate and lactate were measured in response to both cardobondrol methyl and RTA 405 using biochemical assays. The extracellular acidification rate (ECAR) was also measured in response to RTA 405.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.**
**Results:** RTA 405 increased 2-DG uptake in a dose-dependent manner. This effect could be explained by a decrease in GLUT4 to the plasma membrane. Pyruvate and lactate concentrations also increased in a dose-dependent manner following treatment with both bardoxolone methyl and RTA 405. RTA 405 also increased the extracellular acidification rate, further suggesting elevated glycolytic flux.

**Conclusions:** Taken together, these observations are consistent with treatment-induced increases in muscle glucose uptake and anaerobic glycolysis, which could result in enhanced acidification and correlate with muscle spasm, similar to that observed transiently following bouts of extreme exercise.

*Funding:* Pharmaceutical Company Support

**SA-PO2807**

**Progenitor Cell Secretory Products Exert Additive Renoprotective Effects When Combined with ACE Inhibitors in Experimental CKD**

Daren A. Yuen, Yanling Zhang, Kim Connelly, Andrew Advani, Richard E. Gilbert. St. Michael’s Hospital, Toronto, ON, Canada.

**Background:** Bone marrow-derived early outgrowth cells (EOCs) and their secreted factors markedly attenuate the functional and structural manifestations of experimental CKD. However, with blockade of the renin-angiotensin system (RAS) as established therapy in progressive kidney disease, any new therapy would need to show incremental efficiency. Accordingly, we tested whether administration of EOC-derived factors provides additive renoprotective effects when used in combination with RAS blockade for the treatment of late-stage CKD.

**Methods:** Cell free conditioned medium (FCFM) was generated by incubating F344 rat EOCs with serum-free EBM-2 medium to collect their secreted factors. Subtotally nephrectomized (SNX) F344 rats were randomized to a late stage of disease (8 wks post-SNX) to receive enalapril 0.5 mg/L in drinking water or vehicle. 4 wks later, enalapril-treated rats were randomized to receive twice weekly iv injections of FCFM or EBM-2 for 2 wks on top of continued enalapril administration. Three groups were thus studied: (1) No Therapy, (2) Enalapril and EBM-2, and (3) Enalapril and FCFM. GFR, urinary protein, and systolic BP were assessed serially.

**Results:** Compared to vehicle, 4 wks of enalapril treatment lead to a slight reduction in urinary protein at 12 wks post-SNX (vehicle vs enalapril: 117 ± 30 vs 89 ± 20 mg/d). Following treatment with EBM-2, the Enalapril and EBM-2 group experienced a further rise in proteinuria and decline in GFR to levels similar to those of the ‘No Therapy Group’. In contrast, the ‘Enalapril and FCFM Group’ demonstrated a sustained reduction in proteinuria, and a higher GFR. No differences in systolic BP were noted between treatment groups (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>No Therapy</th>
<th>Enalapril and EBM-2</th>
<th>Enalapril and FCFM</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (µL/min/g body wt)</td>
<td>2.14 ± 0.20</td>
<td>2.4 ± 0.3</td>
<td>1.98 ± 0.2</td>
</tr>
<tr>
<td>Urinary protein (mg/d)</td>
<td>2674 ± 602</td>
<td>2536 ± 471</td>
<td>2408 ± 730</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>178 ± 20</td>
<td>183 ± 10</td>
<td>173 ± 10</td>
</tr>
</tbody>
</table>

*p < 0.05 vs No Therapy

**Conclusions:** These data demonstrate that EOC-derived factors exert additive renoprotective effects on top of ACE inhibitor therapy in experimental CKD, providing the rationale for clinical trials of EOC-based therapies for CKD.

*Funding:* Government Support - Non-U.S.

**SA-PO2808**

**Abstract Withdrawn**

**SA-PO2809**

**Repeated Treatment with Progenitor Cell Secretory Products Maintains Long-Term Renoprotection in Experimental CKD**

Darren A. Yuen, Yanling Zhang, Kim Connelly, Andrew Advani, Richard E. Gilbert. St. Michael’s Hospital, Toronto, ON, Canada; St. Michael’s Hospital, Toronto, ON, Canada.

**Background:** Bone marrow-derived early outgrowth cells (EOCs) are thought to mediate organ protection via paracrine effects. Given the dysfunction of autologous cells from patients with CKD and of GLUT4 to the plasma membrane, we considered whether repeated administration of a cell-free but EOC-derived product might provide a viable alternative strategy for kidney protection.

**Methods:** The secretory output of EOCs was harvested by incubation with serum-free EBM-2 medium to generate cell free conditioned medium (FCFM). Subtotally nephrectomized (SNX) F344 rats were randomized 4 wks post-SNX to receive 3x weekly iv injections of FCFM or EBM-2 over 2 wks. Rats were treated if they showed signs of recurrence, as defined by a 4-fold rise in proteinuria above initial post-treatment values.

**Results:** Two groups were studied, according to whether they were administered FCFM: once (Initial Therapy Group), twice (Repeated Therapy Group) or not at all (No Therapy Group). GFR, urinary protein, and systolic BP were assessed serially.

**Conclusions:** Proteinuria rose progressively in the ‘No Therapy Group’ (1740 ± 468 mg/d) but was lower in rats initially treated with FCFM (881 ± 138 mg/d) at 10 wks post-SNX. Following FCFM treatment at this point, the ‘Repeated Therapy Group’ showed sustained improvements in renal function, whereas the ‘Initial Therapy Group’ experienced a fall in GFR and rise in proteinuria to levels similar to those of the ‘No Therapy Group’ at 14 wks post-SNX (Table 1). Systolic BP did not differ between groups.

**Funding:** Private Foundation Support
SA-PO2812

Role of the Transcription Factor ETS-1 in the Regulation of Pro-Inflammatory and Pro-Fibrotic Mediators in Response to Ang II Wenguang Feng,1 Phillip H. Chumley,1 Ping Hua,1 Gabriel Rezoniew,2 Edgar A. Jamieson,2 1Department of Medicine, University of Alabama at Birmingham, AL; 2VA Medical Center, Birmingham, AL.

Background: The transcription factor ETS-1 is an important mediator of growth-related responses and inflammation in different models of injury. We previously demonstrated that ETS-1 mediates macrophage infiltration, cell proliferation, mesangial expansion and oxidative stress in response to Angiotensin II (Ang II) in vivo (AJP '08, ASN '10). Herein, we tested the hypothesis that ETS-1 mediates these effects by regulating the expression of pro-inflammatory and pro-fibrotic mediators upregulated by Ang II.

Methods: C57BL/6 mice (n=6/group) were infused with vehicle (Veh), Ang II (1.4mg/kg/day SQ), Ang II and an ETS-1 dominant-negative peptide (DN, 10 mg/kg/day SQ) or Ang II and an ETS-1 mutant peptide (MU 10 mg/kg/day SQ) for 4 weeks. Kidneys were saved for immunofluorescence (IF), real time PCR and western blot (WB).

Results: Ang II increased the mRNA expression of the pro-inflammatory cytokines IL-4, IL-5, IL-6 and CCL3, the growth factors TGF-β and CTGF and the NADPH oxidase NOX4 (Table). DN reduced the mRNA expression of these mediators (p=0.05 vs Ang II), except IL-6 while MU had no effect. Ang II resulted in a 15-fold increase in TGF-β protein expression (by ELISA), while preserving kidney structure by DO (p=0.01) but not by MU. Ang II also increased CTGF expression (IF): Veh 0.7±0.25, Ang II: 560±299 ARU (p<0.05), which was reduced by 49% by DN (p<0.01) but not by MU. Ang II also increased CTGF expression (WB).

Conclusions: Our studies demonstrate that the transcription factor ETS-1 mediates the expression of several pro-inflammatory and pro-fibrotic mediators induced by Ang II making ETS-1 a potential novel target in the treatment and prevention of end-organ injury in hypertension.

Funding: Veterans Administration Support

SA-PO2813

Conditioned Medium Generated from Early Outgrowth Bone Marrow Cells Reveals Reproductive Effects in Type 2 Diabetes Yanjia Zhang,1 Darren A. Yuen,2 Andrew Advani,1 Kim Connelly,1 Richard E. Gilbert,1 1St. Michael’s Hospital, Toronto, Canada.

Background: Our previous study shows that early outgrowth cells (EOCs) cultured from bone marrow preserve kidney structure and function in chronic kidney disease and also including diabetic nephropathy. The benefits of the cells appear not to be a consequence of engraftment into the kidney, but rather systemic antioxidant and anti-inflammatory effects. Accordingly, we postulated that EOCs might mediate their beneficial effects by secreting soluble reparative factors. To test the hypothesis in the in vivo setting we conducted proof of principle studies using a cell free preparation in which the EOCs had been grown.

Methods: EOCs were grown from donor db/m mice bone marrow and conditioned medium (CM) was generated by incubating EOCs with serum-free EBM-2 culture medium. db/db mice were randomized to receive thrice weekly tail vein injections of either 10 x 10⁶ EOC CM suspended in complete EBM-2 culture medium, 10 x 10⁶ EOB M mice were randomized to receive thrice weekly tail vein injections of either 10 x 10⁶ EOC CM suspended in complete EBM-2 culture medium. db/db mice were randomized to receive thrice weekly tail vein injections of either 1 x 10⁶ EOC CM suspended in complete EBM-2 culture medium.

Results: Ang II increased CTGF expression (IF): Veh 0.7±0.25, Ang II: 560±299 ARU (p<0.05), which was reduced by 49% by DN (p<0.01) but not by MU. Ang II also increased CTGF expression (WB).

Conclusions: Our studies demonstrate that the transcription factor ETS-1 mediates the expression of several pro-inflammatory and pro-fibrotic mediators induced by Ang II making ETS-1 a potential novel target in the treatment and prevention of end-organ injury in hypertension.

Funding: Veterans Administration Support

SA-PO2814

Chronic Nicotine Exacerbates Sub Pressor Ang II (SP-AngII) Induced Hypertension (HTN) and Renal Injury Kiran B. Chandrashekar,1 Andrea P. Soljancic,1 Istvan Arany,1 M. Sood,2 Ian W. Gibson,3 Michael Kulakszewicz,2 Howard Leong-Poi,1 Kim Connelly,1 Richard E. Gilbert,1 Andrew Advani.1 1Keenan Research Centre, St. Michael’s Hospital, Toronto, ON, Canada; 2Health Sciences Centre, University of Manitoba, Winnipeg, ON, Canada.

Background: While the homeostatic chemoactive stromal cell-derived factor-1 (SDF-1) plays an essential role in renal vascular development, its function in the adult kidney and in chronic kidney disease (CKD) is unclear. In the present study, we hypothesized that the pathogenic properties of SDF-1 may be different from its chemotactic properties and may serve to promote microvascular health in both the normal and chronically ischemic kidney.

Methods: SDF-1 expression was determined in human biopsies, rat kidneys and cultured cells. Antagonism and augmentation of SDF-1/CXCR4 signaling were achieved with AMD 3100 (1 µg/ml) or genetic knockdown.

Results: While SDF-1 expression was described by podocytes, fibroblasts, collecting ducts and also corticomedullary proximal tubular cells, its cognate receptor CXCR4 was primarily restricted to endothelial cells of glomerular and peritubular capillaries. Unlike in biopsies from patients with diabetic nephropathy, SDF-1 expression was not upregulated in kidneys from individuals with CKD due to secondary focal segmental glomerulosclerosis (FSGS).

Conclusions: Collectively, these observations indicate that SDF-1 plays a homeostatic role in the adult kidney and in the setting of progressive, proteomic kidney disease. Therapeutic strategies that augment SDF-1 signaling represent a novel approach to slow the decline of kidney function in patients with CKD.

SA-PO2815

Stromal Cell-Derived Factor-1 Preserves Renal Function in Chronic Kidney Disease Li-Hao Cheng,1 Darren A. Yuen,1 Yanling Zhang,1 M. Sood,2 Ian W. Gibson,3 Michael Kulakszewicz,2 Howard Leong-Poi,1 Kim Connelly,1 Richard E. Gilbert,1 Andrew Advani.1 1Keenan Research Centre, St. Michael’s Hospital, Toronto, ON, Canada; 2Health Sciences Centre, University of Manitoba, Winnipeg, ON, Canada.

Background: Smoking, in tandem with HTN, are strong risk factors for the development of isotopically labeled internal standards and determined by flow injection analysis (FIA) bearing microbubbles, attenuated proteinuria progression in SNx rats. Moreover, SDF-1 expression was decreased in renal fibroblasts exposed to the pro-fibrotic growth factor transforming growth factor-β and in the kidneys of corticomedullary proximal tubular cells, its cognate receptor CXCR4 was primarily restricted to endothelial cells of glomerular and peritubular capillaries. Unlike in biopsies from patients with diabetic nephropathy, SDF-1 expression was not upregulated in kidneys from individuals with CKD due to secondary focal segmental glomerulosclerosis (FSGS). Moreover, SDF-1 expression was decreased in renal fibroblasts exposed to the pro-fibrotic growth factor transforming growth factor-β and in the kidneys of corticomedullary proximal tubular cells, its cognate receptor CXCR4 was primarily restricted to endothelial cells of glomerular and peritubular capillaries. Unlike in biopsies from patients with diabetic nephropathy, SDF-1 expression was not upregulated in kidneys from individuals with CKD due to secondary focal segmental glomerulosclerosis (FSGS).

Conclusions: Collectively, these observations indicate that SDF-1 plays a homeostatic role in the adult kidney and in the setting of progressive, proteomic kidney disease. Therapeutic strategies that augment SDF-1 signaling represent a novel approach to slow the decline of kidney function in patients with CKD.

SA-PO2816

Blood or Urine? A Systematic Comparison of Information Content and Analytical Practicality of Different Sample Types for Targeted Metabolomics Uirika Lundin,1 Klaus M. Weinberger.1 1Biores Life Sciences AG, Innsbruck, Austria.

Background: Right now there is a controversy going on since the majority of metabolomics studies are based on either only urine or only blood fluid (serum or plasma) samples. In nephrology, urine might seem like the obvious sample type of choice, because of the metabolic activity of the kidneys and the urine being excreted would show big changes in the composition of its metabolites. We intended to do a systematic review to see which sample type is really the most informative for targeted metabolomics, particularly in nephrology.

Methods: The comparisons between sample types were done in three different studies; on db/db mice, a rodent model of hyperphagia-associated type II diabetes with little kidney damage, nicotine-induced nephropathy in Sprague-Dawley rats and a clinical study on the progression of chronic kidney disease in diabetic and non-diabetic patients. Targeted metabolomics was used to quantify metabolites from plasma and urine including the classes amino acids, biogenic amines, polypeptides, fatty acids/acyl-CoAs, bile acids and energy metabolism intermediates in the presence of isotopically labeled internal standards and determined by flow injection analysis (FIA).
Reduced Klotho Expression Level in Kidney Aggravates Renal Interstitial Fibrosis

**Background:** Klotho is associated with the suppression of several aging phenotypes. In chronic kidney disease (CKD), impaired Klotho expression predisposes the kidney to renal fibrosis, with reduced Klotho expression in a time-dependent manner in epithelial cells.

**Methods:** Expression of fibrotic factors was by Real time PCR, Western blotting, ELISA and immunohistochemical staining.

**Results:** The expression levels of α-SMA (1.6-fold, p<0.05), fibronectin (1.6-fold, p<0.05) and TGF-β1(1.4-fold, p<0.05) were higher and the renal interstitial fibrosis was severer in K+/− mice than those in the wild-type mice. In cultured renal fibroblast cells (NRK49F cells), expression levels of a-SMA and PAI1 (0.5-fold, p<0.05) were significantly suppressed by addition of recombinant Klotho protein to the medium. The expression of a-SMA and PAI-1 were significantly increased in NRK49F cells cultured in the presence of TGF-β for 24 hours, although these elevations in the a-SMA and PAI-1 levels (0.6-fold, p<0.05) were significantly blunted in NRK49F cells cultured in the presence of TGF-β with recombinant Klotho protein. These effects were also suppressed by a TGF-β1 receptor inhibitor (ALK5 inhibitor).

**Conclusions:** Klotho is downregulated by the progression of renal fibrosis and chronic kidney disease via TGF-β1. Reduced Klotho expression levels in the kidney aggravate renal interstitial fibrosis via TGF-β1. Reduced Klotho expression in kidneys has been elucidated.

**Funding:** Government Support - Non-U.S.

**SA-PO2817**

**Xeno-Klotho Administration Retards the Progression of Adriamycin Nephropathy

**Background:** Recent data reveal that elevating free klotho in proximal tubular lumen reduces phosphate reuptake, and suggest that the gain of function in klotho gene slows the progression of chronic kidney diseases, at least partly through the inhibition of TGF-signaling pathway and TGF-β1.

**Methods:** In the present study, effects of exogenous administration of recombinant human klotho protein on renal injury was assessed in two groups of rats (n=6 for each group); male Wistar rats treated with intravenous adriamycin (5 mg/Kg) to induce focal segmental glomerulosclerosis through tail vein as a control (C), those treated with recombinant klotho (10 mg/Kg/day) by unilateral ureteral obstruction (UUO) and compared them with wild-type mice.

**Results:** The results showed that free klotho reduced albumin excretion, showing renoprotective actions in adriamycin nephropathy. These results have provided the evidence that free klotho reduces albumin excretion, showing renoprotective actions in adriamycin nephropathy.

**Funding:** Clinical Research Support

**SA-PO2818**

**Ronacaleret Retards Renal Injury with Preserving Klotho in 5/6-Nephrectomized Rats

**Background:** We have previously reported that vitamin D increases renal expression of klotho in rats with normal kidney function, and that calcimimetics reduces it.

**Methods:** In the present study, effects ofronacaleret, a calcilytic agent, on renal injury was assessed in four groups of rats (n=10 for each group); 5/6-nephrectomized (uninephrectomy with ligation of 2 renal artery branches in contralateral kidney) Wister rats as a control (C), those treated with ronacaleret (150 mg/kg/day) (R), rats treated with calcitriol (30 ng/kg/day, V), and rats treated with both ronacaleret and calcitriol (R+V). Three months later, rats were killed with over-anesthesia, and harvested remnant kidney for analysis.

**Results:** Albuminuria was lower in R (4.2±0.6 mg/day), V (3.8±0.5 mg/day) and R+V (4.2±0.6 mg/day) than C group (13.2±1.4 mg/day, p<0.05). CREB phosphorylation (CRE) was elevated in R (2.2±0.2 mg/ml/min) and V (1.9±0.2 mg/ml/min) than C group (1.5±0.2 ml/min, p<0.05). Serum calcium was increased in R+V (10.1±0.1 mg/dl) than C group (9.4±0.1 mg/dl, p<0.05). Fractional phosphate excretion was increased in R (13.2±2%), V (13.2±2%) and R+V (16±2%) compared to C (7.1±2%, p<0.05), and serum phosphate was reduced in R group (61.0±3 mg/dl vs 78.0±3 mg/dl). FGF23 was lower in R (371±18 pg/ml) and higher in V (675±58 pg/ml) and R+V (671.5±54 pg/ml) than C group (500±33 pg/ml, p<0.05). However, parathyroid hormone did not significantly differ among 4 groups. RT-PCR and western blot analyses revealed that compared to C (p<0.05), renal klotho expression was elevated in R (1.8-2.1 fold) and V (1.7-2.0 fold) groups.

**Conclusions:** The present data indicate that ronacaleret preserved klotho expression and renal function with reduction in serum phosphate and albuminuria in 5/6-nephrectomized rats. Our findings demonstrate that vitamin D prevented declines in klotho expression and renal function suppression, suggesting that immunomodulatory action and protection of genes encoding antioxidant enzymes. Although both Klotho and Nrf2 have an antioxidative effect on kidney, the relationship between these molecules has not been well understood previously.

**Funding:** Clinical Research Support

**SA-PO2820**

**Renal Nrf2 Expression Is Decreased in Klotho Deficient Mouse

**Background:** Klotho is anti-aging humoral factor and abundantly expressed in the kidney and has an antioxidative effect. Nuclear factor erythroid-2-related factor (Nrf2) is a cytoplasmosically localized transcription factor and also has an antioxidative effect. Nrf2 transcription is critical to the nucleus in response to oxidative stress and regulates the expression of genes encoding antioxidant enzymes. Although both Klotho and Nrf2 have an antioxidative effect on kidney, the relationship between these molecules has not been well understood previously.

**Methods:** Tissue extract of kidney was obtained from Klotho deficient and wild-type mice and nuclear Nrf2 expression was assessed by Western blot analysis. The tubular epithelial cells were primarily cultured from these mice and assessed the Nrf2 expression. 300mM of H2O2 was added to these cells for 3 hours and nuclear Nrf2 transcription was assessed by qRT-PCR and immunofluorescence staining. For investigating the association between Nrf2 and Klotho protein further, immunoprecipitation between these molecules were performed.

**Results:** Nuclear Nrf2 transcription was significantly decreased in klotho deficient mouse as compared with wild type mouse (p<0.05). Nrf2 expression in primarily cultured epithelial cells was also significantly decreased in klotho deficient mouse. Nuclear Nrf2 transcription after H2O2 stimulation was remarkably decreased in primarily cultured epithelial cells from klotho deficient mouse compared with wild type mouse. In western blot analysis, the H2O2 stimulation did not affect the Nrf2 expression in klotho deficient mouse, whereas increased in those from wild type mouse. However, immunoprecipitation analysis revealed that klotho protein did not bind to Nrf2.

**Conclusions:** Our data suggested that anti-oxidative effect of klotho was partially and indirectly through Nrf2 expression and its nuclear translocation.
Results: Klotho levels were significantly lower in HD patients than in healthy adults and were moderate CKD (368.3±99.0 pg/ml vs. 468.1±205.8 pg/ml, p<0.01 and 368.3±99.0 pg/ml vs. 498.7±221.9, p<0.01). There was a significant correlation with eGFR (r=0.005, r=0.31). In end stage renal disease there was a weak but significant correlation with bone alkaline phosphatase and 25-OH Vitamin D levels. Correlations to age, calcium or phosphorus levels could not be established. Klotho levels were significantly higher in females (463.0±202.6 pg/ml vs. 387.6±132.0 pg/ml, p=0.02), and acute kidney injury (567.6±294.4 pg/ml vs. 403.5±152.5 pg/ml, p<0.01).±

Conclusions: Serum klotho levels are associated with kidney function. In CKD, impaired klotho levels may contribute to accelerated aging and may be a measure of viable renal tubular cells mass.

Funding: Government Support - Non-U.S.

SA-PO2824

Induction of Phosphaturia Accelerates Chronic Kidney Diseases in Mice Chung-Yi Chen,1 Kuzuhiro Shizizaki,1 Johanne V. Pastor,1 Lei Wang,1 Joel Schwartz-Moretti,1 Addie Dickson,1 Ming Chang Hu,1 Orson W. Moe,1 Kuroko.1 1Pathology, University of Texas Southwestern Medical Center at Dallas, TX; 2Internal Medicine, Taipei Medical University-Wan Fang Hospital, Taipei, Taiwan.

Background: Lowering serum phosphate(Pi) levels by Pi binders improves clinical outcome of chronic kidney disease (CKD). Because serum Pi levels can be lowered also by promoting phosphaturia, we tested if induction of phosphaturia would delay CKD progression in mice. Klotho is expressed in the kidney where it functions as a co-receptor for fibroblast growth factor-23 (FGF23) and plays a critical role in Pi metabolism. The extracellular domain of Klotho is subject to ectodomain shedding and secreted into blood and urine. Secreted Klotho has phosphaturic activity independent of FGF23. Klotho injection ameliorates acute kidney injury (AKI), and transgenic overexpression of Klotho alleviates soft tissue calcification in CKD. This study is to test whether Klotho injection alleviates CKD in mice.

Methods: Eight-week old mice (129S1/SvImJ males) had received uninephrectomy plus ischemic reperfusion injury in contralateral kidney followed by feeding with 2% Pi diet. Mice were treated with secreted Klotho (0.02mg/kg, i.p, every other day) or vehicle for 12 weeks.

Results: Mice given recombinant secreted Klotho exhibited more prominent renal damage than vehicle-treated mice in functional (higher serum creatinine (Cr) and Cr clearance), histological (more severe nephrocalcinosis, renal interstitial fibrosis), and molecular parameters (higher levels of TGF-β1, phosphorylated Smad2/3, α-smooth muscle actin, collagen-1 and p21). Previous animal studies have furnished a model that kidney damage induced by dietary Pi overload correlates better with Pi excretion per nephron than blood Pi. It is conceivable that Klotho injection further increases urinary Pi excretion that is already high due to dietary Pi overload; with increased CaPi crystal formation in the tubules and nephrocalcinosis.

Conclusions: Thus, we propose that a delicate balance between the beneficial effects of phosphaturia v.s. overloading kidney with phosphate should be considered in Pi-lowering therapies.

Funding: NIDDK Support

SA-PO2823

Myocardial Expression of Fibroblast Growth Factor Receptor-1 and Renin-Angiotensin System Genes in Hypertrophic Hearts of Uremic Rats Michael Freundlich,1 Yan Chun Li,1 Jose M. Valdivielso,2 Lourdes Craver,1 Montserrat Martinez-Alonso,1 Adriana S. Dusso,2 Jose M. Valdivielso,1 Elvira Fernandez,1 Nephrology Dept., Arnau Villanova Hospital, Lleida, Spain; 2Estadistic Dept., IRB Lleida, Lleida, Spain; 3Experimental Nephrology, IRB Lleida, Lleida, Spain.

Background: Cardiac hypertrophy (CH), common in CKD, associates with increased mortality. CH causes are multifactorial including disturbances of the renin-angiotensin system (RAS) and fibroblast-growth factor (FGF23). However, expression of RAS components in myocardium (M) of uremic animals has not been studied, furthermore, the co-receptor Klotho, essential for FGF-23 action, is not expressed in M. Since the vitamin D analog paricalcitol (Po) improves uremic CH, suppresses CH in non-uremic animals and interacts with FGF23 in other tissues, we studied Po effects on the expression of these genes in uremic rats.

Methods: CH and left ventricular (LV) gene expression of RAS components and the FGF receptor 1 (r1) were evaluated in 5/6 nephrectomized rats treated 8 weeks with Po 0.3 µg/kg/3x/week IP or enalapril (E) 5 mg/kg/day enterally vs. untreated uremic (U) and sham-operated animals (S).

Results: PC and E improved hypertension (U 204±18 mm Hg; PC 156±8; E 144±9.5; Po 131±11) and LV hypertrophy (U 336±18; P<0.001) and renal insufficiency (creatinine, U 1.67±0.7 mg/ml; PC 0.65±0.11; E 0.78±0.06; P<0.002). Cardiac weight (g) in U was similarly by Pc and E (p=0.02). U mRNA expression of LV angiotensinogen by 30-fold (p<0.01 vs.), attenuated by PC and E (p=0.006); AT1R mRNA by 6.2 by U in E, was suppressed markedly by E and unmodified in Po. ACE mRNA was similar in all groups. Renin mRNA expression and brain natriuretic peptide (BNP) protein in LV/Tn U and suppressed similarly by PC and E (p<0.05). FGFr1 mRNA was 7.6-fold in U, and while Po-only mildly this gene, E-values similar to S.

Conclusions: CH is associated with increased LV renin and angiotensinogen gene expression and M upregulation of FGFr1 mRNA. 2) M renin mRNA and BNP were similarly suppressed by Pc and E. Evaluation of direct effects of PC and E, independent of those related to improvement of uremia and hypertension in this model, requires further studies.

Funding: Clinical Revenue Support, Government Support - Non-U.S.

SA-PO2825

Atheromatous Disease and Resistance to the Phosphaturic Action of FGF23 Contribute to the Severity of Vascular Calcification in Non-Dialyzed Kidney Disease Patients Lourdes Craver,1 Montserrat Martinez-Alonso,1 Adriana S. Dusso,1 Jose M. Valdivielso,1 Elvira Fernandez,1 Nephrology Dept., Arnau Villanova Hospital, Lleida, Spain; 2Estadistic Dept., IRB Lleida, Lleida, Spain; 3Experimental Nephrology, IRB Lleida, Lleida, Spain.

Background: Vascular calcification (VC) is an important contributor to the high mortality rates in chronic kidney disease (CKD), and hyperphosphatemia is a well recognized cause of enhanced VC in these patients. However, a recent study in hemodialysis patients has shown that not only hyperphosphatemia, but also other factors contribute to calcification.

Methods: This cross-sectional study analyzes the impact of abnormalities in phosphate (P) metabolism and atheromatous disease on abdominal aortic calcification (AAC) measured by the Kauppila index (KI) in 186 patients CKD stages 3, 4, and non–dialyzed stage 5.

Results: FGF23 correlated positively to age (P<0.01; r=0.296) and creatinine (P<0.0001; r=0.861) and negatively to hemoglobin (P<0.01; r=−0.340) and albumin (P<0.001; r=−0.861). FGF23 was clearly associated with stage CKD; αKlotho negatively correlated to age (P<0.01; r=−0.368) and positively correlated to hemoglobin (P<0.05; r=0.246). αKlotho had a tendency to associate negatively with serum creatinine. Interestingly, αKlotho levels significantly decreased early phase of CKD (stage 2) as compared with stage1. In addition, αKlotho dramatically decrease according to the progression of CKD, especially stage 4 and 5. However, αKlotho did not show statistically significant correlation with Pi, Ca and FGF23.

Conclusions: Our data indicate that serum αKlotho could be a new predictive marker in progression of CKD, especially in early stage, and αKlotho and FGF23 may play a key role in pathogenesis of CKD-MBD.
Impaired Angiogenesis and Rarefied Peritubular Capillaries Participated in Renal Tubulointerstitial Fibrosis in Experimental Chronic Renal Ischemic Rats
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**Background:** Chronic kidney hypoxia is regarded as a contributing factor in the progression of renal failure, impaired angiogenesis and loss of peritubular capillaries in the late phase of renal diseases gives evidence of renal hypoxia.

**Methods:** Unilateral clamping of the left renal artery to induce chronic ischemia of the kidney tissue was utilized in this study. The experimental rats and respective sham-operated controls were sacrificed at week 1 and 12, and the renal tissues were harvested for histological study.

**Results:** The results showed that the blood pressure and serum creatinine of the experimental rats was elevated at week 6 and keep a high level in late stages. The left renal tissue with artery stenosis showed proximal tubular epithelial vacuole and granulation degeneration with inflammatory cells infiltration in tubulointerstitium in week 1 group. Hypoxic probe labeling showed significant staining in outer medulla and cortex, while the hypoxic probe was only weak stained in outer medulla in the control rats. Interestingly, VEGF and both of its receptors Flk and Flt were markedly upregulated in one week ischemic renal cortex compared with the control(0:1.01). But in advanced stage (week 12), the chronic ischemic renal tissue exhibited atrophic tubular epithelium, widened tubulointerstitium with obvious collagen I expression. The Hypoxprologo-1 labeling showed limited staining only in medulla while negative staining in cortex. Coincidence with Hypoxprobe™-1, VEGF and its receptors staining in cortex weakened significantly compared with that of early stage (week 1 P<0.01). Further investigation revealed that the endothelial cell marker CD31 was markedly reduced and showed a significant loss of peritubular capillaries in cortex area, and also decreased endothelial progenitor cell marker CD34 expression with lack of vascular repair compared with that of one week group.

**Conclusions:** Chronic persistent ischemia would finally destroy the ability of angiogenesis in renal tissues, which accelerated ischemia extent of the kidney, lead to interstitial fibrosis and attributed to renal failure in advanced stages.

SA-PO2827
Paradoxical Pro-Growth Gene Expression Patterns in Rat Kidney Endothelial Cells Characterized by Limited Growth Potential
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**Background:** The renal vasculature is characterized by limited regenerative potential following both acute and chronic injuries, and peritubular capillary loss is a consistent feature in the setting of interstitial fibrosis leading to worsened hypoxia. The current study sought to investigate the growth properties of rat kidney endothelial cells and determine factors that may lead to their impaired regenerative capacity.

**Methods:** Rat kidney endothelial cells (KEC) were isolated by CD31 immuno-isolation techniques and maintained in long-term cultures.

**Results:** When compared with endothelial cells isolated from the pulmonary microvascularity (i.e., PMVEC), KECs did not respond to VEGF, had significantly slower growth rates and limited clonogenic capacity. The expression of VEGF signaling pathway genes was markedly reduced in KEC and PMVEC by quantitative PCR arrays. Despite their low growth rates, KECs showed significant and dramatic enhancement in 18 VEGF pathway genes (log2:2 vs PMVEC, including Flt-1 (~50 fold), FLK-1/KDR (~60 fold) and NOS-3 (~200 fold). In contrast, only 5 genes were expressed at significantly lower levels in KEC vs. PMVEC. These included VEGF-A and Mc. We further screened the expression of 384 rat microRNAs and compared KECs and PMVECs. The vast majority of miRNAs were either undetectable or showed no difference between KECs and PMVECs; However, 4 miRNAs were significantly greater in KECs including the pro-angiogenic miR126, which was expressed at ~10,000 fold greater levels in KEC vs. PMVEC. Moreover, the pro-apoptotic miR24 was highly expressed in PMVECs and was 3-fold lower in KECs.

**Conclusions:** Taken together, rat KECs display a prominent enhancement of genes typically associated with endothelial proliferation and angiogenesis, despite their low growth rates, suggesting that unidentified factors may impede strong negative growth regulation in these cells.

**Funding:** NIDDK Support

SA-PO2828
Reduced γ-Carboxylase Activity in Uremia- A Possible Mechanism of Uremic Vascular Calcification
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**Background:** Vascular Calcification (VC) is present in chronic kidney diseases. This can be inhibited by matrix gla protein (MGP), which achieves full activity by carboxylation by the vitamin K dependent γ-carboxylase. Its inhibition by warfarin leads to augmented vascular calcification. The vitamin K regeneration cycle is formed by DT-diaphorase, VKOR and γ-carboxylase. Vitamin K deficiency is present in dialysis patients and so we investigated whether uremia reduces enzyme activities.

**Methods:** 10 Wistar rats in each group were fed a) standard diet b) 100mg/kg vitamin K2 c) 0.75% adipine or d) 0.75% adipine + 100mg/kg vitamin K2. Finally, serum parameters, extent of VC, uncarboxylated ucMGP and in kidney tissue of divalent of VKOR and γ-carboxylase were measured.

**Results:** After 4 weeks of treatment, creatinine, urea (7-fold) and phosphate were higher in adipine groups (c,d) than in controls (a,b). Aortic calcium content was higher in c,d than in controls. Systolic blood pressure was unaffected in all groups. No changes in adipose tissue content were significantly higher (70, 72%) in groups c,d. VKOR activity was unchanged; γ-Carboxylase was more active in groups c,d significantly. This also led to significantly higher levels of ucMGP (12.2 vs 8.6, 7.4 μM).

**Conclusions:** Experimental uremia inhibits the key enzyme of the vitamin K dependent carboxylation, γ-Carboxylase. This is accompanied by higher levels of ucMGP and calcium deposition in the aorta. Even though there is reduced enzyme activity, vascular calcification and ucMGP can be reduced by dietary vitamin K2 (group d). Our data identifies a new mechanism of uremic vascular calcification and supports the rationale of our vitamin K2 interventional study VitaVask.

SA-PO2829
Low-Dose Erythropoietin Increases Superoxide Production in Normal Rat Aorta and Endothelial Cells

**Background:** High-dose erythropoietin (EPO) affords vascular and tissue protection with its anabolic effects, whereas the controversy on the dose-dependent vaso-protective effect of EPO is beneficial for cardiovascular protection. High-dose EPO has been reported to increase nitric oxide (NO) production via activation of the phosphatidylinositol-3-kinase/Akt pathway. However, the clinical dosage of EPO sometimes leads to an increase in blood pressure in humans. This study was designed to investigate whether the therapeutic dosage of EPO accentuates oxidative stress and modulate endothelial function.

**Methods:** In in vivo experiments, normal male Sprague-Dawley (SD) rats were treated with either EPO (20 IU/kg/week, subcutaneously) 3 times per week or darbepoetin (D-EPO, 0.1 μg/kg/week, subcutaneously) 1 time per week for 4 weeks. The endothelial-dependent vaso-dilatory response, NADPH oxidase activity, and gene expression of ICAM-1 and TNF-α were assessed. To explore whether EPO is involved in superoxide production, in vitro experiments, we stimulated human umbilical vein endothelial cells with EPO and assessed NADPH oxidase activity and NO production.

**Results:** The used doses of EPO and D-EPO had no effect on hemoglobin level. In the normal SD rats, the acetylcholine-dependent vaso-dilatory response decreased significantly in both the EPO and D-EPO treatment groups. NADPH oxidase activity as well as aortic gene expression of ICAM-1 and TNF-α increased significantly, and to the same extent, in both groups. We confirmed EPO-mediated superoxide production in vitro. EPO increased NO levels as previously reported. However, because of parallel superoxide production, NO was consumed during the production of peroxynitrite associated with activation of NADPH oxidase.

**Conclusions:** Administration of EPO and D-EPO increased oxidative stress and impaired endothelial function in normal rats and in human endothelial cells.

**Funding:** Other NIH Support - The Kidney Foundation

SA-PO2830
Hyperphosphataemia Impairs Relaxation in Resistance Vessels; an Effect Which Is Partially Reversed in the Presence of a Phosphodiesterase Inhibitor

**Background:** The mechanism of action of phosphate as a risk factor for cardiovascular disease is unclear. This study looks at the effect of altered phosphate concentration on the function of rat resistance vessels.

**Methods:** Resistance vessels were dissected from the mesentery of 12 week old male WKYs and incubated overnight in physiological saline solution (PSS) with normal (1.18mM) or high phosphate concentration (2.5mM). Vessels were mounted on a wire myograph. Vasoconstriction response to sodium nitroprusside (SNP) was measured. Experiments were repeated in the presence of SNP than those in normal phosphate PSS (p<0.001 and p=0.029). The contractile response to carbachol were measured. L-NAME was added and contractile response to PE measured again. In a separate experiment, following contraction with PE, vasorelaxation response to carbachol was measured. L-NAME was added and contractile response to PE measured again. In a separate experiment, following contraction with PE, vasorelaxation response to sodium nitroprusside (SNP) was measured. Experiments were repeated in the presence of PSS. In the presence of L-NAME, between the 2 groups is similar. In normal phosphate PSS, with the addition of L-NAME, a significant difference is seen in the concentration-response curve to PE (p<0.05). This difference is not seen in the vessels in high phosphate PSS. In the presence of zaprinest, there is improved relaxation in the vessels in high phosphate (p=0.006).

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
Conclusions: Elevated phosphate decreases endothelium-dependent and independent vasoactivity, exacerbates oxidative stress and endothelial dysfunction. The presence of a PDE5 inhibitor, which increases cyclic GMP, improves the relaxation response. Elevated phosphate may result in a combination of reduced production of basal nitric oxide within endothelial cells and cyclic GMP production or guanylate cyclase expression in vascular smooth muscle cells. These experiments mimic a uremic state and may offer an explanation for elevated serum phosphate as a cardiovascular risk factor.

SA-PO2831

CD36-Na/K-ATPase Signaling Complex Mediates a Pro-Inflammatory Signaling Loop in Kidney. David J. Kennedy,1 Wenjing Huang,2 Ting Liu,2 Zi-Jian Xie,3 Joseph I. Shapiro,4 Roy L. Silverstein,1 1Cell Biology, Cleveland Clinic, Cleveland, OH; 2,4Medicine, University of Toledo, OH.

Background: Pro-atherogenic, hyperlipidemic (HL) states are accompanied by increases in circulating ligands for receptor CD36 (e.g. oxLDL) and the signaling Na/K ATPase (e.g. ouabain-like cardiotoxic steroids). These factors increase inflammation, oxidative stress, and progression of chronic kidney disease. We tested the hypothesis that ligands generated in HL accelerate renal inflammation through activation of a CD36-Na/K-ATPase signaling complex, including potentiation of an inflammatory paracrine loop between proximal tubule (PT) cells and their associated macrophages (Mφ).

Methods: CD36 and CD36(+/−) mice on an apoE background were fed a high-fat diet (HFD) for 32wks.

Results: Compared to WT, CD36 kidneys had less glomerular and tubulointerstitial Mφ accumulation, glomerular foam cell formation and mesangiolysis. CD36 Mφ also demonstrated decreased production of proinflammatory cytokines and ROS in response to oxLDL, ouabain, or plasma from HFD mice. Both oxLDL and ouabain increased transduction of WT but not CD36 Mφ to tissue culture plastic. In a modified Boyden chamber migration assay, WT Mφ showed increased migration to cell-free conditioned media from HK2 PT cells treated with either oxLDL or ouabain. OxLDL, ouabain, and HFD plasma stimulated ROS production in LLC-PK1 PT cells and this was inhibited by either N-acetyl-cysteine or NAC. OxLDL-induced ROS were also proportionately attenuated by RNAi-induced knock-down of the Na/K ATPase α1 subunit in A411 cells (40% Na/K-1 knockdown) and PY17 (90% Na/K-1 knockdown). Cells transfected with control vector showed no effect. OxLDL and ouabain increased CD36 and Na/K-1 co-localization in both LLC-PK1 and Mφ as demonstrated by co-IP and a cell surface fluorescence proximity ligation assay. Finally, oxLDL and ouabain increased the content of both CD36 and Na/K-1 in HK2 PT early and late endosomes.

Conclusions: These data suggest that a CD36-Na/K-ATPase signaling complex in both PT and Mφ facilitates the development of chronic inflammation that underlies the renal dysfunction common to HL states.

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SA-PO2832

Selective Estrogen Receptor Modulator Inhibits Fatty Acid-Induced Inflammation in Mice. Yuko Nishi, Minoru Satoh, Naruya Tomita, Tamaki Sasaki, Naoki Kashihara. Nephrology and Hypertension, Kawasaki Medical School, Kurashiki, Okayama, Japan.

Background: Proteinuria is an independent risk factor for progressive renal diseases by initiating or aggravating tubulointerstitial injury. Albumin-bound free fatty acid (FFA) overloaded in proximal tubule evokes inflammatory responses. However, the mechanisms underlying the induction of inflammation have not yet been fully elucidated. Recent study showed that inflammation-dependent inflammatory responses were triggered by FFA and mitochondria-derived reactive oxygen species (ROS) were required for this response in adipose tissue. We hypothesized that albumin-bound FFA would trigger inflammation through mitochondrial ROS production and raloxifene, selective estrogen receptor modulator, could ameliorate tubular injury by reducing inflammations-activation associated with mitochondrial oxidative stress.

Methods: Female ICR-driven glomerulonephritis (ICGN) mice, an inbred strain with hereditary nephrotic syndrome, underwent ovariectomy and treatment with raloxifene. Human renal proximal epithelial cells were cultured with human fatty acid-bearing human albumin (PA-HSA) or human fatty acid free human albumin (Free-HSA) for 24 h with or without raloxifene and antiestrogen, ICI 182,780.

Results: ICGN mice showed tubular activation of inflammations and elevated inflammations-dependent cytokines. Raloxifene attenuated these changes and ameliorated tubular damages. Moreover, raloxifene reduced mitochondrial ROS production, prevented mitochondrial transduction of proinflammatory cytokine, and suppressed expression of fibrotic markers (αSMA and TGFβ 1) and inflammatory cytokines (TNFα and IL-1β) in PA-HSA but not Free-HSA caused loss of mitochondrial membrane potential, increased oxidative stress, and inflammations activation. Pretreatment with raloxifene improved the FA-HSA-induced changes via amelioration of mitochondrial function. These beneficial effects of raloxifene were blocked by co-incubation with ICI 182,780.

Conclusions: Albumin bound FFA activates inflammations in tubular cells through induction of mitochondrial ROS production. Thus, Inflammations could be regarded as a novel and promising therapeutic target for proteinuria-induced renal injuries.

SA-PO2833


Background: Inflammation is a constant features and a key mediator of progression of CKD and its cardiovascular complications. Inflammation in CKD has been attributed to uremic toxins, co-morbidi conditions, infections, dialysis procedures, service, and so on. However, little information has been added to the potential role of the gut and its microbial flora in the CKD-induced inflammation. CKD patients frequently exhibit endotoxemia without detectable infection. Intestinal epithelial and its tight junction form a barrier which prevents entry of microbes and their byproducts in the internal milieu. We tested the hypothesis that uremia may induce inflammatory paracrine function of the colonic epithelial tight junction, facilitating leakage of pro-inflammatory byproducts of the microbial flora in the circulation.

Methods: SD rats were randomized to undergo 5/6 nephrectomy (CKD) or sham-operation (control) and observed for 8 weeks. In a separate experiment SD rats were divided by additional food restriction to their food for 4 weeks and observed for 2 weeks. Rats consuming regular diet served as controls. The animals were euthanized and colon was harvested and processed for expression of the key constituents of the tight junction using RT-PCR, Western blot analysis and immunohistochemistry.

Conclusions: The study revealed for the first time that uremia results in disintegration of the colonic tight junction, a phenomenon which may contribute to the systemic inflammation and common occurrence of endotoxemia in advanced CKD.

SA-PO2834


Background: AKI has been recognized as a risk factor to develop CKD, however the mechanisms involved have not been elucidated yet. We previously demonstrated that Sp prevents renal injury induced by ischemia/reperfusion (I/R). This study was designed to: 1) develop an experimental model that leads to CKD induced by AKI; 2) study the mechanisms by which Sp may lead to CKD and 3) determine if preventing AKI with Sp protects against CKD.

Methods: Forty Wistar rats were divided in 1) Sham-operated, 2) Sp-treated group (20mg/Kg), 3) rats underwent to bilateral ischemia (45') and 4) rats receiving Sp before I/R (Sp+I/R). All groups were followed through 270 days. Proteinuria (UProt) and urinary K-im (Kim) were evaluated every 30 days. At the end, creatinine clearance (CrC) and renal blood flow were measured. Right kidney was used for molecular studies and the left for histopathological analysis.

Results: Rats underwent to I/R exhibited increased mortality rate by 57% and developed CKD characterized by a progressive increase in UProt and Urkim, together with a fall in CrC and RBF. Glomerular hypertrophy and focal sclerosis, extensive tubular dilatation, tubular proliferation and tube-Interstitial fibrosis were also observed. These alterations were associated with an up-regulation of eNOS and TGFβ and its downstream effectors: p-Smad 3, collagen-1, and fibronectin. Also, an up-regulation of TGFβ and inflammatory cytokines were observed. In Sp+I/R group mortality, Urkim, renal dysfunction and structural injury were prevented. Renal architecture preservation was associated with prevention of eNOS up-regulation, reduction of pro-inflammatory cytokines, profibrotic factors and its target genes.

Conclusions: Here we show a new model of CKD induced by AKI. In this model, the mechanisms responsible of CKD progression were mediated by up-regulation of eNOS, TGFβ and TGFβ, together with a greater activation of TGFβ pathway and inflammatory response. Intriguingly, we show for the first time that Sp is a novel treatment to prevent CKD induced by AKI. 2) study the mechanisms by which Sp may lead to CKD and 3) determine if preventing AKI with Sp protects against CKD.

Funding: Government Support - Non-U.S.

SA-PO2835

Clinical Implication of Tubulointerstitial Inflammatory Cell Infiltration in IgA Nephropathy. Haileng Ni, Linti Lv, Bi-Cheng Liu. Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.

Background: IgA nephropathy is the most common glomerular disease which could progressively progress to end stage renal failure. While inflammatory cell infiltration in tubulointerstitium is commonly observed in IgA nephropathy, the role and mechanism of such involvement is not well defined. In the present study, we evaluated the infiltration of inflammatory cells in renal tubulointerstitium with IgA and its clinical implication.

Methods: We have evaluated the infiltrating immune cells in renal biopsies from 31 patients with IgAN using anti-human CD3, CD4, CD8, CD14, CD19, CD20, anti-human Mac-2 (T cells, CD3, CD4, CD8), B cells (CD20), macrophages (CD68) and follicular dendritic

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
cells (CD21). Progression in renal disease was defined as an elevation of serum creatinine above the normal limit and over 20% from baseline.

Results: It was shown that positive rates in progressive disease were 100% for CD3, 100% for CD4, 89% for CD8, 33.33% for CD20, 11.11% for CD21. Those of CD3, CD4, CD8 and CD68 were significantly higher than in Stable disease. Clinical pathologists have confirmed that a positive correlation between the number of CD3+/CD4+/CD8+ and CD68+ macrophages and the level of serum creatinine (p=0.09, p=0.05) and proteinuria (p=0.011, p=0.007) at the time of biopsy was found. Moreover, In the renal tubulointerstitial, the number of CD3+ cells was positively correlated with interstitial fibrosis (r=0.001, p=0.45, p<0.001) and the number of CD8+ cells correlated with segmental glomerulosclerosis (p<0.03). In the multivariate analysis, the number of tubulointerstitial CD3+ and CD68+ cells were independently associated with progressive disease in IgAN.

Conclusions: Our study demonstrated that interstitial CD3+ and CD68+ macrophages play an important role in causing the progression of IgAN. It might hold a great promise in predicting the prognosis of IgAN patients by evaluating inflammatory cells infiltration in renal tubulointerstitium.

Funding: Government Support - Non-U.S.

SA-PO2836

Bardoxolone Methyl Transcriptionally Regulates Transaminase Levels and Increases Glutathione Levels

Gregory A. Miller, Ron Bumeister, Jeffrey Laidlaw, Priam Kambuj, Brandon Pröbst, Deborah A. Ferguson, W. Christian Wigley. Reata Pharmaceuticals, Inc., Irving, TX.

Background: Bardoxolone methyl is the lead molecule from the Antioxidant Information Modulator (AIM) class that potently induces Nrf2, a transcriptional regulator of many antioxidant and cytoprotective genes. In a 52-week randomized, placebo-controlled clinical trial in patients with chronic kidney disease and type 2 diabetes, 98% of patients treated with bardoxolone methyl had transaminase elevations above baseline levels, while 71% had transaminase elevations ≥2X the upper limit of normal. The transaminase elevations were transient, peaking within 2 to 4 weeks of treatment initiation or dose escalation, and generally resolved without drug discontinuation. Transaminase elevations did not recur once resolved and were not associated with liver toxicity.

Methods: Studies were undertaken to investigate the molecular mechanism underlying these observations.

Results: Treatment with bardoxolone methyl and other AIMs increased transaminase mRNA and protein levels in hepatocytes, myocytes, renal cells, and macrophages in a dose- and time-dependent manner. Treatment of HuH-7 human hepatoma cells and AML-12 mouse hepatocytes with bardoxolone methyl resulted in time- and dose-dependent increases in glutathione (GSH) levels. Transaminase enzymatic reactions produce GSH, an essential antioxidant molecule. Consistent with this, microarray analysis of bardoxolone methyl-treated HepG2 hepatoma cells revealed induction of several genes involved in GSH production, as well as the production of GSH precursors. We observed that siRNA knockdown of ALT2 in AML-12 mouse hepatocytes resulted in reduced glutamate levels. Furthermore, siRNA knockdown of ALT2 in HuH-7 human hepatoma cells resulted in up to a 50% reduction in total GSH levels.

Conclusions: These data suggest that transcriptional elevation of transaminases may reflect increased demand for glutamate as a result of bardoxolone methyl-mediated increases in glutathione production.

Funding: Pharmaceutical Company Support

SA-PO2837

CD11c Positive Cells Recruitment Is Critical for the HDF-Induced Kidney Injury

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Background: CD11c is a type I transmembrane protein found at high levels on most human dendritic cells, but also on monocyes/macrophages, neutrophils, and some B cells that induces cellular activation. Recently, a specific subset of CD11c positive macrophages was shown to be recruited to obese adipose and muscle tissue. This subset expresses CD11c and produces high levels of proinflammatory cytokines that are linked to the adipose tissue injury. CD11c+ cell ablation leads to a marked decrease in inflammatory markers, both locally in the peritoneal cavity and reflected by gene expression and protein levels.

Methods: With a method by which mice were subjected to unilateral nephrectomy and with a method by which mice were subjected to unilateral nephrectomy and followed by either high-fat diet (HFD) or normal diet (ND) for 16 weeks, we successfully established a HDF-induced kidney injury model evidenced by significant deposition of lipid, fibronectin, collagen I, and collagen III in the kidneys. This model was used to study the role of CD11c positive cells in the HDF-induced kidney injury.

Results: This HDF-induced kidney injury mice displayed markedly increased plasma levels of MCP-1, IL-6, and TNF-α and reactive oxygen species production in the kidneys compared with ND mice. Interestingly, a significantly increased recruitment of specific subset CD11c positive cells were observed in the kidneys of HDF mice compared to the volume of ND mice. Importantly, when the HDF mice were pretreated with 500 mg of total body irradiation, a significantly decreased numbers of CD11c positive inflammatory cells as well as a markedly ameliorated kidney injury locally, and a marked decrease in proinflammatory cytokines systemically were observed compared to the non-pretreated mice.

Conclusions: Our findings provide new insights into the role of CD11c positive cells in lipid-induced kidney injury, and offer a potential novel target in preventing the progression of chronic kidney disease elicited by HDF-induced hyperlipidemia.

Funding: Government Support - Non-U.S.

SA-PO2838

Differentiating Proinflammatory and Anti-Inflammatory Effects of TGF-β1 by Targeting β-Catenin

Guoping Zheng,1 Xinnui Tian,2 Jianlin Zhang,2 Thian Kui Tan,1 So Ra Lee,1 Tim Tzu-Ting Hsu,1 Ya Wang,1 Qi Cao,1 Dong Zheng,1 Yiping Wang,2 Changqi Wang,2 Vincent W.S. Lee,1 David C. Harris.1 1Centre for Transplantation and Renal Research, University of Sydney, Sydney, NSW, Australia; 2Shanshi Medical University, Taiyuan, Shanxi, China.

Background: TGF-β1 is known to be both anti-inflammatory and profibrotic. Epithelial-mesenchymal transition (EMT) is an important mechanism for TGF-β1-mediated fibrosis, in which β-catenin plays a role. However, the dependence on β-catenin of TGF-β1-induced EMT has never been fully explained. Whether β-catenin plays a role in the anti-inflammatory effects of TGF-β1 is unknown.

Methods: A protein knockdown chimera (F-Trec-Ecad) was used for specific degradation of cytosolic β-catenin in C1.1 renal tubular epithelial cells and J774 macrophages. TGF-β1-induced EMT in C1.1 cells, inhibition of J774 activation by (LPS/IFN-γ), β-catenin/p-Smad/LEF-1 interactions and β-catenin Topflash activity were analysed.

Results: TGF-β1-induced EMT, E-cadherin promoter repression, snail transcription and MMP-9 activity were reduced in C1.1 cells expressing F-Trec-Ecad. F-Trec-Ecad selectively degraded cytosolic β-catenin, and blocked TGF-β1-induced Smad3/β-catenin complex formation with no involvement of β-catenin/LEF-1 complex and Top/Flash activity. In contrast, the steady state level of β-catenin in J774 macrophages was low and was not changed when exposed to IFN-γ or LPS with or without TGF-β1. TGF-β1 inhibition of LPS-induced TNF-α and IFN-γ-stimulated iNOS mRNA expression was not affected in J774 cells expressing wild type β-catenin or F-Trec-Ecad. Neither β-catenin/Smad3 or β-catenin/β-catenin complex formation or β-catenin signaling was involved in the TGF-β1 inhibition of macrophage.

Conclusions: TGF-β1 induces EMT through a β-catenin/p-Smad3 dependent mechanism in C1.1 cells, but inhibits macrophage activation independently of β-catenin in J774 cells. Degradation of cytosolic β-catenin inhibits TGF-β1-induced EMT, but not anti-inflammatory effects of TGF-β1. β-catenin dependency of profibrotic but not anti-inflammatory actions of TGF-β1 expose β-catenin as a key therapeutic target.

Funding: Government Support - Non-U.S.

SA-PO2839

Do Cytokines at Moderately Elevated Concentrations in CKD Really Induce Leukocyte Activity? Nathalie Neirynck, Griet L.R.L. Glorieux, Eva Schepers, Raynond C. Vanholder. Internal Medicine, Nephrology, University Hospital Ghent, Ghent, Belgium.

Background: Oxidative stress induced by uremic retention products is one of the mechanisms involved in the pro-inflammatory status and the cardiovascular morbidity and mortality of CKD. IL6, TNFα, IL1β and IL18 are pro-inflammatory cytokines involved in many aspects of renal disease in IgAN.

In observational studies, IL6 and TNFα were associated with increased cardiovascular mortality in CKD. Although extensively studied at high concentrations as in sepsis, low concentrations as observed in uremia have to the best of our knowledge rarely been evaluated. The present study investigated whether IL6, TNFα, IL1β and IL18, as occurring in CKD, induced oxidative burst in leukocytes.

Methods: Whole blood of healthy volunteers was incubated in vitro with different concentrations of cytokines, ranging from 5 to 120pg/ml for IL6, from 20 to 1400pg/ml for TNFα, from 20 to 400pg/ml for IL1β and from 75 to 1200pg/ml for IL18. Oxidative burst in leukocytes in basal conditions and after stimulation with ILMP, E.coli and PMA, was measured by flow cytometry.

Results: At baseline, TNFα and IL6 increase the percentage of ROS-producing monocytes and granulocytes from the lowest concentration on (P<0.05), while no effects were seen with IL1β and IL18. After stimulation with ILMP, TNFα increased the percentage of ROS-producing monocytes and granulocytes in uremic concentrations, while IL6 suppressed oxidative burst in granulocytes at various uremic concentrations (P<0.05). After stimulation with E.coli, IL18 increased the percentage of ROS-producing cells (P<0.05, all leukocyte cell types). After E.coli stimulation, the mean ROS-production per cell in monocytes increased with IL6 (P<0.05 at 21.9pg/ml and 95.4pg/ml), while it was suppressed by TNFα from 70pg/ml on (P<0.05). After stimulation with PMA there were no significant effects.

Conclusions: Our data indicate that although cytokines appear to influence inflammatory status, there is certainly no consistent evolution in uremic concentrations. First of all, in basal conditions only some cytokines (TNFα and IL6) are pro-inflammatory whereas others (IL1β and IL18) are not. In activated leukocytes, some cytokines appear to have stimulatory and inhibitory effect as well.

Funding: Government Support - Non-U.S.

SA-PO2890

Fibrosis: Stem Cell, Inflammation, and Soluble Factors

Poster/Saturday

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Protective Effect of Electrolyzed Water with High Dissolved Hydrogen (H₂) on the Development of Cardiorenal Syndrome by Aging in Dahl Salt Sensitive Rat Wan-Jun Zhu,1,2 Masaaki Nakayama,1,3 Shigeru Kabayama,1,2 Sadayoshi Ito.1 1Center for Advanced and Integrated Renal Science, Tohko University, Sendai, Japan; 2Department of Blood Purification, Tohko University Hospital, Sendai, Japan; 3Fukushima Medical University, Fukushima, Japan.

Background: Electrolyzed water (EW) exhibits high dissolved H₂ (DH). We recently reported that EW protects kidney and heart tissue from injury induced by ischemia reperfusion. Oxidative stress and inflammation play a crucial role for chronic kidney injury by aging. The present study aims to test the effect of EW drinking on the development of cardiac-renal tissue injury by aging.

Methods: Dahl salt sensitive male rats (n=90) were divided into three groups: filter water (FW; DH 0.0mmol/L) (TI9000, Nihon Trim, Osaka), de-gass EW (DW) as 0.35 mmol/L, and EW (DH 0.0 mmol/L) for ad-lib drinking (n=30 each). They were fed with 0.5% salt diet during the study. Blood pressure (BP) were measured by tail cuff method every-4weeks. Echocardiography, and tissue samplings of kidney and heart, were performed at 16th, 24th, and 48th week.

Results: There were no differences during the study in body weight, water and food consumption among the groups, but the BP was the lowest in EW (p<0.05). Regarding the test parameters, no differences were found at 16th, 24th week, but the following parameter levels or changes (vs. 16th week) were significantly less in FW as compared to counterparts at 48th week (p<0.05); (Heart) left ventricle posterior wall thickness: FW 14.4%, DW 12.7%, EW 6.3%; cardiomyocyte size (um): FW 43.2%, DW 14.0%, EW 0%; heart tissue fibrosis: FW 30.6%, DW 15.5%, EW 25.1%, ED1 staining (number/slice): FW 28.1±1.46, DW 20.5±1.29, EW 12.3±0.41; malondialdehyde (MDA) staining (%/field): FW 63.7±6.24, DW 70.0±3.94, EW 44.6±2.92, (Kidney): ED1 cells in cortex (number/slice): FW 113.5±13.11, EW 79.5±3.04; MDA staining in cortex (%/field): FW 60.5±1.18, DW 66.2±1.48, EW 57.2±1.20.

Conclusions: Ad lib drinking of high H₂ water could suppress the development of cardiac-renal tissue injury of Dahl salt sensitive rat by aging, at least partly, through the mechanism of attenuating inflammation and oxidative stress.

Funding: Pharmaceutical Company Support

Uremic Toxins Inhibit Glucoronidation in Human Proximal Tubule Epithelial Cells Henricus A. M. Mutsaers,1,2 Dorien Reijnders,1 Martijn J. Wilmer,1 Hanneke Wittgen,1 Lambertus Vd Heuvel,1 Joost G. Hoenderop,1 Rosalinde Masereeuw,1 1Pharmacology & Toxicology, Radboud University Nijmegen Medical Centre, Netherlands; 2Physiology, Radboud University Nijmegen Medical Centre, Netherlands; 3Pediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, Netherlands.

Background: Metabolic enzymes play a key role in the clearance and bioavailability of drugs. During chronic kidney disease (CKD), metabolism is affected leading to altered drug disposition. Furthermore, there is a progressive accumulation of uremic retention solutes due to impaired renal clearance. Here we investigated the impact of uremic toxins on the functionality of an important class of phase II enzymes, viz. UDP-glucuronosyltransferases (UGTs).

Methods: Gene expression of phase I and II enzymes in conditionally immortalized renal proximal tubule epithelial cells (ciPTEC) was studied via a qPCR array and UGT protein expression was determined via Western blot. In addition, the glucuronidation activity of a subset of UGTs in ciPTEC, either untreated or exposed to uremic toxins for 48h, was studied by high performance liquid chromatography using 7-hydroxycoumarin as a substrate.

Results: Our results showed that ciPTEC express a wide variety of metabolic enzymes similar to primary proximal tubule epithelial cells, including cytochrome P450 enzymes and UGTs. Especially UGT1A1, 1A9, 2B7 and 2B8 are highly expressed in ciPTEC (Ct: 25, 17, 24 and 25, respectively; Ct GAPDH: 23) and UGTs were demonstrated to be functionally active (7-OCH-glucuronidation Km: 12±2 µM; Vmax: 242±10 pmol/min/mg).

Furthermore exposure of ciPTEC to non-toxic concentrations of indoxyl sulfate, oxalate, putrescine, p-toluenesulfonic acid or a mix of these toxins significantly decreased the glucuronidation of 7-OHC with 20%, 14%, 18%, 16% and 41%, respectively. Moreover, UGT1A and 2B protein expressions remained unaltered following exposure to uremic toxins, suggesting that the observed inhibition occurs via a direct enzyme interaction.

Conclusions: In conclusion, uremic toxins inhibit UGT function in ciPTEC, thereby affecting the metabolic capacity of the kidney. This may have a clinically significant impact on pharmacokinetics in CKD patients.

Funding: Private Foundation Support

Elevated Soluble Flt1 Mediates an Anti-Angiogenic State in Patients with ANCA-Associated Vasculitis Caroline Versel,1 Ruth J. Pepper,2 H. Terence Cook,3 Alan D. Salama,4 Fadi Fakhouri.1 1Nephrology and Immunology, CHU de Nantes, Nantes, France; 2Centre for Nephrology, University College London, London, London, United Kingdom; 3Centre for Complement & Inflammation Research, Division of Immunology and Inflammation, Department of Medicine, Imperial College London, United Kingdom.

Background: Anti-neutrophil cytoplasm antibody (ANCA)-associated vasculitis (AAV) is a group of necrotizing small vessel vasculitis. Little is known regarding endothelium survival and repair in these diseases.

This study aims to demonstrate that elevated levels of sFlt1 (soluble fms like tyrosine kinase1), an inhibitor of VEGF, induce an anti-angiogenic state that could impair vascular regeneration.

Methods: Circulating sFlt1 levels were determined by ELISA, during active disease and remission, in plasma from patients with Proteinase 3 (PR3-AAV)(n=40), Myeloperoxidase(MPO)-AAV (n=23), and healthy controls (n=18).

To assess the anti-angiogenic activity of these plasma, we used the chick chorioallantoic membrane (CAM) model of angiogenesis. To determine the source of sFlt1 in AAV patients, HUVEC and monocytes were incubated with PR3 and MPO-AAV patients’ plasma drawn during acute or remission phase and monoclonal anti-PR3 and anti-MPO antibodies. For each condition, sFlt1-1 level was measured in cells supernatants.

Results: sFlt1 serum levels increased during active AAV in patients with PR3-ANCA(mean 7300 [321-47355]/pg/ml; p=0.001 vs controls(mean 120 [82-168]/pg/ml) and MPO-ANCA(mean 2242 [31-16851]/pg/ml; p=0.001 vs controls). sFlt1 levels decreased during remission. Plasma from patients with acute AAV displayed an anti-angiogenic effect in the CAM model, an effect prevented by incubating with an excess of VEGF.

Monoclonal anti-PR3 antibodies and plasma from patients with acute PR3-AAV induced a significant and sustained sFlt1 release from monocytes. Anti-MPO antibodies and plasma from acute MPO-AAV had no effect.

Conclusions: Anti-PR3 antibodies mediate an increase in sFlt1 during acute ANCA-associated vasculitis that sustains an anti-angiogenic state.sFlt1 may be an optimal tool for the assessment of AAV prognosis and blocking sFlt1 may enhance renal recovery in AAV patients.

Cyclosporine A Treatment for idiopathic Membranous Nephropathy: A Systematic Review and Meta-Analysis Ge Xiao, Liya Yang, Bi-Cheng Liu. 1Institute of Nephrology, Zhongda Hospital, Southeast University School of Medicine, Nanjing, Jiangsu Province, China.

Background: Treatment of idiopathic membranous nephropathy (IMN) is still a challenge for nephrologists. Cyclosporine A (CsA) has recently been used as an commonly immunosuppressant in the treatment of IMN. However, its clinical effect has not been clear. In this study, a systematic review and meta-analysis were performed to evaluate the efficacy and adverse effect of CSA in treatment of IMN.

Methods: Publications in the English literature were searched with the keywords or text words: ‘cyclosporine’, ‘glomerulonephritis’, ‘membranous’, ‘membranous nephropathy’, ‘membranous glomerulopathy’, ‘membranous glomerulonephropathy’, ‘idiopathic membranous nephropathy’, ‘idiopathic membranous glomerulonephritis’ for clinical trials in electronic databases. Primary outcome was relative risks (RRs) of complete and total renal remission at the end of study period. Secondary outcome included RRs of deterioration of renal function, relapse, development of end-stage renal disease (ESRD) and progression.

Results: Five randomized controlled trials (RCTs) and two clinical controlled trials (CCTs) involving 353 patients were included. CSA offers better efficacy in inducing complete renal remission rates compared with control group (RR 1.18, 95%CI 1.18 - 2.18, p<0.002). But CSA did not increase total renal remission rates compared with control group (RR 1.00, 95%CI 0.85 - 1.24, p=0.97) compared with control group. However, there’s no significant difference between two groups in the risks of relapse (RR 1.26, 95%CI 0.35 - 4.49, p=0.72), hypertension(RR 1.50, 95%CI 0.80 - 2.81, p=0.20)and ESRD (RR 0.63, 95%CI 0.25 - 1.56, p=0.32).

Conclusions: This study suggested that CSA would be effective in inducing complete remission in IMN than other regimens. However, CSA appears to cause a decline of renal function which after recovery dosage or discontinuation.

Funding: Government Support - Non-U.S.

New Retinoic Acid Receptor Agonists for Treatment of Kidney Disease Yifei Zhong,1 Ruijie Liu,1 Peter Y. Chuang,1 John C. He.1 1Medicine, Mount Sinai School of Medicine, New York, NY; 2Nephrology, Shanghai University of Traditional Chinese Medicine, Shanghai, China.

Background: There is a lack of the treatment options for kidney glomerular disease including HIV-associated nephropathy (HIVAN). Podocyte injury is a major cause of glomerular disease. Thus, the strategy to develop effective drugs to protect podocytes

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from injury is critical for treating patients with glomerular disease. Retinoic acid reduces proteinuria and glomerulosclerosis in multiple animal models of kidney disease and the clinical studies are limited because all-trans retinoic acid (ATRA) has significant side effects. Animal studies suggest that ATRA attenuates proteinuria likely through protection of podocytes from injury. The protective effects of ATRA are though binding to the retinoic acid receptor-alpha (RARα). We hypothesize that the RARα specific agonists might be potential drugs for treating patients with kidney disease with significantly less side effects than ATRA.

Methods: To test our hypothesis, we first designed a new lead compound (BD4), which can bind to RARα receptors preferentially and has lower toxicity based on the structure prediction and in vitro cell toxicity assay. We examined the effects of BD4 in podocyte differentiation in vitro. We tested the effects of BD4 in vivo by treating the animal model for HIVAN (Tg26) with either BD4 or vehicle from age of 4 weeks to 10 weeks. Then, we assessed podocyturia and kidney histology in these mice.

Results: BD4 is a unique compound as this is the first retinoids containing boronic acid. We found that BD4 induces expression of podocyte differentiation markers including synaptopodin, nephrin, and WT-1 in podocytes similar to the effects of ATRA. We confirmed that BD4 reduces proteinuria and improves kidney injury in HIV-1 transgenic mice, a model for HIV-associated nephropathy. BD4 treated mice did not develop any obvious toxic or side effects.

Conclusions: Our data suggest that BD4 is a potential new RARα agonist with less toxicity for treatment of patients with kidney disease including HIVAN.

Funding: NIDDK Support

SA-PO2845

KLF15 Reduces the Threshold for Podocyte Injury. Sandeep K. Mallipattu, Rutjie Liu, Peter Y. Chuang, Yan Dai, John C. He. Nephrology, Mount Sinai School of Medicine, New York, NY.

Background: Podocyte injury resulting from a loss of podocyte differentiation has been implicated in many glomerular diseases. It has been previously shown that Retinoic Acid (RA) induces podocyte differentiation via stimulation of CAMP/PKA/CREB pathway. Previous computation analysis revealed that Krupper-Like Factor 15 (KLF15), a kidney enriched CREB targeted nuclear transcription factor, is highly regulated in RA mediated podocyte differentiation and binds to the promoter region of many podocyte specific genes. RA was shown to increase KLF15 expression in cultured wild-type and HIV infected podocytes. KLF15 expression was suppressed in in vitro and in vivo models of HIV-associated nephropathy. KLF15 over-expression stimulated the expression of podocyte differentiation markers in wild-type and HIV-infected murine podocytes. KLF15 binds to the promoter region of slit diaphragm proteins in RA treated murine podocytes. Although, KLF15+/− mice have minimal podocyte injury at baseline, we hypothesize that KLF15 may reduce the threshold for podocyte injury.

Methods: We used two known murine models of podocyte injury to test our hypothesis. Initially, wild-type and KLF15+/− mice were administered low dose Lipopolysaccharide (LPS) (10ug/g) and urine was collected and mice were sacrificed at 48 hours. Similarly, wild-type and KLF15+/− mice were administered Adriamycin (20mg/kg) and urine was collected and mice were sacrificed at four weeks.

Results: Compared to the control group, we observed that LPS treated KLF15+/− mice had a 4-5X increase in albuminuria with significant increase in podocyte effacement. Similarly, the Adriamycin treated KLF15+/− mice had a 15-20X increase in albuminuria with significant increase in glomerular and glomeruloid animal models of kidney injury. KLF15+/− mice developed severe podocyte injury with significant proteinuria and significant increase in podocyte injury.

Conclusions: Although a lack of KLF15 expression results in minimal podocyte injury, additional injury (LPS/Adriamycin) results in significant podocyte effacement and albuminuria. This indicates that KLF15 plays a vital role in reducing the threshold for podocyte injury.

Funding: NIDDK Support

SA-PO2846

Podocyturia in Patients Treated with Anti-VEGF Therapy for Cancer Correlates with the Level of Proteinuria. Juan C. Calle, Issamina Craci, Steven Wagner, Aminah Jatoi, Eddie L. Greene, Joseph P. Grande, Veneta D. Garovic, 1Nephrology and Hypertension, Mayo Clinic, Rochester, MN; 2Oncology, Mayo Clinic, Rochester, MN; 3Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

Background: Proteinuria is a recognized side effect of anti-VEGF (Vascular Endothelial Growth Factor) medications. Similarly, podocyturia (excretion of viable podocytes in the urine) has been observed in various proteinuric diseases including patients treated with anti-VEGF therapy. In this study, we report the largest series to date analyzing podocyturia in patients undergoing anti-VEGF therapy with and without proteinuria.

Methods: The aim of the study was twofold: 1. to demonstrate the presence of podocyturia in patients developing proteinuria while undergoing anti-VEGF therapy and 2. to quantify the observed higher levels of viable podocyte excretion in the urine sample. We performed greater than 500 mg of proteinuria/24 hours calculated by the protein to creatinine ratio.

For identification of viable podocytes, overnight cultures of urine sediment followed by staining with podocin antibodies and a secondary FITC-labeled antibody were performed.

Results:

<table>
<thead>
<tr>
<th>Age range (mean)</th>
<th>Female n (%)</th>
<th>Type of cancer</th>
<th>Urinary albuminuria</th>
<th>Urinary podocyturia</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.79 (59) years</td>
<td>19 (68)</td>
<td>46%</td>
<td>51-73 (63) years</td>
<td>1</td>
</tr>
<tr>
<td>41 (7) years</td>
<td>7 (58)</td>
<td>54%</td>
<td>44-79 (59) years</td>
<td>1</td>
</tr>
</tbody>
</table>

Conclusions: Our data demonstrate two major findings: 1. the presence of podocyturia accompanies proteinuria in patients undergoing anti-VEGF therapy regardless of the type of cancer and 2. levels of podocyturia are higher in patients with proteinuria ≥500 mg/24 hour when compared to patients excreting ≤500 mg/24 hours.
Results: Study characteristics were (n=97) MDRD eGFR: 49 ml/min (30–90), protein-creatinine ratio (PCR) 2.0 g/g (0.8–4.4), urate 454 µmol/L (332–545), mean (sd) age 55yrs (20), mean (sd) serum albumin 34g/dl (8.4). 5% had a raised CRP. On separate univariate linear regression models the β (95% CI) for the association with Ln-DDimer was: age (decades) 0.54 (0.02–0.03), eGFR -0.34 (-0.02, -0.005), Ln PCR 0.59, (0.31, 0.65) and serum albumin -0.49 (-0.07, -0.05), all P<0.01. Ln-DDimer was not associated with wbc count, urate, gender, diagnosis of membranous nephropathy, or diabetes mellitus. On multivariate modeling using backward stepwise regression the final model β (95% CI) was: age (decades) 0.36 (0.01, 0.03) p=0.001, Ln-DDimer 0.33 (0.09, 0.39) p=0.002 and Serum Albumin -0.23 (-0.05, -0.004) p=0.002. Ln-DDimer was not independently associated with eGFR (p=0.3). When analysis was performed stratified by DDimer assay type, inPCR correlation with DDimer in both separate analyses (P=0.0001).

Conclusions: The standard reference range of Ddimer for evaluation of thrombosis should not be used for patients with the nephrotic syndrome. Elevated Ddimer levels in the NS is associated with PCR rather than eGFR.

SA-PO2849
1,25-Dihydroxyvitamin D3 Ameliorates Podocytes Injury Via Inhibiting CD80 Expression Junhao Ma, Wei Shi, Wenjian Wang, Shuangxin Liu, Lixia Xu, Zhihuan Li, Zhiming Ye, Yuan Han Chen, Xinling Liang. Nephrological Department, Guangdong General Hospital, Guangzhou, Guangdong, China.

Background: Accumulating studies have demonstrated that 1,25-Dihydroxyvitamin D3 reduces podocytes loss and slows the decline of kidney function in chronic kidney disease. Recent evidences showed that CD80 expressed on proteinuria. It may reduce the amount of prednisone to maintain remission and slow the progression of CKD by inhibiting CD80 expression.

SA-PO2850
Efficacy and Safety of Leflunomide in the Treatment of Steroid Dependent or Resistant Minimal Change Disease: A Single-Centre Experience Jun-Hui Zhu, Yi-Miao Zhang, Gang Liu, Jun Li, Rong Xu, Jing Huang. Renal Division, Peking University First Hospital, Beijing, China.

Methods: Patients with steroid-dependent MCD (minimal change disease) and two patients with steroid-resistant MCD, who had been treated with leflunomide, were retrospectively analyzed. The initial dose of leflunomide was 10-20mg/d combined with cyclophosphamide. This strategy might be usually well tolerated by the patients.

Conclusions: Leflunomide might be effective in steroid-resistant and steroid-dependent minimal change disease. It may reduce the amount of prednisone to maintain remission and reduce relapse rate comparing with prednisone monotherapy and prednisone combined with cyclophosphamide therapy. This strategy might be usually well tolerated by the patients.

SA-PO2851
Synthetic ACTH Is Less Effective Than Cyclophosphamide in Patients with Idiopathic Membranous Nephropathy Julia M. Hofstra,1 Hans S. Brink,2 Jos J. Van de Kerkhof,2 Jack F. Wetzels;1 Nephrology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands;1 Internal Medicine, Medisch Spectrum Twente, Enschede, Netherlands;1 Internal Medicine, Bernhoven Hospital, Veghel, Netherlands.

Background: Therapy with alkylating agents in idiopathic membranous nephropathy (IMN) is effective, but associated with serious side effects. Synthetic ACTH may be advantageous with reported remission rates up to 85% and no significant side effects.

Methods: We conducted a prospective cohort study in patients with IMN and high risk for renal failure (NCT00694863). Patients with IMN, a nephrotic syndrome, eGFR> 60 ml/min and high risk for progression (elevated urinary β2m levels) were treated with i.m. injections of synthetic ACTH during 9 months (m.). Maximal dose was 1 mg tetracosactide-hexacetate (Synacthen Depot®) twice a week. For comparison, we selected historical controls, treated with cyclophosphamide (CP:1.5 mg/kg/day for 12 m.) and steroids, matched for serum creatine, proteinuria, age, sex and previous immunosuppressive treatment.

Results: We compared 16 patients (M/F 13/3, age 52 ±15yr, sCr 105 ±20 µmol/l, sAlb 23 ±7g/L, Prot/Cr Ratio 8.3 ±3.5 g/100ml) treated with ACTH and 16 patients treated with CP (M/F13/3, age 48 ±13 yr, sCr 102 ±23 µmol/l, sAlb 22 ±5 g/L, PCR 9.6 ±3.5 g/100ml). At the end of treatment, 7 (44%) patients treated with ACTH developed a partial remission of proteinuria (PCR < 2.0g/100ml) versus 15 (94%) patients treated with CP (p= 0.02). At the end of follow-up (20.6 m.in the ACTH group and 75±30 m. in the CP group), relapses had occurred in 3 of 7 patients (43%) in the ACTH group and 4 of 15 patients (27%) in the CP group (log rank p<0.01). Although all patients had side effects on ACTH, these were minor and necessitated dose reduction in only one patient compared with 5 patients treated with CP.

Comparing with prednisone combined with cyclophosphamide, the dose of prednisone to maintain remission could be reduced significantly (from median 22.5mg/d to median 5.0mg/d; P=0.003), relapse rate during follow-up time decreased from 100% to 31.3% (P<0.001), the median time before relapse increased from 11.7 weeks to 32.5 weeks.

Conclusions: Leflunomide might be effective in steroid-resistant and steroid-dependent minimal change disease. It may reduce the amount of prednisone to maintain remission and reduce relapse rate comparing with prednisone monotherapy and prednisone combined with cyclophosphamide therapy. This strategy might be usually well tolerated by the patients.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Conclusions: Treatment with synthetic ACTH is less effective than CP in inducing an initial remission in high risk patients with IMN. Sustained remission was induced in only a minority of patients. These data suggest that synthetic ACTH has limited value in the treatment of high risk patients with IMN.

Funding: Private Foundation Support

SA-PO2852

Efficacy of Rituximab in Steroid Resistant & Dependent Nephrotic Syndrome

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Background: To evaluate the efficacy of rituximab (RTX) in inducing & maintaining remission in difficult steroid resistant (SRNS) & steroid dependent nephrotic syndrome (SDNS)

Methods: Data on 74 patients receiving RTX (375 mg/m² IV weekly) for SRNS (4 doses) or SDNS (2 doses) & followed ≥6-months is presented. Therapy with prednisone & calcineurin inhibitors (CNI) was tapered. For SRNS, remission was defined as complete (CR), partial (PR) or non response (NR).

Results: SRNS. Following onset at a mean age of 4 yr, 38 patients received RTX at 10 yr. Failure of IV steroids & failure (26) or toxicity (12) to CNI was present. Histology showed MCD (n=18) or FSGS (n=20). Four weeks post-RTX, 17 (45%) patients showed CR or PR, allowing CNI taper (100%) & discontinuation (61%). Remission rates were similar for initial (15%) or late (50%) steroid resistance & CNI failure (38%) or toxicity (44%). More patients with MCD (60%) than FSGS (24%) attained remission (P=0.04). At 2-yr, 47% had favorable outcome (CR,PR 7, steroid sensitive relapses 11); 25% had impaired GFR.

SDNS. Following onset at a mean age of 3-yr, 36 patients received RTX at 12-yr. Patients had failed therapy with levamisole, cyclophosphamide, MMF or CNI. Following therapy, there was decrease in relapses (difference 3.5 episodes/y, P=0.001). The mean duration of remission was 13 (5-38) months; 3, 18, 37 cases relapsed at ≥6, 7-12 & ≥12 months respectively. Therapy with CNI or MMF was discontinued; steroids were withdrawn in 79%. At 19 months follow-up, 28% had sustained remission, 16% infrequent relapses & 56% frequent relapses. Relapse-free survival was 92%, 32% & 16% at 6, 12 & 18 months respectively. Repeat doses of RTX (n=11) had similar benefit.

CD19 depletion was found in all cases. Conclusions: RTX effectively induced remission in patients with refractory SRNS & maintained 6-months remission in over 90% cases with SDNS. Since a high proportion of patients with SDNS relapsed after 6-months, studies should examine interventions that prevent relapses following RTX.

Funding: Clinical Revenue Support

SA-PO2853

Urinary Exosomes May Represent a New Diagnostic Tool for Early Diagnosis of Renal Damage

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Background: Exosomes are membrane vesicles, that are secreted from the surface of all cell types after fusion of the multivesicular body with the cell membrane. They contain proteins and mRNA of the cells of origin. Because of the surrounding cell membrane intravesicular proteins and mRNA are well protected from digesting enzymes. In the present study we have evaluated the expression profile of intravesicular exosomal mRNAs as a non-invasive tool for the diagnosis of renal disease.

Methods: Toxic podocyte damage was induced by puromycin aminonucleoside in male Sprague Dawley rats. Urinary exosomes were isolated by differential centrifugation at time points 0 (before disease induction), at the onset of proteinuria (day 5) and during maximal disease activity (day 10). Exosomal mRNA was isolated, amplified, and the mRNA species were globally assessed by gene array analysis. Results: Rats treated with puromycin aminonucleoside developed acute kidney damage, indicated by an increase in serum creatinine and BUN and the development of proteinuria. Messenger-RNA isolated from urinary exosomes revealed 887 differentially expressed genes with an at least 1.5-fold change and 77 genes with an at least 3-fold change in their level of expression on days 5 and 10 as compared to baseline expression on day 0. These genes were mainly involved in the maintenance and reorganization of the cytoskeleton, in the response to oxidative stress and in the control of the apoptotic pathway. Out of these genes we identified a number of genes that were regulated only in the initial phase of the disease (day 5), i.e. at the onset of proteinuria, and therefore bear the potential to act as early markers of glomerular damage.

Conclusions: The gene expression pattern in urinary exosomes of rats with puromycin aminoglycosid nephrosis may serve to identify early markers of renal disease that may lend themselves to both diagnostic and pathogenic studies.

Funding: Government Support - Non-U.S.

SA-PO2854

Superior Results with Tacrolimus Than Ciclosporin in Children with Steroid Resistant Nephrotic Syndrome

Mara Medeiros, Yolanda Fuentes, Saul Valverde, Ana M. Hernández. Hospital Infantil de México Federico Gomez, Mexico, DF, Mexico.

Background: The aim of the study was to evaluate if Prednisone (PDN) and Tacrolimus (Tc) therapy administered during a 12 month period achieves a greater rate and prolonged remission of proteinuria in pediatric patients with steroid resistant nephrotic syndrome (SRNS), compared to those treated with the standard prednisone and Cyclosporine (CyA) regimen.

Methods: A comparative, randomized trial was conducted in children with SRNS; the protocol was approved by the IRB, parent and children consent/assent was obtained in all cases. They received prednisone (PDN) 60 mg/m2/day for 1 month and 30 mg/m2/day every 48 hours, for 5 months, group I received CyA 5 mg/kg/day, target levels 100 to 200 mg/ml. Group II received Tac 0.10 mg/kg/day, adjusted to target levels 5-10 mg/ml. Monthly visits were scheduled for clinical and laboratory evaluations (blood and urine).

Results: Twenty patients were included, ten in each group. Clinical laboratory data is depicted in Table 1. All Tac treated patients had complete remission at 6 months of treatment, whereas only four patients in the CyA group.

Table 1. Clinical and Laboratory Data

<table>
<thead>
<tr>
<th>CyA (n=10)</th>
<th>Tac (n=10)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median years, range)</td>
<td>5.9 (2.2, 17)</td>
<td>7.2 (2.7, 15.8)</td>
</tr>
<tr>
<td>Baseline serum albumin (mg/dL)</td>
<td>3.9 (0.7, 3.3)</td>
<td>3.6 (0.4, 3.2)</td>
</tr>
<tr>
<td>6 months serum albumin (mg/dL)</td>
<td>3.6 (2.3, 8.93)</td>
<td>3.7 (3.4, 4.1)</td>
</tr>
<tr>
<td>New-onset hypertension (%)</td>
<td>8 (80%)</td>
<td>1 (10%)</td>
</tr>
<tr>
<td>Partial or complete remission (%)</td>
<td>7 (70%)</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>Complete remission (%)</td>
<td>4 (40%)</td>
<td>10 (100%)</td>
</tr>
</tbody>
</table>

CyA: Cyclosporin, Tac: Tacrolimus, NS: Nephrotic syndrome, P value obtained either by Fisher Exact test or Mann Whitney test.

No difference was found in serum creatinine and eGFR.

Conclusions: The rate of complete remission is higher with tacrolimus than with cyclosporine at six months of treatment in SRNS children.

Patients treated with cyclosporine had significantly higher rate of new onset hypertension.

Tacrolimus seems to be better therapeutic option in SRNS than Cyclosporine.

Funding: Government Support - Non-U.S.

SA-PO2855

Urinary Biomarkers as Predictors of Response to Rituximab in Patients with Membranous Nephropathy

Maria V. Izazabal,1 Alfonso Eirin,1 John C. Liebesc1, Laurent H. Beck,2 John J. Dillon,1 Patrick H. Nachman,1 Sanjeev Sethi,1 Stephen B. Erickson,1 Daniel C. Cattran,3 Fernando C. Ferrenza,1 1Division of Nephrology, Mayo Clinic, Rochester, MN; 2Department of Medicine, Boston University School of Medicine, Boston, MA; 3Division of Nephrology, University of North Carolina, Chapel Hill, NC; 4Division of Nephrology, Toronto General Hospital, Toronto, ON, Canada.

Background: Rituximab (RTX) reduces proteinuria (P) in membranous nephropathy (MN). However, given the cost and potential side-effects, it would be useful to limit treatment to patients likely to respond to RTX. Recent data suggest that urinary markers (e.g. IgG) predict progression in MN, but whether they can predict P response to RTX is unknown.

Methods: Urinary (U) excretion of retinol binding protein (RBP), alpha-1 microglobulin (α1M), albumin (alb), IgG, and IgM at baseline were correlated with P at 12 and 24 mo, in 20 MN patients treated with RTX (375mg/m²x4) with retreatment at 6 mo. Anti-phospholipase A2 receptor antibody (anti-PLA2R) was also tested.

Results: At 24 mo, complete remission (CR=P<0.3g/24h) occurred in 4 patients, partial remission (PR=U=0.3g/24h) in 12, limited response (LR=≤50% UP reduction but U>3.5g/24h) in 2 and non-response (NR=≥50% UP reduction) in 2, and 1 relapsed.

Baseline Ualb (mg/24h), fractional excretion (FE) of IgG, Uα1M (mg/24h) and URBP (μg/24h), significantly correlated with change in P at 12 mo (p=0.04, 0.05, 0.04, and 0.03), but not at 24mo (p=0.55, 0.42, 0.29 and 0.20 respectively). Correlation between baseline UlgG, FeIgG, Uα1B, Fe alb and response at 12 or 24 mo was not significant. In the 16 patients in which the anti-PLA2R antibody were positive, the decline correlated with the reduction in these protein markers.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only; Underline represents presenting author.

774A
### SA-PO2856

**Cd2ap Regulates Actin Cytoskeleton in Podocytes by Regulating RhoA Activity**

**Hani Suleiman, Andrey S. Shaw, Department of Pathology and Immunology, Washington University, Saint Louis, MO; Institut für Anatomie und Zellbiologie, Universitätmedizin Greifswald, Greifswald, Germany.**

**Background:** Cd2ap-associated protein (CD2AP) is a scaffold protein which plays a critical role in the maintenance of the kidney filtration barrier. The integrity of the kidney filtration barrier is regulated through the fine organization of the actin cytoskeleton. Since CD2AP has been implicated in actin regulation, we were interested to determine whether CD2AP deficient podocytes exhibit any changes in actin-myoosin machinery and whether these changes in actin-myoosin machinery could explain the kidney phenotype found in the Cd2ap(-/-) mice.

**Methods:** Imaging, FRET sensor based live imaging, Immunohistochemistry, Western blot, Immunoprecipitation.

**Results:** We found that podocytes lacking Cd2ap(-/-) have less prominent actin stress fibers, and disorganized localization of Non-muscle myosin IIa. Consistent with the defect in Non-muscle myosin IIa, when we measured the actin-myosin cytoskeleton force across the cells, we found that podocytes lacking Cd2ap exhibit less cell tension. Biochemically, Cd2ap(-/-) cells had reduced myosin light chain phosphorylation (pMLC) on Ser19, a key regulator of non-muscle myosin II activity. We measured the RhoA activity RhoA using a FRET sensor and found that RhoA activity is being reduced, which could explain the pMLC defect. Potentially explaining the defect, the RhoGAP, p190, was found to be hyperphosphorylated in Cd2ap deficient cells. Furthermore, we have evidence suggesting that Cd2ap and p190RhoGAP interact together, which could be responsible for proper activation/inhibition of p190RhoGAP.

**Conclusions:** We conclude that Cd2ap regulates the actin-myoosin machinery by regulating RhoA activity.

### SA-PO2857

**The Occurrence of Cancer in Patients with Membranous Nephropathy**

**Sophia Lionaki, Sean Barbour, Yichu Hu, Susan L. Hogan, Caroline E. Jennette, Ronald J. Falk, Daniel C. Catran, Patrick H. Nachman, Leather N. Reich, UNCI Kidney Center, University of North Carolina; Nephrology, Laiko Hospital, Greece; University of Toronto, Canada; Contributed equally.**

**Background:** To describe the frequency and character of cancers (CA) in patients with membranous nephropathy (MN), in relation to clinical parameters, and long term outcomes.

**Methods:** We studied 898 patients with MN from the Glomerular Disease Collaborative Network (N=412) and the Toronto Glomerulonephritis Registry (N=486). Clinical and laboratory values at biopsy were included in the analysis. Smoking and immunosuppressive therapies for MN were studied as predisposing factors for cancer. Among 898 patients with MN, a total of 85 malignancies were recorded in 64 patients (7.1%) [56 patients (6.2%) excluding non-melanoma skin CA]. Of these, 39 (45.9%) occurred within 5 years of biopsy diagnosis. The most common cancers were of the skin (20%), prostate (10%), breast (9%), lung (9%), GI tract (6) and hematologic (6). Excluding non-melanoma skin CA, patients with CA were older.

**Results:** Of 898 patients with MN, 42 (4.6%) received renin-angiotensin-aldosterone therapy. There were no statistical differences in serum albumin, eGFR adjusted for age, proteinuria or duration of proteinuria prior to biopsy between those with and without CA. Cancers occurred in 3.6% of patients <60 y.o. and 12.9% in those ≥ 60 y.o.

**Conclusions:** There is an increased frequency of cancers among patients with MN older than 60 years. We found no distinguishing clinical features at presentation between patients with and without cancer. Presence of cancer did not affect renal survival.

**Funding:** NIDDK Support, Government Support - Non-U.S.

### SA-PO2858

**Performance of Anti-Phospholipidase A2-Receptor Antibody Testing for Membranous Nephropathy in Routine Clinical Practice**

**Ulf Schoenermarck, Thomas Eichhorn, Timo Wendlter, Volker Vielhauer, Stephan R. Lederer, Kai Fechner, Michael Fischieder, Medical Clinic I, Nephrology Div., University Hospital Munich-Grosshadern, Munich, Germany; Institute of Clin. Chemistry, University Hospital Munich, Munich, Germany; Medical Policlinic, Dept. of Nephrology, University Hospital Munich-Innenstadt, Munich, Germany; KKH Nierenzentrum Laim, Munich, Germany; Institute of Experimental Immunology, Euroimmun AG, Luebeck, Germany.**

**Background:** Autoantibodies against the M-type phospholipase A2 receptor (anti-PLA2R) have been recently identified in patients with primary membranous nephropathy (MN). Aim of the study was to evaluate the measurement of anti-PLA2R-antibodies with a commercially available serologic test in routine clinical practice.

**Methods:** All patients with non-muscle biopsies proven MN seen between October 2010 and 2011 were tested for the presence of anti-PLA2R-antibodies. As control group patients with secondary forms of MN and other proteinuric renal diseases were analysed. For detection of circulating anti-PLA2R-antibodies an indirect immunofluorescence assay was used (EUROIMMUN AG, Lübeck, Germany). A specific cytoplasmic fluorescence of transfected HEK 293-cells at a dilution of 1:10 higher was considered to be positive.

**Results:** The anti-PLA2R-antibody test was negative in all patients (n=10) with secondary forms of MN (hepatitis B, n=1, tumor-assoc., n=1, assoc. with connective tissue disease and SLE, n=7, MPO-ANCA-assoc. MN, n=1) and patients with other proteinuric diseases (n=14). 11 of 16 patients with primary MN were positive for anti-PLA2R-antibodies. The antibody titer ranged from 1:10 to 1:3200 and was present up to 8 years after last diagnosis of MN. There was no correlation between the antibody level and proteinuria or renal function. Further 3 patients with primary MN tested at time or after kidney transplantation were also negative for anti-PLA2R-antibodies, although one patient presented with nephritic proteinuria due to bioptronically proven recurrent MN.

**Conclusions:** Anti-PLA2R-antibodies have a high sensitivity and 100% specificity for primary MN. The serologic test appears to be a useful tool in routine clinical testing.

### SA-PO2859

**The Role of Gene Polymorphisms of the Renin-Angiotensin-Aldosterone and Inflammation Pathways in the Progression of Glomerular Diseases:**

**Isabelle Chapdelaine, Remi Goupil, Jean-Philippe Rioux, Stephanie Raymond-Carrier, Francois Madere, Stephan Troyanov, Experimental Immunology, Hôpital du Sacré-Coeur, University of Montreal, Montreal, QC, Canada.**

**Background:** Clinical risk factors of progression in glomerular diseases such as proteinuria and blood pressure predict outcome imperfectly. Previous studies have proposed various polymorphisms of genes implicated in the pathophysiology of glomerulopathies as clinical tools in risk assessment. We sought to validate their impact on the rate of renal function decline in a prospective cohort study receiving usual anti-hypertensive and anti-inflammatory treatment.

**Methods:** Using Medline, we identified 9 candidates polymorphisms of known genes, mostly of the renin-angiotensin-aldosterone and inflammation pathways: MCP-1 A2518G, TGF-β1 T869C and C-509T, ACE I/D, AGT M235T, AT1R A1166C, TSC-22 A-396G, eNOS 4b/a C344T.

**Results:** We prospectively recruited 93 predominantly male (73%) and Caucasian (85%) patients with an age of 64 ± 13 years and eGFR of 34 ± 21 mL/min/1.73m2 at baseline. Patients were matched to 50 healthy controls of similar ethnic background. Sixty-one percent of patients had diabetic nephropathy, almost all received renin-angiotensin-aldosterone blockade (91%) and none immunosuppressive therapy. The average MAP during follow-up was 94 ± 7 mm Hg with a urinaly protein to creatinine ratio of 0.15 (interquartile range from 0.05-0.30) g/mol. The rate of renal function decline was -2.9 ± 4.5 mL/min/1.73m2 or over a median 34 months period. Proteinuria and blood pressure strongly predicted progression.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**Underline represents presenting author.**
SA-PO2860
The Serum Cholesterol, a Marker of Cholesterol Absorption, Is Elevated in Patients with Nephrotic Syndrome. Masahiro Kikuchi, Shouichi Fujimoto, Yuji Sato, Kazuo Kitamura. University of Miyazaki Hospital, Faculty of Medicine, Miyazaki, Japan.

Background: In generally, nephrotic syndrome-associated hypercholesterolemia is considered due to increased production of lipoprotein by the mechanism of compensation for hypoproteinemia. But, there have been reports that cholesterol synthesis was not increased in nephritic animal or human. There is still no convincing evidence for this hypothesis, especially in human.

Methods: In the present work, we investigated the serum cholesterol, carotenoprotein and sitosterol, as markers of cholesterol absorption, and lathosterol as a marker of cholesterol synthesis in nephrotic patients not treated with a cholesterol lowering drug. Furthermore, we did same examinations in patients including complete remission. We made a comparison those results in patients in the nephrotic state (NS, n=19) and those in patients in the complete remission state (CR, n=8).

Results: The serum lathosterol, a marker of cholesterol synthesis, was elevated in NS, compared to CR (NS vs. CR, 3.85±0.42 vs. 2.39±0.32 µg/ml, p<0.04). As for markers of cholesterol absorption, the serum carotenoprotein and sitosterol were not significantly different in both state, but the serum cholesterol, which is less affected by diet, were significantly elevated in NS, compared to CR (NS vs. CR, 5.52±0.50 vs. 3.55±0.34 mg/dl, p<0.01). The serum lathosterol and carotenoprotein were not correlated with the severity of nephrotic syndrome (ie: serum total protein and albumin, urinary protein concentration, serum total, and low-density lipoprotein cholesterol, etc.).

Conclusions: In nephrotic syndrome, not only synthesis but also absorption of cholesterol should contribute to hypercholesterolemia.

SA-PO2861
Development of Chronic Kidney Disease Was Not Predicted by Urinary Testing or Renal Biopsy at Liver Transplantation for Hepatitis C Virus-Induced Cirrhosis. David Patrick Newton, Brendan M. McGuire, Bruce A. Julian. Medicine, University of Alabama at Birmingham, AL.

Background: We have shown that urinalysis and quantitative proteinuria are often normal despite biopsy-proven immune complex glomerulonephritis at the time of liver transplantation for cirrhosis due to chronic infection with hepatitis C virus (HCV) (Ann Intern Med 2006;144:735). It is uncertain whether the renal outcome for such transplant recipients can be predicted based on urinalysis or renal histology at the time of transplantation.

Methods: We reviewed the long-term clinical follow-up of 30 patients who had undergone renal biopsy at time of liver transplantation for HCV cirrhosis in 2004-2005. Results: Four patients died within 2 years of transplantation, including 1 on dialysis at engraftment. The second patient on dialysis at transplantation now has eGFR 65 mL/min/1.73 m2. Seven patients have developed stage 4 chronic kidney disease at last follow-up. The Table compares this subgroup to the 19 other surviving patients. Microscopic hematuria was defined as >4 rbc/hpf; proteinuria was defined as dipstick 1+ or urinary protein/creatinine ratio at least 0.30.

<table>
<thead>
<tr>
<th>eGFR at last follow-up</th>
<th>&lt; 30 mL/min/1.73 m2</th>
<th>≥ 30 mL/min/1.73 m2</th>
<th>n</th>
<th>AT LIVER TRANSPLANTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr, mean</td>
<td>50.3</td>
<td>55.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender, male, n (%)</td>
<td>17 (89)</td>
<td>2 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race, Caucasian, n (%)</td>
<td>16 (84)</td>
<td>6 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR, mL/min/1.73 m2 (±SD)</td>
<td>62.2 (15)</td>
<td>61 (131)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV viral load, copies/mL, mean</td>
<td>599,910</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteinuria, n (%)</td>
<td>4 (21)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematuria</td>
<td>10 (55)</td>
<td>2 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal histology, n (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranoproliferative glomerulonephritis</td>
<td>9 (47)</td>
<td>2 (29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>3 (16)</td>
<td>3 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesangiproliferative glomerulonephritis</td>
<td>5 (26)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minor glomerular changes</td>
<td>2 (11)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Conclusions: Development of chronic kidney disease was common within a relatively short interval after liver transplantation for HCV cirrhosis. This complication was not predicted by severity of urinary abnormalities, eGFR, or renal histological features at the time of liver transplantation.

Funding: NIDDK Support, Pharmaceutical Company Support

SA-PO2862
Antiproteinuric Action of Cinacalcet in Children with Steroid-Sensitive Nephrotic Syndrome. Retti Schafer, Jun Oh, Franz S. Schafer, Burkhard Toenhoff, C. P. Schmitt. 3rd Pediatric and Adolescent Medicine, Heidelberg; 4th University Children’s Hospital, Hamburg.

Background: CaSR is expressed in human podocytes. In vitro, exposure of podocytes to the calcium-imaging R-568 stabilizes the actin cytoskeleton and reduces PAN induced apoptosis. In vivo it attenuates proteinuria, podocyte and GFR loss and glomerulosclerosis. Clinically, this role has not yet been evaluated.

Methods: 4 children (3-9 years) with idiopathic nephrotic syndrome (NS), who showed up with initial manifestation (n=2) and second relapse (n=2), respectively, and who objected to steroid therapy, were treated with the calcimimetic Cinacalcet (Cin). Initial dose was 15 mg/m²BSA/d, gradually increased by 5 mg/m²BSA according to the antiproteinuric effect. Calcium was supplemented at an initial dose of 2x500 mg/m²BSA/d.

Results: The first patient received a single dose of Cin (5 mg/m²). Protein- and albuminuria were reduced (570 to 398 and 378 to 255 g/mol/crea) within 24 hours (normal range). In the remaining 3 patients proteinuria was 1618, 821 and 763 g/mol crea and declined by 92, 71 and 73% to a nadir of 130, 235 and 209 within 8, 5 and 3 days. Albuminuria was 1347, 648 and 746 g/mol crea and declined by 96, 73, and 76% to a nadir of 58, 172 and 178. Serum albumin was 26.7, 26.9 and 29.1 g/l and increased to a maximum of 27.4, 28.6 and 31.8. Oedema disappeared in all 3 patients within 6-13 days. Ca²⁺- and phosphate excretion did not change; serum Ca²⁺ remained in the normal range. The maximal doses of Cin were 26.1, 21 and 28.3 mg/m²BSA, the treatment was well tolerated. All 3 children experienced a relapse after 10, 23 and 19 days which was associated with an increase in proteinuria. In the last patient Cin was discontinuated and prednisolone therapy initiated, which induced remission in all 3 patients within 7-9 days.

Conclusions: Cinacalcet markedly reduces proteinuria in children with idiopathic NS, albeit without inducing a complete and stable remission.

SA-PO2863

Background: Developing therapeutics for glomerular disease requires testing in preclinical models, often relying on albuminuria as an indicator of efficacy. Podocyte loss has recently been recognized as a critical step in glomerulopathy progression, and measurement of podocyte number has consequently emerged as an additional disease endpoint. However, the correlation between podocyte loss and albuminuria has not been carefully explored.

Methods: To better understand the relationship between these two endpoints, we carried out a glomerulonephritis study in a rat model of monoclonal PAN (a rat model of glomerular injury. Uninephrectomized rats were dosed with PAN or with PBS as a control. Urine, serum, and kidneys were collected at weeks 1-5, 7, and 12.

Results: Average ACR in PAN-treated rats peaked at 4 weeks, after which ACRs declined but remained elevated relative to PBS controls. Podocyte loss is evident in PAN-treated rats over the course of the study, measured as number of WT-1 positive nuclei per glomerular area. A 42% reduction in podocyte density was observed at week 12 relative to controls or to PAN-treated rats at week 1. The largest drop in podocyte density was observed between weeks 2 and 3, slightly preceding the peak in average ACR. When all samples are analyzed together, ACR and podocyte number show a modest but statistically significant correlation (R²=0.158, P<0.002). However, no correlation between ACR and podocyte number is observed when early PAN-treated samples (from weeks 1-5, peak proteinuria) are compared separately, and early PAN data are statistically distinct from late PAN- (weeks 7-12) and PBS-treated samples (P<0.01). Accordingly, the ACR/podocyte loss correlation improves when early PAN-treated samples are removed (R²=0.472, P<0.001).

Conclusions: Taken together, these data suggest that the high levels of albuminuria seen in the early weeks of toxin-induced renal injury models may not reflect long term disease progression, and that care must therefore be taken in using such models to predict the efficacy of candidate therapeutics for renal disease.

Funding: Pharmaceutical Company Support

SA-PO2864
Treatment of Resistant Glomerular Diseases with ACTH Gel: A Prospective Trial. Andrew S. Bomback, Pietro A. Canetta, Jai Radhakrishnan, Gerald B. Appel. Columbia University Medical Center, New York.

Background: Adrenocorticotropic hormone (ACTH) has shown promising results as second- and third-line therapy for idiopathic glomerular diseases resistant to conventional therapies, but the data reported to date has solely been from retrospective, observational studies.

Methods: In this prospective, open-label, pilot study (NCT01129284), 15 patients with resistant glomerular diseases were treated with ACTH gel (80 units SC twice weekly) for 6 months. Resistant membranous nephropathy (MN) was defined as failure to achieve sustained remission with at least 2 immunosuppressive regimens, resistant MCD/FSGC was defined as failure to achieve sustained remission with corticosteroids and at least 1 other immunosuppressive regimen, and resistant IgA nephropathy was defined as ≥1 g/day proteinuria despite effective ren angiotensin system blockade. Complete remission was defined as stable or improved renal function with ≥50% reduction in proteinuria and final proteinuria 500-3500 mg/day.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

776A
Results: The study included 5 patients with resistant MN, 5 patients with resistant MCD (n=2) and FSGS (n=3), and 5 patients with resistant IgA nephropathy. Two resistant MN patients were in partial remission at the end of 6 months of therapy, although 3 achieved immunologic remission of disease (PLA2R antibody disappeared by 4 months of therapy). One patient with resistant FSGS achieved complete remission at 6 months; one patient with resistant MCD achieved a partial remission at 6 months but relapsed within 4 weeks of stopping ACTH. Three of 5 patients with resistant IgA nephropathy demonstrated ≥50% reductions in proteinuria while on ACTH, with proteinuria consistently <1 g/day by 6 months. Three of 15 patients reported significant steroid-like adverse effects with ACTH, including weight gain and hyperglycemia, prompting early termination of therapy without any signs of clinical response.

Conclusions: ACTH gel is a promising treatment for resistant glomerular diseases. This therapeutic option should be studied further in randomized, controlled trials against currently available therapies for resistant disease.

Funding: Pharmaceutical Company Support

SA-PO2865

Antiviral Therapy in Hepatitis B-Associated Membranous Nephropathy
In O Sun, Yu Ah Hong, Hyun Gyung Kim, Hoon Suk Park, Sun Ryong Choir, Byung Ha Chung, Cheol Wее Park, Chloe Woo Yang, Yong-Soo Kim, Bumsoon Choi. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: Lamivudine is effective for treatment of hepatitis B-associated membranous nephropathy (HBV-MN), but prolonged use leads to the emergence of drug-resistant variants. Also, new drugs such as entecavir, adefovir or clevudine have been introduced to treat the chronic hepatitis B, but there are few data on its efficacy in HBV-MN. We describe our experiences about treatment of HBV-MN with various anti-viral drugs.

Methods: From 1996 to 2010, biopsy-proven MN was diagnosed in 89 patients, and 10 patients had HBsAg. We investigated the clinical courses and therapeutic responses, and propose of patients with HBV-MN.

Results: The incidence of HBV-MN was 10.1%. The mean age of the patients was 33 years (range, 19-55), and 8 patient (80%) were men. All patients had HBsAg, hepatitis B e antigen (HBsAg) and HBV DNA. Of the patients, 6 received anti-viral drugs, and 4 were treated by supportive care. One out of four patients who received supportive care had a spontaneous remission. Out of six patients who received anti-viral drugs, four were treated by lamivudine, and the other two by entecavir. Two of the four patients treated by lamivudine achieved complete remission with seroconversion to anti-HBsAg, whereas the other two patients experienced lamivudine-resistant strains with mutations at the the tyrosine-methionine-aspartate-aspartate (YMDD) motif of DNA polymerase, which were detected at 22 and 23 months after lamivudine treatment, respectively. Therefore adefovir was added in one patient, and lamivudine was switched to clevudine in the other patient. After treatment, two patients were kept in remission of proteinuria. Two patients who received entecavir as an initial therapy went into complete remission, and resistance to entecavir didn’t occur during follow-up period. No side-effects were seen in the patients who received anti-viral drugs.

Conclusions: New nucleoside analogues such as entecavir, adefovir or clevudine could be effective at treatment of HBV-MN including lamivudine resistant strains.

SA-PO2866

Factors Influencing Treatment Choice in Idiopathic Membranous Nephropathy (IMN)
Shannon L. Mahoney,1 Vimal K. Derebali,2 Andrea Biddle,1 Yichun Hu,1 Michelle A. Hladunewich,2 Ronald J. Falk,1 Daniel C. Cattran,2 Heather N. Reich,1, 3 Patrick H. Nachman,1 1UNC Kidney Center; Chapel Hill, NC, 2University of Toronto, ON, Canada; 3Contributed Equally.

Background: The treatment of patients with IMN may include immunomodulation in patients resistant to conservative therapy alone, or perceived at high risk of progression. This study assesses the factors influencing the decision to treat with immunomodulators and the choice of treatment between cyclophosphamide (CP) and calcineurin inhibitors (CNI).

Methods: An inception cohort of 720 adults with biopsy-proven IMN (from 1976-2005) was derived from the Glomerular Disease Collaborative Network (N=328) and the Toronto Glomerulonephritis Registry (N=392). Subjects were allocated to groups based on initial treatment: no immunotherapy, CP, CNI, glucocorticoids (GC) only, or other second treatment was considered in patients who initially received GC alone. Two of the four patients treated by lamivudine achieved complete remission with seroconversion to anti-HBsAg, whereas the other two patients experienced lamivudine-resistant strains with mutations at the the tyrosine-methionine-aspartate-aspartate (YMDD) motif of DNA polymerase, which were detected at 22 and 23 months after lamivudine treatment, respectively. Therefore adefovir was added in one patient, and lamivudine was switched to clevudine in the other patient. After treatment, two patients were kept in remission of proteinuria. Two patients who received entecavir as an initial therapy went into complete remission, and resistance to entecavir didn’t occur during follow-up period. No side-effects were seen in the patients who received anti-viral drugs.

Conclusions: New nucleoside analogues such as entecavir, adefovir or clevudine could be effective at treatment of HBV-MN including lamivudine resistant strains.

SA-PO2867

Urinary Interleukin-6 (IL-6) and Epidermal Growth Factor (EGF) as Independent Risk Factors for the Progression of Primary Chronic Glomerulonephritis (CGN)
Hiona Idasisiak-Piechocka1, Elżbieta Pawlizcak2, Andrzej P. Oka.1 1Nephrology, University of Medical Sciences, Poznan, Poland; 2Nephrology, University of Medical Sciences, Poznan, Poland; 3Nephrology, University of Medical Sciences, Poznan, Poland; 4Nephrology, University of Medical Sciences, Poznan, Poland.

Background: The aim of the study was to estimate whether initial urinary IL-6 and EGF excretions in patients with newly diagnosed CGN are associated with clinical course and the outcome of the disease during 4-year follow-up.

Methods: 150 Caucasian patients (114 men and 36 women) with biopsy proven primary CGN were included into the study. One day before kidney biopsy urinary excretion of IL-6 (UIL-6) and EGF (UEGF) were measured using the ELISA methods. R&D System. 106 patients were treated with steroids alone and in 78 patients immunosuppressive therapy was included.

Results: UIL-6 excretion was significantly higher (p=0.0000) than in healthy subjects. Significant negative correlation between initial UIL-6 and UIBR was found in patients with CGN (SR = -0.40).UIL-6 was significantly higher but UEGF excretion was significantly lower in patients with eGFR < 60 ml/min/1,73m2 when compared to patients with eGFR > 60 ml/min/1,73m2. After 4-year of follow-up, patients were divided into two groups: progressors (PG) - loss of eGFR > 5 ml/min/1,73m2/year and nonprogressors (NP) with stable kidney function (loss of eGFR < 5 ml/min/1,73m2/year). UIL-6 was significantly higher in PG when compared with the NPG (p=0.0005) and control subjects. On the contrary, UEGF excretion was significantly lower in PG in comparison to NPG patients (p=0.02).

Conclusions: Logistic regression analysis of the UIL-6 and UEGF excretions and traditional risk factors for the progression of the disease (eGFR > 60ml/min/1,73m2, male gender, age > 60 years, glomerulosclerosis > 30% and interstitial fibrosis 2+) showed that the most important independent risk factors for the deterioration of renal function are initial high (>11,8 mg/gCr) UIL-6 excretion, initial low (<15,468 mg/gCr) UEGF excretion and male gender. The initial value of IL-6/EGF 0.46 increased the risk of progression of the disease 7,8 times in patients with newly diagnosed primary CGN.

Funding: Clinical Revenue Support

SA-PO2868

Clinical Outcome of Renal Primary Systemic Amyloidosis after Stem Cell Transplantation
Claire Tinel1, Laurent Martin,2 Denis Caillot, Jean-Michel Rebibou,1 1Nephrology, University Hospital, Dijon, France; 2Pathology, University Hospital, Dijon, France; 3Hematology, University Hospital, Dijon, France.

Background: Regarding the treatment strategy of primary amyloidosis, autologous stem cell transplantation after high dose melphalan has similar patient survival than melphalan plus dexanethasone, but little is known on the renal outcome when complete remission is achieved.

Methods: Nine patients (sex-ratio F/M=3/7; age 60±9) with biopsy-proven AL amyloidosis and renal manifestations (defined by proteinuria ≥ 0,5 g/d or nephrotic syndrome, and/or a glomerular filtration rate (GFR) ≤ 30 ml/min) were treated in our center. 8 patients received high-dose melphalan and autologous stem cell transplantation.

Results: Renal manifestations included proteinuria (n=8) (median 10 g/d [range 1,5-47]), nephrotic syndrome (n=6) and GFR ≤ 30 ml/min (n=2). Multiple myeloma was associated in 6 patients (75%), at stage II (n=5) and III (n=1) according to the International Staging System. The mean follow-up was 62 ± 19 months; 2 patients died without any hematological or renal response. Among the 6 surviving patients, 4 achieved stringent complete remission (SCR), 1 reached partial remission (PR), and 1 relapsed after 4 years (digestive tract relapse without renal involvement). The patient showing PR, reached terminal renal failure after 33 months and initiated hemodialysis. Renal response defined as a proteinuria ≤ 0,5 g/d was achieved by the 5 other patients, in a mean time of 21 months. All renal responders experienced transitory renal impairment. By month 12, the lowest kidney function was noted with a mean GFR of 35 ml/min (n=5), as compared to 76 ml/min (n=5) on diagnosis. We observed a late improvement with a mean GFR of 54 ml/min (n=4) after 6 years.

Conclusions: In primary systemic amyloidosis treated with high-dose melphalan followed by autologous stem cell transplantation, achieving complete hematologic response is associated with an excellent renal outcome. Our unique patient with partial remission doesn’t allow us to draw conclusions on the renal prognosis in this event. Interestingly we observed worsening renal function during the 1st year, followed by a late improvement.

Funding: NIDDK Support, Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Membranoproliferative Nephropathy Associated with Monoclonal Immunoglobulin-M

**Background:** Membranoproliferative nephropathy (MPN) is the most common cause of primitive nephrotic syndrome in adults. MPN is characterized by IgG and C3 deposits on urinary side. Membrane repair is seen in 70% of the cases. Membranous 30% of MPN without aetiology. The aim of our work is to study the association of monoclonal gammapathy with MPN.

**Methods:** Retrospective study of all the MPN diagnosed in an university hospital in France. The time lapse of the study was from January 2000 to July 2010. A monoclonal gammopathy, lupus bi markers, hepatitis B and C, and HIV serology were systematically searched for all the patients.

**Results:** We identified 94 patients with MPN, 11 MN were associated with lupus. In the remaining 83 patients, 58 males and 25 females of mean age 53.5 years were studied. In 7 patients we found a monoclonal gammapathy. All the 7 were IgM. In two cases, Waldenström’s disease (WD) was diagnosed, in the remain 5 cases, it was concluded to a IgM MUGS.

The two patients with WD were treated with success and the MN is in full remission. One patient received a Ponticelli protocol with success on MN associated with a decrease in IgM level. One patient received only rituximab without effect on MN and IgM level. The last 3 patients received supportive care and experienced partial remission. On kidney biopsy we identified IgM deposits associated with IgG and C3 deposits in 5 cases.

**Conclusions:** Our data suggested that monoclonal IgM could be associated with MPN. Full remission of nephrotic syndrome was obtained only when level of monoclonal IgM decreased and we identified IgM deposits in the kidney, thus suggesting a relationship between the hematological disease and nephropathy. The prevalence of MUGS IgM in our cohort of MN is 6% compared to 0.5% in general population in favor of a none fortuitous association between monoclonal IgM and MN.

In conclusion, monoclonal IgM could play a role in pathogenesis of MN.

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**SA-PO2870**

**Renal Involvement in Patients with Primary Myelofibrosis: Fact or Fiction?**

Sandra Herrmann, 1 Sahim H. Nasr, 2 Samar M. Said, 2 Nelson Leung. 1 Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; 2 Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN.

**Background:** Some small reports suggest that primary myelofibrosis (PMF) can be associated with renal dysfunction. However, the prevalence of renal disease including glomerulopathy in PMF remains poorly defined.

**Methods:** Retrospective cohort analysis of all patients with PMF listed in the Mayo Clinic Myelofibrosis Database. Patients without renal function data or those who underwent stem cell transplantation were excluded.

**Results:** Among a total of 565 patients with PMF (mean age 65.12 years, 366 men), the mean serum creatinine (Scr) at referral was 1.16±0.36 mg/dL, eGFR was <60 mL/min/1.73 m2 in 210 patients (37%). Follow-up data were available for 383 patients, and over a mean follow-up period of 34±36 months, the average Scr increased from 1.13±0.35 to 1.71±0.6 mg/dL (p<0.05). Only 22 (5.7%) had a Scr ≥2 mg/dL and 5 (1.3%) had ESRD requiring hemodialysis. A total of 241 patients had complete urine analysis at the end of the follow up, and the mean proteinuria wash 686±213 mg/day. Proteinuria ≥1 g/day and ≥3 g/day was seen in 29 (12%) and 13 (5.3%) patients, respectively. Survival data were obtained by chart review for the follow-up patients. Mortality rates were not different for patients without and with an eGFR <60 mL/min/1.73m2 from the time of the initial visit (mean follow-up time 63±50 months). However, mortality rates from the time of their last follow-up visit (mean follow-up time 29±42 months) were significantly higher for patients who sustained a decrease in eGFR ≥30 mL/min/1.73m2 from the initial to the last follow-up visit (63 vs. 38%, p<0.01).

Five kidney biopsies were reviewed. Myeloproliferative-neoplasm (MNP)-related glomerulopathy was the most common finding in four. One biopsy showed ATN. In two patients, the biopsies also showed extramedullary hematopoesis.

**Conclusions:** This is the largest study on renal function in patients with PMF and indicates that a decline in eGFR may predict mortality. Contrary to plasma cell dyscrasias, glomerulopathy was the most common finding in four. One biopsy showed ATN. In two patients, the biopsies also showed extramedullary hematopoesis.

**Funding:** Private Foundation Support

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**SA-PO2869**

**Rituximab vs. Calcineurin Inhibitors in Steroid Dependent Nephrotic Syndrome**

Aditi Sinha, Ashima Gulati, Arvind Bagga. 1 Division of Nephrology, Children’s National Medical Center, Washington, DC.

**Background:** Children with steroid resistant nephrotic syndrome (SRNS) typically have FSGS, but may rarely have minimal change histology. These patients are resistant to immunosuppressive (IS) drugs with poor long-term outcome. Immune mediated FSGS has been associated with the presence of a circulating permeability factor (FSPF), thought to increase glomerular permeability to albumin leading to proteinuria and focal sclerosis. Galactose binds to FSPF in vitro, but the effect in vivo is unknown. We prospectively investigated the effect of oral galactose on change in FSPF and clinical response in children with SRNS.

**Methods:** 52 children with primary SRNS or biopsy proven FSGS were screened. Children meeting inclusion criteria (nephrotic proteinuria (UPC) ≥2) despite IS therapy and ACE/ARB more than 12 weeks, stable Cr and estimated GFR above 60 mL/min/1.73 m2 were tested for FSPF. Those with positive FSPF were treated with oral galactose(0.2g/kg dose BID) for 16 weeks. Pre and post treatment FSPF levels, UPC, serum albumin(SAlb), and eGFR were assessed.

**Results:** FSPF was tested in 6 children(8.8±3.8 years old; 3 male, 3 female). Of these, 5 had FSGS (1 with post transplant recurrence) and 1 had minimal change. Patients with FSGS had positive FSPF, whereas patient with MCNS was negative for FSPF. Three children with baseline eGFR 93.9±26.7 completed 16 weeks of therapy without any side effects. These children were resistant to previous treatments with tacrolimus (3), ACE (2), rituximab (2), cyclosporine (1), and MMF (1). Time from onset of NS to FSPF was 31.8±7.8 months. Post-therapy, FSPF decreased from 0.74±0.16 to 0.35±0.21 and became negative in 2 of 3 children. UPC did not improve after therapy (12.6±9.4 pre and 27.4±31.9 post-therapy). Pre and post treatment eGFR (93.9±26.7 versus 89.4±50.7) and SAlb (2.4±1.3 versus 2.2±0.6) remained unchanged. One child with post-transplant recurrent FSGS entered chronic dialysis at the end of therapy.

**Conclusions:** Oral galactose was well tolerated in 3 children with SRNS and was able to decrease FSPF to negative range in 2 children. However, no clinical improvement was observed. It remains to be determined whether galactose will be clinically beneficial if used early in the course of SRNS.

**Funding:** Private Foundation Support

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**SA-PO2873**

**Columbia FSGS Classification Predicts Renal Outcome**

Aline Lázara Resende, 1 Silvia M. Titan, 2 Leonardo Abreu Testagrossa, 2 Vinicius Colares, 1 Denise Maria Avancini Costa Malheiro, 3 Rui Toledo Barros, 1 Viktoria Woronik, 3 & Rui Toledo Barros, 1

1 Nephrology, Division, Hospital das Crianças, Sao Paulo University Medical School, Sao Paulo, Brazil; 2 Pathology Division, Hospital das Crianças, Sao Paulo University Medical School, Sao Paulo, Brazil.

**Background:** Columbia classification (CC) has been proposed as a predictor of worse renal outcome in FSGS. We have analyzed clinicico-laboratorial features and renal survival of Brazilian patients according to CC.

**Methods:** Inclusion criteria were primary FSGS from 99-09, no dialysis at presentation, and follow-up time >4m. Of 80 patients, 57 had available material for CC reclassification. Data was retrospectively collected. Primary outcome (PO) was defined as ESRD or creatinine doubling.

**Results:** 27 patients received CNI (CsA 14, Tac 13) and 10 RTX. Baseline features were similar (Table).

<table>
<thead>
<tr>
<th>Feature</th>
<th>Baseline</th>
<th>Rituximab, N=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at onset, yr</td>
<td>2.5 (1-7)</td>
<td>4.3 (1-6)</td>
</tr>
<tr>
<td>Age at therapy</td>
<td>0.0 (1-7)</td>
<td>12 (8-15)</td>
</tr>
<tr>
<td>Relapses/yr</td>
<td>3 (1-7)</td>
<td>3 (1-5)</td>
</tr>
<tr>
<td>Cumulative prednisone, mg/kg/sm</td>
<td>70 (7-138)</td>
<td>69 (16-127)</td>
</tr>
<tr>
<td>Body mass index (BMI) SDS</td>
<td>1.8 (0.6-4.3)</td>
<td>2.0 (0.5-3.6)</td>
</tr>
<tr>
<td>Effect of therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sustained remission at 1 yr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to first relapse, months</td>
<td>11 (1-35)</td>
<td>6 (0.60)</td>
</tr>
<tr>
<td>Kaplan-Meier</td>
<td>2 (7-22)</td>
<td></td>
</tr>
</tbody>
</table>

The median follow up was 26 (range 12-50) months in CNI & 14 (12-36) months in RTX groups. Patients treated with RTX had a shorter duration of remission (P=0.3). Relapse rates & proportions of patients with sustained remission of frequent relapses were similar.

In conclusion, monoclonal IgM could play a role in pathogenesis of MN.

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
**Underline represents presenting author.**
Results: Of 57 cases, 23 (40.3%) had collapsing or cellular (COL-CEL), 23 (40.3%) had mem-<ref>hilar or not otherwise specified (NOS-PH) and 11 (19.4%) had tip lesion variants (TIP). Baseline variables were similar between groups. The PO was significantly related to age, renal fibrosis, non-response to treatment and to CC. Cox regression showed that CC was significantly associated with PO, even after adjustments for age and fibrosis. Logistic regression models on the risk of ESRD or creatinine doubling endpoint was regarded as complete remission of proteinuria (CR), and the secondary endpoint was effective for treating IMN in some populations. This study was undertaken to test the hypothesis that children with NS have higher PWV than healthy controls.

Methods: Carotid-radial PWV was measured using applanation tonometry (ATCor Medical) in 35 subjects with NS and 25 healthy controls. Variables associated with increased pulse wave velocity in subjects with NS were evaluated by Pearson correlation analysis. The diagnosis of FSGS is associated with higher PWV and may place these patients at increased risk for cardiovascular disease (CVD). Arterial stiffness is an independent risk factor for CVD and is associated with proteinuria and hyperlipidemia; however, the relationship between arterial stiffness and childhood NS has not been explored. The objective was to investigate arterial stiffness measured by pulse wave velocity (PWV) and to test the hypothesis that children with NS have higher PWV than healthy controls.

Results: NS subjects had greater BMI (r = 0.37, p = 0.03), systolic BP (r = 0.56, p < 0.001), diastolic BP (r = 0.5, p < 0.001), and diagnosis of FSGS vs other NS (r = 0.37, p = 0.03). Cholesterol, proteinuria, and time since diagnosis and medications were not associated with PWV.

Conclusions: In the majority of patients, there was no concordance in FSGS variants between repeated biopsies or between native and transplant kidney biopsies. These data suggest that the morphological variants may reflect different stages in the evolution of lesions.

Conclusions: CC was an independent predictor of renal outcome in our study.

Conclusions: In the majority of patients, there was no concordance in FSGS variants between repeated biopsies or between native and transplant kidney biopsies. These data suggest that the morphological variants may reflect different stages in the evolution of lesions.

Conclusions: In the majority of patients, there was no concordance in FSGS variants between repeated biopsies or between native and transplant kidney biopsies. These data suggest that the morphological variants may reflect different stages in the evolution of lesions.
A Steroid-Sparing Therapeutic Option for Minimal Change Disease in Adults: An Initial Report of Tacrolimus Combined with Low-Dose Corticosteroid Treatment

Jeroen Deegens, Jack F. Wetzels.

Methods: Eighteen patients (mean age ± SD, 39.1 ± 19.3) with biopsy-proven MCD (mean serum albumin, 2.25 g/dL; mean urine protein-creatinine ratio (UPCR), 10.46) were enrolled. Tacrolimus was given orally at a dose of 0.03 to 0.05 mg/kg/day (target trough level, 5-10 ng/dL) for 16 weeks. Oral prednisolone was also simultaneously prescribed at a dose of 0.3 to 0.5 mg/kg/day with slow tapering.

Results: Proteinuria was significantly and consecutively reduced starting from the first week (median [range] of UPCR, 10.45 [3.1-18.4] at baseline, 3.07 [0.03-13.82] at 1 week, 0.14 [0.02-8.28] at 2 weeks; P<0.01). Complete remissions (UPCR < 0.3) were attained in 16 out of 17 patients after 16 weeks of the treatment and mean time to complete remission was 3.5 weeks (range, 1 to 16 weeks). One patient dropped out of the study. No significant adverse events were observed.

Conclusions: The therapeutic effect of tacrolimus plus low-dose corticosteroid regimen was shown early with an excellent response rate. This regimen can be considered as a steroid-sparing therapeutic option for MCD in adults.
occurred in 66%, median number of treated relapses was 2 (range 1-14).

**Conclusions:** Acute renal failure is a serious complication of adult-onset MCNS. Long-term outcomes are good. Spontaneous remission rate is high. CS treatment results in complete remission in the majority of patients. Characteristics at onset (mean ± SD)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>48.1 ± 17.5</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>24/12</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>101 ± 68</td>
</tr>
<tr>
<td>gGFR by modified MDRD (ml/min per 1.73 m²)</td>
<td>79 ± 11</td>
</tr>
<tr>
<td>Serum albumin (g/d)</td>
<td>1.4 ± 0.6</td>
</tr>
<tr>
<td>Urine protein (g/d) (n=84)</td>
<td>10.4 ± 5.3</td>
</tr>
</tbody>
</table>

SA-PO2881

Comparative Response of immunosuppressive Treatment on Proteinuria in Adults with Primary Focal and Segmental Glomerulosclerosis

**Diana Elvia Ceja Villanueva.** Nephropathy and Hemodialysis, IMSS, D.F., Mexico.

**Background:** The objective was to determine the difference in proteinuria in patients with GSFs treated with cyclophosphamide, mycophenolate mofetil or cyclosporine at 12 and 24 weeks of treatment, reduction according to treatment response and improvement of serum albumin.

**Methods:** Retrospective clinical study conducted in the Nephrology department of the Specialty Hospital Raza, IMSS. We analyzed 58 cases of patients registered with FSGS in a period of five years. The cases were divided into three different groups depending on the treatment received. Cyclophosphamide, mycophenolate mofetil or cyclosporine. Albumin and albuminuria were compared to 12 and 24 weeks of treatment, and response to treatment as partial, full or no response.

**Results:** The improvement of the proteinuria was significant in each group compared with the CSA group. The improvement of the proteinuria, serum albumin and complete response rates were > 0.01.

**Conclusions:** The CSA group improved more than the other groups, showing better response rates. Cyclophosphamide was the most effective treatment for the improvement of proteinuria and serum albumin.

**Funding:** Clinical Research Support

SA-PO2882

Interstitial Nephritis Is the Commonest Renal Pathology in HIV Infected Patients in West London

**Jeremy B. Levy.** 1Heather Isenman, 1Herence Cook, 1Rachael Jones. 1Imperial College Renal & Transplant Centre, Imperial College NHS Trust, London, United Kingdom; 2Dept of HIV, Chelsea & Westminster NHS Foundation Trust, United Kingdom.

**Background:** The survival of patients with HIV has been transformed by highly active anti-retroviral therapy (HAART): patients survive longer, develop non-HIV related chronic disease, and are exposed to potentially toxic therapies.

**Methods:** We retrospectively reviewed the data on all patients with HIV in West London who had a renal biopsy over the last 12 years. Results: 39 patients had a renal biopsy between 1998 & 2010 (10 female, 29 male; mean age 44). Mean creatinine at presentation was 3.4 mg/dl (sd 3.6). Mean time from diagnosis of HIV to renal biopsy was 8.6 years but 9 patients presented with renal disease as the indicator illness of HIV (diagnoses HIV associated nephropathy (HIVAN); 2, tubulo-interstitial nephritis (TIN); 4, immune complex nephritis (ICN); 2, advanced scarring (1)). The most common diagnosis overall was acute TIN: 8 patients (21%) as primary diagnosis and 5 (13%) as a major but probably secondary feature. 92% of patients with TIN were taking either AZT or abacavir and mean creatinine was 3.4 mg/dl. 3/13 required dialysis but the remainder recovered renal function with steroids and change of ART. Acute tubular damage was the 2nd commonest finding (6 patients) 7-22 years after HIV diagnosis, all with controlled HIV on ART. Mean creatinine 6.4 mg/dl, 1 required acute dialysis and all recovered renal function. All were taking protease inhibitors and 3/6 tenofovir. HIVAN was uncommon and only seen in 5 patients (13%): all black, mean creatinine 4.5 mg/dl with heavy proteinuria, all uncontrolled HIV with viral loads > 50. None recovered renal function. Other renal diagnoses included IgA, immune complex GN, FSGS, membranous, advanced scarring, focal necrotising GN.

**Conclusions:** Renal biopsy findings in HIV are very variable with important prognostic features: in West London acute TIN and tubular damage are the commonest findings mostly related to drug toxicity and with a good outcome. There may be an association of AZT & abacavir with TIN, and PIs with ATN. HIVAN is rare in W London and has a poor outcome. Other glomerular diseases are uncommon.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.**

781A
SA-PO2885

Treatment with Mizoribine Followed by Low-Dose Prednisone Is Highly Effective in Patients with Idiopathic Membranous Nephropathy and Nephrotic Syndrome

Background: Idiopathic membranous nephropathy (IMN) patients with persistent high-grade proteinuria are at the highest risk of developing end-stage renal failure. We have previously reported the effects of treatment with mizoribine followed by low-dose prednisone in 4 INN patients with nephrotic syndrome. The purpose of this study was to elucidate these results by using a larger study group consisting of patients who have been recently diagnosed with IMN and nephrotic syndrome.

Methods: We selected 12 consecutive patients who visited our hospital from 2005 to 2010 and were diagnosed with IMN and nephrotic syndrome. After 2 months of observation without any other treatment, mizoribine was started at a dose of 150 mg/day. After 2 months of mizoribine monotherapy, 20 mg/day prednisone was combined with 150 mg/day mizoribine. After that, the dosage of prednisone and/or mizoribine was tapered gradually according to the urinary protein-to-creatinine ratio (P/C), which is closely correlated to the daily protein excretion. After initiating combination therapy, the urinary P/C and the levels of serum albumin were measured on a monthly basis for 12 months.

Results: Before treatment, the P/C and serum albumin levels of the patients ranged from 3.7 to 15.9 g/dl and from 1.6 to 3.4 mg/dl, respectively. Although no patient showed a decrease in the P/C during mizoribine monotherapy, all patients showed a decrease in the P/C with time during combination therapy. After 1, 3, and 9 months after combination therapy, a P/C of <1.0 was observed in 17%, 25%, and 75% of the patients, respectively. By month 12, the P/C decreased to <0.4 g/dl in 10 patients, and the remaining 2 patients showed a P/C of 1.1 and 1.4 g/dl; in addition, the serum albumin levels increased to >3.5 g/dl in 9 of 12 patients.

Conclusions: The addition of prednisone after an initial treatment with mizoribine alone would be valuable in all IMN patients with nephrotic syndrome. The risks associated with immunotherapy can be decreased by initially using mizoribine alone, which acts as a base for establishing therapy, followed by low-dose prednisone.

SA-PO2886

Fabry Disease in Shanghai—A Survey from Chinese Single Center

Background: Fabry disease is an X-linked lysosomal storage disease with morbidity of 1/4,000 in male newborns. The deficiency of lysosomal hydrolase a-galactosidase A (a-Gal A) leads to accumulation of globotriaosylceramide in multiple organs including kidney, skin, cornea and heart which results in variable clinical manifestations.

Methods: Since 2002, 25 cases of Fabry disease have been recruited in Nephrology Department of Ruijin hospital in Shanghai, China. 207 related family members have been screened and 85 living patients have been diagnosed finally from 18 provinces in China. 39 hemizygotes and 46 heterozygotes were identified in all pedigrees. Among 39 probands, 31 were male and 8 were female with average age of 29.7 ys and 22 cases were biopsy-proven.

Results: In patients with Fabry disease 47% were male and 53% were female. The mean age of female and male was 30 and 25 years respectively. Patients were classified according to their slope of kidney injury. Kidneys with a low GD may be a potential characteristic of individuals who are susceptible to obesity-induced glomerular enlargement and subsequent renal injury.

Conclusions: Kidneys with a low GD may be a potential characteristic of individuals who are susceptible to obesity-induced glomerular enlargement and subsequent renal injury.

SA-PO2889

Albumin Excretion Fraction for Monitoring Proteinuria When Protein Plasma Level Is Manipulated

Background: Urinary protein/creatinine ratio (pU/Cr) or protein excretion rate are the most common indicators for monitoring nephrotic syndrome and the efficacy of treatments. However, these indicators are very much influenced by changes in plasma protein level induced by PEX itself. We examined the basic before the laboratory basic before, during and after the PEX treatment. After the 3rd PEX the efficacy of the treatment was clearly detected by FeAlb but not by uPr/Cr (line 4 in table).

Methods: A 19 yo girl with FSGS unresponsive to standard treatment (steroids, tacrolimus) was addressed to high volume PEX with albumin solution 4.5 gr/dl (thrice weekly).

Results: Following the first few sessions of PEX, uPr/uCr ratio increased (from 11 to 15) as a consequence of the increase in plasma protein level induced by PEX itself. Following the PEX, it decreased to 0.78. Treating the 3rd PEX treatment, the efficacy of the treatment was clearly detected by FeAlb but not by uPr/Cr (line 4 in table).

Conclusions: In conclusion in selected conditions (whenever plasma protein level is manipulated), FeAlb seems a better indicator of protein loss than protein excretion rate or uPr/uCr. More studies are needed to test its efficacy.

SA-PO2888

Low Glomerular Density with Glomerulomegaly Characterizes Renal Biopsies of Obesity-Related Glomerulopathy

Background: Obesity-related glomerulopathy (ORG) is a secondary form of glomerular disease that may occur in individuals with obesity. It is histologically characterized by a dense mesangial proliferation associated with a concomitant glomerulomegaly. The presence of mesangial cell proliferation, the increase in extracellular matrix, and the accumulation of lipids in the mesangial area are generally considered to be the hallmarks of obesity-related glomerulopathy. However, there is likely to be factors other than obesity that contribute to the development of this renal complication since only a minority of obese individuals actually manifests renal injury. This study examined the glomerular density (GD, non-sclerotic glomerular number/renal cortical area of biopsy) in ORG to determine if the difference in the glomerular number is associated with the pathogenesis.

Methods: Obesity and overweight were defined as BMI≥25 and BMI≥30 kg/m², respectively. ORG was morphologically defined as obesity-associated glomerulonephritis with conspicuous FSGS lesions. Patients with any evidence of other renal diseases or eGFR<60 ml/min/1.73 m² at biopsy were excluded. The GD and the glomerular volume were measured using a computed imaging analyzer.

Results: The distribution of the GD in the biopsies of ORG was extremely low (1.7±0.6/mm² in normotensive, 0.19±0.09/mm² in hypertensive). This was quite different than the widely-distributed GD in biopsies of kidney transplantation donors (3.1±1.0/mm², n=20). However, an analysis of autopsy cases without renal diseases showed that the distribution of the GD in overweight (2.9±0.7/mm², n=15) or obese (3.1±1.1/mm², n=8) subjects was similar to that in the non-obese subjects (3.1±0.6/mm², n=25). The biopsies of patients with ORG showed marked glomerulomegaly (6.3±1.8/mm²) compared with those of kidney transplantation donors (2.4±1.6/mm²), However, only a modest increase in glomerular size was found in the overweight (3.8±1.5/mm²) or obese (3.7±1.3/mm²) autopsy kidneys without renal diseases. The comparison of the GD in the normotensive ORG patients and hypertensive ORG patients did not show significant difference.

Conclusions: Kidneys with a low GD may be a potential characteristic of individuals who are susceptible to obesity-induced glomerular enlargement and subsequent renal injury.

SA-PO2889

Influence of a Functional Polymorphism of Aldosterone Synthase Gene on Membranous Nephropathy

Background: Aldosterone synthase (CYP11B2) plays a critical role in the renin-angiotensin system. Genetic polymorphisms in CYP11B2 have been associated with the risk and progression of renal diseases. The comparison of the GD in the normotensive ORG patients and hypertensive ORG patients did not show significant difference.

Methods: A-PO2889

Inference of a Functional Polymorphism of Aldosterone Synthase Gene on Membranous Nephropathy

Results: The distribution of the GD in the biopsies of ORG was extremely low (1.7±0.6/mm² in normotensive, 0.19±0.09/mm² in hypertensive). This was quite different than the widely-distributed GD in biopsies of kidney transplantation donors (3.1±1.0/mm², n=20). However, an analysis of autopsy cases without renal diseases showed that the distribution of the GD in overweight (2.9±0.7/mm², n=15) or obese (3.1±1.1/mm², n=8) subjects was similar to that in the non-obese subjects (3.1±0.6/mm², n=25). The biopsies of patients with ORG showed marked glomerulomegaly (6.3±1.8/mm²) compared with those of kidney transplantation donors (2.4±1.6/mm²), However, only a modest increase in glomerular size was found in the overweight (3.8±1.5/mm²) or obese (3.7±1.3/mm²) autopsy kidneys without renal diseases. The comparison of the GD in the normotensive ORG patients and hypertensive ORG patients did not show significant difference.

Conclusions: Kidneys with a low GD may be a potential characteristic of individuals who are susceptible to obesity-induced glomerular enlargement and subsequent renal injury.

SA-PO2887

Albumin Excretion Fraction for Monitoring Proteinuria When Protein Plasma Level Is Manipulated

Background: Urinary protein/creatinine ratio (pU/Cr) or protein excretion rate are the most common indicators for monitoring nephrotic syndrome and the efficacy of treatments. However, these indicators are very much influenced by changes in plasma protein level induced by plasmapheresis or albumin infusion as well as by plasmaexchange (PEX). In these conditions, it becomes difficult to detect improvement, as a response to treatment, since the increase in plasma protein per se causes an increase in proteinuria.

As an example, in a 12 yo boy with clinically stable FSGS without specific treatment but weekly albumin infusion, uPr/Cr was 15, 95 and 34 at baseline, 1 hr and 24 hrs after albumin infusion, respectively. In the present paper we explore the working hypothesis that albumin excretion fraction (FeAlb) is more accurate for monitoring proteinuria whenever plasma protein level is manipulated.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only; Underline represents presenting author.
SA-PO2890

Abstract Withdrawn

SA-PO2891

Effect of the Initial Type of Hemodialysis Access Planned in the Elderly on Mortality Outcomes Ranil N. Desilva, Bhau K. Patibandla, Yael Vin, Akshita Narra, Alexander S. Goldfarb-Rumyantzev, Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Background: Patients with fistulas that are functional at the time of hemodialysis initiation are known to have superior survival compared to those with grafts and catheters. Because the use of AV fistulas and AV grafts fail to be functional at the time of dialysis start, we measured outcomes associated with the type of access first placed in preparation for hemodialysis (as opposed to the access used at hemodialysis initiation) in the elderly population.

Methods: We included incident hemodialysis patients who started dialysis from 2005 to 2008, >67 years of age, from the United States Renal Data System. Medicare Claims from 2003-2008 were used to identify placement of the first vascular access. Primary variable of interest was the type of vascular access first placed (i.e., fistula, graft, or central catheter), with primary outcome of all-cause mortality (time to death) measured from the first out-patient hemodialysis. Data analyzed using Cox regression model adjusted for age, sex, race, co-morbidity index, BMI, cause of ESRD, and diabetic status.

Results: 115,425 subjects were identified with first access placed being AV fistulas (n=21,436), AV grafts (n=3,472), and catheters (n=90,517). Significant mortality benefit was found in those with fistula or graft placed as a first vascular access compared to those with catheters [HR 0.506, p<0.001 and HR 0.562, p<0.001 respectively]. Cohort was then stratified by age into three groups: 67-79, 80-89, and ≥90. Each of these three groups demonstrated statistically significant benefit from initial placement of an AV fistula or AV graft compared to catheter: for AVF [HR 0.506 (p<0.001), HR 0.527 (p<0.001), and HR 0.509 (p<0.001), respectively for each age group] and for AVG [HR 0.562 (p<0.001), HR 0.513 (p<0.001), and HR 0.522 (p<0.001), respectively for each age group].

Conclusions: First access placed in an elderly prospective hemodialysis patient being an AV fistula or AV graft is associated with better mortality outcomes compared to catheters.

SA-PO2892

Effect of the Initial Type of Hemodialysis Access Used in the Elderly on Mortality Outcomes Ranil N. Desilva, Guruprasadh Singh Sandhu, Jalaj Garg, Alexander S. Goldfarb-Rumyantzev. Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA.

Background: Though the “fistula first” initiative is intended to be applied across all population subgroups, we hypothesized that certain subpopulations (i.e., elderly and those with greater co-morbidity) might not benefit from it.

Methods: Study cohort included incident hemodialysis patients from 2005 to 2007 ≥70 years old, derived from the United States Renal Data System. Primary variable of interest was the type of vascular access used at first outpatient hemodialysis (i.e., fistula, graft, or central catheter), with primary outcome of all-cause mortality (time to death measured from the first outpatient hemodialysis).

Results: Of the 82,202 elderly patients, 82.2% were dialyzed initially by catheter, 13.5% by AV fistula, and 4.2% by AV graft. Cohort was stratified by age (70-79, 80-89, and ≥90). Each of these age groups demonstrated a survival benefit with use of an AV fistula compared to catheter [HR 0.56 (p<0.001), HR 0.55 (p<0.001), and HR 0.69 (p=0.007) respectively].

Conclusions: While fistulas and grafts are generally advantageous to survival, there are subgroups of hemodialysis patients (>90 years old with peripheral vascular disease, malignancy, or diabetes), where their use may not be of clear benefit compared to catheters.

SA-PO2893

Vascular Access Type and Early Death in the First Two Years of Hemodialysis Treatment Lilia L. Lukowsky,1,2 Leeka I. Khieftes,1 Onyebuchi A. Azu,1 Allen R. Nissenson,3 Kamyrz Kadantar-Zadeh,1,2 Harold Simonovis Center, Torrance, CA; 1 UCLA School of Public Health, Los Angeles, CA; 2 DaVita Inc., Denver, CO.

Background: Mortality is exceptionally high during the first year of hemodialysis treatment. There are limited data about the role of dialysis access type in early mortality of hemodialysis patients. We hypothesized that central venous catheter (CVC) is associated with stronger death risk in the first several months of dialysis therapy initiation.

Methods: We identified 18,707 incident MHD patients, who had started MHD treatment from the first week of therapy in a DaVita clinic (prior to Gambro acquisition) between 7/1/2001 and 6/30/2006. We calculated the risk of death at the time periods of 0-3, 4-6, 7-12, and 13-24 months after starting of dialysis therapy comparing patients with CVC to those with different types of vascular access.

Results: The incident MHD patients had a mean age of 63±15 years and included 45% women, 24% African Americans and 14% Hispanics. Use of CVC was associated with increased mortality during all time periods, but the death hazard ratio was even higher during the first 3 months of the therapy (2.7 [95% CI: 2.3-3.1]) compared to all other types of vascular access; it was 3.0 (2.5-3.6) compared to arteriovenous fistula (AVF) and 2.5 (1.9-3.2) compared to graft.

SA-PO2894

The Effect on Cardiac Function of AV Fistula Flow in Hemodialysis Patients Misaki Morishii,1 Hideki Kawanishi,2 1 Internal Medicine, Tschiya general hospital, Hiroshima, Japan; 2 Surgery, Tschiya general hospital, Hiroshima, Japan.

Background: Cardiac failure is majority of deaths in hemodialysis patients. Some studies demonstrated that increase of the blood flow by AVF flow might cause left ventricle overload, resulting left ventricular failure. This study was to evaluate if AVF flow contributes to an increase in left ventricular mass (LVM).

Methods: 43 hemodialysis patients with ejection fraction (EF) more than 50% who had first AVF access created, entered the study. All were performed echo cardiography prior to AVF access creation and again at 12, 24 months post-hemodialysis initiation. Blood flow of brachial artery (BA Flow) as AVF blood flow was measured by echography prior hemodialysis initiation and again at 12, 24 months post-hemodialysis initiation. Date were analysed using Student’s t-test, correlation coefficients and regression.

Results: The mean BA Flow was 420.1±175.6 mL/min prior hemodialysis initiation, and increased to 486.5±233.2 mL/min at 24 months post-hemodialysis initiation (p<0.001). The mean LVM was not changed at 24 months post-hemodialysis initiation (prior : 251.6±89.2 g , post-24 months 232.±84.3 g ). BA Flow at 24 months did not correlate with percent change in LVM. Percent change in LVM correlated with percent change in EF.

Conclusions: Our study shows that blood flow of AVF had not an effect on left ventricular mass in hemodialysis patients with normal cardiac function.

SA-PO2895

Effect of the “Fistula First” Paradigm on the Population Attributable Fraction (PAF) for Mortality from Vascular Access (VA) in the Australian and New Zealand (ANZ) Hemodialysis (HD) Population Mark R. Marshall,1 Stephen P. McDonald,2 Peter G. Kerr,3 Kevan R. Polkinghorne.1 1 Department of Renal Medicine, Counties Manukau District Health Board, Auckland, New Zealand; 2 Australia and New Zealand Dialysis and Transplant Registry (ANZDATA), Adelaide, Australia; 3 Department of Nephrology and School of Medicine, Monash Medical Centre and University, Clayton, Australia.

Background: Arteriovenous fistulas (AVFs) are associated with lower mortality than arteriovenous grafts (AVGs) and central venous catheters (CVCs). The “Fistula First” paradigm emphasizes the need to increase use of AVFs. We determined the PAF for mortality attributable to AVGs and CVCs pre and post “Fistula First” in prevalent HD patients in ANZ.

Methods: Using the ANZDATA Registry, we included all adults commencing renal replacement therapy (RRT) between 1999-2007 and on HD at 1 year post-RRT inception. We defined the primary exposure as VA recorded in the next ANZDATA survey.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Poster/Saturday
categorized patients into eras (1999-2002, 2003-7). The primary outcome was death. We used Cox regression with shared frailty by hospital, to determine PAF attributable to AVGs and CVCs in each era adjusting for baseline characteristics.

**Results:** We analyzed 1936 deaths in 5079 pts (1850 pt-years) from 70 hospitals. Compared to 1999-2002, there was a lower prevalence of AVGs but a higher prevalence of CVCs in 2003-2007. Adjusting for age, sex, ethnicity, late referral, eGFR at RRT inception, BMI, fluid, HD time & dose, primary renal disease, diabetes and co-morbidity, the HR for death (relative to AVFs) was 1.64 (1.41-1.90) for AVGs and 1.78 (1.10-2.87) for CVCs. 354 of the deaths (PAF 18.3%) were attributable to VA other than AVFs:

<table>
<thead>
<tr>
<th></th>
<th>Pre “Fistula First” (1999-2002)</th>
<th>Post “Fistula First” (2003-2007)</th>
<th>PAF (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All pts</td>
<td>2243</td>
<td>2666</td>
<td></td>
</tr>
<tr>
<td>AVF</td>
<td>1,373 (71.6)</td>
<td>1,099 (71.9)</td>
<td></td>
</tr>
<tr>
<td>AVG</td>
<td>268 (11.4)</td>
<td>203 (7.6)</td>
<td></td>
</tr>
<tr>
<td>CVC</td>
<td>418 (17.25)</td>
<td>544 (20.48)</td>
<td></td>
</tr>
</tbody>
</table>

**Conclusions:** The lower PAF due to less AVGs post “Fistula First” use has been offset by the higher PAF due to more CVCs. We suggest a complementary paradigm of “Line Last,” and re-emphasize establishing AVFs before HD inception to avoid ongoing CVC use.

**SA-PO2897**

Haemodialysis (HD) Via a Functioning Native Arteriovenous Fistula (AVF) Is Associated with a Lower Erythropoietin (EPO) Requirement in Camparison to HD Via a Central Venous Catheter (CVC) Muhammad Umair Sharif,1 David Lappin,1 Donal N. Reddan.2 1Nephrology, Mayo General Hospital, Castlebar, Ireland; 2Nephrology, University College Hospital, Galway, Ireland.

**Background:** EPO use is a significant cost in requiring long-term haemodialysis (HD). Higher doses of EPO are known to be an independent predictor of mortality & morbidity in this patient population. Due to difficulties in obtaining and maintaining a functioning native AVF many patients in our HD centre dialyse using a CVC. In this study, we examined the relative amount of EPO use in HD patients dialysing either via an AVF or CVC.

**Methods:** EPO requirement for 53 maintenance HD patients was calculated & then matched with their dialysis access, whether native AVF or CVC. The following conversion factors were applied when converting other forms of EPO to similar dose equivalents of Epoetin alpha or beta. Epoetin = Epoetin alpha = Epoetin beta 1 mg of Darbepoeitin (Aranesp) = 200 units of Epoetin 1 microgram Methoxy polyethylene glycol-epoetin beta (MIRCERA) = 200 units of Epoetin.

**Results:** In our study patients with a native AVF required an average of 7919 Units of Epoetin/week as compared to patients dialysing via a CVC who required an average of 10125 units of Epoetin/week.

<table>
<thead>
<tr>
<th></th>
<th>AVF</th>
<th>CVC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55± 27</td>
<td>62± 28</td>
</tr>
<tr>
<td>No. of Patients</td>
<td>32</td>
<td>21</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>11.1± 2.1</td>
<td>11.6± 2.7</td>
</tr>
<tr>
<td>Average EPO Requirement/Week/Patient</td>
<td>7919 Units</td>
<td>10125 Units</td>
</tr>
</tbody>
</table>

**Conclusions:** HD via a functioning native AVF, in comparison to HD via a CVC, results in significant cost savings of approximately 72 euros per patient per month or 850 euros per patient per year.

**SA-PO2898**

Non-Programmed Vascular Access Is Associated with Greater Mortality in Patients Who Return to Hemodialysis with a Failing Renal Graft Gustavo Laham, Carlos H. Diaz, Gervasio Soler Pujol, Mario Davalos, Ana M. Cusumano, Antonio R. Vilches. Nephrology Section, Department of Medicine, CEMIC, Buenos Aires, Argentina.

**Background:** There is an increasing number of Pts entering dialysis because of a failing graft. Although the use of a catheter for vascular access (VA) in Pts entering a hemodialysis population (HD) is associated with increased mortality it is not known whether this association also holds in the case of failed renal transplants (Tx). The purpose of our study was to assess the relationship between the type of VA and mortality in Tx patients re-entering our HD program.

**Methods:** Between 1/1995 and 05/2010, 131 incident Pts started HD after a failed Tx. The cohort was divided into 2 groups according to the type of VA: 1) Planned VA (PVA), A-V fistula or a graft; and 2) Unplanned VA (UPVA), (cather). Pts were censored at the time of re-transplantation or loss of follow up (FU). Co-morbid conditions were weighted using Khan’s index based on age, presence of diabetes and organ-specific comorbidities. Cox regression analysis was used to establish mortality predictors and Kaplan-Meir’s method for survival comparisons.

**Results:** Mean age was 44 years; 67.9% were males and median follow up was 52 months, IQR 14-105. Median Tx survival was 106 months, IQR 62-162, and the serum creatinine at the start of HD was 6.2 mg/dl +/- 2.4. 48/131 Pts (36.6%) died during FU. There were significant differences in age in p<0.004), Khan index (p<0.008), time on HD after re-started on the program (p<0.0001), and survival when the PVA (n=82) and UPVA (n=49) groups were compared. Mortality was 26.8% and 54.2 %, respectively, Log Rank test p=0.0001. Multivariate Cox regression analysis showed that catheter use was independently associated with a greater mortality after adjusting for the other variables also associated with mortality in this cohort, such as Khan Index and donor type; Odds ratio 6.5; 95%. Confidence Interval 2.9-14.1.

**Conclusions:** In this retrospective study patients entering our institutional hemodialysis program after a failed renal Tx using a catheter, as opposed to a previously fashioned permanent access, showed a greater all cause mortality.

**SA-PO2899**

Hemodialysis Vascular Access Patency: Results from a Single Centre Initiative Janet Lynn Graham, Peter Magner, Swapnil Hiremath. Division of Nephrology, Ottawa Hospital, Ottawa, ON, Canada.

**Background:** Guidelines recommend the arteriovenous (AV) fistula as the vascular access of choice. The data on patency rates is from studies done over two decades ago; since then the hemodialysis population has changed with increasing proportion of older patients with diabetes and vascular comorbidities. At our academic centre, we have an active vascular access monitoring program, comprising of regular access flow surveillance, weekly rounds with interventional radiology +/- vascular surgeons and early intervention. The aim of our study was to examine the patency rates of AV access with this system of active surveillance.

**Methods:** This was a retrospective cohort study examining all patients who had creation of a vascular access at our centre over the period 2003 to 2010. Data was abstracted from our administrative database. Access survival was calculated as time from access placement to abandonment, including intervening manipulations (surgical or endovascular). Intervention-free survival was measured as the time from access placement to any intervention designed to maintain or re-establish access patency. Kaplan-Meier survival analysis and the life table method were used to calculate access patency rates.

**Results:** 1064 patients, with 62% men with a mean age of 63 +/- 18 years had a first vascular accesses created over the period of study, with 960 AV fistula and 104 grafts. 319 accesses failed over the period of study with thrombosis being the commonest cause (68.5%). The median survival for fistula at 6.21 years was much longer than grafts at 1.05 years. 175 patients had a 2nd attempt at a vascular access (83% fistula, 17% graft), 35 patients had a 3rd access (83% fistula, 17% graft) and 5 patients had a 4th vascular access (all fistula). 482 patients (45%) required at least one intervention to assist in maintaining patency, an angioplasty being the commonest intervention (418/482, 87%). The intervention-free survival of the vascular accesses was also higher for fistula (4.4 years) compared to grafts (0.58 years).

**Conclusions:** A system of active vascular access surveillance results in a high access survival rate despite the increasing vascular comorbidity burden in the hemodialysis population.

**Funding:** Clinical Revenue Support

**SA-PO2899**

Who Is Eligible for a Fistula? A Survey of Vascular Surgeons Andrea Nica,1 Charmaine E. Lok,2 Timmy C. Lee,1 Jeremy R. Harris,1 Michele H. Mokrzycki,1 Ivan D. Maya,2 Miguel A. Vazquez,2 Louise M. Moist.1 1London Health Sciences Center, Victoria Hospital, London, ON, Canada; 2University of Toronto, ON, Canada; 3University of Cincinnati, OH; 4Albert Einstein College of Medicine, NY; 5University of Alabama, AL; 6UT Southwestern Medical Center, TX.

**Background:** The arteriovenous fistula (AVF) is the recommended vascular access (VA). Currently there are no criteria to determine which patients are eligible for an AVF.

**Methods:** We conducted an international survey of VA surgeons to assess the patient, vessel and process considered to decide the type of VA created, as well as contraindications and barriers to VA.

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Conclusions: This study demonstrates great variability in the pre-operative assessment of patients, and in the criteria used to determine which type of VA a patient receives. Establishing guidelines for VA access eligibility is an important future step in optimizing patient care.

SA-PO2900

Surgical Specialist Practice Pattern Variation in AVF Placement across ESRD Networks for Late Stage CKD and ESRD Medicare Patients

Background: Given variation in practice patterns in many areas of medical care we inquired into the possible existence of similar variation among specialists treating ESRD patients. If specialists of fistula placements can be distinguished within ESRD Networks, it may be possible to increase referrals for such placements and increase fistula placement rates.

Methods: We reviewed over 600,000 part B Medicare claims for late stage CKD and ESRD patients who had hemodialysis access placements and/or access related diagnostic, maintenance or revision procedures during 2009. Results: The variation in the ratio of surgeon AVF placement procedures over all procedures done by surgeons is over 2.5, from 13.4% to 34.1%. Which physician specialists are doing more AVF placements than others? General surgeons do 46% of all AVFs, followed by vascular surgeons, who do 38%. NW variation revealed rates from 33% to 64% done by general surgeons (See display), and rates from 20% to 54% done by vascular surgeons across regions. What variation exists in the availability of surgical specialists who perform permanent access placements across ESRD Networks? The presence of Ambulatory surgeons and Physician Assistants doing AVF procedures varies considerably with more of these specialties active in rural western regions.

Conclusions: What surgical specialists do varies considerably across the 18 ESRD Networks. Practice patterns and concentration within a practice vary over two fold within the same surgical specialty depending on regional distinctions. Funding: Other U.S. Government Support

SA-PO2901

Arteriovenous Fistula for the 80 Years and Older Patients on Hemodialysis: Is it Worth It?
Annie-Claire Nadeau-Fredette, Remi Goupil, Bernard Montreuil, Annie Carignon, Martine Leblanc. Hôpital Maisonneuve-Rosemont, Université de Montréal.

Background: Over the last years, the proportion of patients older than 80 years with end-stage renal disease has been constantly growing. Arteriovenous fistula (AVF) is known as the best vascular access in hemodialysis (HD) population but the evidence for its added value is lacking for older patients. We evaluated patients aged 80 years and older (>80 yo), in whom a new AVF had been installed and compared their outcome to a group of HD patients between 50 and 60 years.

Methods: For both groups, we identified every new vascular access (AVF and central venous catheter [CVC]) created or installed between June 2005 and June 2008. We collected demographic and clinical data as well as radiological and surgical interventions from June 2005 to April 2010. We calculated primary failure rates (AVF never used after 6 months from creation), primary patency (intervention-free survival) and secondary patency (survival until definitive failure) durations for every new AVF.

Results: In our study, 55 and 57 patients had a new vascular access (AVF or CVC) in the 50-60 yo and >80 yo groups respectively. Among these, 41 and 26 were new AVF in the younger and older groups. As recognized in the literature, primary failure was significantly more frequent in the older group than in the younger group (40% vs 17%, p = 0.0449). When excluding primary failure, the primary patency was not significantly different in both groups (median 8 vs 16 months, p = 0.32). However, the secondary patency was significantly shorter for the older group (p = 0.04). During the 58 months of observation, the mortality rate was 24% in the 50 to 60 yo group while it was 54% for the > 80 yo patients. Among the younger group, the presence of an AVF was associated with a significant lower rate of mortality (12% vs 43%, p = 0.008) but not in the older group (46% vs 60%, p = 0.28).

Conclusions: In conclusion, knowing the advantages of AVFs in the HD population and keeping in mind the similar primary patency duration shown here between the older and younger groups, patients of 80 yo should probably be considered just as younger patients for AVF creation for hemodialysis.

SA-PO2902

Hemodialysis Patients’ Preference for Thigh Grafts – A Cross-Sectional Survey
Zulqarnain Abro,1 Sunanda J. Ram,1 Neville R. Dossabhoy.1,2 Nephrology, LSUHSC, Shreveport, LA; 1Nephrology, VA Medical Center, Shreveport, LA.

Background: Recent studies have shown that survival and complication rates of thigh grafts are similar to those of arm grafts and fistulas. However, there is little information in the literature regarding patients’ preference for thigh grafts.

Methods: This IRB-approved cross-sectional survey was conducted on patients currently on hemodialysis (n=159), who were queried regarding their preference for access location (arm access vs. thigh graft) and details of their dialysis access history. Data on age, race, gender and educational level was collected. Data is presented as means or percentages and analyzed using unpaired t test or chi square test. Significance set at P< 0.05.

Results: 93% were African American, 52% were female, 94% had been on dialysis ≥1 year. Most patients (79%) had completed high school or higher education. Most patients’ current access was arm fistula (54%), followed by catheter (30%) and arm graft (13%). Only 3% had arm grafts currently. 5.7% had previously had thigh grafts. Overall, 90% patients preferred arm accesses, but 10% said they would go for a thigh graft if there was no arm site available. The patients’ preferences for access location was not influenced by their level of education, nor by a prior history of placement of arm access. Patients’ age, gender, particular dialysis unit and dialysis vintage did not seem to influence patient preference. Those patients with a current (3.1%) or prior thigh graft (5.7%) were more likely to prefer a thigh graft for their next access, when compared to those without (P<0.05). However, even in this subset, the majority still preferred arm access (approximately 60% to 40%).

Conclusions: The likelihood of preference for placement of a thigh graft is increased by patients having a current or prior thigh graft - apparently influenced by possibly favorable
experience with thigh grafts. Other demographic factors and dialysis history seemed not to influence patient preference. As thigh grafts are a better option than tunnelled dialysis catheters in patients who have exhausted all arm access sites, more efforts at educating patients on their benefits in this setting are warranted.

SA-PO2903
Contemporary Assessment of Incident Dialysis Patients Starting Dialysis with Catheters through 2010
Vincent Mor,1 Franklin W. Maddux,2 Mahesh Krishnan,3 Brown Center for Gerontology & Health Care Research; 2Fresenius Medical Care; 3DaVita, Denver, CO.

Background: The Performance Excellence and Accountability in Kidney Care (PEAK) initiative is a program sponsored by Kidney Care Partners. Its goal is to reduce first year patient mortality by 20% from 2007 to 2012. Since catheterization at dialysis is initiated is associated with infections, we examined changes in incident patient vascular access for first outpatient dialysis sessions among new ESRD patients.

Methods: In order to get the most recent data, we used the Renal Management Information System (REMIS) available under a Data Use Agreement from CMS through December 2010. Beginning dates of entry of patients (from Medical Evidence form 2728), Patient demographic and clinical characteristics (2728/Patient Master file), and date of death (From Patient Master file, Form 2746, Social Security Master Death File (SSMDF)) were obtained. Initial vascular access type was obtained through December 2010 from the completed 2728 forms for all new entrants initiating dialysis.

Results: The percentage of patients dialyzing with catheters as their primary access on first date of dialysis is recorded below.

<table>
<thead>
<tr>
<th>Year</th>
<th>Percentage of Patients Dialyzing with Catheters</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>20.2%</td>
</tr>
<tr>
<td>2008</td>
<td>18.6%</td>
</tr>
<tr>
<td>2009</td>
<td>18.1%</td>
</tr>
<tr>
<td>2010</td>
<td>17.6%</td>
</tr>
<tr>
<td>2011</td>
<td>17.2%</td>
</tr>
</tbody>
</table>

Conclusions: Through 2010, over 80% of incident patients were documented to dialyze via a catheter on their first dialysis session. This was in spite of significant improvements in prevalent catheter rates as a result of efforts such as the Fistula First Breakthrough Initiative. While some of these patients may be dialyzing with a catheter as a temporary bridge access while a permanent access matures, their risks of infectious complications are directly correlated to catheter days. These data suggest that additional effort is needed to improve incident vascular access placement with sufficient time for maturation as a means to reduce a measure of incident patient risk.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

SA-PO2904
Differences in Vascular Access Use in Incident U.S. Hemodialysis Patients – Ethnic Disparities in the Optimal Delivery of Timely Nephrology Care
Maria Cristina Arce, Aya Alice Mitani, Benjamin A. Goldstein, Wolfgang C. Winkelmayer. Division of Nephrology, Stanford University, Palo Alto, CA.

Background: Hispanics are the largest and fastest-growing minority in the U.S. and comprised 16.3% of the US population in 2010. Hispanics are at double the risk of end-stage renal disease (ESRD) compared with non-Hispanics. Numerous studies have demonstrated the poor access to health care and the low quality of care received among Hispanic individuals; yet, relatively little is known about the preparation of Hispanic patients with predialysis nephrology care.

Methods: We used data from the Medical Evidence Report form CMS-2728-U3 (version 06/04) for the ascertainment of all patient characteristics and outcomes. All adult patients initiating hemodialysis (HD) between 7/1/2005 and 12/31/08 were evaluated. Incident arteriovenous vascular access use was considered present if either an arteriovenous fistula (AVF) or graft (AVG) was noted. We used a log binomial model with state as a confounder to evaluate associations. We used national data to investigate any differences by Hispanic ethnicity in the rate of incident arteriovenous access.

Results: Among 321,996 U.S. patients initiating hemodialysis (HD) between 7/1/2005 and 12/31/08 were evaluated. Incident arteriovenous vascular access use was considered present if either an arteriovenous fistula (AVF) or graft (AVG) was noted. We used a log binomial model with state as a confounder to evaluate associations. We used national data to investigate any differences by Hispanic ethnicity in the rate of incident arteriovenous access.

Conclusions: Hispanics are less likely to use arteriovenous vascular access during the initiation of hemodialysis. While differences in clinical and socioeconomic characteristics explained some of the difference, access to adequate predialysis nephrology care explained more than half of the underuse of these more desirable access options.

Funding: NIDDK Support

SA-PO2905
Effect of Interventions in Hemodialysis Outcomes with Clinical Coordinated Care Model in Renal Therapy Service Colombia Network. Angela S. Rivera, Medical Director, Baxter, Bogota, Cundinamarca, Colombia.

Background: Renal Therapy services (RTS) is a network of renal units in Colombia with national coverage in 49 renal units with a Clinical Coordinated Care Model focused on clinical quality assurance process (standardized clinical protocols, ongoing education and training), internal national and regional clinical audits for protocol compliance, disease management (anemia, hypertension, diabetes, nutrition, mineral & bone disorder, hepatitis B vaccination and Dialysis management including in center hemodialysis with single use dialyzers) and quality standards adherence monitored by nephrologists and nurses.

Methods: A retrospective analysis of hemodialysis prevalent patients older than 18 years was performed from 2006 to 2010. There were analyzed trend of achievement of standards: Kt/V sp, hemoglobin, vascular access, adverse patient’s occurrences (including clotted dialyzer rate) and outcomes as mortality rate. The data collection was made from clinical record.

The interventions implemented were:
- On-site nephrologists
- Monthly comprehensive evaluation by nephrologists
- Dedicated and trained professional nurses to hemodialysis
- Attention by nutrition, psychology and social work according to risk

Results: Incentive and continuing education program in vascular care access for nurses
- Strengthening quality process and define key performance indicators of care vascular access including monthly reports

Conclusions: The comprehensive model of RTS implemented in Colombia has resulted in consistently improvement of achievement of dialysis standards and a trend of reduction of mortality rate during 5 years of implementation.

Funding: Pharmaceutical Company Support

SA-PO2906

Background: Patients with CKD should have no PICC lines or IV lines in their non-dominant arm. A prior survey of vein protection practices in our hospital suggested potential for improvement. Effects of a year-long educational initiative to improve compliance by increasing Nephrologist awareness of these guidelines were assessed, compared to prior results.

Methods: Estimated GFR (eGFR) was assessed on all hospital patients on a single day. Patients with eGFR <60 were examined for the locations of all intravenous lines and limb protection bracelets. Device rates were compared from before (2010) and after (2011) the initiative.

Results: For 415/420 patients, eGFR could be determined; 107 (26%) had eGFR <60. 41 had eGFR between 45-59, 29 had eGFR 30-44, and 37 had eGFR <30, of whom 15/37 were dialyzing. CKD rates differed widely within the hospital, present in more than 50% of patients in medical and thoracic ICU’s and stepdown units, but <10% in Neurological, Neurosurgical, and Orthopedic areas. Med-Surg, Cardiology, and Oncology had CKD rates of 20-40%.

Limb protection bracelets were found on 19 patients, who represented a 9% improvement in the protection rate. 33% of dialysis patients had bracelets, and 10-12% of all other CKD groups. One PICC line and one peripheral IV were found in bracelet-protected arms. Among the 88/107 patients without protection bracelets, 86 had some IV device, of which 5 were central lines.

Conclusions: The comprehensive model of RTS implemented in Colombia has resulted in consistently improvement of achievement of dialysis standards and a trend of reduction of mortality rate during 5 years of implementation.

Funding: Pharmaceutical Company Support
Conclusions: Raising awareness of vein protection among nephrologists resulted in a modest increase in the placement of limb bracelets, particularly among patients already on dialysis, but did not reduce the rate of PICC placement or prevent invasion of non-dominant arms in patients with CKD stages 3-5. Our results suggest that other factors may influence choice and placement of vascular access devices, which are now virtually universal in inpatients. Effective measures to protect veins in CKD must involve multiple stakeholders in the hospital community.

SA-PO2907
Fistula First Breakthrough Initiative (FFBI): Lessons about AVF Prevalence Goals
Andrew D. Howard,1,2 Robin S. Howard,3 Stuart Goldstein,4 Klemens B. Meyer,5,6 1Metropolitan Nephrology Asso, Alexandria, VA; 2Clinical Investigation, Walter Reed Army Medical Center, Washington, DC; 3Tufts Medical Center, Boston, MA; 4Forum of ERSD Networks, Richmond, VA.

Background: FFBI is a successful quality improvement collaboration between the Centers for Medicare & Medicaid Services (CMS) and ERSD Networks (NW). In 2005, CMS set a 2009 prevalent national arteriovenous fistula (pAVF) goal of 66%, 13/18 Networks failed to meet their individual 2009 pAVF goals. The Forum of ERSD NW analyzed these outcomes and proposed alternative approaches to goal-setting.

Methods: In 2006, CMS defined the Annual Improvement Target for each NW as (66% NW’s March pAVF rate) x 20%; with a maximum rate 4%, minimum 1%. We proposed a regression model based on 2004-09 data, relating pAVF changes to an annual average of each NW’s pAVF rate, and compared model predictions to actual 2010 and 2011 pAVF changes for each of the 18 NW. Differences between achieved pAVF rates and model predictions vs CMS targets were compared using the Wilcoxon signed ranks test.

Results: The 2009 likelihood of failure was associated with pAVF rate. The CMS formula and NW pAVF rates <50% were unable to meet goal, and the 5 NW with pAVF rates >50% met their goals. The CMS formula assumes linear improvement, but the data suggest non-linear pAVF change. For 2010-11, the non-linear model more accurately predicted 26 of 36 pAVF rates (72%) than did the CMS formula (p=0.001, Wilcoxon signed ranks test).

Conclusions: CMS goals are based on March data only and do not reflect NW differences in prevalent and incident AVF rates, and in the number, demographics and characteristics of patients served. We suggest a more robust methodology using all data. Vascular access may become the next measure in the CMS Quality Incentive Program, and can be expected to incorporate goals for chronic venous catheters and pAVF rates for both facilities and NWs. We propose an adaptive approach to goal-setting, similar to that described by the FDA with reference to clinical trials, which includes prospectively planned opportunities for modification of one or more specified goals based on periodic analysis of data.

SA-PO2908
Reasons for Unavailability of AV Fistula at the Initiation of Hemodialysis in Pakistan
Svee Rizwan Bokhari,1 Hafiz I. Ahmad,2 Department of Nephrology, Allama Iqbal Medical College/Jinnah Hospital, Lahore, Pakistan.

Background: Chronic kidney disease (CKD) is a challenging condition in the developing countries such as Pakistan. In our set up a great majority of patients presenting with CKD does not have an arteriovenous fistula (AVF) at the time of initiation of dialysis.

Methods: In this study, we investigated the reasons for unavailability of an AVF. One hundred consecutive CKD patients presenting for chronic hemodialysis through outpatient and emergency department during a period of three months (March to May, 2011) were included in this study. Patients were interviewed according to a standardized questionnaire.

Results: The demographic characteristics revealed that 52 males were (52%), with mean age 34.2 years. None of the patients had an AVF at the time of initiation of hemodialysis. 36% had received the advice to get AVF but refused the surgery. 31% were not aware of their pre-existing renal disease. Although 17% had prior knowledge of their kidney disease they were not referred to a nephrologist or a surgeon. 8% did not have the availability of surgeons trained in vascular access creation. 7% mentioned poverty/lack of resources. 6% had unsuccessful AVF surgery. Only 2% presented with presumed ARF. They received dialysis through temporary catheter and did not get AVF for 3 months in hospital stay.

Conclusions: The two most frequent reasons for unavailability of AVF were the refusal to get permanent vascular access and the presence of advanced CKD (stage 5) without having a prior knowledge of renal disease. Both are modifiable risk factors and highlight the issue of lack of public awareness and health education and underscore the importance of robust efforts on part of medical community and health authorities to educate the masses about kidney diseases.

Funding: Private Foundation Support, Government Support - Non-U.S.

SA-PO2909
Variability in Vascular Access Management for Canadian Home Hemodialysis Patients Undergoing Intensive Hemodialysis
Deborah Lynn Zimmerman,1 Robert P. Paul,2 Paul Komenda,3 1Medicine, University of Ottawa, ON, Canada; 2Medicine, University of Alberta, Edmonton, AB, Canada; 3Medicine, University of Manitoba, Winnipeg, MB, Canada.

Background: Despite growing interest in intensive hemodialysis (HD) (more frequent and/or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns.

Methods: Survey development was completed with the assistance of physician and allied health experts in intensive home HD. The survey underwent several iterations based on reviews of the face expert panel prior to a face-to-face meeting with the physician experts before final instrument changes were made. The survey was completed on line. This abstract summarizes vascular access practice patterns.

Results: Of the 19 physicians contacted, 17 participated in survey development and provided program information. The preferred vascular access is the AVF in 14/16 programs. Lack of an AVF (6/16) might delay training but would not prevent training for any intensive HD program. The majority of programs are using the buttonhole technique for AVF needleling (8, 100%-99%, 4, 61-80%; 1, 21-40%; 1, 1-20%). One set of buttonholes is usually developed during training (12/17) but most programs have had to establish new buttonholes post training (14/16). Steel needles are used in 14/16 programs; 2/16 programs use angiocatheters. Routine monitoring of access flow is not done by the majority of programs. For patients on nocturnal HD, the majority of patients with AVF or AVG use single needle HD in 3 and 5 programs respectively. For patients with CVCs, 5 programs do not have patients use any type of safety ‘connectology’; 6 use lockboxes and 11 are using devices like the TEGO. Locking solutions were heparin and citrate for 6 and 11 programs respectively.

Conclusions: There is significant variability among vascular access type, cannulation approaches, and utilization of access safety devices in Canadian home HD programs. Such variability may, in part, explain perceived differences in access complication rates, but further prospective study is necessary.

Funding: Clinical Revenue Support

SA-PO2910
Early Versus Late Arteriovenous Fistula Placement and Use in the First Year after an Acute Inpatient Start of Chronic Hemodialysis
Stacy L. Andersen,1 Yue-Fang Chang,2 Patricia A. Seddon,3 Susan C. Martin,4 Kevin Ho,5 Renal Electrolyte Division, University of Pittsburgh, PA; 2Dale W. Wolf, Jr. Center for Quality Improvement and Innovation, University of Pittsburgh Medical Center, PA; 3University of Pittsburgh, PA.

Background: Tunnelled dialysis catheter (TDC) conversion to an AV fistula in incident hemodialysis (HD) is associated with a 30%/+ decrease in first-year mortality risk. But early AV fistula or graft (AVF/G) placement may not necessarily lead to successful use.

Methods: We studied if early AVF/G placement in adult incident HD inpatients is associated with less TDC use in the first 365 days (d) of outpatient HD. 84 such patients were discharged over 26 months(m) to 8 clinics affiliated with our academic hospital and transplant center. 30 had Early AVF/G, placed <90d after inpatient HD began; and 40 had Late AVF/G, placed >90d. 14 patients with <90d HD were excluded. To gauge AVF/G placement and use, we compared outpatient mean HD Hemoglobin (Hb) (Hbmean=total HD treatments via TDC/total HD treatments) at 3, 6, and 12m for Early vs. Late AVF/G.

Results: 46% of patients (mean age 55 years) had diabetes, 36% were female; 16% had PD just before HD, and 19% had a failing renal allograft. 26% had other non-renal transplants. For 89% of patients, HD began via TDC, 10d (median) before hospital discharge. Those who eventually had an AVF/G placed took 101d (median) to do so. Of 59 non-ESRD patients, once became dialysis-independent by 90d (5.1%); one had prior CKD. Early AVF/G resulted in a mean Hbmean=57% from 3 to 6m (n=29), compared with a Hbmean=91% for Late AVF/G (n=38) (p=0.0004). From 6 to 12m, the Hbmean for Early AVF/G was 33% (n=28) versus 74% for Late AVF/G (n=33) (p=0.0004). Early AVF/G is associated with progressively less TDC use. However, we were not able to correlate differences between Early and Late AVF/G Hbmean with differences in hospital days or mortality.

Conclusions: Early AVF/G placement even in incident HD inpatients is associated with earlier AVF/G function and less TDC use in the first year of HD, providing a basis for inpatient vascular access planning.

SA-PO2911
Central Venous Catheter (CVC) Incidence at Hemodialysis Initiation: Is It the Right Metric for Provider Quality of Care? Karthik K. Tennakone,1 Steven D. Soroka,2 Bryce A. Kibler,3 Medicine, Dalhousie University, Halifax, NS, Canada.

Background: High CVC rates for incident dialysis patients may indicate indicate poor quality of provider care. Nurse lead multi-disciplinary kidney clinics might reduce CVC starts.

Methods: We studied consecutive incident ERSD patients (nemo, PD and pre-emptive transplantation) over 36 months at a single center. Patients were excluded if their first presentation was to hospital with AKI.

Results: There were 3 nurse practitioners (NP) and 9 nephrologists (NEPH) involved with CKD multi-disciplinary clinic patients. NPs were less likely to start hemopatients

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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with a CVC (51% [20/39]) compared to NEPHs (65% [104/160]). However, NP2 were more likely (p=0.004) to see patients followed for greater than 2 years (80%) compared to physicians (62%) and 35 of the 36 CKD patients referred late (<0.5 year) were seen only by NEPHs. In a logistic model, the only predictor of CVC start was time of referral, not provider type. Patients referred <0.5 years were 8.3 (OR, 95% CI 3.1-23) more likely to start with a CVC.

Figure 1 shows the difference in CVC vs fistula rates considering hemodialysis alone. Rates among the 3 NP's differed (NP1 had highest and NP2 the lowest CVC rate) whereas the rates for the 2 selected NEPHs were similar and high. However figure 2 shows that when PD starts and pre-emptive transplant patients were included, all NP's were equivalent but the 2 physicians differed. NP1 and NEPH B had high pre-emptive transplant and peritoneal dialysis rates but only 40% of their starts were with a CVC when considering all patients.

**SA-PO2913**

**Nephrologist Care for 12 Months or More Increases Hemodialysis Initiation with Permanent Vascular Access**

Daisuke Inaguma, Masato Ikeda, Nobuhiko Joki, Takashi Shigematsu.

**Background:** The objective of this study was to evaluate the effect of early referral (ER) to nephrologists on the type of vascular access (VA). In patients who have been followed by nephrologists for less than 3 months, management before the initiation of hemodialysis (HD) is often insufficient and urgent initiation of HD is often necessary; therefore, patients in this study were limited to those who had been followed for at least 3 months by nephrologists.

**Methods:** 940 patients at 9 institutions were enrolled in the study. The study was a retrospective observational study. We defined the ER group as patients followed up by nephrologists for at least 12 months, the late referral (LR) group as followed between 3 and 12 months, and the type of VA available was compared between the groups.

**Results:** ER was found to be significantly associated with the availability of a permanent VA at the time of initiation of HD (OR, 1.705; 95% CI 1.001-2.89). A multivariate analysis also revealed ER to be significantly associated with the availability of a permanent VA.

**Logistic regression analysis of initiation of hemodialysis with permanent VA**

<table>
<thead>
<tr>
<th>Variables</th>
<th>OR</th>
<th>95% CI</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cofade</td>
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<td>ER</td>
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<td>E-creactive protein (1/mg/dl)</td>
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</tr>
</tbody>
</table>

**Conclusion:** ER is advantageous for increasing the likelihood of availability of a permanent VA even after patients who had been followed up for less than 3 months by nephrologists were excluded.

**SA-PO2914**

**Low Renal Recovery in Inpatients Starting Hemodialysis and Transitioning to Outpatient Dialysis: Priority Chronic Kidney Disease and Vascular Access Planning**

Stace L. Andersen, Yue-Fang Chang, Patricia A. Seddon, Kevin C. Martin, Donald D. Wolff, Jr. Center for Quality Improvement and Innovation, University of Pittsburgh Medical Center, PA; Donald D. Wolff, Jr. Center for Quality Improvement and Innovation, University of Pittsburgh Medical Center, PA; University of Pittsburgh, PA.

**Background:** 81% of our local incident outpatient hemodialysis (HD) actually begins in hospital, yet little is known about renal recovery and vascular access (VA) planning for incident HD inpatients who then start outpatient HD. It is difficult to know which incident HD inpatients will need chronic HD and benefit from early VA planning.

**Methods:** We examined renal recovery (dialysis independence by 90d), AVF placement, and pre-ESRD renal care in 84 adult incident HD inpatients discharged from our tertiary care and transplant center to 8 affiliated HD units over 26 months (m). Inpatients were on HD neither at hospital admission nor in the preceding 12m. We defined Early AVF as access placement <90d and Late AVF as >90d after incident HD began. We calculated the Early Access Rate (EAR=Early AVF/G patients/All AVF/G patients) for each CKD stage.

**Results:** Of 59 non-ESRD incident HD inpatients discharged to outpatient HD, only 3 (5.1%) had renal recovery. All 48 inpatients with prior CKD remained dialysis-dependent at 3m: 1 CKD1, 7 CKD3A, 14 CKD3B, 19 CKD4, 7 CKD5. 5 patients had no CKD data. Of 6 patients with no prior CKD, 2 recovered. 89% of patients began HD via a TDC. Those without prior AVF/G who got one had it after 101d (median). 79% of CKD4 patients had prior renal care (mean≈254d) with EAR=53%; 86% of CKD5 had renal care (mean≈290d) with EAR=33%; 43% of CKD3B and 29% of CKD3A had renal care (mean≈274d and 291d) with EAR=33% and 29%. For 15 ESRD patients with a failing renal graft, EAR=57%; and for 10 PD patients, EAR=50%. 95% of CKD patients had ≥1 and 53% ≥2 of the following: DM, age 65, AKI in the past 12m, or tacrolimus use.

**Conclusion:** Given these low renal recovery rates, CKD status may help to predict which incident HD inpatients will require chronic HD post-discharge and benefit from inpatient VA planning.
SA-PO2915

Geographic Variability in Rate of Improvement in Incident and Prevalent AVF Is Not Strongly Correlated


Background: Substantial regional variation in the rate of change of incident and prevalent AVF at the start of hemodialysis is noted in the United States. This report examines the degree to which the improvement in incident and prevalent AVF use are similar and correlate to the baseline AVF prevalence.

Methods: ESRD Networks collect a census of the vascular access currently in use of each patient within all treatment centers within their region. The date that hemodialysis for ESRD began is also recorded. We defined a patient as incident during the 91 days after the start of treatment and prevalent otherwise. Data aggregated at the network level was used to calculate slopes of change in prevalent and incident AVF for the period from March 2007 to February 2011. The recorded AVF rates for March 2007 were taken as baseline AVF rate.

Results: Incident and prevalent mean (SD) and median rates for all Networks were 0.0578% (month 0.02), 0.0586%/month and 0.27%/month (0.05) and 0.27%/month (Figure). Network incident and prevalent AVF slopes were modestly correlated (Pearson correlation=0.2875). The Figure shows that the rates of incident and prevalent AVF improvement were inversely associated with baseline AVF rate.

Conclusions: Incident and prevalent AVF rates increased in all networks, with the largest increases in AVF prevalence occurring in networks with the lowest baseline AVF rates.

Funding: Other U.S. Government Support

SA-PO2916

The Majority of Autogenous Dialysis Access (AVF) Is Now Constructed with Advanced Surgical Techniques

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Background: Concept 5 of the FFBI Change Package urges surgeons to “utilize current techniques for AVF placement including vein transposition.” While there has been a progressive increase in the proportion of AVF construction among incident and prevalent HD patients, there are few data to show the contribution of changes in surgical practice to this result.

Methods: We reviewed CY 2009 claims submitted to the Centers for Medicare and Medicaid Services (CMS) for primary hemodialysis (HD) access procedures performed in NW5. Data were grouped by self-designated CMS surgical specialty codes (General Surgery 2 [GS], Thoracic Surgery 33 [TS] & Vascular Surgery 76+77[VS]) and primary access procedures were grouped into direct AVF (36621), complex AVF (36818, 36819, 36820, 36825) and AVG (36830).

Results: In CY 2009, 4747 long-term surgical HD accesses were constructed in NW5. Self-designated VS performed 62% of cases, and GS performed nearly one third. Surgeons constructed autogenous AVFs in 64% of cases and complex AVFs accounted for 52% of all AVFs. Although direct AVF as a proportion of all access was similar among GS and VS, the ratio of complex AVF to all AVF was higher among GS.

Conclusions: Although construction of AVG remains high, these data clearly show significant adoption of advanced AVF construction techniques by surgeons in NW5 and that these approaches contributed significantly to the rate of AVF construction in NW5. Subspecialty contributions must be interpreted with caution since this is a self-designated parameter. Claims data provide no information regarding the eventual maturation or function of these accesses. Regardless, these data support the success of the “spread” methodology of the FFBI as regards surgical practice.

Funding: Other U.S. Government Support

SA-PO2917

Starting Long-Term Hemodialysis with an Arterio-Venous Fistula: The Importance of a Formal Patient-Based Chronic Kidney Disease Course

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Background: Starting long-term hemodialysis (HD) with an arterio-venous fistula (AVF) is critically important, but most patients in this country start with a catheter. We analyzed data from our renal clinics to determine which factors were significant in predicting the initiation of HD with an AVF or having an AVF placed.

Methods: CQI data of all new patient HD starts within an urban academic medical center over a 12 month period were examined. We eliminated patients who had no prior renal care or were not seen in our Nephrology clinics. Data analyzed included: demographics, insurance, med history, creatinine levels, number of clinic visits, documents of physician counselling, Chronic Kidney Disease (CKD) education course participation, access vein mapping, and placement of AV shunt. Binary Logistic Regression modeling was used to find significant associations.

Results: 110 patients were started on HD. We excluded (67%) those who had no prior renal care, were seen in other clinics, or were prevalent to HD. 37 patients (33%) were cared for in our Nephrology clinics prior to HD – these were the subjects of our analyses. 10 patients initiated HD with an AVF. In 27 patients who started with a catheter, 6 had AVF’s in place but not ready to use. Binary logistic regression for start of HD with AVF showed that attending our CKD education course was significantly associated with that outcome (0.021, 0.001 to 0.51; p=0.018). When we analyzed for the outcome of having any AVF placed prior to HD, regardless of whether it was used or not, having attended the CKD education course was also favorably associated (0.02, 0.00 to 0.86; p=0.042).

Conclusions: A large percentage of patients who started long-term HD presented with no prior renal care and started HD with a catheter. Of patients who were seen in the Nephrology clinics, those who had participated in a formal CKD education course that is led by a team consisting of an experienced Renal nurse specialist, an Access nurse coordinator and a Renal dietician, were more likely to have an AVF in place at the start of HD and to initiate HD with an AVF.

Funding: Clinical Revenue Support

SA-PO2918

Patients with Cardiac Failure and Female Gender Is Most Likely To Start Hemodialysis Using a Double Lumen Catheter

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Background: Vascular access is the key step for a successful hemodialysis (HD) treatment. Early Nephrologists referral provide better options for a long term HD vascular access. Despite this important issue, a number of patients are without a permanent vascular access at HD initiation. The aim of the study was to assess whether age, gender and primary renal disease associate with differences in the type of vascular access placement.

Methods: We reviewed the records of 145 patients on HD 44 female and 101 male with mean age 64±14.5 on HD for 46±9±12 months (range 1-252). The primary renal diseases were diabetes, (23.4%), hypertention (17.2%.) glomerulonephritis (25.7%), cardiac diseases were diabetes, (23.4%), hypertention (17.2%.) glomerulonephritis (25.7%), cardiac failure (6.9%) and others (26.8%).

Results: Double lumen catheter and arteriovenous fistula was the first vascular access in 109 (75%) and 35 patients (24) patients respectively. Grafit was the first vascular access in 1 patient (0.5%). Arteriovenous fistula was the fist choice for 29.7% of the male patients and 9% of the female. Double lumen catheter was the first choice for 70.3% of the male (subclavian 50.5%, femoral 4.95% , and jugular 14.85%) and 86.4% of the female (subclavian 75%, femoral 2.3%, and jugular 9.1%) (p=0.04). Double lumen catheter was the first choice for the 79.7% of the patients older than 65 years old of age and 70.4% for the patients <65 years old (p=0.19). Double lumen catheter was the first choice for all the patients with cardiac failure as primary cause of renal disease.

Conclusions: Double lumen catheter was the first vascular access for the majority of our patients, probably due to late Nephrologists referral. Cardiac failure as primary cause of renal disease, Female gender, but not the age seems to influence this choice.
SA-PO2919
A Randomized, Controlled, Prospective, Double-Blinded Trial Investigating the Prevention of Catheter-Caused Infections Via a Coating Containing Bismuth
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1Nephrology, St. Joseph Krankenhaus (Hospital), Berlin, Germany; 2Gambro Medical & Safety Office, Gambro Dialysatores GmbH, Hechingen, Germany.

Background: Hemodialysis (HD) catheter-related bacteremia is a major cause of increased morbidity and mortality of patients with acute renal failure (ARF) and chronic renal failure (CRF).

Methods: We conducted a randomized, controlled, prospective, double-blind clinical study investigating the efficacy of a new non tunnelled HD-catheter with a surface coating containing bismuth in patients in need of temporary short-term vascular access. Implanted was a standard catheter (GamCath™, SC) or a bismuth-containing surface coated catheter (GamCath™ Dolphin® Protect, BCC) both with identical standard design. After removal of the catheter due to medical indication, both arterial and venous lumina were rinsed and the fluid cultured for detection of bacterial colony-forming units (CFU). The catheter tip was placed in a tube containing sterile saline, ultrasonicated for 2 min at 35 KHz and shaken at 300 rpm for 10 min. The filtrate was assayed for colonization.

Results: 88 patients received a catheter. 76 suffered from ARF, 12 from CRF (the catheter was implanted due to initiation of HD or shunt dysfunction). 58 (66%) catheters were removed due to no further need. 5 SC and 3 BCC were removed after assumed infection. The time to catheter removal for any reason was shorter for SC with a mean dwell time of 13 days vs 21 days for BCC.

Bacterial colonization over cut off 100 CFU/mL was not different, both for collected catheter tips as well as for rinsing fluids. However, 19 (50%) of collected SC were not colonized vs 23 (59%) of BCC. Mean predialytic CRP was significantly lower for BCC treatments (p<0.001) and procalcitonin values tended to be lower for BSC treatments. The last CRP value during catheter dwell time of each patient was significantly lower for BCC treatments (p=0.034).

Conclusions: Surface modification with bismuth film offers a new promising alternative to reduce HD catheter-related septicemia in patients who need temporary non-tunnelled central venous catheters.

Funding: Pharmaceutical Company Support

SA-PO2920
Prevention of Tunneled Cuffed Hemodialysis (HD) Catheter-Related Thrombosis and Bacteremia by the TEGO® Connector: A Monocentric Randomized Controlled Study
Florence Bonkain,1 Judith Racape,1 Isabelle Goncalvez,1 Micheline Moerman,1 Olivier Denis,1 Nadia Gammam,1 Karine Gastaldello,1 Joelle L. Nortier,1
1Nephrology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium; 2Microbiology, Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium.

Background: The TEGO® connector (ICU Medical) is a closed positive pressure system for tunneled cuffed catheter (TCC) attached on each hub washed with 0.9% NaCl, and used during 3 consecutive HD sessions. We conducted a randomized controlled trial, approved by our Ethical Committee, comparing the incidence of TCC-related thrombosis and bacteremia in patients carrying a TEGO® connector vs controls receiving trisdium citrate 46.7% as locking solution (Citralock®).

Methods: All adult HD patients of our unit, prevalent or incident, were included, excluding patients with a mature AV fistula and with a dysfunctional catheter. Patients who developed TCC thrombosis or bacteremia were censured. The time of TCC use was calculated and the incidence of complications was expressed per 1,000 TCC days.

Results: Sixty-six patients were followed during 9,194 days. Baseline characteristics were similar in both groups. The combined primary outcome was not significantly different in the TEGO® group vs controls (3.15 vs 4.36 /1,000 TCC days, p=0.34). TCC-thrombosis rates were equivalent (2.96 vs 3.15 /1,000 TCC days, p=0.87). Only 6 TCC-bacteremia episodes were identified, 1 in the TEGO® group (0.19 vs 1.2 /1,000 TCC days, p=0.06). The total cost of the TEGO® procedure was significantly lower than the Citralock® one (560 vs 1,086 $ per patient).

Conclusions: This study confirms the non-inferiority of the TEGO® connector in preventing TCC-related complications compared to citrate locking solution. Strict nursing procedures and TCC hub manipulations can explain our low bacteremia rate. The TEGO® connector seems to be a promising and attractive device.

Funding: Clinical Revenue Support

SA-PO2921
Randomised Control Trial Comparing Bacteraemia Rates in Closed Luer Lock Access Devices (TEGO) with Standard Access Devices in the Outpatient Haemodialysis Setting
Frank J. O'Brien, Claire Kennedy, Peter J. Conlon. Department of Nephrology, Beaumont Hospital, Dublin, Ireland.

Background: Catheter related bloodstream infections (CRBSI) are the second commonest cause of death in haemodialysis patients. Patients with permanent indwelling catheters have a 50% higher risk per year of developing systemic sepsis compared to patients with arteriovenous fistulae. The international rates of CRBSI’s are 2.5-5 episodes per hundred catheter days. This study hypothesizes that reduced handling of central catheters will reduce rates of CRBSI

Methods: A randomised controlled trial is being conducted from April 2010 to March 2012. This compares CRBSI rates in patients who have closed luer lock access devices placed at the end of their central catheters to those patients receiving current gold standard central catheter access techniques as per the current Beaumont Hospital protocol. All patients attending out patient haemodialysis units at Beaumont Hospital are eligible for inclusion in the study. This study highlights interim results at twelve months.

Results: Sixty-five patients were recruited in the first twelve months. 27 in the TEGO group and 31 in the control group. 36 patients completed follow up at month 12. 11 episodes of CRBSI were noted in the control group, and four in the TEGO group, (p=0.159).

Conclusions: Preliminary twelve month follow up data on the use of closed luer lock access devices in reducing CRBSI shows a reduction in episodes of infection, but was not statistically significant. There was no difference in flow rates or thrombosis rates between the groups.

Funding: Pharmaceutical Company Support

SA-PO2922
TEGO® Connectors Reduce Heparin Use without Affecting Blood Flow Rate Compared to Traditional Central Venous Catheter Locks
Mahesh Krishnan, Tracy Jack Mayne, Carol Farthing, Shaun S. Collard, Allen R. Nissenson. DaVita Inc, Denver, CO.

Background: The TEGO® Connector is a heparin-free device developed to reduce catheter-related infections and clots associated with central venous catheters (CVCs). We compared the efficacy and cost effectiveness of the TEGO Connectors and saline CVC locks to traditional heparin CVC locks, and compared both to the costs of using rt-PA.

Methods: We conducted a pre/post study comparing conversion from traditional CVC locks to TEGO Connectors in hemodialysis patients. The pre-period was defined as 90 days before, and the post-period 90 days after TEGO Connector conversion. Outcomes included monthly blood flow rate and heparin use (efficacy analysis); and cost of heparin, connectors, syringes, active, sodium citrate, medication and equipment (cost analysis). Both CVC locks and TEGO Connector costs were compared to the cost of using rt-PA.

Results: Blood flow rate remained the same over the course of the evaluation period (Table). Total heparin use decreased nearly 2000 units 3 months after TEGO conversion. The 6-month cost of traditional locks + caps + heparin + syringes was $127.92 per CVC patient compared to $111.54 for TEGO Connectors. Both are significantly less than rt-PA ($1834).

Blood Flow Rate and Heparin Use (before and after conversion to TEGO Connectors)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Days Prior to Conversion</th>
<th>Days After Conversion to TEGO Connectors</th>
</tr>
</thead>
<tbody>
<tr>
<td># Facilities</td>
<td>90.61</td>
<td>60.31</td>
</tr>
<tr>
<td># Patients</td>
<td>2,040</td>
<td>2,302</td>
</tr>
<tr>
<td>Blood Flow Rate (ml/min)</td>
<td>343.8 ± 41.5</td>
<td>343.8 ± 42.6</td>
</tr>
<tr>
<td>Total Heparin Units / Treatment</td>
<td>6,177</td>
<td>6,782</td>
</tr>
</tbody>
</table>

Note: the observed periods pivot around the date the dialysis facility switched to TEGO Connectors.

Conclusions: Use of TEGO Connectors decreased heparin use without affecting blood flow rate at a lower monthly cost. Cost of both TEGO and saline CVC locks were significantly lower than use of rt-PA. TEGO Connectors are a viable and cost-effective alternative to traditional locks.

Funding: Clinical Revenue Support
SA-PO2923
Comparison of Trisodium Citrate and Heparin as Catheter Locking Solution in Hemodialysis without Anticoagulant
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Background: Bleeding complications are common in hemodialysis patients, especially for those with high risk. To prevent the hemorrhagic events, adjustment for the anticoagulant in catheter locking solution should be considered. Because of the property of local antitriglycerol, trisodium citrate has recently become an ideal capping solution for hemodialysis.

Methods: The efficacy of different locking solutions were prospectively explored in a randomized, controlled, single blind trial. All enrolled patients used temporary central venous catheter as dialysis access. They were prescribed no-anticoagulant hemodialysis due to hemorrhagic tendency. These patients were randomly assigned to three groups according to the locking solution: Group A (routine heparin,416U/ml), Group B (low heparin, 2084U/ml), Group C (4% trisodium citrate). The incidence of bleeding, index of coagulation function and catheter patency during one observational period were evaluated. The observational period indicated the interval between two consecutive hemodialysis sessions. Prothrombin time (PT), activated partial thromboplastin time (APTT) and antiplatelet aggregation were determined at 0, 0.5, 2, and 24 hours after the locking procedure.

Results: Ninety patients were enrolled and thirty in each group. There were no significant differences in patient and catheter characteristics on inclusion. Both PT and APTT prolonged distinctly at 0.5 hour and 2h after the locking in Group A (P<0.01), then regressed to normal at 24h. In Group B, just APTT at 0.5h prolonged significantly (P<0.05) and recovered rapidly to normal at 2h. No significant changes on coagulation index were observed in Group C. During the observational period, six hemorrhagic cases occurred in Group A (20%), just one in Group B (3.3%) and no bleeding in Group C. The incidence is apparently higher in Group A (P<0.05). Besides, two cases of minor catheter clotting were observed in Group B.

Conclusions: To prevent post-hemodialysis bleeding, adjustment for the concentration or type of the locking antagonist should be considered. Due to the better outcomes of local antitriglycerol, trisodium citrate can be advocated as a safe and less expensive alternative to heparin.

Funding: Clinical Revenue Support

SA-PO2924
Peripherally Inserted Central Catheters Use in Patients with Acute and Chronic Kidney Disease: Single Center Experience
Mireille El Tors, Andrew D. Rule, Amy Mahon, Bernice (Bonnie) M. Jenson, Amy W. Williams, Sanjay Misra, Robert C. Albright, Sandra J. Taler, Marie C. Hogan. Div of Nephrology, Mayo Clinic; Dept of Radiology, Mayo Clinic, Rochester, MN.

Background: Peripherally inserted central catheter (PICC) use has increased significantly due to many perceived advantages in acutely ill patients. Their potential to cause central venous thrombosis/stenosis led to efforts to limit their use in the CKD population. We implemented a new PICC line order set in our institution in 2010 listing elevated creatinine (sCr) as relative contraindication for PICC placement and studied the impact of changes to this order set before & after on physician prescribing patterns of PICCs at our center.

Methods: In a cross-sectional study, the medical records of a total of 550 patients undergoing PICC placement (equally divided before and after revised orders implementation) were reviewed to determine sCr levels at the time of PICC request. The PICC RN is required to contact ordering physician to discuss complications before proceeding with the procedure. Acute kidney injury (AKI) was defined as ≥sCr of 25% from baseline at the time of the PICC placement and CKD defined as ≥sCr >1.3mg/dL in women and ≥1.5mg/dL in men.

Results: Of 275 patients undergoing PICC placement before the order set change, 52 met the criteria for ≥sCr (18.9%). After the revised order set was implemented 275 individuals 66 met the criteria for ≥sCr (26.4%). The percentage of patients with CKD in the sample prior to orderset implementation was 18/275 (6.5%) compared to 36/275 (13.1%). No statistically significant difference was noted between the two samples both for ≥sCr and CKD (p=0.145 and 0.09 respectively).

Conclusions: Despite efforts to increase awareness of the risks associated with PICC use in the CKD population, this venous access continues to be used frequently due to its ease and convenience. In fact we observed an increasing trend toward more CKD patients among those receiving PICC lines even after listing it as a relative contraindication. Effective processes should be developed to promote alternative venous access in our CKD population, including small bore internal jugular access, in order to preserve future venous access.

SA-PO2925
Improvements in Clinical and Operational Outcomes for a Cohort of Patients Converted from Central Venous Catheter Access
Andrew Glowaala, Andrew Barba, Randy Smith, Chan Basho, Abbe Volz. DaVita Inc, Denver, CO.

Background: Improvements in mortality and morbidity related to the reduction of central venous catheters (CVCs) in end-stage renal disease (ESRD) patients have been reported, however, little has been described regarding improvements in surrogate biochemical outcomes and operational parameters. Given the economic incentives associated with the new ESRD bundled payment system, improvements in operational and surrogate biochemical outcomes may be an important ancillary driver to CVC reduction efforts.

Methods: We assessed operational and biochemical outcomes of patients after implementation of a CVC reduction program called Cathaway™ which significantly reduced CVC rates from 2008 to present. Data from 6 months pre and post CVC conversion were assessed in a cohort of patients who converted from CVCs between October 2008 and June 2010 (n=3235). The analysis had 2 components, (i) impact on relevant clinical parameters including albumin, Kt/V, hemoglobin (Hb), and average blood flow rate (BFR) and (ii) impact on operational dialysis parameters including heparin use, tissue plasminogen activator (tPA) use, and missed treatments. Three month averages for the cohort from months -6 to -4 were compared to months +4 to +6.

Results: Improvements were noted in most of the clinical parameters including an improvement in BFR. These clinical improvements were achieved with more efficient resource utilization, specifically heparin and tPA (Table). The impact of changes to this orderset before and after on physician prescribing patterns of PICCs at our center.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Months -6 to -4</th>
<th>Months +4 to +6</th>
<th>P-value</th>
<th>% Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (mg/dL)</td>
<td>3.67±0.45</td>
<td>3.86±0.38</td>
<td>&lt;0.0001</td>
<td>5.2%</td>
</tr>
<tr>
<td>Hb (mg/dL)</td>
<td>11.63±1.08</td>
<td>11.59±0.87</td>
<td>0.10(NS)</td>
<td>0.34%</td>
</tr>
<tr>
<td>BFR</td>
<td>11.59±0.87</td>
<td>11.44±2.02</td>
<td>0.001</td>
<td>1.70±0.34</td>
</tr>
<tr>
<td>tPA (mg/tx)</td>
<td>0.072±0.24</td>
<td>0.085±0.07</td>
<td>&lt;0.0001</td>
<td>0.92±1.72</td>
</tr>
<tr>
<td>Missed tx/month</td>
<td>0.85±1.66</td>
<td>0.85±1.66</td>
<td>0.10(NS)</td>
<td>92.9%</td>
</tr>
</tbody>
</table>

Conclusions: We demonstrate that tangible benefits exist both in terms of patient outcomes and operational parameters with the successful conversion of patients from CVCs as a method of vascular access. These findings add to the compelling rationale for continuing to reduce the prevalence of CVC access in ESRD patients.

Funding: Clinical Revenue Support

SA-PO2926
Outcomes from a Multi-Center Catheter Reduction Collaborative Program
Alex J. Rosenblum, Eduardo K. Lacson, Shu-Fang Lin, Jill A. Hall, Raymond M. Hakim. Fresenius Medical Care, North America, Waltham, MA.

Background: In 2010, Fresenius Medical Care, North America (FMCNA) implemented a pilot Catheter Reduction Collaborative (CRC) based on the Institute for Healthcare Improvement (IHI) Framework for Spread Model to reduce prevalent hemodialysis catheter rates.

Methods: Medical Directors and their facility team from high-catheter facilities were invited to participate in the CRC. They were expected to adopt and implement a list of ten clinical and operational “best practices” and engage in six months of peer to peer learning and calls. The proportion of patients dialyzing without a central venous catheter (CVC) was tracked. Overall rates of positive blood cultures are also being collected.

Results: 164 facilities volunteered to participate. The group improved percentage of patients without a catheter in 2010 by 6.2%, from 61.2% to 67.4% by year-end.

Conclusions: We demonstrate that tangible benefits exist both in terms of patient outcomes and operational parameters with the successful conversion of patients from CVCs as a method of vascular access. These findings add to the compelling rationale for continuing to reduce the prevalence of CVC access in ESRD patients.

Funding: Clinical Revenue Support

SA-PO2927
Accuracy of Blood Culture Results from the Hemodialysis Circulation Based on Guidelines for Diagnosing Catheter-Related Blood Stream Infections
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Background: Most hemodialysis (HD) units diagnose HD catheter-related bloodstream infections (CRBSI) by obtaining blood cultures (BC) from the HD bloodstream concurrent with clinical exclusion of other sources of infections.

Funding: None
The recently updated guidelines from the Infectious Diseases Society of America (IDSA) recommend making the diagnosis of CRBSI by cultivating the same organism from a peripheral vein and from the catheter hub meeting criteria of differential time to positivity. The IDSA criteria for CRBSI have not been validated in HD patients. Our hypothesis is that a BC taken from a peripheral vein during HD will yield the same result as a BC taken from the dialysis circulation or the catheter hub. We further hypothesize that obtaining peripheral vein BC will be challenging (<50%) and may limit application of IDSA guidelines.

Methods: Four adult sets of BC (from a peripheral vein, from both catheter hubs and from the dialysis line) were obtained from patients who were suspected to have a CRBSI. Data was collected on the challenges of obtaining peripheral BC.

Results: To date, 40 patients who presented with signs and symptoms of CRBSI have been enrolled in this study. Bacteremia was found in 45% of these patients and all BC consistently grew the same bacteria from all culture sites. In 55% with undetectable bacteremia, all BC were negative for bacterial growth. Peripheral vein BC were obtained in 77.5% of the suspected CRBSI, 28.6% had >1 attempt before successful blood flow; 10% required a second nurse to obtain the peripheral blood. 1 Patient refused peripheral blood sample drawing, and in one case the blood sample did not yield the appropriate amount for a BC set due to collapsing peripheral veins.

Conclusions: More than ¾ of HD patients had successful peripheral vein BC drawn. 100% of organisms identified in peripheral vein samples were identical to those obtained from the dialysis bloodstream, suggesting that the current standard of practice of obtaining BC from the HD bloodstream concurrent with clinical exclusion of any other sources of infections is a valid method of diagnosing CRBSI.

Funding: Clinical Revenue Support

SA-PO2928

Attributed Mortality of Catheter Use in Incident Dialysis Patients: The Impact of an Acute Dialysis Start
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Nephrology, Dalhousie University, Halifax, NS, Canada.

Background: Central venous catheter (CVC) use as incident dialysis access is associated with mortality. However, some patients unexpectedly progress to end stage renal disease prior to reaching a GFR at which one would expect to establish alternative access. The purpose of this study was to identify the impact of this “acute start” on attributed mortality.

Methods: We studied 406 incident dialysis patients from 1/2006 to 12/2009. Patients were classified as “acute start” if the MDRD eGFR was >25 ml/min/1.73 m², ≤3 months prior to dialysis initiation and declined after an acute event (n=48). RPGN/myeloma patients without prior GFR measurements were also included (n=10). All remaining patients were classified as “CKD start” (n=348).

Results: 58.7, 23.6 and 17.7% of patients initiated dialysis with a CVC, fistula and peritoneal dialysis catheter, respectively. 81% (20%) were referred late (within 90 days of dialysis start). At 2 years, there were 103 deaths. The mortality of acute start patients was significantly higher than CKD start patients (figure 1, p<0.001). In a univariate Cox survival analysis, acute start (HR 3.44, p<0.001), CVC use (HR 1.88, p=0.004), and late referral (HR 2.52, p<0.001) were associated with mortality. Adjusting for age, gender, Charlson Comorbidity Index and albumin, only acute start remained associated with mortality (HR 2.50, 95% CI 1.57-3.99, p<0.001). Restricting the analysis to CKD start patients on hemodialysis (n=276), CVCs were not associated with mortality (HR 0.86, 95% CI 0.49-1.53, p=0.614).

Conclusions: A significant proportion of early dialysis mortality occurs after an “acute start”. Most of this mortality is related to the acute event itself, as opposed to CVC use or late referral. Dialysis registry analyses may overestimate attributed CVC mortality without considering the impact of an “acute start”.

Figure 1. Cumulative survival for “Acute Start” patients, log rank p<0.001

Funding: Clinical Revenue Support

SA-PO2929


Background: This open, prospective cohort study aims to evaluate the difference in the incidence of catheter related infections (CRI) with sodium citrate 4% compared to heparin 5,000 units/ml as a catheter locking solution in hemodialysis (HD) patients. Secondary outcomes include hospitalization, mortality, and catheter patency.

Methods: The use of citrate locks were compared to a historical control using heparin locks before September 2009. Medical records of chronic HD patients with permanent catheters between the periods of July 2008 - July 2009 and September 2009 - December 2010 were reviewed. CRI incidence was calculated as the number of infections per total number of catheter days. Pertinent information on patient medical history, thrombosis, infections, hospitalization, and mortality were collected.

Results: Mean age was above 60 years, and more than 90% of the patients were male. The major causes of ESRD were diabetes and hypertension in the 2 cohorts. A total of 412 and 441 patient-months were included in the heparin and citrate groups respectively. There were 22 CRIs with heparin and 11 CRIs with citrate (p = 0.013). CRI for heparin was 1.76 infections per 1,000 catheter days compared to 0.82 infections per 1,000 catheter days in the citrate group. Hospitalization occurred in 17 of the 22 CRIs in the heparin group and 9 of 11 CRIs in the citrate group (p = 0.086). The heparin group had 41 incidents of thrombosis compared to 40 in the citrate group (p = 0.69). CRI and thrombosis led to catheter exchange or removal in 36 cases in the heparin group and 18 cases in the citrate group (p = 0.09). There was no statistical difference in 6-month mortality for CRI between the treatment groups (p = 0.27).

Conclusions: This study showed a statistically significant difference in the incidence of CRI using sodium citrate versus heparin as a locking solution. Sodium citrate is also effective as heparin as an antithrombotic agent. Secondary outcomes were also comparable between sodium citrate and heparin. Results of this study support the use of sodium citrate as an alternative to heparin for the maintenance of catheter patency and prevention of CRI.

Funding: Veterans Administration Support

SA-PO2930

1 Department of Internal Medicine, Division of Nephrology and Hemodialysis, Medical University of Graz, Graz, Austria; 2 Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria.

Background: Between haemodialysis treatments catheters are locked with a locking agent. Because of its antimicrobial properties hypertonic trisodium citrate has become popular. This solution is not only spilled when injected, but is in part exchanged against whole blood due to its high density. Plasma proteins are therefore exposed to hypertonic trisodium citrate.

Methods: In vitro, whole blood and trisodium citrate (concentrations ranging from 4.7 to 46.7%) mixtures were used to assess protein precipitation. In vivo, listed filling volumes of hemodialysis catheters locked with trisodium citrate 4% (n=10) or 46.7% (n=10) were aspirated and then analyzed for protein precipitation.

Results: During in-vitro tests with hypertonic trisodium citrate protein precipitation was observed at concentrations exceeding 12%. When catheter locks were aspirated in-vivo, precipitated protein could only be separated and analysed in catheters locked with 46.7%. The main constituent was albumin.

Conclusion: Literature search revealed data confirming precipitation of proteins by different salts. ‘Salting out of plasma proteins by sodiumcitrate’ has become a common method for serum protein purification since the 19th century. Nevertheless, none of these papers has considered its relevance with respect to clinical application in hemodialysis patients.

Conclusions: Hypertonic trisodium citrate is exchanged against whole blood even up to the highest point in the catheter. During this process plasma proteins come into contact with hypertonic citrate and subsequently precipitate. Therefore, hypertonic trisodium citrate lock solutions are potentially dangerous and may be the underlying cause for reported embolic complications in patients with central venous catheters. Based on our findings we suggest that only commercially available trisodium citrate 4% lock solution can be used safely.

SA-PO2931

Gentamicin Catheter Locks: Trends and Markers for Development of Resistance and Clinical Outcomes in One Urban Outpatient Dialysis Center Sudhir B. Vyakaranam, Heather Duncan, Karthikeyan Meganathan, Kotagal Shashi Kant, Int Med, Div Neph & HTN, Univ Cincinnati Coll Med, Cincinnati, OH; HTN, Univ Cincinnati, Cincinnati, OH.

Background: Catheter Related Bacteremia (CRAB) and associated deep infections remain the second leading cause of hospitalization and mortality for patients dialyzing with tunnelled catheters (TC). Antibiotic catheter lock solutions (CLS) reliably reduce CRBs. Several studies report development of resistance to gentamicin (GR). To address these concerns we examined CLS (gent 2.7mg/mL heparin) use in one large urban dialysis facility
SA-P02932
Comparative Evaluation of Shower-Washing Technique and Aseptic Technique for Exit-Site Care of Tunneled Cuffed Venous Catheters

Methods: The subjects were 100 hemodialysis patients who had begun to use a TCC in our institutions from January 2005 to January 2010. Fifty subjects used shower-washing technique (Group S) and 50 used the aseptic technique (Group A). The shower-washing technique in Group S involved washing the exit site of the TCC with tap water immediately after TCC insertion and at every dialysis session. Moisture could be wiped away with non-sterile gauze. No antiseptic was applied. The aseptic technique in Group A was a conventional exit-site care method using povidone-iodine. In both groups we evaluated the skin conditions of the exit-site at the time of removal of TCC and the incidence of catheter-related infections, and compared the white blood cell (WBC) count and C-reactive protein (CRP) at the time of insertion with those at the time of removal of TCC.

Results: No significant difference was observed in the skin conditions of the exit site at the time of removal of TCC. Two exit-site infections (ESIs) and two catheter-related blood stream infections (CRBsis) occurred in Group S, and three ESIs and two CRBsis in Group A. There was no significant difference in WBC count and CRP between the time of insertion and at removal of TCC in both groups.

Conclusions: The shower-washing technique for exit-site care of TCC is simple, effective and as safe as the aseptic technique. Future studies are needed to compare these approaches in a larger number of subjects.

SA-P02935
Citrate (46%) Based Catheterlock Solution Reduces the Incidence of Catheter Related Sepsis in Dialysis Patients as Compared to Heparin, but Might Induce a Change in Pathogens

Methods: We tested the effect of citrate (46%) on 7 species of pathogenic bacteria, using the Kirby-Bauer method. All tunneled dialysis catheters used in our centre from 1-1-2000 until 7-1-2009 were reviewed. Episodes of sepsis were identified and responsible microorganisms were recorded. On 10-1-2003 citrate replaced heparin as a catheter lock solution. The incidence of sepsis before and after this switch was calculated. Sepsis free survival of the catheters was compared using Kaplan Meijer analysis.

Results: In vitro, citrate was bactericidal for Staph. aureus and S. pneumoniae. Enterococci and Gram-negative species were not affected. 438 tunnelled dialysis catheters in 330 patients were studied. Heparin was used in 175 catheters; citrate in 263 catheters. The number of catheter days per catheter in both cohorts was similar. The sepsis rate was

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Systemic Anticoagulation as a Result of Heparin Locking of Temporary Hemodialysis Catheters

**Background:** Bleeding is an important problem in critically ill patients with AKI. Temporary hemodialysis (HD) catheters require locking with heparin to maintain patency. In permanent tunneled catheters, there is evidence that heparin leaks into the circulation in concentrations that are high enough to produce anticoagulation. Impact of heparin locking on systemic coagulation has not been tested with temporary catheters.

**Methods:** We performed a prospective study of 20 patients requiring HD, and used a 1Fr, 20 cm double lumen temporary catheter (Balton Co., Warsaw, Poland). Catheters were locked using unfractionated heparin 5,000 U/ml according to manufacturer’s instructions. We obtained peripheral blood draws before catheter use for HD and 10 minutes after the catheter was locked post-HD. HD was performed without heparin. Coagulation parameters measured included activated partial thromboplastin time (aPTT), prothrombin time (PT) and platelet count.

**Results:** We recruited 17 men and 3 women aged 60±16 years (range 21-85). All patients had normal baseline coagulation parameters. Baseline aPTT was 38±3.8 seconds, whereas the mean aPTT 10 min after locking was 87±27 seconds. All patients had an increase in aPTT; the average increase was 130% from baseline (p<0.01). Three of the 20 patients had bleeding complications in the 24h period following testing. All 3 had post-locking aPTT levels >100 seconds. Patients with femoral catheters were associated with significantly higher post-lock aPTT levels as compared with jugular catheters despite identical catheter size and heparin dose.

**Conclusions:** Temporary double lumen catheter locking with heparin increases aPTT to levels capable of inducing systemic anticoagulation. This must be considered an important risk factor for bleeding in patients undergoing HD through a catheter in the acute setting.

**Funding:** Private Foundation Support.

Mathematical Modeling of In Vitro Data Provides an Accurate Method for Comparing the Performance of Dialysis Catheters

**Background:** Patient outcomes depend on catheter blood flow rate (Qb); therefore a method is needed to compare catheter performance since they vary in length and design. Modeling of in vitro data was used to calculate negative arterial pressure (AP) at any Qb or hematocrit (HCT), allowing comparison of catheters from different manufacturers.

**Methods:** Palindrome™ (PAL) (19, 33, 55 cm, 14.5 F) and Equistream™ (EQ) (23, 27, 35, 42 cm, 14.5 F) catheters were evaluated in an in vitro dialysis circuit. Blood was adjusted to HCTs of 40%, 35%, 30%, 24% using plasma. Height difference (H) from AP for PAL 33cm and EQ 35 cm are shown in Fig. left. EQ (23cm) measured AP and PAL values from AP in HCTs were shown in Fig. right. Mean difference EQ AP - PAL AP for Qb = 200, 300, 400 and 500 was -25.4±14.8, -33.6±15.4, -46.7±14, and -55±10.

**Results:** For all Qb and HCT values, EQ catheters had greater negative APs than PAL. Modeling of in vitro data provides an accurate method for comparison of catheter performance regardless of variations in catheter length and design.

Impact of Co-Morbidity and Type of Access on Subsequent Hospitalisation Following a Bacteremic Episode – Observational Study in a Hemodialysis Cohort

**Background:** Patients on hemodialysis (HD) are at risk of bacteremia and this is a major cause of morbidity and mortality. The type of dialysis access is a major factor, with HD lines being more likely to become infected than AV fistulae. Co-morbidities may increase the risk of hospitalisation, and the length of stay (LOS) in hospital.

**Objective:** To assess the effect of a bacteremic episode on morbidity (as defined by the need for hospitalisation), and to see whether this is influenced by the type of HD access or co-morbidity in HD patients.

**Methods:** Data were obtained on patients who had HD in 2009. We studied two groups - those who experienced a bacteremia in 2009 (group A) and patients who did not (group B). Data on the source of the infection, HD access at the time of infection, Davis co-morbidity score, and diabetes status were analysed, as was the total in-patient days in the 12 months following the bacteremic episode.
Results: 720 patients were included in the study. 618 (85%) in group A and 659 (91.5%) in group B. In group A, 36% were dialysing via a line, and 34% were dialysing through an AVF. Mortality within 12 months: group A - 16 (26%), group B - 88 (13.3%). Co-morbidity scores were similar in both groups.

Hospitalisations: group A - 92%, group B - 50%. Average LOS - group A - 40.9 days, group B - 9.4 days.

Conclusions: Morbidity was higher in the subsequent 12 months in patients who had a bacteremia, as compared with those who did not. The LOS in hospital was also greater in patients who had a bacteremia. Patients in group A were more likely to be dialysing via lines. Co-morbidities were similar in both groups, but diabetics had an increased risk of bacteremia and re-admissions.

Costs of treating bacteremias, and the increased LOS in hospital add to the financial burden on the health service. One modifiable risk factor is the type of HD access, and we feel our study adds weight to the need to reduce the number of patients on HD via lines.

SA-PO2940
Factors Associated with Bacterial Endocarditis in Dialysis Patients

Darren Green, Paul Hand, Philip A. Kalra. Department of Renal Medicine, Salford Royal Hospital, United Kingdom.

Background: Following a cluster of sub-acute bacterial endocarditis (SBE) in a nephrology unit, we reviewed who was most at risk.

Methods: Most SBE patients were on hemodialysis via a catheter. We reviewed data from the prospectively collected audit of tunnelled dialysis catheters inserted at this centre. We hypothesised that those most at risk had underlying structural heart disease or immunosuppression.

Results: 13 patients were treated for SBE over 2 years.

Table 1. Characteristics of SBE in dialysis patients

<table>
<thead>
<tr>
<th>Age</th>
<th>Organism</th>
<th>Vegetation</th>
<th>Duke</th>
<th>Access</th>
<th>Complication</th>
<th>Pre-existing valve changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>79</td>
<td>Staph Aureus</td>
<td>TUNN</td>
<td>No</td>
<td>Yes</td>
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<td>No</td>
</tr>
<tr>
<td>72</td>
<td>Culture-ve</td>
<td>AV</td>
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<td>Yes</td>
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<td>No</td>
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<tr>
<td>66</td>
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<tr>
<td>58</td>
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<td>42</td>
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<tr>
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</tr>
<tr>
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</tbody>
</table>

Conclusions: Age, presence of native valve disease or concomitant conditions which predispose to SBE may be associated with development of SBE. Further work is needed to determine whether there is any correlation between catheter use and the development of SBE.

SA-PO2941
Abstract Withdrawn

SA-PO2942
The Risk of Peritonitis after Exit Site Infection in Peritoneal Dialysis: A Contemporaneous Analysis

Alissa Lloyd, Sharon Nessim, Leigh Anne Shafer, Jeffrey Perl, Mauro Verrelli, Claudio Rigatto, Paul Komenda, Manish M. Sohd, 1 1Department of Medicine, Wanninger, MB, Canada; 2Department of Medicine, McGill University, Montreal, QC, Canada; 3Medicine, University of Toronto, ON, Canada.

Background: Peritonitis remains a leading cause of morbidity and technique failure among Peritoneal Dialysis (PD) patients. It is generally accepted that the development of PD catheter exit site infections (ESIs) directly lead to peritonitis with the same organism, however, the evidence to support this conclusion is limited.

Methods: The cohort consisted of 991 incident adult patients (≥ 18 years of age) residing in the province of Manitoba, Canada who received PD during the period from 2000-2009. 975 (98%) of patients were included where complete data was available. All ESIs were treated with oral or local antibiotics. Patients who experienced an ESI were matched by time on dialysis to those with no ESI and the probability of subsequent peritonitis was determined using generalized estimating equations (GEE) at 3, 6, and 9 months.

Results: There were a total of 1,002 ESIs and 1,228 episodes of peritonitis among 692 individuals. Nine hundred and forty-seven matched pairs were analyzed. Patients with an ESI on the same day were excluded. When the organisms were classified as gram positive, gram negative, culture negative or fungal, the risk of peritonitis following ESIs with the same class of organism was significantly increased at 3, 6, and 9 month intervals post-ESI (3 mths: 2.02 CI 1.13-3.48, 6 mths: OR 1.81 CI 1.20-2.75, 9 mths: 1.84 CI 1.30-2.64). When individual organisms were examined, the risk of peritonitis due to Staphylococcus aureus or Pseudomonas species was significantly increased up to 9 month post-ESI due to the same organism. In all analyses, the effects persisted after adjustment for diabetes, Aboriginal status and gender.

Conclusions: Patients with one or more ESI had an increased likelihood of developing peritonitis following the ESI. This increased risk occurred despite antibiotic treatment of the ESI suggesting biofilm formation, occult tunnel infection or poor technique.

SA-PO2943
Left Atrial Enlargement Is Associated with a Rapid Decline in Residual Renal Function in ESRD Patients on Peritoneal Dialysis

Mi Jung Lee, Dong Ho Shin, Dong Eun Yoo, Hyung Jung Oh, Seung Hyook Han, Tae-Hyun Yoo, Shin-Wook Kang. Dept. of Int. Medicine, College of Medicine, BK 21, SBSI, Yonsei Univ., Seoul, Korea.

Background: Left atrial volume (LAVI) has been considered an indicator of diastolic dysfunction and an independent predictor of mortality in patients with end-stage renal disease (ESRD). Residual renal function (RRF) has also been recognized as a significant predictor of morbidity and mortality in these patients. However, little is known on the relationship between ESRD and RRF in patients on peritoneal dialysis (PD).

Methods: One hundred and twenty-one incident PD patients were included. Within 2 months after PD initiation, LAVI was determined by echocardiography and RRF by 24-hour urine collection. Subsequently, RRF was measured every 6 months. Patients were divided into two groups according to the presence of LAVI > 32 mL/m², and the clinical and laboratory data, including the rates of decline in RRF, were compared between the two groups.

Results: Patients with LAVI tended to have higher baseline RRF, but RRF at 24-month was significantly lower in patients with LAVI (P=0.014). The overall rates of decline in RRF were significantly greater in patients with LAVI compared to those without LAVI (0.17±0.18 vs -0.07±0.16 mL/min/1.73m², P=0.002). Moreover, there was a significant inverse correlation between the slope of the decline in RRF and LAVI (0.026, P=0.018) along with the presence of diabetes (P=0.051, P=0.016) and baseline RRF (P=0.129, P=0.001).

Conclusions: This study shows that a higher LAVI is independently associated with a more rapid decline in RRF in ESRD patients, suggesting that volume and pressure control may help to preserve RRF in these patients.

SA-PO2944
Automated Wearable Artificial Kidney (AWAK): Feasibility of Removing Protein-Bound Toxins

Martin Roberts,1-3 1John F. Collins,3 2Marjorie Wai Yin Foo,2 Siti Noor Huda,3 Christian G. Bluchel,3 4David B. Lee, 5,6 5VAGLA Healthcare System, Los Angeles, CA; 6David Geffen School of Medicine at UCLA, Los Angeles, CA; 2AWAK Technologies, Singapore; 6Auckland City Hospital, Auckland, New Zealand; 7Renal Medicine, Singapore General Hospital, Singapore; 8Temasek Engineering School, Temasek Polytechnic, Singapore.

Background: Current dialytic modalities do not remove protein-bound (P-B) uremic toxins. Using digoxin as a surrogate P-B uremic toxin, we examined whether our peritoneal dialysis (PD)-based AWAK removes this ligand from spent peritoneal dialysate (SPD's).

Methods: From a CAPD patient taking 62.5 µg of digoxin orally every other day, the SPDs drained from an overnight dwell contained [digoxin] and [protein] concentrations (per L) of 0.5 µg and 0.82 g, respectively. This SPDs was flow through (single-pass) a reduced-size AWAK sorbent cartridge (SC), to test the potential of a regular SC and at a proportionately reduced dialysate flow rate of 200 mL/h.

Results: [Digoxin] dropped to undetectable levels (<0.2 µg/L) in the first effluent emerging from the SC and remained undetectable through a 6-hour study. Initial effluent [digoxin] dropped to 0.49 µg/L, but returned to its pre-SC levels within 15 min and thereafter. These observations were duplicated in 3 additional studies using synthetic SPDs with the addition to each L of 6 µg digoxin and [protein] concentrations (per L) of 0.5 µg and 0.82 g, respectively. This SPDs was flow through (single-pass) a reduced-size AWAK sorbent cartridge (SC), to test the potential of a regular SC and at a proportionately reduced dialysate flow rate of 200 mL/h.

Conclusions: Our results indicate that the AWAK SC removes the protein-bound ligand, digoxin, but does not remove dialysate protein and thus has the potential of removing protein-bound uremic toxins. The toxin-free protein is returned to the patient where it can bind more toxins.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

795A
SA-PO2945

Predictors of Peritoneal Dialysis (PD) Failure in Incident U.S. Patients Jenny Shen, Aya Alice Mitani, Benjamin A. Goldstein, Wolfgang C. Winkelmayer. Stanford University, Palo Alto, CA.

Background: Switching from PD to hemodialysis (HD) is undesirable, due to complications from temporary vascular access, disruption of daily routine, and higher costs. Little is known about the role social factors play in technique failure.

Methods: We identified and followed for 5 yrs a U.S. cohort of 1588 patients who initiated PD (1996-97). Morality failure was defined as any switch from PD to HD. Follow-up was censored at transplantation, death, loss to follow-up. We used Cox regression to examine associations among sociodemographic, medical, and healthcare related factors and the outcome. We estimated hazard ratios (HR) with 95% confidence intervals (CI).

Results: In a multivariate analysis, female sex (HR=0.78; 95%CI: 0.66-0.92) and older age (per 10 yrs; HR=0.89; 95%CI: 0.83-0.96) were associated with lower risks of technique failure. Coronal artery disease (HR=1.38; 95%CI: 1.15-1.65), heart failure (HR=2.20; 95%CI: 1.01-4.77), and receiving Medicaid (HR=1.50; 95%CI: 1.23-1.82) were associated with higher risks of failure. Subjects who completed high school were more likely to fail than those who did not complete high school (HR=1.33; 95%CI: 1.08-1.62). Separated, divorced, and widowed subjects also had a higher risk of failure than married subjects (HR=1.30; 95%CI: 1.03-1.60). Virtually all other levels of employment had a greater risk of failure than those who worked full time (Table 1). In an analysis restricted to subjects who had pre-dialysis care information, early referral to a nephrologist (> 3 months) and the primary decision maker of dialysis modality (physician vs. patient vs. shared) were not significantly associated with technique failure.

Adjusted hazard ratio for technique failure by level of employment

<table>
<thead>
<tr>
<th>Level of Employment</th>
<th>Hazard ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full Time</td>
<td>1</td>
</tr>
<tr>
<td>Part Time</td>
<td>1.51 (1.05-2.15)</td>
</tr>
<tr>
<td>Homemaker</td>
<td>1.53 (1.07-2.20)</td>
</tr>
<tr>
<td>Retired</td>
<td>2.01 (1.52-2.65)</td>
</tr>
<tr>
<td>Unemployed</td>
<td>1.30 (0.94-1.81)</td>
</tr>
<tr>
<td>Disabld</td>
<td>1.50 (1.16-1.95)</td>
</tr>
<tr>
<td>Other</td>
<td>1.41 (1.02-1.95)</td>
</tr>
</tbody>
</table>

Conclusions: Our study confirms that in addition to demographic and medical factors, several social factors are also associated with technique failure. They emphasize the importance of social and financial support in maintaining peritoneal dialysis.

Funding: Private Foundation Support

SA-PO2946

Understanding the Variability in Ultrafiltration Obtained with Icodextrin – From Theory To Bedside Zanze Yu,1,2 Mark Lambie,1,2 Simon J. Davies,1,2 1Department of Nephrology, University Hospital of North Staffordshire, Stoke on Trent, Staffordshire, United Kingdom; 2Institute of Science and Technology in Medicine, Stoke on Trent, Staffordshire, United Kingdom.

Background: There is considerable between patient variability in ultrafiltration (UF) obtained with icodextrin (ICO) that is not fully understood. Modelling of individual patients using the 3-pore model suggests that both hydrostatic and oncotic pressure differences as well as membrane characteristics need to be considered. The purpose of this study was to elucidate clinical predictors of the variability in UF including indirect measures of these additional factors.

Methods: Net UF obtained during ICO dwell was recorded as well as membrane characteristics and clinical factors every 6 monthly. Multi-level analysis was used to identify the predictor of UF taking account of within subject correlations.

Results: 690 dwellts in 202 patients were analysed, among which 280 were CAPD (typically 9 hours overnight dwell), 289 APD long day exchanges (typically 15 hours), and 126 in APD patients using an additional day-time exchange (typically 9 hours day time dwell). In multi-level mixed linear modelling, on CAPD predicted 160mls more UF compared with APD, no matter 9 hours or 15 hours. High input volume (2.5L) was related to an 11mls less UF compared with 2L. The UF negatively correlated to time on PD therapy and serum albumin. D/P creatinine, UF capacity (UF in PET) and BMI contributed positively to UF.

Conclusions: These observations fit with the theoretical modelling. They confirm the impact of membrane characteristics on UF that fast transport status and better UF capacity indicate more UF and prolonged time on PD decreases UF. They also clarify that factors which are likely to affect the oncotic pressure gradient (plasma albumin) and hydrostatic pressure gradient (input volume, patient position, BMI and gender) are more important than the dwell length in explaining UF variability. These observations have clear implications for dialysis prescription.

Funding: Government Support - Non-U.S.

SA-PO2947

Stability and Maintenance of Hemoglobin Levels with a Once Monthly Subcutaneous Administration of CERA in Chronic Kidney Disease Patients on Peritoneal Dialysis: The MISTRAL Study Raymond Azar,1 Jean-Philippe Ryckelynck,2 Philippe Rieu,3 Pierre Yves Durand,5 Bertrand Morel,6 Robert Milongo,7 Ali Aizei,1 David Pau,1 Christian Verger,1 CH Dunkerque; CHRU Caen; CHU Reims; CH Quimper; CH Chambery; Agliche Grenoble; Auar Reunion; Roche, Neullly sur Seine; CH Pontoise, France.

Background: Maintaining the stability of hemoglobin (Hb) level is a major goal of anemia treatment. The new European recommmendations (ERBP 2009) indicate that Hb level should be in the target range of 11-12 g/dl without intentionally exceeding 13 g/dl. CERA, continuous erythropoetin receptor activator, corrects anemia and maintains the stability of Hb level with a once monthly administration. This French multicenter clinical trial aims to provide more data about CERA in peritoneal dialysis (PD).

Methods: Patients (pts) without any absolute iron deficiency and stable with darbepoetin alfa, epoetin alfa or beta, received subcutaneous once monthly CERA during 44 weeks. The dose of CERA was adjusted to maintain the Hb level within a range of ± 1 g/dl and in the target range 10-12 g/dl. The primary endpoint was the proportion of pts maintaining Hb level within [10-12] g/dl during the evaluation period (weeks 16, 20 and 24).

Results: 95 PD patients (men: 60%) were analyzed (age: 67±16 years). Main etiologies of chronic kidney disease were hypertension (32%), glomerular diseases (22%) and diabetes (20%). Mean duration of dialysis was 2.4±1.3 years. At baseline, mean dose of previous ESA was 3903±2502 IU/week. Mean Hb level was 11.3±0.6 g/dl at baseline and 11.6±1.0 g/dl during the evaluation period. 51% of pts were within [10-12] g/dl during the evaluation period and 71% of pts were within the target range newly recommended by the ERBP 2009. 74% of pts had a variation of Hb ≤ 1 g/dl in baseline or their Hb between 10-12 g/dl during the evaluation period. Mean monthly dose of CERA was 133±30 µg at baseline and 127±76 µg after 6 months. 71 serious adverse events were reported in 47 pts (49%) and none were related to CERA.

Conclusions: A once monthly administration of CERA is safe and effective in maintaining the stability of Hb level in PD patients.

Funding: Pharmaceutical Company Support

SA-PO2948

Further Development of an Efficient Dialysate Delivery Protocol for Application in a Peritoneal Dialysis-Based Automated Wearable Artificial Kidney (AWAK) Marjorie Wai Yin Foo,1 John F. Collins; 2 Martin Roberts,1,2 Siti Noor Huda,1 Christian G. Bluchel,1 Kok-Seng Wong,1 David B. Lee.1,3 Renal Medicine, Singapore General Hospital, Singapore; 2Auckland City Hospital, Auckland, New Zealand; 1VAGLA Healthcare System, Los Angeles, CA; 3David Geffen School of Medicine at UCLA, Los Angeles, CA; 1AWAK Technologies, Singapore; 2Temasek Engineering School, Temasek Polytechnic, Singapore.

Background: We have reported high dialysate (D) flow rate (4, 5 L/h), using tidal PD (TPD) with low tidal volume (TV) and reserve volume (RV). We now report solute exchanges and ultrafiltration (UF) at several D FRs.

Methods: 5-hour TPD (TV 250, RV 500 mL) was conducted in 4 high transporters (HT) and 4 low transporters (LoT), using Baxter Home Choice system and pH neutralized D. Clearance (µL/min for urea [Cµ], creatinine [Ccr], phosphorus (Cp) and beta-2 microglobulin (Cb)) glucose uptake (G uptake, g/5h); and UF (ML/5h) were measured in each subject at D FR of 1, 2, 3 or 4 L/h, respectively.

Results: 1. Cµ is FR-dependent (FR-D) and not membrane-dependent (M-D), i.e., similar between HT and LoT. Calculated Kt/V range is 2.5±4.3. 2. Cp and Cb are M-D and relatively FR-independent. Extrapolated peritoneal Cp (L/week)/1.73m2 were 64 (at 1L/h, HT & LoT) and 111 (HT) and 95 (LoT) at 4L/h. 3. G uptake was both FR-D and M-D and varied from 106-190 g/day. 4. UF in LoT is higher (500-690 vs 200-580 mL/5h) in HT) and is relatively FR-independent, while UF in HT is FR-D.

Conclusions: 1. Dialysis adequacy is maintained with FR as low as 1L/h. A lower FR and lighter sorbent cartridge may be used during day time and a higher FR, heavier cartridge for night time. 2. Adequate UF is obtained at all FRs in both HT and LoT using only 1.5% D. G uptake, compared to CAPD (100-150 g/d) is not excessive. 3. Cp exceeds those reported (CJASN, 6:591. 2011) for CAPD, 43/34 (HT/LoT), and APD, 44/28.

Funding: Private Foundation Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

796A
SA-PO2949

Background: Maintaining euvolemia is an important goal in peritoneal dialysis (PD) patients. Adequate assessment of volume status in clinical practice is difficult. A new multifrequency bioimpedance tool has recently been validated for volume assessment.

In a longitudinal observation, we evaluated the association of hypervolemia on cardiac biomarkers, morbidity and mortality in PD patients.

Methods: In 54 PD patients Body Composition Monitoring (BCM; Fresenius Medical Care, Bad Homburg, Germany) and measurement of plasma troponin T (cTNT) and cardiac-specific pro brain natriuretic peptide (NT-proBNP) was performed. Clinical overhydration was defined as an overhydration-to-extracellular water ratio of > 0.15. Mortality was assessed over a follow-up period of 24 months.

Results: Clinical overhydration was found in 29.6% of the PD patients. Patients with overhydration had significantly higher cTNT and NT-proBNP levels compared to euvolemic patients (cTNT median 0.089 ng/mL, interquartile range 0.041-1.40 ng/mL vs. 0.028 ng/mL, p < 0.001; NT-proBNP median 13976 pg/mL, interquartile range 4336-31095 vs. 2286 pg/mL, p < 0.001). Chronic PD patients with clinical overhydration >0.15, cTNT >0.049 ng/mL and NT-proBNP >7266 pg/mL were more likely to die in the follow-up period.

Conclusions: Although much clinical attention is paid to volume status, almost one third of PD patients still have clinically relevant volume overload. Overhydration assessed by BCM, increased NT-proBNP and cTNT are strongly associated with adverse outcome in PD patients, and are useful tools for risk stratification.

SA-PO2950
A Reduced Ultrafiltration Rate in Children Undergoing Peritoneal Dialysis and a History of Peritonitis Depends on a High Intraperitoneal Pressure Rainer Buescher, Anja K. Büscher, Anika Von Gliszczynski, Peter F. Hoyer. Pediatrics II, Pediatric Nephrology, University of Duisburg-Essen, Essen, Germany.

Background: The adequacy and efficiency of peritoneal dialysis (PD) treatment in children is a dynamic process and depends on the administered intraperitoneal volume (IPV), dwell times, intraperitoneal pressure (IP) and maintenance of peritoneal function following complications such as episodes of peritonitis. While optimal IP ranges are well defined for pediatric and adult PD patients under stable clinical conditions, little is known about changes of IPV and ultrafiltration rate (UFR) in children following one or more episodes of peritonitis.

Methods: IPV was measured in 19 children (10 males and 9 females, mean age 12.0 ± 1.8 years (range 2 - 18 years), mean weight 31.4 ± 19.4 kg, (range 8.1 - 60.2 kg) as standard procedure every six months and in addition one month following each peritonitis. Seventeen patients were on automated peritoneal dialysis (APD) and two on continuous ambulatory peritoneal dialysis (CAPD). All children underwent (n = 104), two (n = 3) or three (n = 2) episodes of peritonitis. Patients were stratified into two groups according to their initial IPV pressure (IPV < 10 cm H2O, n = 11 and IPV > 10 cm H2O, n = 8) and UFR was monitored.

Results: Mean initial IPV was 95.9 ± 28.1 cm H2O/mL BSA, mean IPV prior to the first peritonitis 620.8 ± 335.6 mL/m2BSA/24 h and mean IPV for an inflow volume of 0 mL was 9.1 ± 4.3 cm H2O. IPV increased linearly by 1.8 ± 0.5 cm H2O when the IPV was increased by 250 mL/m2BSA. One month after the first peritonitis, UFR did significantly drop in those patients with an initial IPP > 10 cm H2O (IPP = 10 cm H2O: 637.3 ± 253.1 mL/m2BSA/24h vs. IPP > 10 cm H2O: 364.6 ± 307.7 mL/m2BSA/24h, p = 0.036). Hemiarrows were not observed within the study population.

Conclusions: We observed a drop of UFR after one peritonitis in those patients who already presented with a high IPP. Whether this effect is due to a irreversible fibrotic remodeling of the peritoneum or a transient phenomenon needs to be investigated in future studies.

Funding: Private Foundation Support

SA-PO2951

Background: Adequate peritoneal access is a prerequisite for good peritoneal dialysis (PD) treatment. Functional catheter problems constitute a major challenge for every PD program.

Methods: We reviewed the files of patients who received a PD catheter between 01/2005 and 08/2010. Only swan neck double cuffed curled catheters (SWAN NECK™ Missouri) were used and all were placed by open surgical technique. The “internal memory” of these catheters requires two features to be taken into account for good function: (1) swan neck angle and (2) correct inclination of catheter through the abdominal wall. Based on plain abdominal X-ray post-implantation, we defined the following categories: swan neck angle in PA view (<45°, 45°-90° or >90°), inclination of catheter through abdominal wall in lateral view (angle between intramural part of catheter with imaginary horizontal line <30° or >30°). In addition, we evaluated the position of the internal cuff relative to the spine (L1-2, L3-4 or lower) and the position of the pigtail (hypogastric, umbilical or subcostal zone) in PA view. Endpoints: occurrence of clinically overt functional catheter problems and need for surgical reintervention.

Results: 110 patients (64 male, mean age 56±16 years) were studied. During follow-up (till October 2010), there was at least one documented episode of mechanical catheter dysfunction in 42 patients (38%). 21 patients (19%) needed surgical repositioning of the catheter. Swan neck angle (larger vs. smaller angle, HR 1.58 [1.10-2.28]) and inclination through abdominal wall (<30° vs. >30°, HR 1.99 [1.57-2.50]) were associated with clinically overt functional catheter problems. The inclination through abdominal wall (>30° vs. <30°, HR 2.80 [1.18-6.60]) was associated with need for surgical reintervention.

Conclusions: Respecting the so-called “internal catheter memory” is of utmost importance to avoid functional PD catheter problems. Plain abdominal X-ray post-implantation is an easy tool to evaluate these parameters and may serve for educational and internal audit purposes.

SA-PO2952
Different Influence of Peritoneal Dialysis Duration on Outcome of Peritonitis between First and Successive Episode Peritonitis Rong Xu, Yanjun Li, Jie Dong. Institute of Nephrology, Peking University.

Background: The leading cause for patients dropout from long-term PD therapy is peritonitis. We aim to explore whether there is difference between first and successive-episodes peritonitis in the influence of peritoneal dialysis duration on outcome of peritonitis.

Methods: A total of 248 episodes of peritonitis (124 first-episodes and 124 successive-episodes) were observed between 1st January 2008 and 30th April 2011. In first-episodes and successive-episodes respectively, patients were divided into “shorter duration” group and “longer duration” group according to the median of PD duration when peritonitis occurred. The baseline demographic data and latest biochemical parameters measured no more than 3 months before the peritonitis were recorded. Multivariate logistic regression analysis was used to find the predictor of peritonitis outcome.

Results: Both in first-episodes and successive-episodes, the age, serum albumin, hemoglobin, total Kt/V, bacterial distribution, dialysate white cell count on 3 day and subsequent episode respectively, patients were divided into “shorter duration” group and “longer duration” group according to the median of PD duration when peritonitis occurred. The baseline demographic data and latest biochemical parameters measured no more than 3 months before the peritonitis were recorded. Multivariate logistic regression analysis was used to find the predictor of peritonitis outcome.

Conclusions: Compared with peritonitis occurred at shorter PD duration, peritonitis occurred at longer PD duration had similar outcome in first-episode peritonitis, but poor outcome in successive episodes, which couldn’t be explained by difference in demographic characteristics and biochemical parameters.

SA-PO2953

Background: Colonic diverticulitis is an important cause of polymicrobial peritonitis, which requires surgical treatment and cessation of peritoneal dialysis (PD). The aim of this study was to examine whether plain abdominal computed tomography (CT) is useful for evaluating colonic diverticulitis at renal replacement therapy (RRT) modality selection and to explore whether colonic diverticulosis is a risk factor for enteric peritonitis.

Methods: Subjects were 137 consecutive chronic kidney disease patients (stage 4 or 5) who were candidates for PD from February 2005 to November 2009. Abdominal CT without contrast media was performed in all PD candidates. Average individual peritonitis rate, enteric peritonitis rate, and cumulative survival and technical failure survival rate were assessed.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only. Underline represents presenting author.
Results: Diverticula of the colon were detected by plain CT in 57 cases (41.6%). The number of diverticula tended to increase with age. There was a common site of diverticulosis at the descending colon (70.1%). In patients treated with PD, the incidence of peritonitis was higher in patients with diverticulosis than in those without diverticulosis (p=0.004). However, only one episode of enteric peritonitis was observed among patients with diverticulosis. The cumulative survival and technical failure survival rate was not different between the diverticulosis-positive and -negative groups. PD was not selected in 4 cases due to a high frequency of diverticula with episodes of abdominal pain. Two of these cases developed severe diverticulitis with peritonitis and underwent removal of the colon.

Conclusions: Our study suggests that plain CT examination is useful for detecting diverticulosis at modality selection for RRT. Silent diverticulosis is not a risk factor for peritonitis. PD may be contraindicated in cases having frequent diverticulosis with episodes of lower abdominal pain.

SA-PO2954
The Use of Tidal Peritoneal Dialysis in Northern Ireland and the Effect on Residual Renal Function
Agnes Masengu, 1 Ronan Cunningham, 2 Robert Mullan. 3 Nephrology, Belfast City Hospital, Belfast, United Kingdom; 2 Nephrology, Antrim Area Hospital, Antrim, United Kingdom.

Background: In Northern Ireland (NI) there has been a steady increase in the percentage of patients performing automated peritoneal dialysis (APD). As a consequence of this more patients are being placed on tidal peritoneal dialysis (TPD), meaning that only a certain percentage of dialysis fluid is drained after each cycle. The aims of this study were to review the indications and prescription practices for TPD and determine if any important patient associations exist.

Methods: All NI patients on APD in December 2010 with at least one previous PD adequacy test had relevant data collected from clinical notes and electronic records. A questionnaire concerning TPD prescription and monitoring was also sent to each PD nurse specialist. Statistical survival analysis was performed using GraphPad Prism version 5.

Results: Of the 54 APD patients identified 93% were receiving TPD. In 68% the indication for TPD was to alleviate pain during drainage. All PD nurses reporting prescribing TPD with no direct input from the patient’s supervising nephrologist. There appeared to be a regional variation as to how the patient’s dialysis prescription was altered after the initiation of TPD, with only some nurses making adjustments to account for alterations in dialysis dwell time that can occur with TPD. No association was detected between the amount of TPD prescribed versus physical status and dialysis adequacy. However there was a significant association between TPD and residual renal function (RRF). Patients on >75% TPD (i.e. <25% dialysis fluid not drained after each cycle) had a higher residual urine output compared to patients on >75% TPD (mean RRF 1530 mls +/- 222 mls vs 922.5 mls +/-112 mls respectively, p<0.02).

Conclusions: The vast majority of APD patients are now prescribed TPD by nurses staff mainly to alleviate drainage pain. Theoretically TPD may alter solute clearance but this was not demonstrated in our study. The amount of TPD prescribed did have a significant effect on urine output and therefore raises the intriguing possibility that TPD plays a role in the preservation of RRF, the mechanism for which remains unclear.

SA-PO2955
Characteristics of Eosinophilic Peritonitis in 19 Children Receiving Peritoneal Dialysis
Masako Ikemiyagi, Yoko Hamasaki, Takeshi Yamada, Naoko Morita, Ryoko Urushitani, Chizuru Honda.

Nephrology, Tokyo metropolitan Children’s Medical Center; Fuchu, Tokyo, Japan.

Background: Peritonitis is a common, yet potentially serious complication in patients who are receiving peritoneal dialysis (PD). Eosinophilic peritonitis (EP) is usually benign and often resolves without intervention. It is essential to distinguish EP from bacterial and fungal peritonitis to avoid unnecessary treatments. We retrospectively analyzed children with EP to better characterize the condition.

Methods: EP was defined as >100 white cells per milliliter of dialysate effluent, of which eosinophils constitute >10% of the all white cells.

Results: Between January 2000 and April 2011, 72 children commenced PD. During follow-up, we identified 27 episodes of EP in 19 children (median 2.7 years). One child had 4 episodes, which was the maximum. The study group comprised 11 boys and 8 girls, with a median age of 3.0 years (4 months-13 years). The median interval from the initiation of PD to the first episode was 12 days (0-232 days). The median leukocyte count of the dialysate was 640 µl, and the eosinophil count was 287 µl. The median leukocyte count of peripheral blood was 7554 µl, the median eosinophil count was 554 µl, and the CRP was 0.02 mg/ml. Twenty-four episodes of EP resolved without antibiotics, and 3 were treated until effluent cultures became negative. Four episodes were associated with physical symptoms (fever; 2 episodes; abdominal pain, 2 episodes). Other episodes were unassociated with physical symptoms. Seventeen of the 27 episodes were associated with mechanical trauma: 11, involved catheter insertion; 5, enteral resection; and 1, herniorrhaphy. Five episodes were associated with intraperitoneal administration of antibiotics. Eighty-nine percent of the 27 episodes occurred within 14 days after the apparent cause of EP. Among the 27 episodes, 24 resolved spontaneously within 1 week. The possibility of EP should be considered in children who present with cloudy dialysate within 14 days after mechanical trauma, even in the absence of physical symptoms.

SA-PO2956
Peritoneal Dialysis Is a Good Option for Patients with Polyctic Kidney Disease Patients
Anna M. Tato, 1 Jose Portoles, 2 Paula Lopez, 2 Maire Rivera. 3 ‘Nephrology, Hospital Universitario Fundacion Alcorcon, Alcorcon, Madrid, Spain; 4Nephrology, Hospital Puerta de Hierro, Majadahonda, Madrid, Spain; 5Nephrology, Hospital Ramon y Cajal, Madrid, Spain.

Background: Polyctic kidney disease (PKD) is considered a partial contraindication for peritoneal dialysis (PD) because of concern about hernias and peritonitis eventually would end in technique failure.

Methods: Multicentre observational study of patients starting PD between 2003 and 2007 and follow them up to January 2010.

Outcomes: Patient survival, technique survival; peritonitis rate.

Results: The PD-centre-group’s data base (GCDP) includes 882 incident patients (2003-07) from 19 public hospitals. Patients are free to choose the renal replacement therapy, and they mainly opt for hemodialysis (HD) (regional-registration-data: 83.6% HD, 14.9% PD, and 1.5% pre-emptive-transplantation). PKD patients seem to prefer PD rather than HD (12% vs 7.5%). They are younger and have less morbidity than nonPKD patients. Although the transfer rate to hemodialysis is similar in both groups, in PKD patients is more often due to abdominal space related complications. Evolution of PKD patients vs nonPKD patients is summarized in table 1.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
798A
SA-PO2958
Diastolic Dysfunction as a Cause of Rapid Decline of Residual Renal Function in Patients with Peritoneal Dialysis Jwa-Kyung Kim, Jung-Woo Noh, Ja-Ryong Koo, Young Rim Song. Department of Internal Medicine & Kidney Research Institute, Hallym University Medical Center, Korea.

Background: Diastolic dysfunction is frequently observed in dialyzed patients; however, the significance of diastolic dysfunction has not been clearly determined. We evaluated whether diastolic dysfunction could influence the rate of RRF loss in 82 ESRD patients starting peritoneal dialysis.

Methods: This study performed at the Hallym University Hospital between April 2005 and March 2009, and subjects with pre-existing systolic dysfunction were excluded. Tinted urine collections were performed at baseline (within 1 month of start) and at 6-month intervals thereafter. Loss of renal function was defined as urine output less than 200mL/day.

Results: The mean follow-up period was 22.9±7.3 months. The median slope of RRF decline was -0.076L/min/week/month/1.73m² and the rate was significantly faster in diabetes compared to that of non-diabetes (-0.085 v. -0.049). Multivariable analysis showed that the rate of RRF loss had the highest correlation with diabetes (p=0.314, p=0.004), followed by LAVI (left atrial volume index) (r=0.276, p=0.015), increasing age (r=0.256, p=0.020), LV hypertrophy (r=-0.236, p=0.032) and E/E' ratio (early peak transmural inflow velocity to peak mitral annulus velocity) (r=-0.221, p=0.048). Forty-five patients (54.9%) had fast RRF decline (<0.076L/min/week/month/1.73m²) that had higher prevalence of diabetes (68.9%) and showed significantly elevated baseline LAVI, LAD (left atrial diameter) and E/E' ratio (p<0.05). However, LV ejection fraction and other laboratory parameters were comparable between the two groups. In unadjusted logistic regression, the presence of diabetes (OR 4.159 [95% CI 1.65-10.44], baseline RRF (OR 0.575 [95% CI 0.39-0.82], LAVI=32 mL/m² (OR 3.945 [95% CI 1.52-10.21) and eccentric LV hypertrophy (OR 5.50 [95% CI 1.26-23.94) was closely associated with the increased risk of accelerated loss of RRF. After adjustment for other clinical variables, LAVI=32 mL/m² was an independent predictor for RRF loss.

Conclusions: In conclusion, left ventricular diastolic dysfunction could be the main determinant for loss of RRF in ESRD patients starting peritoneal dialysis.

SA-PO2959
Good Glycemic Control Is Associated with Better Survival in Diabetic Patients on Peritoneal Dialysis Dong Eun Yoo, Tae Ik Chang, Mi Jung Lee, Dong Ho Shin, Seung Jun Kim, Hyung Jung Oh, Seung Hyeok Han, Tae-Hyun Yoo, Shin-Wook Kang. 1Dept. of Int. Medicine, College of Medicine, BK21, SBSI, Yonsei Univ., Seoul, Korea; 2Dept. of Int. Medicine, NHIC Ilsan Hospital, Goyang, Korea.

Background: Previous studies have demonstrated that strict glycemic control was associated with slow progression of diabetic nephropathy, and with higher survival rates in diabetic patients on hemodialysis. However, the impact of glycemic control on outcomes in patients with diabetes(DM) on peritoneal dialysis(PD) has largely been unexplored. This prospective observational study was undertaken to clarify whether good glycemic control is associated with a better outcome in DM patients on PD.

Methods: We conducted a prospective observational study, in which 140 DM patients who started PD between Jan 2001 and Dec 2008 were recruited. Patients were divided into tertiles according to the means of quarterly HbA1C levels measured during the first year of patients the statin group presented lower levels of TC (p=0.011), triglyceride (p=0.007), LDL-C (p=0.001), Apo-B (p=0.001), absolute amount of sd-LDL are lower than HD patients, likely due to peritoneal dialysate. Statins are able to decrease all LDL subclasses levels, but it is not known whether HD patients can benefit from the reduction of sd-LDL.

Results: Few studies have been performed in HD patients and none in PD patients. We compared the lipid profile and the effect of statins on it in HD and PD patients.

Methods: We enrolled 101 and 79 patients undergoing HD and PD at St Bortolo Hospital in Vicenza. The mean age of HD and PD patients was 66±13.9 and 58±15.9 (yr). Men were 67.3% and 59.5% in the 2 groups. 83.2% of HD and 81% of PD patients had hypertension. 27.7% of HD and 19% of PD patients were diabetic. We divided HD and PD patients into 2 groups according to statin therapy.

Conclusions: Our study suggests that statins may reduce the absolute amount and the proportion of sd-LDL in patients with a more abnormal lipid profile, such as PD patients. Statins do not alter sd-LDL levels in HD patients due to the higher number of patients with hypertension and diabetes, which can influence their generation.

SA-PO2962
Lateral Atrium Volume Index as Useful Predictor for Decline of Residual Kidney Function in Peritoneal Dialysis Patients Takeshi Yokoyama, Katsuo Satomi, Tsutomu Sakurada, Yugo Shibagaki, Yusuke Komoto, Takashi Yasuda, Kenjiro Kimura. 1Division of Nephrology and Hypertension, Kawasaki Municipal Tama Hospital, Kawasaki, Kanagawa, Japan; 2Division of Nephrology and Hypertension, Department of Internal Medicine, St. Marianna University School of Medicine, Kawasaki, Kanagawa, Japan.

Background: Preservation of residual kidney function (RKF) has been recognized as an independent predictor of survival in peritoneal dialysis (PD) patients. Although heart failure is known to associate with decline of RKF, little is known which parameters of ultrasound cardiology (UCG) are helpful to predict decline of RKF.

The aim of present study is to clarify the relationship between the rate of decline of RKF and the parameters of UCG.

Methods: UCG was performed in 15 PD patients at the initiation of PD, the following parameters such as left atrial dimension (LAD), left atrial volume index (LAVI), left ventricular end-diastolic dimension (LVEDd), left ventricular internal dimension in systolic (LVESd), left ventricular mass index (LVMI) were recorded. We calculated index (LVMI) were calculated as the half of the sum of weekly urea and creatinine clearances normalized to 1.73m². RKF was defined as urine output less than 200mL/day.

Results: Average RKF at the initiation of PD was 36.11± 19.14/Lweek/1.73m² and average rate of decline of RKF was -0.16 ± 2.07/Lweek/1.73m²/month. Rate of decline of RKF was significant correlated with LAD (r=-0.546), LVEDd (r=-0.544), LVMI (r=-0.473)
Use of Dressing with Polyurethane Absorbent Foam with Chlorhexidine Glucconate (BIOPATCH®) To Reduce Exit-Site Infection and Peritonitis in Patients Undergoing Peritoneal Dialysis

Background: Peritoneal dialysis (PD) catheter insertionrelated infections cause significant morbidity and often force the removal of the catheter.

Methods: We gathered data on 59 patients who had a PD catheter inserted over a year before introduction of Biopatch and 70 patients who had PD catheter inserted over a year afterwards. Our practice is to use Biopatch dressing at the time of catheter insertion and change it weekly for 6 weeks. Data was recorded on ESI, tunnel infection, PD peritonitis, catheter loss and mortality during first 12 weeks after catheter insertion.

Results: 17 patients could not complete the study period in pre-Biopatch group (3-died, 8 - catheter malfunction, 1 - transplant, 2 - transferred out & 2 - lost due to infection). 10 patients could not complete the study period in post-Biopatch group (2-died, 5-catheter malfunction, 1-transferred out & 1 -lost due to infection).

Incidence of infection leading to catheter loss was not statistically different in 2 groups. (p=0.11)

ALL INFECTION EPISODES:

<table>
<thead>
<tr>
<th>ESI</th>
<th>0-7 days</th>
<th>8-28 days</th>
<th>29-42 days</th>
<th>42-84 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE BIOPATCH</td>
<td>1 (1.7%)</td>
<td>4 (6.8%)</td>
<td>1 (1.7%)</td>
<td>5 (8.5%)</td>
<td>11 (18.6%)</td>
</tr>
<tr>
<td>POST BIOPATCH</td>
<td>0 (0%)</td>
<td>5 (7.1%)</td>
<td>1 (1.4%)</td>
<td>7 (10%)</td>
<td>13 (18.6%)</td>
</tr>
</tbody>
</table>

p = 0.12

PD peritonitis

<table>
<thead>
<tr>
<th>0-7 days</th>
<th>8-28 days</th>
<th>29-42 days</th>
<th>42-84 days</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRE BIOPATCH</td>
<td>0 (1.0%)</td>
<td>0</td>
<td>2 (3.8%)</td>
<td>3 (5.1%)</td>
</tr>
<tr>
<td>POST BIOPATCH</td>
<td>0</td>
<td>0 (1.4%)</td>
<td>0</td>
<td>0.041</td>
</tr>
</tbody>
</table>

p = 0.02

Unplanned PD with bleeding in 5 (24%) vs none (p=0.09). There was no exit site infection nor peritonitis. Late mechanical complications occurred in 13 (18%) of unplanned pts (leakage in 1, hernia in 2) and 10 (18%) of planned pts (leakage in 4, hernia in 7); p=0.9. PD catheter migration was noted in 1 (5%) urgent and 9 (15%) elective started pts; p=0.2.

Conclusions: The PD can be a safe alternative to HD in acute start allowing to avoid a temporary cannulation of large veins.

SA-PO2965

Does the Glomerular Filtration Rate at Initiation of Peritoneal Dialysis Affect the Nutritional Status and Survival of Patients? Narayan Prasad, Nephrology, SGPGIMS, Lucknow, UP, India.

Background: The effect of initial GFR on clinical outcomes on follow-up in Indian PD patients has not been studied. We aimed to study the effect of baseline GFR on nutritional status and survival of PD patients.

Methods: We included 342 PD patients (age 51.51±4 yrs,250 male,179 diabetics) and followed for 21.61±4.4 patient-months. Nutrition status of patients was assessed by anthropometry, biochemical parameters, diet-diary and Subjective Global Assessment(SGA).

GFR was calculated by using Cockcroft-Gault (CG) formula. Patients were classified into 3 groups.grp: G1: GFR<5ml/min, Grp II GFR 5-10 ml/min and Grp III GFR>10ml/min.

The clinical outcome and nutritionparameters at baseline and follow up were compared between three groups

Results: The variables between 3 grp are shown in the table.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kt/V urea</td>
<td>1.29±0.41</td>
<td>1.39±0.46</td>
<td>1.91±0.44</td>
<td>0.76</td>
</tr>
<tr>
<td>ESR (L/week)</td>
<td>3.09±11.75</td>
<td>6.07±11.74</td>
<td>10.57±14.07</td>
<td>0.04</td>
</tr>
<tr>
<td>D/P Cr</td>
<td>0.67±0.10</td>
<td>0.70±0.11</td>
<td>0.69±0.11</td>
<td>0.58</td>
</tr>
<tr>
<td>Age (Years)</td>
<td>56.3±114.4</td>
<td>51.8±15.2</td>
<td>69.46±23.54</td>
<td>0.04</td>
</tr>
<tr>
<td>S. Albumin (g/dL)</td>
<td>3.06±0.67</td>
<td>3.20±0.53</td>
<td>3.5±0.5</td>
<td>0.04</td>
</tr>
<tr>
<td>SGA score</td>
<td>3.5±1.4</td>
<td>4.4±1.6</td>
<td>4.4±1.7</td>
<td>0.81</td>
</tr>
<tr>
<td>NRI score</td>
<td>86.7±11.5</td>
<td>90.1±6.7</td>
<td>91.3±19.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Energy(Kcal/Kg/day)</td>
<td>17.9±5.8</td>
<td>20.9±6.5</td>
<td>31.6±6.0</td>
<td>0.06</td>
</tr>
<tr>
<td>Protein (g/kg/d)</td>
<td>0.7±0.30</td>
<td>1.1±0.29</td>
<td>0.84±0.27</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Conclusions: Initial GFR is an important factor to affect the nutritional status and survival of PD patients.

Funding: Government Support - Non-U.S.

SA-PO2966

Assessment of Hydration State in Peritoneal Dialysis Patients by Two Different Bioimpedance Methods-Body Composition Monitor and Calf Bioimpedance Measure Sri Ramachandra Gunturi, Ismail Kocyigit, Narayan Prasad, Erciyes University, Kayseri, Turkey.

Background: Our aims were to evaluate hydration state in peritoneal dialysis (PD) patients using body composition monitor (BCM) and calf bioimpedance (CBM), and compare both methods.

Methods: 58 PD patients studied. Overhydration (OH) was measured by BCM device (normal limits:1.1 to 1.1). OH was also measured using BCM and Calf hypervolemic groups according to OH value. Normalized calf resistivity (nRho) was calculated from resistance at 5 KHz using CBM. The mean value of 3 BP measured at different time on study day were used.

Results: Mean age was 47.2±10.12, 41 (70.7%) male. Clinical characteristics of OH groups were given in the table.

Table 1. Clinical Characteristics of Patients Grouped by BCM Data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Hypervolemic (n=24)</th>
<th>Normovolemic (n=34)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>51.36±14.9</td>
<td>44±12.1</td>
<td>0.015</td>
</tr>
<tr>
<td>OH (L)</td>
<td>2.58±0.92</td>
<td>3.0±116.5</td>
<td>0.009</td>
</tr>
<tr>
<td>nRho</td>
<td>13.7±2.4</td>
<td>16.1±3.3</td>
<td>0.005</td>
</tr>
<tr>
<td>D/P Cr</td>
<td>4.2±3.3</td>
<td>3.2±1.6</td>
<td>0.04</td>
</tr>
<tr>
<td>D/P Creatinine</td>
<td>0.09±0.17</td>
<td>0.06±11.1</td>
<td>0.50</td>
</tr>
<tr>
<td>No of antihypertensives</td>
<td>1.5±1.06</td>
<td>0.97±0.93</td>
<td>0.035</td>
</tr>
<tr>
<td>Edema + pts (n, %)</td>
<td>11 (45.8%)</td>
<td>9 (17.6%)</td>
<td>0.042</td>
</tr>
</tbody>
</table>

Conclusions: The difference between rel.OH and nRho is (p<0.01) (Fig1b).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Effect of Intra-Peritoneal Dialysate on Evaluation of Volume Status by Bioelectrical Impedance Analysis in the Peritoneal Dialysis Patients

Background: Direct segmental multi-frequency bioelectrical impedance analysis (DSM-BIA) has been proposed as a tool for adequate assessment of fluid status in continuous ambulatory peritoneal dialysis (CAPD) patients.

Methods: We examined the influence of intra-peritoneal dialysate on total body water (TBW) and the accuracy of DSM-BIA to estimate TBW. We measured the change of TBW by DSM-BIA (TBW_{DSM}) and compared TBW_{DSM} with TBW by Watson formula (TBW_{Watson}) under influence of dialysate in 55 CAPD patients.

Results: After infusion of dialysate, TBW_{DSM} increased, the index of hydration (TBW/weight(WB), %), and obesity (fat mass(FM)/WB, %) were significantly increased (p < 0.001), but the index of edema (extracellular fluid(ECF)/TBW) was not changed. TBW_{DSM} had fairly strong correlation with TBW_{Watson} before and after infusing dialysate (r = 0.946 or 0.940, p < 0.001), but TBW_{DSM} was overestimated versus TBW_{Watson} (p = 0.001). The difference between two methods was correlated with index of hydration (r = 0.529 or 0.542, p < 0.001) and obesity (r = -0.515 or -0.538, p < 0.001).

Conclusions: The dialysate influences the evaluation of TBW, however, we were able to use the edema index to evaluate fluid status of CAPD patients without the effect of dialysate. DSM-BIA overestimated TBW in hydrated patients and underestimated it in obese patients when compared with the Watson formula.

Funding: Government Support - Non-U.S.

SA-PO2968
Better Technique Survival with an Alternative Treatment Strategy of PD Related Peritonitis

Background: ISPD PD-related infections recommendations suggest cather removal in refractory, relapsing and enteric peritonitis. We compared the outcomes of all PD-peritonitis episodes (1998-2008) treated with different treatment strategies in two university hospitals.

Methods: Hospital 1: initial treatment: intraperitoneal (i.p.) rifampicin and gentamicin, if need be adapted to culture results. Decision to remove PD-catheter guided by ISPD recommendations.

Hospital 2: Patients ≤ 50 yrs: i.p. cefepime; Patients >50 yrs: considered at risk for enteral peritonitis. Assuming that PD hinders omental sealing of (micro)perforations, PD was discontinued and meropenem given intravenously and intra-peritoneal. In cases of enteric peritonitis continued for 1 week, then restart of PD with 1 week i.p. meropenem. If cultures yielded non-enteral organisms, PD was resumed with appropriate i.p. antibiotics. Fungal peritonitis was treated with i.p. fluconozol, oral flucytosine and intra-peritoneal amphotericin B. Enteral or fungal peritonitis were no indication for catheter removal.

Results: 323 peritonitis episodes in Hospital 1 and 251 in Hospital 2. Patient and peritonitis episode characteristics were similar. Fungal episodes occurred in 7.4% vs. 3.2%.

Antibiotics alone resulted in cure in 79.9% of episodes in Hospital 1 vs. 92.8% in Hospital 2. PD-catheter removed in 16.7% vs. 2.4% of episodes. Recovery after cather removal in 11.4% vs. 1.6%, with patient returning to PD in 3.4% vs. 0.0%, permanently switching to HD in 8.0% vs. 1.6% of episodes. Technique survival thus was 83.3% vs. 92.8% (OR 2.6, 95%CI 1.48-4.56).

Respectively in 5.0% and 0.8% of episodes patients died despite catheter removal and in 3.7% vs. 4.8% with cather in place. Patient survival thus was 91.3% vs. 94.3% (OR 1.61, 95%CI 0.83-3.10).

Conclusions: A peritonitis treatment strategy with interrupting PD, intravenous and intra-peritoneal meropenem, tailored to patient age and causative micro-organism and particular fungal peritonitis treatment resulted in better PD-technique survival than a strategy consisting of intraperitoneal antibiotics and catheter removal according to ISPD-recommendations.

SA-PO2969
Outcomes of Peritonitis in Incident Peritoneal Dialysis Patients

Background: We evaluated the incidence and outcomes of peritonitis among peritoneal dialysis (PD) patients who begin home dialysis within 90 days of first (ever) dialysis.

Methods: All 1,984 incident PD patients admitted to Fresenius Medical Care North America facilities from January 1 to December 31, 2009 were followed for one year from their first home PD treatment. We tracked patients that developed peritonitis and investigated outcomes within 30 and 90 days thereafter.

Results: 557 patients (28.1%) developed peritonitis over a median time of 123 days (139±98 days). Compared to patients without peritonitis, mean ages were similar (56.8 vs. 56.3 years, p=0.58), male gender (59.5% vs. 55.2%, p=0.11), white race (69.1% vs 76.7%, p=0.001), diabetes (53.7% vs 53.5% p=0.93), mean BMI (33.3 vs 31.9, p<0.05), medians 30.5 vs 29.3). Overall technique failure rate in patients who had peritonitis was 28.4% compared to patients who did not have peritonitis at 18.4% (p<0.0001). Subsequent hospitalization rates were higher for peritonitis patients as well (72% vs. 52.7%, p<0.0001). Odds ratios for technique failure and hospitalization for patients with peritonitis were 2.31 (95%CI 1.76-3.04) and 2.81 (95%CI 1.67-4.70) respectively in 5.0% and 7.7% of episodes patients died despite catheter removal and in 3.7% vs. 4.8% with cather in place. Patient survival thus was 91.3% vs. 94.3% (OR 1.61, 95%CI 0.83-3.10).

Conclusions: A peritonitis treatment strategy with interrupting PD, intravenous and intra-peritoneal meropenem, tailored to patient age and causative micro-organism and particular fungal peritonitis treatment resulted in better PD-technique survival than a strategy consisting of intraperitoneal antibiotics and catheter removal according to ISPD-recommendations.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

801A
SA-PO2970

Development and Evaluation of a Novel Semi-Long Peritoneal Dialysis Catheter for Upper Abdominal Exit-Sites

Deceased Membranous Group P

Myeloma kidney

Malignancy

Wael F. Hussein, Mohamed Shantier, Catherine A. Wall,

0.34

Group S

10 (15.9%)

Still on PD

Endometrial (2010)

4 (6.3%)

IgA nephropathy

Multiple Myeloma

17.2

Drug induced renal failure

12 (18.8%)

Secondary drainage failure

5 (7.9%)

0.09

Results: 64 percutaneous and 63 surgical catheter insertions were analysed. PDC related complications are shown. Complications of PDC insertions:

<table>
<thead>
<tr>
<th>Complications</th>
<th>Group S</th>
<th>Group P</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peritonitis (patient-months per episode)</td>
<td>17.2</td>
<td>20</td>
<td>0.66</td>
</tr>
<tr>
<td>Exit leak</td>
<td>4 (6.3%)</td>
<td>10 (15.9%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Poor initial drainage</td>
<td>7 (10.9%)</td>
<td>6 (9.5%)</td>
<td>0.34</td>
</tr>
<tr>
<td>Secondary drainage failure</td>
<td>12 (18.8%)</td>
<td>5 (7.9%)</td>
<td>0.09</td>
</tr>
</tbody>
</table>

There were no differences between the two groups in peritonitis rates, leaks, poor initial drainage and secondary drainage failure. Technical survival for group P catheters compared favourably with group S (P=0.35).

Background: Conventional Swan-Neck peritoneal dialysis catheters (PDC, 43.5cm) have been lutherto used in Japan. Recently several ultra long PDC (80 cm) designed for the prestral or upper abdominal exit-site were established to avoid infectious complications. However, it is not easy to handle these long catheters at the PDC implantation procedure. Furthermore, the problem of dislocation and omental wrapping remain unsoled.

Methods: We developed a newly designed Flexible-Neck semi-long PDC (JBS-2) to reduce the incidence of catheter-related problems including dislocations and omental wrappings. JBS-2 consists of 65 cm of lengths (5 mm of diameter) with 34 smaller side-holes (0.5 mm) on the four longitudinal slit lines. The tube around the peritoneal cuff (8.0 cm between the peritoneal and subcutaneous cuffs) was thickened and reinforced to prevent dislocation. We implanted JBS-2 for 16 patients and assessed implantation procedure and inflow-/outflow-time with 1500 ml of peritoneal dialysis fluid (PDF). The incidence of dislocation and omental wrapping were also recorded.

Results: Owing to the length of PDC, JBS-2 made the implantation technique easier than the ultra long catheters. We could provide upper abdominal exit (UA) without cutting PDC. It took 7.5 minutes of mean inflow time and 15 minutes of mean outflow time. No omental wrapping and no PDC dislocation has been observed in patients with JBS-2.

Conclusions: Our newly developed PDC (JBS-2) was appropriate for UAE without catheter-related problems including dislocations, omental wrappings and inflow-/outflow-delays.

SA-PO2971

Patient Recruitment to Home Hemodialysis (HHD) in Canada

Robert P. Paul1, Paul Komenda, Deborah Lynn Zimmerman.

1University of Alberta, Canada; 2University of Manitoba, Canada; 3University of Ottawa, Canada.

Background: There is growing interest in HHD though little is published on establishing and maintaining an HHD program. Canada is a recognised leader in HHD delivery and the purpose of this study was to leverage Canadian expertise and survey HHD programs nationwide to describe practice patterns in a variety of domains. The current abstract focuses on patient recruitment to HHD.

Methods: A comprehensive questionnaire of HHD practice patterns was developed by an expert panel with input from allied health services and underwent multiple modifications. It was distributed and completed online between July and December 2010; data reflect practices during this time period.

Results: Seventeen of 19 (90%) programs responded. Thirty-six of 17 (76%) have a specific recruitment strategy including special modality education classes (11/17 ~ 65%), a designated modality educator (12/17 ~ 71%), posters advertising HHD (11/17 ~ 65%), an information video on HHD (10/17 ~ 59%), discussion of potential HHD patients at patient care rounds (12/17 ~ 71%), and active recruitment of failing peritoneal dialysis patients (14/17 ~ 82%). Many programs restrict HHD access to patients expected to remain on HHD for at least 6 months (12/17 ~ 71%) or at least 12 months (6/17 ~ 33%), while other programs have no such policy and will train patients even if they are expected to exit the program sooner (eg. for planned living donor transplantation). Ten of 17 programs (59%) mandate an HHD assistant for selected patients; all programs allow family members to act as HHD helpers, and 4 of these 10 (40%) allow paid assistants. Ten of 17 (59%) estimate that <10% of pre-dialysis clinic patients choose HHD as their initial modality and 13/17 (76%) estimate <10% of unplanned (precipitated) dialysis starts will choose HHD.

Conclusions: Canada is a recognized leader in HHD recruitment strategies may facilitate increased uptake.

SA-PO2972

Comparison of Percutaneous and Open Surgical Techniques for First-Time Peritoneal Dialysis Catheter Placement in the Unrenched Peritoneal

Samar A. Medani, Wael F. Hussein, Mohamed Shantier, Catherine A. Wall, George Mellotte. Nephrology, Adelaide & Meath Hospital, Dublin, Ireland.

Background: The percutaneous seldinger method of peritoneal dialysis catheter (PDC) insertion has gained favour over recent years whereas traditionally it was reserved for patients unfit for general anaesthesia. This blind technique is believed to be less safe in patients with previous abdominal surgery therefore a criticism of this method is selection bias. In those with no history of abdominal surgery the optimal method of insertion has been established.

Methods: We retrospectively compared the outcomes of first-time percutaneous (group P) and surgical (group S) PDC placements in our centre between January 2003 and April 2010 in patients without a history of abdominal surgery. We reviewed outcomes and complications of PDCs until April 2011. Kaplan Meier curves were generated to represent survival of catheters for the first 12 months after insertion.

Conclusions: There were no significant differences between the two groups in peritonitis rates, leaks, poor initial drainage and secondary drainage failure. Technical survival for group P catheters compared favourably with group S (P=0.35).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

SA-PO2973

Peritoneal Dialysis Patients with Cancer; the Experience of One Centre

Harsha Wodeyar, Kaegeu Surenradhan, Muhammad Imran, Gordon M. Bell, Hameed Anijeet, Pearl Pai. Nephrology, Royal Liverpool University Hospital, Liverpool, Merseyside, United Kingdom.

Background: Peritoneal dialysis (PD) is often regarded as an inferior modality of renal replacement despite many advances in PD technology and solution formulation. There is also concern whether cancer patients should access chronic dialysis therapy. We report our experience of cancer patients on PD over the last 7 years in a well established PD unit in the north-west of England.

Methods: We reviewed the data of our PD population and the clinical details of those suffering from malignant disease. Our PD programme (n=80) includes 8 patients suffering from malignant disease in the last 7 years. Their details are below:

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Renal Diagnosis</th>
<th>Malignancy (time year)</th>
<th>PD vintage (months)</th>
<th>Current status</th>
</tr>
</thead>
<tbody>
<tr>
<td>25/Male</td>
<td>Drug induced renal failure</td>
<td>Neuroendocrine tumour (2007)</td>
<td>17</td>
<td>Not on dialysis due to sufficient renal recovery</td>
</tr>
<tr>
<td>54/Female</td>
<td>Obstructive nephropathy</td>
<td>Endometrial (2010)</td>
<td>26</td>
<td>Still on PD</td>
</tr>
<tr>
<td>62/Female</td>
<td>Membranous glomerulonephritis</td>
<td>Breast and bladder (2010)</td>
<td>5</td>
<td>Still on PD</td>
</tr>
<tr>
<td>40/Female</td>
<td>Diabetic nephropathy</td>
<td>Malignant melanoma with lymphode metastasis (2005)</td>
<td>24</td>
<td>Still on PD</td>
</tr>
<tr>
<td>65/Female</td>
<td>Myeloma kidney</td>
<td>Multiple Myeloma (2007)</td>
<td>18</td>
<td>Deceased</td>
</tr>
<tr>
<td>70/Female</td>
<td>Myeloma kidney</td>
<td>Multiple Myeloma (2005)</td>
<td>24</td>
<td>Deceased</td>
</tr>
<tr>
<td>20/Male</td>
<td>IgA nephropathy</td>
<td>Osteosarcoma with lung metastasis (2004)</td>
<td>24</td>
<td>Deceased</td>
</tr>
<tr>
<td>24/Male</td>
<td>Myeloma kidney</td>
<td>Multiple Myeloma (2009)</td>
<td>15</td>
<td>Deceased</td>
</tr>
</tbody>
</table>

Results: Our cohort had good nutritional status (mean albumin= 38.5 g/L) and range=37 to 45 g/L) and positive feedback with no patient withdrawal from treatment during the study period.

Conclusions: Many of these PD patients with cancer will never be on transplant waiting lists. In the United Kingdom, the use of a cycler (automated PD) frees restrictions and allows the patients to dialyse at their home. The quality of dialysis and life of cancer patients on PD would appear to be just as good as in non-cancer patients.
Home Hemodialysis (HHD) and Peritoneal Dialysis (PD) in Canada: The Status of Co-Existing Home Therapies in 2010  Robert P. Paulus,1 Paul Komenda,2 Deborah Lynn Zimmerman,3 1University of Alberta, Canada; 2University of Manitoba, Canada; 3University of Ottawa, Canada.

Background: Canada has historically treated a relatively high proportion of ESRD patients with PD. Canada is also a leader in the delivery of HHD, particularly with respect to nocturnal hemodialysis (NHD), short-daily hemodialysis (SDHD), and variations on these prescriptions. Uncertain is the extent to which these two home modalities can successfully co-exist in individual renal programs.

Methods: Between Jul and Dec 2010, 19 dialysis programs (14 university-based centers and 5 community centres known to provide all options of home dialysis therapies) were surveyed to determine the relative proportion of HHD and PD.

Results: Seventeen of 19 programs responded. Uptake of home therapies (HHD and PD) averaged 24.8±7.2% (range 9-41%) of prevalent ESRD patients; the balance was conventional thrice weekly in-centre or satellite HD. One of 17 (6%) programs had a combined HHD and PD population ≥40%, 4 of 17 (24%) had a combined home therapies proportion ≥30%, and 15 of 17 (88%) had a combined population ≥20%. Of patients receiving HHD, the median proportion (and range) receiving some form of NHD (6-8 hours on ≥3 nights per week) was 74% (24-94%), while a median of 4% (0-41%) received SDHD (1.5-3 hours on 6-7 days per week); non-NHD, non-SDHD dialysis prescriptions were also common in the home setting. Program 1 (graph) represents a suburban community dialysis centre, while program 3 represents an urban academic centre.

Conclusions: The predominant HHD modality in Canada is NHD. High uptake of HHD does not necessarily equate to poor PD prevalence; in fact, several programs with HHD uptake >10% also have high PD uptake suggesting that both home dialysis paradigms can successfully co-exist in both academic and nonacademic, urban and suburban settings.

SA-PO2975
Frequency of Payments for More Than Thrice Weekly Dialysis for Home Hemodialysis (HHD) Richard Hirth,1 Kathryn Sclerman,1 John R.C. Wheeler,2 Marc Turcotte,2 Wei Zhang,1 Adam S. Wilk,3 Joseph M. Messana.4 1Kidney Epidemiology & Cost Center, University of Michigan; 2Arbor Research.

Background: The limitation to three paid dialysis treatments weekly has been cited as a barrier to the diffusion of HHD, which is often performed on a daily basis. However, the regional fiscal intermediaries (FIs) and Medicare Administrative Contractors (MACs) that pay Medicare claims can authorize the payment of additional treatments based on medical necessity. It is unknown how often additional payments have been authorized and how this varies across FIs/MACs.

Methods: We identified all Medicare HHD patients in 2009 by FI/MAC. The average number of paid HHD treatments per month across the 19 FIs/MACs.

Conclusions: Results of regression analysis suggest that only some of this variation in HHD treatment frequency is predicted by demographics and patient comorbidities. The FI or MAC responsible for administering dialysis facility claims is an independent predictor of number of paid HHD sessions, potentially contributing significantly to the variation in HHD practice.

Funding: Other U.S. Government Support

SA-PO2976
Survival of Patients Receiving Home Daily Hemodialysis: A Multinational Cohort Study Rita Sun,1 Lihua Li,1 Robert M. Lindsay,1 Amit X. Garg,1 Louise M. Mois,1 Peter Austin,2 Cecille Coudoud,3 Ronald L. Pisoni,4 Bruce M. Robinson,5 Meaghan S. Cuerden,5 Gihad E. Nesaiballah,1 1University of Ottawa; 2ICES; 3Biomedicine Agency; 4Arbor Research.

Background: Increasing hemodialysis (HD) frequency from 3 to 6 times per week improves left ventricular mass and quality of life, but effects on survival are unknown.

Methods: We identified 177 patients from France, the US, and Canada in the International Quotidien Dialysis Registry, who received home daily HD ≥2 times/wk from 2001-2010. Using propensity-score based matching techniques, we matched 106 of these patients to 240 contemporaneous patients receiving in-center conventional, 3 times weekly HD in the Dialysis Outcomes and Practice Patterns Study. HD session times were <5 hrs. We compared mortality rates between groups using Cox proportional hazards regression. Because the proportional hazards assumption was not met, we divided the followup time into fixed periods and calculated hazard ratios (HR) for each.

Results: The daily group received 5.6±0.5 sessions/wk. Mean weekly treatment time was 16.1±4.8hrs (daily gp) and 11.5±1.3hrs (conv gp). After matching, there were no significant differences in baseline characteristics between groups, except more daily patients had HTN (68%/vs.55%), while more conventional patients had grafts (19%/vs.5%). Mean age was 53±14, 71% were male, 32% had diabetes. During 668 patient-yrs, 59/346 patients died. There was a trend toward higher risk of death with home daily HD until 1 yr, after which the risk was less than with in-center conventional HD. HR of Death with Home Daily HD

Conclusions: Compared to in-center conventional HD, the risk of death associated with home daily HD changed over time. Despite rigorous matching, it is possible that our results are affected by indication bias as we did not know reasons for daily HD initiation in this retrospective cohort. Previous studies have had similar limitations. As large RCTs of daily HD have not been feasible, well-conducted prospective studies are needed to better assess the effects of daily HD on survival.

SA-PO2977
Daily Hemodialysis (DHD) Improves Overall Quality of Life (QOL) and Physical Intimacy: Interim Results from the FREEDOM Study Michael A. Kraus,1 Fredric O. Finkelstein,1 Rachid Daoui,1 Janice P. Lee,2 Yoonjin Lee,2 Brigitte Schiller,3 Isaac Teitelbaum,1 Bertrand L. Jaber,1 1IU, IN; 2Tufts, CT; 3Rabin, NY; 4Emory, GA; 5Tufts, MA; 6Satellite, CA; 7U Colorado, CO.

Background: The FREEDOM study, an ongoing prospective cohort study investigating the clinical and economic benefits of DHD, has demonstrated improvements in various QOL measures following initiation of DHD, including SF-36, depressive symptoms, post-dialysis recovery time, sleep and restless leg symptoms.

Methods: In this a priori planned interim analysis, results of the following 3 special study questions (SSQ) from the QOL survey are reported on a 10-point Likert scale (0=worst, 10=best) for the Per Protocol (PP) and Intention-To-Treat (ITT) populations:

1) Considering all aspects of your life, physical, emotional, spiritual, and financial, how would you rate your overall QOL?
2) How satisfied are you with your degree of physical intimacy over the last 4 weeks?  
3) If you were given a choice of changing back to the previous regimen you were receiving prior to DHD, how likely would you be to change? (0=extremely likely, 10=strongly opposed)  

Results: Of the 299 enrolled pts, 165 completed 12-month f/u. Mean age was 53 yrs; 65% were male, 70% white, 58% used an AVF, 45% had diabetes and 26% CHF. Mean estimates (with 95% CI) of the PP analysis are shown below:

<table>
<thead>
<tr>
<th></th>
<th>Overall QOL</th>
<th>Month-4 score</th>
<th>Month-12 score</th>
<th>Global P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline vs. month-4</td>
<td>-3.9 (-6.2, -1.5)</td>
<td>5.7 (5.1, 6.3)</td>
<td>4.9 (4.3, 5.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>All baseline vs. month-12</td>
<td>-3.9 (-6.2, -1.5)</td>
<td>5.7 (5.1, 6.3)</td>
<td>4.9 (4.3, 5.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

**Summary effect on other cardiac and BP parameters**

<table>
<thead>
<tr>
<th></th>
<th>Change (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-hypertensive drugs</td>
<td>-0.8 (-1.2, -0.5)</td>
<td>0.012</td>
</tr>
</tbody>
</table>

**Conclusions**: Conversion from conventional three-weekly to frequent or extended thrice-weekly HD is associated with an improvement in LVM and other cardiac and BP parameters, which may have a potential longterm cardiovascular benefit.

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**SA-PO2980**

**Nephroptin-4 Is a Negative Regulator of Hippo Signaling**

**Sandra Habbig,** 1 Malte P. Bartram, 1 Roman-Ulrich Mueller, 1 Ricarda Schwarz, 1 Max C. Liebau, 1 Thomas Benzing, 1 Bernhard Schermer. 1 1 Renal Division, Department of Medicine and Center for Molecular Medicine Cologne, University of Cologne, Germany; 2 Department of Pediatrics, University of Cologne.

**Background:** Nephroptin (NPIH) is the most common genetic cause for endstage renal disease in children and adolescents. NPH is characterized by renal fibrosis and cytoskeletal remodeling, hence in contrast to ADPKD the size of the kidneys is normal or even reduced.

**Aim:** To analyze whether different disease causing NPH variants have been identified recently, there is little known about the function of the related NPH-proteins (NPH1-11).

**Results:** We report that NPH4 negatively regulates Hippo signaling. The Hippo pathway has recently emerged as a potent regulator of cell proliferation and organ size. NPH4 directly interacts with LAT1, the central kinase of the pathway, and prevents the inactivation of the downstream effectors YAP and TAZ. In the presence of NPH4, TAZ is released from 14-3-3 binding and translocates to the nucleus thereby promoting TAZ/TAZ-dependent pro-proliferative transcriptional activity. Consistently, knockdown of NPH4 results in reduced transcription of TAZ/TEAD target genes and diminishes cell proliferation in human kidney epithelial cells and several tumour cell lines. Currently, we are investigating the influence of other nephrocystins on Hippo signaling.

**Conclusion:** Our data suggest that NPH4 promotes cell proliferation through the control of Hippo signaling. Loss of NPH4 in NPH patients might result in hyperactive Hippo-signaling and reduced pro-proliferative transcriptional activity. These changes might be critical in the pathogenesis of NPH characterized by small-sized kidneys and atrophic tubular epithelium. In contrast, loss of Hippo signaling activity might be associated with polydysgenic kidney diseases characterized by massive proliferation such as ADPKD.

**Funding:** Government Support - Non-U.S.

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**SA-PO2981**

**3-D-Spheroid Defects in NPH Knockdown Cells Are Rescued by the Somatostatin Agonist Octreotide**

**Amiya K. Ghosh,** 1 Toby W. Hurd, 1 Friedhelm Hildebrandt. 1, 2 Department of Pediatrics, University of Michigan, Ann Arbor, MI; 2 Howard Hughes Medical Institute, University of Michigan, Ann Arbor, MI.

**Background:** Ciliopathies are a clinically and genetically heterogeneous group of diseases that exhibit shared clinical phenotypes, including renal cysts, retinal degeneration, mental retardation and obesity. Nephroptin (NPH) is one such ciliopathy characterized by kidney cysts at the cortico-medullary border. Among the 10 different disease-causing genes (NPH1-11), mutations in NPH3, NPH6 or NPH8 cause the ciliopathy variants of NPH, Joubert syndrome and Meckel Syndrome.

In this study, we hypothesized that loss of function of NPH3, NPH6 or NPH8 leads to morphological defects in a 3-dimensional (3-D) renal cell (IMCD3) culture model by either lack of cilia formation and/or cell polarity defects.

**Methods:** Stable shRNA knockdown cells of NPH3, NPH6 and NPH8 were generated using a retroviral transduction method. Knockdown cell lines were examined in 3-D spheroid culture followed by rhodamine-phalloidin staining to assess spheroid architecture.

**Results:** We observed significantly higher percentages of abnormal spheroids for all three stable cell lines (NPH3: 72%, NPH6: 80% and NPH8: 50%) compared to control shRNA cells (25%). Recent studies indicated that the somatostatin receptor agonist octreotide decreased cAMP level and inhibited cyst formation in PKD model systems. In our study, we observed that treatment of knockdown spheroids with octreotide (2mM) reduced the percentage of abnormal spheroids to 30% (NPH3), 36% (NPH6), and (NPH8) 35% (NPH8) respectively, whereas vehicle control abnormal spheroids were 66% (NPH3), 91% (NPH6), 60% (NPH8).

**Conclusions:** These results show that the lack of NPH3, NPH6 or NPH8 leads to cell polarity defects resulting in spheroid abnormalities which can be rescued by inhibiting cAMP levels with octreotide treatment. Our study confirms that manipulation of CAMP-pathway could be one of the therapeutic approaches in treating patients with kidney cysts.

**Funding:** Other NIH Support - Advances in Polycystic Kidney Disease.
SA-PO2982
Ciliated Cell-Type Specific Functions of Cep290/NPHP6 Christina Austin,1 Iain A. Drummond.1,2 1Department of Genetics, Harvard Medical School, Boston, MA; 2Nephrology Division, Massachusetts General Hospital, Charlestown, MA.

Background: Mutations in the centrosomal protein Nephrocystin 6 (NPHP6/Cep290) are implicated in a broad spectrum of genetic disorders featuring polycystic kidneys in addition to pleiotropic cilophaty phenotypes. A recent study finds that Chlamydomydous reinhardtii Cep290 mutants have truncated cilia with abnormal protein content, which was attributed to defective protein gating at the basal body transition zone. Nevertheless, cilia structural defects have not been observed in vertebrate models of Cep290 loss of function.

Methods: We set out to determine whether the Cep290 protein is, in fact, required for vertebrate ciliogenesis using the zebrafish Danio rerio as a model system particularly amenable to the study of cilia in vivo. Cilia of the photoreceptors, pterynephrons, and Kupffer’s vesicle (KV) were characterized after disruption of Cep290 expression by injection of antisense morpholino oligonucleotides.

Results: In contrast to IFT protein mutants, we find that a subset of single cilia, which includes sensory cilia, are truncated in Cep290 morphants, while the length of motile, multiciliated bundles is unchanged. Despite the apparent restriction of cilia structural changes to singly ciliated cells, immunostaining with anti-zebrafish Cep290 antibody revealed that the protein localizes to the basal bodies of all ciliated cells types in the zebrafish embryo. Furthermore, immunoelectron microscopy demonstrated that Cep290 localization is not restricted to the transition zone but also accumulates on the basal body and ciliary rootlet, implying that Cep290 may play roles independent of protein entry into the cilium.

Conclusions: In summary, we have characterized a vertebrate Cep290 loss-of-function model that implicates the basal body-localized protein, Cep290, in the structural maintenance of a select class of cilia.

Funding: NIDDK Support

SA-PO2983
Abelson Helper Integration Site-1 Knockdown in Zebrafish Models the Ciliopathy Joubert Syndrome and Is Associated with Altered Left-Right Assembly.

In the recent years, there has been increasing evidence to suggest that the ciliary and the apical cell polarity signaling pathways, both controlled by the conserved scaffold protein, Par6, are critical for the regulation of the left-right asymmetry during embryonic development. Joubert syndrome (JSRD) is a group of congenital disorders characterized by a set of ciliated dysfunctions, hypotonia and abnormal development of the cranial nerves. The proteins involved in the generation of the cilia asymmetry, and the organization of the left-right asymmetry are not well characterized.

Methods: To identify genes involved in the left-right asymmetry, we performed a genetic screen using the TPR repeat protein, Fleer. After identification of an enhancer-like deletion, we characterized the role of Fleer in ciliogenesis in vivo.

Results: In zebrafish embryos, the knockdown of Fleer results in various ciliopathy phenotypes, such as curved body axis; hydrocephalus; heart edema; and diaphragm defects, which are consistent with the phenotype of JSRD patients. Moreover, the knockdown of Fleer in the posterior lateral line (PLL) is associated with defects in pronephric cysts and neuromasts of the posterior lateral line (PLL). The knockdown of Fleer in the PLL is associated with defects in pronephric cysts and neuromasts of the PLL. Following immunofluorescence using anti-acetylated tubulin, cilia were observed in zebrafish embryos, and defects in single cilia were observed in the PLL. Furthermore, the knockdown of Fleer results in defects in single cilia in the PLL. The knockdown of Fleer in the PLL is associated with defects in pronephric cysts and neuromasts of the PLL. Moreover, the knockdown of Fleer in the PLL is associated with defects in pronephric cysts and neuromasts of the PLL. These results suggest that the Fleer protein is essential for the structural and functional integrity of single cilia in vivo.

Conclusions: The knockdown of Fleer in the PLL results in defects in single cilia, which are consistent with the phenotype of JSRD patients. These results suggest that the Fleer protein is essential for the structural and functional integrity of single cilia in vivo.

Funding: NIDDK Support

SA-PO2985
The Centriolar Satellite Protein Wtip Regulates Cilia Mediated Processes By Modulating Non-Canonical Wnt Signaling. Tomoko Ohba, Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK.

Background: Mutations in the centrosomal protein Nephrocystin 6 (NPHP6/Cep290) are implicated in a broad spectrum of genetic disorders featuring polycystic kidneys in addition to pleiotropic cilophaty phenotypes. A recent study finds that Chlamydomonas reinhardtii Cep290 mutants have truncated cilia with abnormal protein content, which was attributed to defective protein gating at the basal body transition zone. Nevertheless, cilia structural defects have not been observed in vertebrate models of Cep290 loss of function.

Methods: We set out to determine whether the Cep290 protein is, in fact, required for vertebrate ciliogenesis using the zebrafish Danio rerio as a model system particularly amenable to the study of cilia in vivo. Cilia of the photoreceptors, pterynephrons, and Kupffer’s vesicle (KV) were characterized after disruption of Cep290 expression by injection of antisense morpholino oligonucleotides.

Results: In contrast to IFT protein mutants, we find that a subset of single cilia, which includes sensory cilia, are truncated in Cep290 morphants, while the length of motile, multiciliated bundles is unchanged. Despite the apparent restriction of cilia structural changes to singly ciliated cells, immunostaining with anti-zebrafish Cep290 antibody revealed that the protein localizes to the basal bodies of all ciliated cells types in the zebrafish embryo. Furthermore, immunoelectron microscopy demonstrated that Cep290 localization is not restricted to the transition zone but also accumulates on the basal body and ciliary rootlet, implying that Cep290 may play roles independent of protein entry into the cilium.

Conclusions: In summary, we have characterized a vertebrate Cep290 loss-of-function model that implicates the basal body-localized protein, Cep290, in the structural maintenance of a select class of cilia.

Funding: NIDDK Support

SA-PO2986
The von Hippel-Lindau Tumor Suppressor Is Required for Proximal Tubule/Glomerular Integrity during Zebrafish Development and Regulates Renal Endosomal Trafficking. Rachel H. Giles,1 Ellen Van Rooijen,2,3 Ive Logister,1 Emile E. Voest,2 Stef Schulte-Merkert.1 1Nephrology and Hypertension, University Medical Center Utrecht, Netherlands; 2Medical Oncology, University Medical Center Utrecht, Netherlands; 3Hubrecht Institute, Utrecht, Netherlands.

Background: The von Hippel-Lindau (VHL) tumor suppressor gene is commonly mutated in hereditary and sporadic clear cell renal cell carcinoma (c RCC). We set out to determine whether the zebrafish homolog, vhl, is required in a developmental ciliopathy model.

Methods: We generated two stable zebrafish lines with null alleles of vhl and no detectable protein. Because the proxenpors are externally visible in transparent zebrafish embryos, we could observe traumatic events in situ over time. Analysis of the kidney phenotype was performed by immunohistochemistry, confocal microscopy in living fish and electron microscopy. Human kidney epithelial renal cells knocking down endogenous VHL by siRNA were recaptured in the in vivo phenotype.

Results: Here, we report that loss of vhl in zebrafish embryos results in severe periaptic phenotypic changes. In vivo, the glomerular architecture is widened and dilated cxxcr4a-positive capillary loops are observed. While siblings exhibit a single layer of cuboidal cells comprising the proximal tubule, vhl-/- tubule cells are irregularly shaped with a defect in exocytosis. VEGF receptor inhibition confirms that neovascularization of the vhl mutant pronephros is defective. vhl-/- tubule cells are irregularly shaped with a defect in exocytosis. VEGF receptor inhibition confirms that neovascularization of the vhl mutant pronephros is defective. vhl mutants display a phenotype similar to pkd2 knockdown related to human ciliopathies. These phenotypes were recapitulated in a mammalian context using the vhl-/- transgenic mouse.

Conclusions: The vhl tumor suppressor gene is essential for ciliogenesis and is required for normal renal development.

Funding: NIDDK Support

SA-PO2987
The Centriolar Satellite Protein Wtip Regulates Cilia Mediated Processes By Modulating Non-Canonical Wnt Signaling. Tomoko Ohba, Cell Biology, University of Oklahoma Health Science Center, Oklahoma City, OK.

Background: Mutations in the centrosomal protein Nephrocystin 6 (NPHP6/Cep290) are implicated in a broad spectrum of genetic disorders featuring polycystic kidneys in addition to pleiotropic cilophaty phenotypes. A recent study finds that Chlamydomonas reinhardtii Cep290 mutants have truncated cilia with abnormal protein content, which was attributed to defective protein gating at the basal body transition zone. Nevertheless, cilia structural defects have not been observed in vertebrate models of Cep290 loss of function.

Methods: We set out to determine whether the Cep290 protein is, in fact, required for vertebrate ciliogenesis using the zebrafish Danio rerio as a model system particularly amenable to the study of cilia in vivo. Cilia of the photoreceptors, pterynephrons, and Kupffer’s vesicle (KV) were characterized after disruption of Cep290 expression by injection of antisense morpholino oligonucleotides.

Results: In contrast to IFT protein mutants, we find that a subset of single cilia, which includes sensory cilia, are truncated in Cep290 morphants, while the length of motile, multiciliated bundles is unchanged. Despite the apparent restriction of cilia structural changes to singly ciliated cells, immunostaining with anti-zebrafish Cep290 antibody revealed that the protein localizes to the basal bodies of all ciliated cells types in the zebrafish embryo. Furthermore, immunoelectron microscopy demonstrated that Cep290 localization is not restricted to the transition zone but also accumulates on the basal body and ciliary rootlet, implying that Cep290 may play roles independent of protein entry into the cilium.

Conclusions: In summary, we have characterized a vertebrate Cep290 loss-of-function model that implicates the basal body-localized protein, Cep290, in the structural maintenance of a select class of cilia.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Conclusions: Our data indicate that vhl is required to maintain pronephric tubule and glomerular integrity during zebrafish development possibly through endothosome homeostasis.

SA-PO2987

Functional Analysis of Renal Ciliopathy Disease Alleles in 3D Spheroid Culture. Rachel H. Giles, Liyun Song, Peter K. Jackson. Nephrology and Hypertension, University Medical Center Utrecht, Netherlands; 2Cell Regulation, Genentech, South San Francisco, CA.

Background: Renal cystic diseases such as Bardet-Biedl, nephronophthisis (NPHP), Joubert, and Meckel-Gruber syndromes are phenotypically and genetically overlapping diseases displaying variable mental retardation, blindness, cerebellar ataxia, and renal anomalies. Genes known to be mutated in these disorders often code for proteins associated with cilia and/or basal bodies.

Methods: Standard 2D immunofluorescence often does not reveal clear effects on renal cilia when we reduced cellular levels of a panel of 21 cilia-associated proteins by siRNA knockdown, which were validated by qPCR. To improve the sensitivity of our readout we generated a more physiological setting by growing siRNA-treated murine IMCD cells in a 3D matrix consisting of matrigel and collagen.

Results: We observe reduced apical-basal polarity, lumen irregularities, and small but significant changes in cilia numbers or length, depending on the target. Reconstitution experiments with a subset of the proteins using siRNA-insensitive alleles confirmed specificity of the effects in all cases tested. Live cell imaging of the vasopressin receptor in these 3D spheroids reveals functional transport of AQP2 to the apical membrane upon exposure to arginine vasopressin. Furthermore, IMCD3 stable lines expressing ciliary GFP- or RFP-labeled serotonin receptor 5-HT6 grown in 3D spheroids revealed ciliary dynamics including rubbing, membrane shedding, and extended contact with debris in the lumen. Chimeric GFP- and RFP-spheroids can be generated such that one cell type has been treated with siRNA, but is surrounded by wild-type sister cells. We demonstrate this principle by evaluating the cell-autonomous effects of siRNA of Nphp1, Nphp2, Bbs1, Bbs2, Kif12, or Mks1 using real-time imaging of 3D spheroid cultures.

Conclusions: We conclude that this assay is a robust and sensitive read-out for loss of function or hypomorph mutations involving ciliopathy disease alleles.

Funding: Pharmaceutical Company Support

SA-PO2988

Kinesin Family Member 12 Localizes to Primary Apical Cilium and Correlates with Structural and Developmental Expression Patterns of Key Cystogryphy Genes. Michal Mrug, Bruce Aronow, Lisa M. Guay-Woodford.

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Background: We have mapped a major modifier of polycystic kidney disease (Mpkd) locus to <14 Mbp interval with 69 RefSeq genes. Among them, 32 were candidate genes for these loci, we crossed CAST/Ei-derived Mpkd1-2 interval allowed the use of congenic recombinants to fine map the transduction of the hyperosmolal microenvironment to cell cycle arrest.

Results: Cilia-proficient 176-5 cells experienced arrest of cell proliferation upon adaptation to hyperosmolal NaCl or urea, whereas cilia-deficient 176-5a cells continued to proliferate in spite of hyperosmolal adaptation. Expression of PCNA, a marker of cell proliferation, was unchanged in osmotically adapted 176-5a cells, whereas 176-5c cells demonstrated a reduction in PCNA under hyperosmolal conditions. Interestingly, activation of p53 was not present in either cell following adaptation to hyperosmolal conditions.

Conclusions: These results indicate that cilia are required for reduction in cell proliferation following adaptation to hyperosmolal conditions, and this reduction appears to be ATM-independent. Further studies are needed to determine the role TRPV1 plays in the transduction of the hyperosmolal microenvironment to cell cycle arrest.

Funding: NIDDK Support

SA-PO2990

Loss of the Primary Cilia Leads to Elevated Activity of the Disintegrin Metalloenzyme ADAM17. Monika Gooz, May Y. Amria, Yujiang Ding, Binlin Song, P. Darwin Bell.

Medicine, Medical University of South Carolina, Charleston, SC.

Background: Epidermal growth factor receptor (EGFR)-dependent signaling pathways are among the most important regulatory circuits that induce and maintain cellular proliferation. In polycystic kidney disease (PKD) abnormal expression/function of EGFR and its substrate growth factors have been described. Since ADAM17 is the main shedding enzyme responsible for activation of EGFR ligands, we investigated whether primary cilia dysfunction leads to increased ADAM17 activity, which in turn maintains an autocrine signaling loop resulting in the proliferative phenotype found in PKD.

Methods: We used a tamoxifen-inducible Cre recombinant adult mouse model of autosomal recessive PKD that had the conditional floxed allele for the orpk mouse (Tg737 gene), a component of the primary cilium.

Results: We observed significant cytos development in cilia (-) animals compared to cilia (+) animals. There were no significant differences in overall ADAM17 expression using whole kidney lysates from cilia (+) compared to cilia (-) mice by Western blot. However, using immunohistology, there was intense ADAM17 staining in the epithelium that lined adult cysts in cilia (-) mice. This staining was localized mainly to the apical surface of these cells. In cilia (+) kidneys, ADAM17 staining was distributed more evenly across apical and basolateral membranes of epithelial cells. Interestingly, we observed strong ADAM17 staining in the primary cilium. Since cyst development is localized mainly to the collecting duct, we next used the cilia (+) and cilia (-) collecting duct cell lines PCaPNA and BAP2 originating from the orpk mouse (Tg737 hypomorph) to compare ADAM17 activity. Using a fluorogenic substrate we observed increased ADAM17 activity in cilia (-) cells. Phorbol ester (PMA) treatment elevated ADAM17 activity by 2-fold in cilia (+) cells but, interestingly, PMA-induced ADAM17 activity was attenuated in cilia (-) cells.

Conclusions: We conclude that in PKD, ciliary dysfunction induces localized ADAM17 activity in the kidney, resulting in continued up-regulation of EGFR activity and maintenance of the proliferative phenotype of cystic epithelium.

Funding: NIDDK Support, Veterans Administration Support

SA-PO2991


Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, Tyne and Wear, United Kingdom.

Background: Joubert syndrome (JBTS) is an inherited ciliopathy leading to a cerebellar-motor-renal syndrome. Recent genetic advances have allowed positional cloning and identification of several JBTS genes. AHI1 encodes Jouberin and CE290 (alias NPHP6) encodes nephrocystin-6. Mutations in AHI1 account for ~12% of patients with JBTS and mutations in CE290 account for ~7% of patients with JBTS.

Objectives: Here, we use human embryonic renal tissue from the MRC/Wellcome Trust-funded Human Developmental Biology Resource (www.hdbr.org), we reveal an early
its interaction with PLCE1, loss of cell polarity may be underlying the DMS phenotypes

Pronephric glomeruli using podocin promoter, we showed by confocal microscopy that the pronephric glomeruli position 63 in the zebrafish ortholog of PAR6gb (2). The mutated protein partially lacks calcium binding protein bodies belonging to non-motile cilia. Our current hypothesis is that cell line with stunted cilia. We confirmed potential changes in localization and expression in comparison to our candidate proteins in CD cell lines with normal length cilia and in a cell line with stunted cilia. We confirmed potential changes in localization and expression of these basal body proteins by immunofluorescence in both cell types. Results: We provide novel evidence for α-actinin4 localization to the basal body in normal collecting duct cells. Evidence suggests cilia along the nephron function as a fluid sensor. Cilia signaling events are associated with the cell cycle and growth, events leading to the development of a polarized apical and basolateral membrane through the organization of the cytoskeleton Numerous reports suggest a link between dysfunctional cilia of the collecting duct and Polycystic Kidney Disease. Methods: Coimmunoprecipitation experiments with a specific antibody to α-tubulin, a basal body component, helped identify potentially novel basal body components. To monitor changes in BB composition in a PKD cell model, Western Blot analysis was used to determine cellular expression of proteins known to localize to the basal body in comparison to our candidate proteins in CD cell lines with normal length cilia and in a cell line with stunted cilia. We confirmed potential changes in localization and expression of these basal body proteins by immunofluorescence in both cell types.

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Underline represents presenting author.

SA-PO2994
Generation and Analyses of AQP11 BAC Transgenic Mice Yuichi Inoue,1 Eisie Sohara,2 Katsuki Kobayashi,3 Tatetsui Rai,1 Kenichi Ishibashi,4 Sei Sasaki,1 Shinichi Uchida.1 1Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan; 2Division of Molecular Genetics, Clinical Research Center, Chiba-East National Hospital, Chiba, Japan; 3Medical Physiology, Meiji Pharmaceutical University, Kyiose, Tokyo, Japan.

Background: Aquaporin 11 (AQP11) belongs to superaquaporins since it has Asn-Pro-Pro-Asn motif instead of the Asn-Pro-Pro-Asn motif typically found in aquaporins. In transgenic mice and humans, the expression of AQP11 gene in mice resulted in polycystic kidneys following vacuolization of the proximal tubular cells. However, mechanism of the cyst formation and the vacuolization has not yet been clarified partly because of lack of an antibody good for detecting endogenous AQP11 in mouse tissues.

Methods: To enable the analyses of AQP11 in mice at the protein level, we decided to generate AQP11 BAC transgenic mice that expresses AQP11 tagged with 3×HA sequences at its N-terminus. By injecting the transgene containing whole exons of mouse AQP11 with in situ injection region into fertilized eggs of C57BL/6 mice, we could obtain seven lines of transgenic mice with different copy numbers.

Results: In two lines carrying three and ten copies of the transgene respectively, we first performed immunofluorescence in the kidney, and found that 3×HA-AQP11 was mainly localized in the cytoplasm of proximal tubules. Double immunofluorescence with an antibody specific for nuclear tubulin revealed that AQP11 was partially colocalized with KDEL, an ER marker, suggesting that AQP11 is an aquaporin of intracellular organelles. In addition to kidney, we investigated the expression of AQP11 in other organs. Immunoblot of various mouse organs with anti-HA antibody revealed a single 27kDa band in brain, liver, and lung, which is consistent with the previous Northern blot of AQP11. Moreover, we detected abundant 3×HA-AQP11 in thymus, spleen, stomach, small intestine and colon, which has not been identified before.

Conclusions: Thus, we confirmed in vivo intracellular localization and tissue distribution of AQP11. AQP11 transgenic mouse will be useful tools to clarify the physiological role of AQP11 as well as the pathogenesis of polycystic kidney in the AQP11 knockout mice.

Funding: Government Support - Non-U.S.

SA-PO2995
CFTR Is Highly Expressed in the Cyst-Lining Epithelial Cells of the AQP11 Knockout Mouse Kidney Katsuki Kobayashi,1 Shinichi Uchida,2 Sei Sasaki.1 1Division of Molecular Genetics, Clinical Research Center, Chiba-East National Hospital, Chiba City, Chiba, Japan; 2Department of Nephrology, Tokyo Medical and Dental University, Bunkyo-ku, Tokyo, Japan.

Background: AQP11 knockout mouse suffers from polycystic kidney disease and dies of renal failure at the weaning stage of the neonatal development. A large number of studies have established that the aberrant cell proliferation of the cyst epithelial cells and the abnormal secretion of fluid into the cyst cavity are the essential mechanisms of the cyst formation and growth in the polycystic kidney disease. We have previously reported that cell proliferation induced by ER stress is involved in the cystogenesis of the AQP11 knockout mouse. In this study we examined the cystic fibrosis transmembrane conductance regulator (CFTR) C1 channel, supposed to be the channel playing the central role in the fluid secretion, in the AQP11-null mouse kidney.

Methods: To elucidate the mechanism that stained the wild-type mouse and AQP11 knockout mouse kidneys with the anti-CFTR antibody, produced in rabbit, affinity-isolated (HPA021939, Sigma-Aldrich, St. Louis, MO). For the functional test we subcutaneously administered the CFTR inhibitor (CFTR inh)-172, Sigma-Aldrich, St. Louis, MO) to the AQP11-deficient mice for one week starting at the postnatal day 14 (5 mg/kg of body weight/day).

Results: Immunohistochemistry using the specific antibody against the CFTR protein displayed that CFTR was expressed in the proximal tubular cells deeply located in the cortex of the wild type mouse and in the mutant mouse CFTR was markedly expressed in the cyst-lining epithelial cells. This result prompted us to administer the CFTR inhibitor to AQP11 knockout mouse in order to investigate its effect on the cyst growth. Subcutaneous administration of the CFTR inhibitor, however, produced no significant effect on the cyst growth in the AQP11 knockout mouse.

Conclusions: These results collectively suggest that CFTR could function as the main channel via which chloride ion is secreted into the cyst cavity in the AQP11 knockout mouse, but further experiments are needed to determine its significance in the cystogenesis.

Funding: Government Support - Non-U.S.

SA-PO2996
P-Glycoprotein Expression and Function in Cystinotic Proximal Tubular Cells Elena N. Levtchenko,1 Karen Peeters,2 Martijn J. Wilmer,3 Rosalinde Masereeuw.4 1Dept of Pediatric Nephrology/Laboratory for Pediatrics, EULeuvven, Leuven, Belgium; 2Dept of Pharmacology and Toxicology, Radboud University Nijmegen Medical Center, Nijmegen, Netherlands.

Background: P-glycoprotein (P-gp) is an ATP-dependent organic cation transporter localized on the apical membrane of the proximal tubules, that plays a role in the efflux of endogenous waste products and xenobiotics into urine. Studies in mice deficient for P-gp showed proximal tubular dysfunction and ATP deficiency combined with swollen mitochondria, resembling the phenotype of patients with cystinosis. We now have investigated whether the proximal tubular efflux transporter P-gp is affected in cystinosis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Methods: We used conditionally immortalized (ci) proximal tubular cells (ciPTC) obtained from patients with chronic kidney disease (CKD) and ciPTC were cultured to determine P-gp expression by Western blot and P-gp gene expression was assayed by qPCR. P-gp- mediated transport was measured by using the fluorescent P-gp substrate calcine in the presence and absence of the P-gp-inhibitor PSC833. Additionally, the effect of the cystine dependent uptake of the P-gp substrate OAG was determined by the calcine assay.

Results: The activity of P-gp, in presence or absence of PSC833, in control cell lines is comparable to the activity in cell lines of cystinotic patients. Cysteine depletes cystine accumulation in cystinotic cell lines and having a stimulatory effect on P-gp activity. PO, upshift of pH, L-cysteine and cystine and decreased cystine transport rates in ciPTC. The uptake was sodium-dependent and in the majority of the cells and conditions tested, both PSC833 and cysteine (pre)incubation resulted in significant decreased uptake of phosphate.

Conclusions: Inhibition of P-gp activity in ciPTC resulted in decreased uptake of phosphate comparable to the phenotype of P-gp deficient mice and to that of patients with cystinosis. Although cysteine depleted cystine accumulation in cystinotic cells and increased P-gp activity, it inhibited phosphate uptake in ciPTC. This observation is compatible with the presence of renal Fanconi syndrome in vivo under cysteine therapy and requires further study.

Funding: Private Foundation Support

SA-PO2997
Pathology and Clinical Manifestations in Patients with Uremic Tumor Calcification Undergoing Hemodialysis Rikako Hiratsuka, Keichi Sumida, Masayuki Yamanouchi, Yoshifumi Ubara. Nephrology Center, Tokyo Metropolitan Hospital, Tokyo, Minato-ku, Japan.

Background: Uremic tumor calcification (UTC) is a rare complication in patients undergoing hemodialysis (HD) and its clinical manifestations are poorly understood. This study aimed to elucidate clinical manifestations and pathological features in cases with UTC.

Methods: The subjects were 8 HD patients (1 male and 7 females; age, 41-75 years) with UTC, who visited our hospital between 1999 and 2011. HD duration was 6 years or shorter in 87.5%.

Results: Calcification was observed in about 75% of large joints, such as hips, wrist and shoulders. Mean serum adjusted Ca and P were 10.7±0.6 mg/dl and 7.0±1.1 mg/dl, and the mean Ca x P was 72±11.7 mg²/dl. Mean serum CRP was 1.46±2.1 mg/dl. Intact-PTh (iPTH) was 247±325 pg/ml, and iPTH (iPTH<100 pg/ml) was seen in 37.5%. Suggested secondary hyperparathyroidism, while low iPTH (iPTH<100 pg/ml) was seen in 37.5%. All cases with high iPTH showed positive CRP. The aortic calcification index (ACI) was 16.7% on average and 0% in 3 cases with high iPTH. In cases with high iPTH, parathyroidectomy with Ca x P control eliminated UTC and CRP became negative. Biopsy was performed in all cases of UTC and only calcium deposits were observed in 75%, while the remaining two cases showed ectopic bone tissue with positive CRP and fibrous bone. Of the 5 cases undergoing right iliac bone biopsy, there were 2 high iPTH cases with osteitis and 3 low iPTH cases with adynamic bone.

Conclusions: UTC was observed around large joints in cases with short HD duration and high Ca x P with hyperphosphatemia. Parathyroid hyperfunction may contribute to large UTC, while hypoparathyroidism may lead to small UTC. Serum CRP was positive in cases with secondary hyperparathyroidism, which suggested that tumor calcification induced an inflammatory reaction that in turn led to ectopic bone formation. Vascular calcification was mild and did not always present in UTC. It is conceivable that different onset mechanisms and factors other than Ca x P and iPTH are involved in UTC.

Funding: Private Foundation Support

SA-PO2998
Recovery of Renal Function in Incident Hemodialysis Patients Initiating Dialysis Therapy in the Hospital Sanjay Chaudhary, LaTonya J. Hickson, Andrew D. Rule, John J. Dillon, Robert C. Albright, Amy W. Williams.

Background: Patients initiating renal replacement therapy (RRT) may have acute or chronic renal failure. The rate at which renal function recovers in patients dismissed to a chronic hemodialysis unit following RRT initiation in the hospital is not well known.

Methods: The rate at which renal function recovers in patients dismissed to a chronic hemodialysis unit following RRT initiation in the hospital is not well known.

Results: Of 173 incident patients; mean age 64 years, 64% males, 87% Caucasian, 51% diabetic, 47% coronary artery disease (CAD), 47% congestive heart failure (CHF). Baseline estimated GFR (eGFR) was 14±13 ml/min with eGFR<45 in 36%. Over a mean period of 1.1±1.1 years, 46 recovered renal function. Kaplan-Meier estimate of cumulative renal recovery rate at 6 months was 27%. Baseline eGFR (HR=1.01 per ml/min, C1.01-1.02, p=0.001) was a strong positive predictor of renal recovery. eGFR>45 was also associated with renal recovery. HR=0.63, C1.3±1.42, p=0.001 (see figure). Also in univariate analysis, diabetes (HR=0.56, C1.03-0.98, p=0.049), CAD (HR=0.54, C1.29-0.98, p=0.044) and CHF (HR=0.40, C1.21-0.75, p=0.003) were negative predictors of renal recovery. In multivariate analysis, only eGFR was associated with renal recovery (HR=1.01, C1.00-1.02, p=0.001), while diabetes, CAD, and CHF were not.

Conclusions: Recovery of renal function in the chronic hemodialysis unit setting following dialysis initiation in the hospital is not infrequent. Patients with normal to even moderately impaired function at baseline are more likely to recover.

SA-PO2999
Long Term Use of Trisodium Citrate for Regional Anticoagulation in Chronic Intermittent Hemodialysis in Patients at Risk of Bleeding Birgit Doris Bader, Anna Weber, Christiane M. Erley. Department of Medicine II, St. Joseph Hospital Berlin-Tempelhof, Berlin, Germany.

Background: Hemodialysis patients suffer often from bleeding complications. Their management is difficult. Frequently the bleeding is associated with heparin anticoagulation during dialysis treatment. An alternative to heparin is the regional citrate anticoagulation (RCA), described first in 1961 and in wider clinical use since 1983. Normally RCA is used in short term and in continuous renal replacement therapy. This retrospective study documents the chronic use of RCA (trisodium citrate) in intermittent hemodialysis.

Methods: 6 patients (5 women, 1 man; aged 46-80 years) with bleeding complications were followed up from 12/2005 over 10-28 months. They received >1000 intermittent hemodialysis treatments with RCA. Initially the patients got a trisodium citrate 4% solution by an infusion rate of 250-280 ml/min with a blood flow of 200-350 ml/min, since 09/2010 a trisodium citrate 30% solution was used (infusion rates of 30-70 ml/min with a blood flow of 200-250 ml/min). Citrate flow was adapted to achieve post-filter ionized calcium of 0.5-0.7 mmol/L. Calcium substitution was adapted to maintain the patients' serum calcium levels within the physiological range. Low calcium dialysis fluid (1.00 – 1.25 mmol/L) was used.

Results: Within the extracorporeal blood circuit a successful regional anticoagulation was achieved while the coagulation of all 6 patients remained within physiological ranges. Only one patient showed clotting problems with RCA, solved by increasing the citrate dose and changing the filter from high to low flux. Adverse events didn’t occur, safety laboratory measurements stayed within normal ranges. In all 6 patients the bleeding complications decreased or stopped during dialysis with RCA. The rate of transfusion was reduced markedly.

Conclusions: The long term use of trisodium citrate (4% or 30%) for regional anticoagulation in chronic intermittent hemodialysis in patients with risk of bleeding is feasible, safe and effective.

SA-PO3000
A Quality Audit Analysis of Hemodialysis Circuit Clotting in an Inpatient Dialysis Unit Merlake Sennett,1 Mary Ann Ryan,2 Robert C. Albright,1 John J. Dillon,1 Sandra J. Talier,1 Marie C. Hogan,1 Nephrology, Division, Dept of Internal Medicine; Dept of Nursing, Mayo Clinic, Rochester, MN.

Background: Quality of delivery of inpatient hemodialysis (HD) in the acutely ill patient has been subject to little attention & no clinical performance measurement guidelines exist. HD circuit clotting has adverse effects on the process and quality of dialysis. Past studies reveal clotting can occur in up to a quarter of heparin-free dialysis treatments. The purpose of this audit was to evaluate HD circuit clotting rates in acutely hospitalized patients, & to determine if heparin use rates could be safely increased.

Methods: We audited heparin use & dialyzer clotting rate during HD before & following a practice intervention in an inpatient dialysis unit. The intervention was to notify nephrologist to prescribe heparin on all dialysis patients, if not contraindicated. The dialysis unit was located in a 1500 bed acute surgical & medical hospital which runs >6000 HD treatments yearly. All non-ICU inpatient HD runs were included. Clotting and heparin use rates were recorded for 2 weeks pre-intervention & 4 weeks post intervention & medical records reviewed.

Results: Of 178 pre-intervention HD treatments, 111 (62%) were in a non ICU setting. Heparin was used in 17 (15%) these HD treatments. Clotting of the HD circuit occurred in 12 (10.8%) treatments. Of those whose HD circuit clotted, 58% were on chronic HD, 83% had a tunneled dialysis catheter & 42% had a documented reason for not using heparin. Of the 549 post intervention HD treatments, 317 (58%) were in a non ICU setting. Heparin use increased to 23.2% (p=0.08) & HD circuit clotting decreased to 2.2% (p=0.0005) of treatments.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
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Conclusions: At our center, heparin use in acutely ill surgical & medical inpatients in the sample group was low & dialyzer clotting rates were higher than anticipated. We demonstrated that heparin prescribing can be modified when the prescribing nephrologist is made aware of the extent of the problem of HD clotting rates in their patients. Following this quality intervention, we increased heparin prescribing rates without negative effects and further audits are ongoing.

SA-PO3001
Heparin-Free Hemodialysis: A Multi-Center, Prospective, Randomized, Crossover Study between Two Hemodialyzers VIE Versus EVODIAL

Background: Several strategies of Heparin-free hemodialysis (HfH) were performed in patients with high risk bleeding problems. In this study, we tested the non-inferiority of the hemodialyzer VIE (vitamin E coated) having anti-oxidative and anti-thrombotic activities compared with EVODIAL (heparin coated) having anti clotting properties in a HfH strategy.

Methods: (NCT01221337): 32 patients aged 68±18, with well functioning fistulas or long-term catheters and no major hemodynamic or inflammatory disorders, were assigned to be dialedyzed in random order with VIE or EVODIAL. The two study periods consisted of 4 hemodialysis (HD) sessions of 4-hours, separated by a washout period of two HD sessions with the patient’s usual hemodialyzer. During each study period, the usual heparin dose was reduced to 50% for the 1st HD session, to 25% for the 2nd, followed by 2 successive HfH sessions. The primary endpoint was the success rate with no clotting event leading to a premature end of the HD session. Secondary endpoints included total HD duration without clotting, the number of saline flashes, the bubble trap and the hemodialyzer restituation qualities (1-4 grades), eKt/V (Daugirdas) and hemoglobin levels.

Results: The success rate with VIE (81%) was not inferior to EVODIAL (78%, p = NS). Restitution quality was better with VIE compared to EVODIAL (p = 0.002).

Conclusions: Both VIE and EVODIAL permitted to perform 4-hour HD sessions with reduced dose or no heparin. As the mechanisms of clot prevention are different these two hemodialyzers, further large-scale studies are required.

Funding: Pharmaceutical Company Support

SA-PO3002
Effects of Hydration Status and Relative Blood Volume Changes on Peripheral Skin Blood Flow During Hemodialysis
Sylvie Sulikova, Roman Safranek, Michaela Kubisova, Lydia Habanova, Petr Moucka, Erik Mistrik, Katerina Petranova, Lubos Sobotka. Department of Nephrology, Gerontology and Metabolic Care, Medical Faculty and Teaching Hospital, Hradec Kralove, Czech Republic.

Background: In chronic hemodialysis patients, disorders of perfusion of peripheral tissues are very common, clinically important and may for example aggravate healing of frequent skin defects. The aim of our study was to investigate effect of hemodialysis procedure (HD) on peripheral skin blood flow.

Methods: Forty-one clinically and hemodynamically stable hemodialysis patients (22 males, 19 females, 64 (29-84) years) underwent routine HD (4h isometric HD, low-flux dialyzer FX10 1.8m², QB 300ml/min, ultrafiltration to “dry weight” set by clinician). Skin blood flow was measured noninvasively at 1.5 mm depth on the principle of backscattered light from moving red blood cells using Laser Doppler Line Scanner. Skin blood flow was estimated on hands and feet in every patient before HD, at 30, 90, 240min of HD, and 30 min postHD. Using Cell-line we assessed relative blood volume changes during HD. Bioimpedance spectroscopy (Body Composition Monitor) was used to assess hydration status.

Results: Skin perfusion progressively declined during HD (p<0.001), being decreased by 39% in toes, and 35% in fingers at the end of HD. 30 minutes after the end of HD, skin perfusion was still decreased; by 35 and 30% in toes and fingers respectively. Skin perfusion decreased in patients with observed either decrease or increase in blood pressure during HD. Lower hydration at the end of HD was associated with more pronounced decrease in peripheral perfusion (correlation coefficient 0.51-0.65 for different areas of perfusion measurement).

Conclusions: HD significantly reduces peripheral skin blood flow. Drop in peripheral perfusion is dependent on relative blood volume decrease during HD and hydration status. Change in blood pressure during HD cannot be used as a simple tool to assess peripheral perfusion.

Funding: Government Support - Non-U.S.

SA-PO3003
Serum Sodium Concentration and Interdialytic Weight Gain in a Cohort of Hemodialysis Patients

Background: Patients on hemodialysis (HD) have high rates of cardiovascular mortality and morbidity. Important risk factors include hypertension and fluid retention, measured as interdialytic weight gain (IDWG). This study aimed to describe the distribution of pre-dialysis serum sodium(SS) concentrations and to investigate the relationships between SS and IDWG.

Methods: This retrospective analysis determined the relationship with monthly SS levels and 48 hour subsequent IDWG. We reviewed the records of 260 patients on HD over a period of 12 months. IDWG, SS concentration, age and gender were tabulated. The mixed procedure method in SAS was utilized with a mixed effect model to capture the individual differences, and the fixed effect model to see the relationship between SS and IDWG.

Results: 260 patients, 106 male and 154 female, with total of 5054 dialysis sessions in 12 months period were analyzed. Average SS for the group was 137.2 ± 2.1 and average IDWG was 2.42 ± 1.42 KG. Data showed that IDWG and SS level are inversely related to each other in the entire male cohort. Subgroup analysis shows a significant relationship in all patients >80 years. SS expressed as mean mEq/L ± SD and IDWG as mean Kg± SD.

Conclusions: For a given patient, a decreasing sodium level is predictive of a greater subsequent IDWG. This is most prominent in male patients and in all patients above the age of 80. This finding, if confirmed, will have implications for guiding patient treatment and dietary counseling. Further evaluation as to mechanism is needed.

SA-PO3004
Dialysate Sodium and Mortality in Hemodialysis
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Background: Dialysate sodium concentration has been associated with increased thirst, weight gain and higher blood pressure. The relationship between dialysate sodium and outcome, and influence on serum sodium, has not yet been thoroughly investigated.

Methods: We studied a cohort of 2272 subjects from a medium-sized dialysis provider. Available data included demographic, laboratory and clinical parameters, detailed information of the dialysis prescription and 2.5 year follow-up for all subjects. Patterns of dialysate sodium prescription were examined within and between centers. Cox regression models, stratified on clinical center, were used to compare all-cause mortality according to dialysate sodium concentrations. Interaction was examined for in relation to serum sodium, interdialytic weight gain and pre-dialysis systolic blood pressure.

Results: The mean pre-dialysis serum sodium was 136.1 mEq/L, without significant difference across dialysate sodium concentrations. There was evidence for interaction between serum and dialysate sodium and their relationship with all-cause mortality (p<0.04). The hazard ratio for death for higher dialysate sodium (>140mEq/L or sodium modeling) versus lower dialysate sodium (≤140mEq/L) was 1.05 (0.85, 1.30) at serum sodium of 134mEq/L; 1.15 (0.94, 1.40) at serum sodium of 136mEq/L; and 1.26 (1.01, 1.58) at serum sodium of 138mEq/L.

Conclusions: The dialysate sodium varies within and between centers; does not appear to affect the pre-dialysis serum sodium concentration; but has a variable influence across dialysate sodium concentrations. There was evidence for interaction between serum and dialysate sodium and their relationship with all-cause mortality (p<0.04). The hazard ratio for death for higher dialysate sodium (>140mEq/L or sodium modeling) versus lower dialysate sodium (≤140mEq/L) was 1.05 (0.85, 1.30) at serum sodium of 134mEq/L; 1.15 (0.94, 1.40) at serum sodium of 136mEq/L; and 1.26 (1.01, 1.58) at serum sodium of 138mEq/L.

Funding: Private Foundation Support
SA-P03065

The Effect of Isotonic Sodium Dialysate Prescription in Conventional In-Center Hemodialysis Patients: A Case Series

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Background: Recent studies have focused on the association between sodium (Na⁺) dialysate prescriptions and interdialytic weight gain (IDWG). We report on a case series of 13 HD patients undergoing IDWG weekly, conventional, in-center hemodialysis with an individualized, isotonic sodium dialysate prescription.

Methods: Isotoniaemia was achieved in all patients through a stepwise weekly reduction of the standard Na⁺ dialysate prescription (140 mEq/L) by 2 mEq/L until reaching a Na⁺ gradient of >2 mEq/L. HD patients were assigned a Na⁺ prescription for the following week (averaged over the preceding 3 months). Dialysis logs from six consecutive treatments with the two different Na⁺ prescriptions (standard and isotonic) were reviewed. Changes were assessed for the following measures: interdialytic weight gain corrected for dry weight (IDWG0), blood pressure, and proportion of treatments of hypotension, hypotension requiring intervention.

Results: The average age of the patients was 62 years; 10 of 13 were male. The pre-dialysis plasma Na⁺ concentration ranged from 132 to 141 mEq/L. The mean pre-dialysis plasma Na⁺ was unchanged (135.5 ± 3.7 mEq/L vs. 134.9 ± 3.9 mEq/L p=0.43) but the mean post-HD plasma Na⁺ concentration was significantly reduced (137 ± 3.1 mEq/L vs. 134.3 ± 3.4 mEq/L; p=0.03). IDWG0 was decreased with isotonic dialysate (3.4 ± 1.6 vs. 2.5 ± 1.0%; p=0.003) without affecting pre- or post-HD blood pressure (all p>0.05). No significant changes in the proportion of treatments with cramps (6% vs. 13%), hypotension (62% vs. 65%) or hypotension requiring an intervention (29% vs. 33%) were noted.

Conclusions: Individualized isotonic sodium dialysate prescriptions reduced IDWG without increasing the incidence of cramps or hypotension.

SA-P03066

Interaction of Potassium, Sodium with Higher Magnesium Dialysate on Muscle Cramps in Chronic Hemodialysis Patients

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Background: We reported an inverse relationship between pre-dialysis serum magnesium (Mg) and muscle cramps in chronic hemodialysis (HD) patients (JASN 21:436A, 2010). However, the relationship of potassium and sodium with different dialysate Mg concentrations and muscle cramps in ESrD patients is scarce.

Methods: 62 ESrD patients (Mean age 60, range 25-87 years; 36 males, 26 females) on HD were studied. The patients were hemodialyzed initially with a dialysate Mg of 0.75 mEq/L and then with a dialysate Mg of 1.00 mEq/L. The patients received HD with each dialysate for at least 3 months. Monthly pre-HD laboratory data, before and after the change in dialysate Mg, were used for analysis. A single nephrology fellow conducted an in-person questionnaire on 62 patients twice. The severity of cramps was evaluated on a 5-point scale.

Results: 48 out of the total 62 ESrD patients had muscle cramps with dialysate Mg of 0.75 mEq/L; vs only 35 had muscle cramps with dialysate Mg of 1.00 mEq/L (p=0.01). The frequency of muscle cramps significantly decreased with higher dialysate Mg, (p=0.01). The frequency of muscle cramps significantly decreased with higher dialysate Mg (1.00 mEq/L in patients with serum potassium of ≥4 mEq/L or ≥4 mEq/L and serum sodium of <135 mEq/L or ≥135 mEq/L and sodium ≥135 mEq/L, vs only 35 had muscle cramps with dialysate Mg of 1.00 mEq/L. The patients received HD with each dialysate for at least 3 months. Monthly pre-HD laboratory data, before and after the change in dialysate Mg, were used for analysis. A single nephrology fellow conducted an in-person questionnaire on 62 patients twice. The severity of cramps was evaluated on a 5-point scale.

Conclusions: Individualized isotonic sodium dialysate prescriptions reduced IDWG without increasing the incidence of cramps or hypotension.

SA-P03067

Fluid Distribution in the Chest and Calf in Hemodialysis Patients

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Background: Knowledge of body fluid status is essential in the management of hemodialysis (HD), but excess fluid volume may not be uniformly distributed in the body. This study compares fluid removal from the chest and calf during HD.

Methods: Thirty-two HD Patients underwent HD after the long (LINT) and short (SINT) interdialytic interval using 2 bioimpedance techniques, the ZOE fluid status monitor (NMT) for chest impedance (Zo), and Hydra 4200 for calf bioimpedance spectroscopy (cBIS) (Xitron Technologies). Calf and chest resistivities were determined and normalized to body mass index (pNa chest, pNa calf).

Results: We studied 15 patients. During HD weight decreased in both LINT and SINT groups, the decrease was greater in LINT than SINT (LINT -21.3 ± 1.3; SINT -2 ±1.1; P<0.001). In LINT, pNa calf, chest Zo, and pNa calf increased during HD.

SA-P0307

Intradialytic Hypotension (IH): Variation in Dialysis Facilities

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Background: Lowering dialysate temperature may improve outcomes for patients undergoing maintenance chronic hemodialysis.

Objectives: To evaluate the effect of cooling dialysate compared to normal temperature dialysate on important outcomes in chronic hemodialysis patients

Methods: We searched the Cochrane Central Register of Controlled Trials, OVID MEDLINE, EMBASE and Pubmed (from inception to January 2011). We included all prospective randomized controlled trials that evaluated the effect of any method of cooling dialysate in adult patients receiving chronic hemodialysis. Two authors independently assessed study quality and extracted data in duplicate. The GRADE methodology was followed to assess bias in individual studies and across studies for each outcome. We pooled data using a random effects model. We measured heterogeneity using the y² and I² statistics. We registered this systematic review at PROSPERO: International prospective register of systematic reviews. 2011 (CRD420111104)

Results: We included twenty-six cross-over studies. Cool dialysis significantly reduced the rate of intradialytic hypotension (IDH) by 70% (95% CI 49%-89%; I² = 0%). Also it significantly increased mean arterial pressure (MAP) 14 mm Hg (95% CI 10–18 mm Hg; I²=89%).

Conclusions: The overall quality of studies was poor. The effect on symptoms of discomfort were poorly studied. None of the studies reported long-term patient important outcomes

SA-P03088

Intradialytic Hypotension (IH): Variation in Dialysis Facilities

Jeffrey J. Sands, 1,2 Len A. Usvyat, 1,2 Sandi Moore, 1,2 Mary T. Sullivan, 1,2 Jonathan H. Segal, 1,2 Paul M. Zabetakis, 1,2 Sudhir Movva, 1,2 Fadi Bdair, 2 Elie Akik, 1,2 Gihad E. Nersallah, 1,2 Amit X. Garg, 1,2 Holger Schunemann. 1,2 Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, Canada; 2Department of Medicine, State University of New York Downstate, Brooklyn, NY; 2Department of Medicine, McMaster University, Hamilton, ON, Canada; 3Department of Medicine, University of Western Ontario, London, ON, Canada; 2Department of Nephrology, Humber River Regional Hospital, Toronto, ON, Canada.

Background: Intradialytic hypotension (IH) occurs in 10-30% of HD treatments (Tx) but is not routinely reported or aggregated to facilitate evaluation and prevention.

Methods: We studied 26 cross-over studies. Cool dialysis significantly reduced the rate of intradialytic hypotension (IDH) by 70% (95% CI 49%-89%; I² =0%). Also it significantly increased mean arterial pressure (MAP) 14 mm Hg (95% CI 10-18 mm Hg; I²=89%).

Conclusions: Limitations: The overall quality of studies was poor. The effect on symptoms of discomfort were poorly studied. None of the studies reported long-term patient important outcomes

SA-P03059

Intradialytic Hypotension (IH): Variation in Dialysis Facilities

Jeffrey J. Sands, 1,2 Len A. Usvyat, 1,2 Sandi Moore, 1,2 Mary T. Sullivan, 1,2 Jonathan H. Segal, 1,2 Paul M. Zabetakis, 1,2 Sudhir Movva, 1,2 Fadi Bdair, 2 Elie Akik, 1,2 Gihad E. Nersallah, 1,2 Amit X. Garg, 1,2 Holger Schunemann. 1,2 Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, ON, Canada; 2Department of Medicine, State University of New York Downstate, Brooklyn, NY; 2Department of Medicine, McMaster University, Hamilton, ON, Canada; 3Department of Medicine, University of Western Ontario, London, ON, Canada; 2Department of Nephrology, Humber River Regional Hospital, Toronto, ON, Canada.

Background: Intradialytic hypotension (IH) occurs in 10-30% of HD treatments (Tx) but is not routinely reported or aggregated to facilitate evaluation and prevention.

Methods: Intradialytic hypotension (IH) occurs in 10-30% of HD treatments (Tx) but is not routinely reported or aggregated to facilitate evaluation and prevention.

Results: Intradialytic hypotension (IH) occurs in 10-30% of HD treatments (Tx) but is not routinely reported or aggregated to facilitate evaluation and prevention.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

810A
Reference: Muck, female; non-diabetic, AM shift; MWF, AVF/VG; Tx in clinic 4

Conclusions: IH is common and highly variable by patient and facility. Increased risk of IH was associated with older age, diabetes, higher UVF, albumin and post HD weight-EDW difference, lower pre-HD SBF and being dialedyzed in clinic 4. Additional evaluation of facility practice patterns and modifiable risk factors is needed to decrease the frequency of IH.

SA-PO3010

Dialysate Calcium Concentration and Intradialytic Hemodynamic Stability

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Background: The interplay of correct sodium (Na+), potassium (K+) and total calcium (Ca++) concentrations in dialysate fluids (Na+:K+:Ca++) has a major impact on the physiological and hemodynamic changes in patients during HD, so that superior preventative strategies for IH can be uncovered.

Methods: Twenty-two stable anuric uremic patients underwent three 4h-bicarbonate HD sessions, each with one of the three dialysate Ca++ concentrations. Vos, and dialysate Na+, K+, and bicarbonate concentrations were prescribed to be the same (respectively, 140, 2.0 and 35 mmol/l). Hourly measurements of plasma water (pw) ionized Ca (Ca++), Na+, and K+, blood pH and plasma bicarbonate concentrations were measured from the dialysate side (GENUS bath dialysis system, FMC, Germany). Intradialysis systolic, diastolic and mean arterial pressure (SBP, DBP and MAP, respectively) and heart rate (HR) trends were analyzed.

Results: A statistically significant difference was observed among the mean hourly pwCa++ concentrations, being significantly higher with a dialysate Ca++ concentration of 1.50 (P < 0.0001). Mean tCa++Ms were positive (diffusion gradient from the dialysate to the patient), being more and more higher by increasing dialysate Ca++ concentrations (+ 75 ± 122 mg, + 182 ± 125 mg, + 293 ± 228 mg, respectively) (P < 0.0001). No statistically significant difference was observed when comparing pw concentrations of Na+ and K+, blood pH and bicarbonate levels during the three different sessions (repeated measures ANOVA stratified for treatments). Mean Na+Ms/K+Ms and K+Ms/BMs were measured from the dialysate side (GENUS bath dialysis system, FMC, Germany). Intradialysis systolic, diastolic and mean arterial pressure (SBP, DBP and MAP, respectively) and heart rate (HR) trends were analyzed.

Conclusions: These highly controlled experiments show that hemodynamic stability does not appear to be statistically significantly influenced by any specific dialysate Ca++ concentration in this peculiar subset of patients.

Funding: Clinical Revenue Support

SA-PO3011

The Effect of Pneumatic Compression Devices on Hemodynamic Parameters in Hemodialysis Patients: A Randomized Crossover Trial

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Background: Intradialytic hypertension (IDH) is the most common complication of chronic hemodialysis (HD) therapy, leading to increased morbidity and mortality. The pathophysiology of IDH is multifactorial and poorly understood. Studies have shown that central blood volume (CBV) is maintained in stable HD patients. Pneumatic compression devices (PCDs) are thought to improve venous return by preventing pooling of blood in the lower extremities. Thus, PCDs could potentially improve CBV and intradialytic blood pressure (BP) in HD patients.

Methods: We performed a randomized, two-period crossover trial to determine the effect of PCDs, compared with control, on central blood volume (CBV) in HD patients. Patients on intermittent HD ≥ 3 times per week for ≥ 3 months were eligible for study inclusion. The study period consisted of 2 consecutive mid-week HD sessions. Patients were randomized to begin with either 1 mid-week HD session with PCDs (the intervention), or 1 mid-week HD session without PCDs (the control), stratified by whether or not they were IDH-prone.

Results: 51 patients (75%, male 49%, diabetic) with a mean age of 65 ± 14 years and mean HD vintage of 44 ± 37 months were randomized. Forty-six patients completed the study. During HD, the mean change in CBV in the control and intervention sessions was +4.3% vs +0.8% (P=0.78). Similarly, comparing central and control intervention sessions, there were no differences in any challenges in cardiac output (+0.5% vs -0.3%, P=0.83) and systemic vascular resistance (+1.5 mmHg/L/min vs +1.0 mmHg/L/min, P=0.67). Post HD systolic blood pressures (SBP) and minimum intradialytic SBPs were similar in the control and intervention sessions (-1.26 mmHg vs 128 mmHg, P=0.08), and (112 mmHg vs 108.5 mmHg, P=0.19), respectively.

Conclusions: Compared with standard of care, PCDs have no effect on hemodynamic parameters, including CBV, during HD. Further studies are required to better understand the physiological and hemodynamic changes in patients during HD, so that superior preventative strategies for IDH can be uncovered.

Funding: Pharmaceutical Company Support

SA-PO3013

Continuous Recording Reveals Extreme Blood-Pressure Variability in Nominally Stable Dialysis

Scott Wilson, Gavin J. Becker.
Royal Melbourne Hospital, Australia.

Background: The prediction and avoidance of haemodynamic instability during haemodialysis (HD) is important to minimize circulatory stress and maintain efficacy. Intradialytic hypotension or hypertension is defined as a symmetrical change in systolic blood pressure (SBP) of ≥ 20 mmHg and associated with increased morbidity and mortality. In clinical practice, intradialytic blood pressure records are used to adjust HD, ultrafiltration, and antihypertensive prescription. Clinicians assume this record captures the true haemodynamic profile and the descriptor “stable dialysis” is routinely drawn from such observation. We sought to investigate the actual SBP profile during HD using continuous recordings and compare these with standard clinical measures and changes in relative blood-volume (RBV).

Methods: Continuous measure of SBP by plethysmograph using the Finometer system and RBV was recorded in 6 “stable” midweek HD outpatients. In parallel, 5 arm-cuff SBP measurements were made by HD staff blind to the continuous record. Time-series data from beat-to-beat recording (~17,000 data points per HD) was used examining a heart-rate dependent median-hybrid filter.

Results: Concordance between the plethysmograph and simultaneous arm measures of SBP was excellent (mean difference <5mmHg). The time-series record revealed a slow increase in SBP through HD with a mean change from start (127mmHg) to end (167mmHg) of 40mmHg. Continuous recordings revealed significant asymptomatic hypertensive (SBP >160mmHg) in SBP that weren’t associated with variation in RBV. The mean difference between minimum (94mmHg) and maximum (199mmHg) SBP during HD was 105mmHg. SBP was beyond 2 standard deviations from baseline for an average of 18% of HD time, typically in the hypertensive range. Among the 6 dialyses only a single symptomatic event was observed.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Effect of Oxygen Therapy on Hemodynamic Stability during Hemodialysis with Continuous Blood Volume and O2 Saturation Monitoring

Nupur Jhawar, Kiran M. Goli, Paul F. Visintainer, Jason D. Cooney, Joy WhirlBeck, Michael J. Germain. Baystate Medical Center/Tufts University School of Medicine, Springfield, MA.

**Background:** Intradialytic hypotension (IDH) is the most common complication of hemodialysis (HD). The effects of oxygen administration on IDH during HD are currently unknown. To address this question, a randomized-controlled clinical trial (RCT) of the effects of oxygen(O2) administration on hemodynamic stability during HD is planned. Prior to initiating the clinical trial, we present results of a preliminary pilot study.

**Methods:** This pilot study was a non-randomized, prospective and two group study that included 24 patients receiving HD in an inpatient HD unit with either an AV fistula or a venous catheter. The study compared hemodynamic stability in 12 patients receiving 2 liters of O2 via nasal cannula during HD to 12 patients not receiving O2 during HD. BP was recorded every 30 minutes. RBV, Hct and O2 saturation were recorded continuously by Crit-line III monitor.

**Results:** Results showed that O2 administration during HD significantly stabilized systolic blood pressure (SBP) over time (average change in SBP = 1.10 mmHg; p = 0.037). Furthermore, O2 administration resulted in substantially lower variability in the individual readings in the treatment group compared to the placebo group (SDp = 8.9 vs. SDtx = 17.2; p < 0.001), suggesting that O2 may stabilize SBP over time during HD.

Finally, log-RBV slope showed a significantly steeper decline compared with placebo (average change in log-RBV every 30 minutes for treatment vs. placebo: -0.018 vs. -0.003, p = 0.005).

**Conclusions:** Greater hemodynamic stability and fewer episodes of IDH are achieved in the treatment group compared to the control group. These results need to be validated in a subsequent RCT.

**SA-PO3016**

Associations of the Malnutrition-Inflammation Score with Depressive Symptoms and Kidney-Disease Targeted Health-Related Quality of Life Measures in a Brazilian Sample of Hemodialysis Patients


**Background:** The malnutrition-inflammation-score (MIS) has been largely used to evaluate nutritional status in maintenance dialysis (MHD) patients. There is a lack of studies, however, to assess associations of the MIS with kidney-disease targeted health-related quality of life (KDT-HRQOL) measures and depression symptoms in MHD patients. This study assessed associations of MIS with scores of KDT-HRQOL and depressive symptoms in MHD patients. This study assessed associations of MIS with scores of KDT-HRQOL and depressive symptoms in MHD patients.

**Methods:** Cross-sectional study of 632 prevalent MHD patients enrolled in the Prospective Study of the Prognosis of Chronic Hemodialysis Patients (PROHEME) developed in Salvador, Brazil. MIS-6 defined worse nutrition status. The Kidney Disease Quality of Life Short-Form was used to determine scores (range: 0 to 100) for the eleven KDT-HRQOL scales. The Center for Epidemiological Studies Depression scale was used for depression symptoms scores (range: 0-60). Linear regression with adjustments for numerous covariates were used to determine differences in scores of HRQOL and depression symptoms by MIS categories.

**Results:** Patients with MIS≥6 (compared with MIS<6) were found to have significantly (P<0.05) lower adjusted scores for the following KDT-HRQOL: symptoms/problems (difference=-5.35), effects of kidney disease (difference=-4.28), burden of kidney disease (difference=-4.45), cognitive function (difference=-4.87), social interaction (difference=-4.37) and sleep (difference=-4.08). Depression symptoms scores were significantly (P<0.05) higher for patients with MIS ≥6. Gastrointestinal symptoms and functional capacity were the MIS components more strongly associated with poorer HRQOL and depression symptoms by MIS categories.

**Conclusions:** The data suggest that MHD patients with higher MIS have clinically significantly lower scores for several HRQOL components (both generic and kidney-disease targeted) and higher probability of depression.
SA-PO3017

Depression and Its Management in Maintenance Haemodialysis Patients
Marguerite McCloskey, Ronan Cunningham, Robert Mullan, Agnes Masengu, Camille Harron. Renal Unit, Antrim Area Hospital, Antrim, Northern Ireland, United Kingdom.

Background: The purpose of this audit was to determine the prevalence of depression within our haemodialysis population and review treatment strategies in accordance with National Institute of Clinical Excellence (NICE) guidance.

Methods: A total of 126 haemodialysis patients (71 male, 55 female, median age 66.9) were asked to complete a voluntary depression questionnaire, namely the patient health questionnaire 9 (PHQ-9), which has been validated for use by general practitioners.

We also identified those patients on antidepressant medication using our computerised database.

Results: 76.2% (96/126) completed the questionnaire, 15.9% (20/126) refused, 0.8% (1/126) were unable to complete secondary to language barrier, and 6.3% (8/126) were unable to complete secondary to cognitive impairment. 42.7% (41/96) were classified as mildly depressed, 35.7% (10/28) of whom were on an antidepressant. 83.3% (8/9) were classified as moderately depressed, 25.2% (2/8) of whom were on an antidepressant. 4.2% (4/96) were classified as moderately severely depressed, 73.3% (3/4) of whom were on a antidepressant, and one patient was classified as severely depressed and was already on an antidepressant.

32.5% (41/126) were already on an antidepressant at the time of assessment, 80.5% (33/41) of whom were on a selective serotonin reuptake inhibitor (SSRI), 12.2% (5/41) were on an SSRI or a different antidepressant, 4.2% (4/96) were classified as moderately severely depressed, 73.3% (3/4) of whom were on antidepressant, and one patient was classified as severely depressed and was already on an antidepressant.

Conclusions: Depression is common in the haemodialysis population and the majority of those with moderately severe and severe symptoms were appropriately on medication. The majority of those treated were on low dose SSRIs. Those who were mildly/moderately depressed did not appear to be identified and treated for depressive symptoms as readily. This audit also highlights the use of antidepressant medication in this population group, and the difficulties in management that may be related to side effects.

The results were reviewed and consideration made to the use of alternative approaches, such as counselling and art therapy.

SA-PO3018

Buddhist Intrahemodialytic-Insight Meditation Improves Depression in Hemodialysis Patients
Kriensak Vareesangthip, Renal Division, Department of Medicine, Faculty of Medicine Siriraj Hospital, Bangkok, Thailand.

Background: End stage renal disease (ESRD) patients can live longer with renal replacement therapy, including hemodialysis, continuous ambulatory peritoneal dialysis and kidney transplantation. Living on hemodialysis is a perpetual challenge, due to the demanding treatment schedule, dietary restrictions, and changes in function. The life expectancy of dialysis patients is 1.3 to 1.6 that of general population. Depression has long been identified as the primary mental health problem of patients with ESRD. It has been clearly shown that Buddhist meditation can improve the depression state of chronic illness patients. We, therefore, hypothesized that buddhist intradialytic-Insight Meditation for 10 weeks could calm down the severity of depression in hemodialysis patients.

Methods: Twenty stable hemodialysis patients who have been dialysed three times a week, and all have Kt/V ≥ 1.2. All patients were trained to practice Insight Meditation schedule in the term of Anapanasati during hemodialysis for 30 minutes in each hemodialysis session for 10 weeks. The severity of depression was assessed at pre and post period of insight meditation schedule by using Thai Depression Inventory. The Thai Depression Inventory was developed as a self-rating instrument for evaluating the severity of depression. The scale was tested with the Hamilton Rating Scale for Depression.

Results: The depression score in hemodialysed patients was significant improved after practicing the buddhist insight meditation (pre and post depression score, 15.0 ± 7.2 vs. 10.3 ± 7.8, p = 0.01).

Conclusions: The buddhist insight meditation can be used to improve the depression and would provide good quality of life in hemodialysis patients.

Funding: Private Foundation Support

SA-PO3019

Anxiety during Dialysis in Maintenance Dialysis (MD) Patients: A Highly Prevalent Co-Morbidity
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Background: Maintenance dialysis (MD) patients have a high prevalence of anxiety and depression. We have begun to study the frequency of anxiety episodes related to regular dialysis sessions and factors which may engender anxiety in MD patients.

Methods: 170 patients, 155 undergoing Maintenance hemodialysis (MHD)patients and 5 undergoing continuous ambulatory dialysis, were examined.Inclusion criteria included dialysis vintage of at least six months,Patients completed the Beck Anxiety Inventory and Beck Depression Inventory and questionnaires that examined their feelings of anxiety related to individual dialysis sessions.

Results: Of 170 patients, 29 had no dialysis in the last 16 years, dialysis vintage, 55±48 months, 46% were female. Anxiety and depression were found in 53% and 36% of patients, respectively.
showed insomnia was more common and decreased mobility less common in the youngest age group (p<0.01 for both, respectively). Global symptom score showed significant but weak negative correlation with area reduction ratio (coeff -0.13, p=0.016).

Conclusions: Data reveal symptoms are highly prevalent among dialysis patients. Global symptom score does not correlate with age or dialysis vintage, and specific symptoms are more prevalent in certain age and diagnostic groups. Aggressive symptom management will improve quality of life and could be targeted by age and diagnostic group. This work is part of a project led by NYS Kidney Care.

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SA-P0302

Daytime Intradialytic Sleep, Nocturnal Sleep, and Mortality Risk Among Hemodialysis Patients in the CDS

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Background: Increased sleepiness during HD may reflect treatment-induced alterations in arousal status and/or thermoregulatory processes (Sleep 23:887-891, 2000). We explored intradialytic sleep, nighttime sleep, and survival among HD patients in the Comprehensive Dialysis Study (CDS).

Methods: Incident dialysis patients aged ≥18 from 296 randomly selected clinics were surveyed. 1,439 HD patients reported how long they typically dozed off/slept during HD, usual nighttime sleep hours, and trouble with waking up at night (nocturnal sleep fragmentation). These variables, and age, gender, race, education, employment status, diabetes, cardiovascular comorbidity, BMI, early nephrology care, SF-12 PCS score, and reported RLS, were included in a Cox proportional hazards model predicting mortality from dialysis start date to September 30, 2009. Patients were censored at change to PD or transplant.

Results: Younger age and diabetes were associated with greater doze/sleep time. 684 patients (47.5%) slept 6 or fewer hours at night, and 755 patients (52.5%) slept >6 hours at night. More patients who slept 6 or fewer hours reported always/sometimes experiencing nocturnal sleep fragmentation (73% vs. 47%; p<0.0001), and they reported more intradialytic doze/sleep time than patients who slept >6 hours at night (0.97 [0.94] vs. 0.85 [0.90] hour; p<0.01). In the multivariable Cox model, mortality risk increased as HD doze/sleep time increased among patients with 6 or fewer nightime sleep hours (HR 1.23 [95% CI 1.06-1.44]; p=0.008), but among patients with >6 hours nighttime sleep, greater HD doze/sleep time was not associated with increased mortality risk (HR 1.02 [95% CI 0.86-1.20]; p=0.85).

Conclusions: Consistent with evidence that subjective sleepiness increases during HD treatment, intradialytic sleep was common. Patient and treatment factors that may be associated with different intradialytic and nighttime sleep quantity/quality patterns may have important clinical implications.

Funding: NIDDK Support

SA-P0303

John Henryism Active Coping, Perceived Health, and Depression Symptoms in Brazilian Hemodialysis Patients: The PROHENO Study

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Background: John Henryism refers to a strong behavioral predisposition to actively cope with difficult psychosocial stressors. It is measured by the 12 item John Henryism Active Coping Scale (JHAC). Sample JHAC questions are: 1) In the past, even when things got really tough, I never lost sight of my goals; 2) Once I make up my mind to do something, I stay with it until the job is completely done. Persons who score high on the JHAC believe they can overcome difficult problems through determination and hard work. This study assessed if JHAC scores were associated with perceived general health (HRQOL) and depressive symptoms in maintenance hemodialysis (MHD) patients.

Conclusions: Cross-sectional analyses were conducted on 585 patients from phase II of the PROHENO Study developed in Salvador, Brazil. John Henryism was assessed by the scores of the 12-item JHAC (range 12-60). The SF-36 was used to determine general health score (range 0-100); and the Center for Epidemiologic Studies Depression (CESD) scale to depression symptoms score (range 0-60). Linear regression was used to test associations of JHAC score with general health, and depression symptoms scores, adjusting for several sociodemographic, treatment and comorbidity factors.

Results: Mean age was 48.29±13.6 yr, 61.3% males, 19.4% diabetics and 88.5% non-whites. Median JHAC=52. Patients with JHAC score above the median had higher mean general health score (difference+6.36 points, P=0.002) and lower mean depressive symptoms score (difference -2.43, P=0.013). The differences in scores after adjustments for covariates were +6.75 (P=0.002) for general health and -2.55 (P=0.011) for depression symptoms. Similar patterns of associations were observed by subgroups of age (<60 and ≥60 yr), race, gender and diabetic status.

Conclusions: These results suggest that MHD patients with high JHAC scores are more likely to report better general health and lower depression symptoms than patients with low JHAC scores, independently of sociodemographic factors and comorbidities.

Funding: Government Support - Non-U.S.

SA-P0304

Predictive Characteristics of Successful Hemodialysis Patient Survival in the First 120 Days of Treatment

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Background: In 2007, mortality rate in the first 3 months of dialysis was more than double compared to the overall annual mortality rate of 19% of prevalent hemodialysis patients (pts) [USRDS]. We aim to understand some of the specific factors that impact success during this period of time.

Methods: We reviewed data of all HD pts who were admitted to the RRI clinics within 30 days from their first date of dialysis b-n 2001 and 2010. Survival was assessed in the end of the incident dialysis period at 120 days.

2 analyses were conducted:

1. Analysis 1 (pre-dialysis “PRE-D” factors) looked at success factors at the initiation of dialysis
2. Analysis 2 (incident dialysis “INC-D” factors) looked at success factors present at the end of the incident period

Cox proportional hazards models were constructed for PRE-D and INC-D adjusted for: gender, race, ethnicity, age, comorbid conditions, facility effect, access type, BMI, presence of cardiac medication [beta blockers, ACEs, ARBs] and nutritional supplements, EPO dose, blood pressure, albumin, online clearance [OLC], neutrophil to lymphocyte ratio [NLR] and target weight.

Results: We analyzed data of 11,727 pts for PRE-D. Table 1 summarizes the outcomes for Cox models for PRE-D and INC-D. Only significant predictors are shown (p<0.05). Other covariates described above were not significant.

Funding: None

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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### SA-PO3028

**Economic Impact of Reduced HBV Testing among Hemodialysis Patients in the New Environment of Bundling: Results of a Study of De Novo HBV Infection Rates in a Mayo Clinic Hemodialysis Population – A Call for Changes in Current US CDC Guidelines on HBV Testing Protocols**

**Background:** Hepatitis B Virus (HBV) infection is a serious public health issue. Hemodialysis (HD) exposes ERSD patients to significantly higher HBV risks. Therefore, current US CDC guidelines call for monthly HBsAg tests for all HD patients. The charge to Medicare per HBsAg test is about $100 per patient per month. In the new environment of Medicare Bundling, this is unwiseful and wasteful if the de novo HBV infection is rare.

The aim of this study is to determine de novo HBV infection rates among HD patients in a Mayo Clinic HD population between July 2000 to July 2010.

**Methods:** A retrospective analysis of electronic databases of all relevant HBV serology and clinical data from patients attending five Mayo Clinic HD units between July 2000 and July 2010 was carried out to identify de novo HBV infection.

**Results:** A total of 965 HD patients were studied. There was one de novo HBV infection - a case incidence rate of 0.1%. He was a 54-year old Caucasian male with a known history of IV drug abuse, and previous hepatitis C carrier. Transient asymptomatic Recombinant post-vaccination HBsAgAgenemia (false positive HBsAg) was identified in another patient, two days following an Engerix B HBV booster vaccination.

**Conclusions:** Our study demonstrated that de novo HBV infection among HD patients is not as common as feared. We recommend 3-monthly HBsAg testing for all HD patients, but to continue current monthly testing for IV drug users and other high-risk groups. There exists no solid evidence-base for the efficacy of monthly HBsAg testing. At the national level, with over 500,000 HD patients, this would translate to a mind-boggling 40 billion annual savings in Medicare + private. The US CDC current guidelines on HBV serology testing among US HD patients, last revised in 2001, are outdated, and must be revised to fall in line with current clinical realities on the ground.

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### SA-PO3026

**Drop-Out Rates in Home Hemodialysis (HHD): Understanding the Challenges**

**Background:** Advanced technology has led to the re-emergence of HHID in the US. However, more frequent dialysis schedules may limit acceptance of HHID due to the increased burden of therapy on patient and/or partner. Limited data exist on modality failure with HHID. We report HHID drop-out rates from a dialysis provider with dedicated home training centers caring for about 200 HHID patients.

**Methods:** We performed a retrospective analysis of patients undergoing HHID with the North Stage System One (NCS Medical, Lawrence, MA) who dropped out during a 3-year period (2008-2010) to further understand reasons for drop out.

**Results:** The annual drop-out rate for HHID patients was 48% over the 3 years. 186 events of discontinuation were noted in a total of 182 patients. Reasons are shown below:

- Transferred to center hemodialysis (CHD) = 84 (45%)
- Died = 31 (17%)
- Transplanted = 34 (18%)
- Transferred to peritoneal dialysis = 15 (8%)
- Transferred to another home program = 13 (7%)
- Failed to complete training = 9 (5%)

**Reasons for modality switch to CHD were documented for 72/84 (86%) events; 12 were unknown. Primary reason for modality switch to CHD was found to be “burn-out” in 34 patients (47%). Either HHID became overwhelming, strained the patient-partner relationship or resulted in care partner’s fatigue. Medical, non-dialysis related reasons caused drop-out in 16 patients (22%), while access issues were responsible in 12 patients (17%). Acute medical issues of the care partner resulted in 4 patients (6%) transferring to CHD. Lack of access to a home dialysis provider after moving resulted in 4 patients (6%) changing to CHD. Non-adherence and lifestyle issues resulted in transfer of 2 patients (3%). One third of the transfer to CHD occurred within the first 90 days after starting HHID.

**Conclusions:** Drop-out rate in HHID was found to be substantial and caused by a variety of reasons. The primary cause was the perceived burden of HHID and subsequent fatigue of both patient and partner. The impact of close attention to possible “burn-out” symptoms and respite care options needs to be evaluated.

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### SA-PO3027

**Which Dialysis Facilities Chose to Transition into the Expanded Prospective Payment System (PPS)? Implications for Access to Care**

**Background:** The China Renal Data System (CRDS) is built to understand the situation of hemodialysis in China. This is the first survey on the treatment of hemodialysis in China. We have had an overview of situation of hemodialysis in China. We want to have an overview of situation of hemodialysis in China. We have had an overview of situation of hemodialysis in China. **Funding:** Government Support - Non-U.S.

**Results:** There were 3583 hemodialysis centers in China registered in CRDS. The total number of hemodialysis patients at the end of 2010 was 221 628. The mean age of patients was 53 and the male to female ratio was 1.44:1. The mean duration of hemodialysis was 3.03 years. Primary diseases were primary glomerular diseases (58.76%), diabetic nephropathy (16.83%), hypertensive nephropathy (10.7%), polycystic kidney disease (3.54%), renal calculus (2.39%) and others (7.78%). The standard-achieving rate of blood pressure in predialysis (<=140/90 mmHg) was 39.08%. The average dose of erythropoietin was 7408.33IU/week and the standard-achieving rate of hemoglobin in predialysis (<110g/L) was 18.56%. The level of serum calcium was 2.1 to 2.37mmol/L in 36.73 percent of patients. There were 13.08% patients whose serum phosphorus was 1.13 to 1.78mmol/L. The standard-achieving rate of calcium-phosphorus product (<55%) was 51.08%. There were 24.22% patients whose serum phosphorus was 1.13 to 1.78mmol/L. The HBsAg positive rate was 7.81% and the HCV antibody positive rate was 33.64%.

The new hemodialysis patients in 2010 was 58732. The mean age was 52.18 and the male to female ratio was 1.5:1. The diseases were primary glomerular diseases (55.74%), diabetic nephropathy (17.29%), hypertensive nephropathy (9.62%), renal calculus (2.96%), polycystic kidney disease (2.67%) and others (4.36%). 6424 hemodialysis patients died in 2010, the mean age was 59. The mean duration of hemodialysis was 3.4 years. The causes of death were cardiovascular events (45.78%), cerebrovascular events (18.05%), infection (14.7%). In 2010, 1377 hemodialysis patients received kidney transplant treatment, and 653 patients changed to peritoneal dialysis.

**Conclusions:** This is the first survey on the treatment of hemodialysis in China. We have had an overview of situation of hemodialysis in China. **Funding:** Government Support - Non-U.S.
The Changing Pattern of Primary Renal Disease in the Haemodialysis Population: A Forty Year Retrospective Review

**Background:**
There is established geographical variation in the epidemiology of primary renal disease worldwide, but less is known about the temporal change in patterns of primary renal diagnoses over time. The aim of this study is to identify the variation in the pattern of primary renal diagnoses among incident chronic haemodialysis (HD) patients over the last four decades.

**Methods:**
All 4142 patients that were established on the chronic haemodialysis programme in Northern Ireland (NI) between 1970 and 2009 were included in this study. Clinical information was obtained from a prospectively recorded database. Each patient had a primary renal diagnosis classified according to the European Dialysis and Transplant Association (EDTA) coding system. The study period was divided into four decades: A (1970-79), B (1980-89), C (1990-99), and D (2000-09) in order to assess the change in patterns over time. Statistical analysis was performed using SPSS software and Cochran-Armitage Chi-square test was used for analysing trends.

**Results:**
The greatest change was the proportion of people with diabetic nephropathy (type 1 diabetes rising from 0% in the 1970s to 9.5% in 2000s, p<0.001) and type 2 diabetes from 0.5% to 9.4%, p<0.001). There was also significant increases in the number of patients with chronic renal failure of unknown aetiology (from 17.8% to 29.1%, p<0.001), and renovascular disease (from 2.7% to 10.6%, p<0.003).

Conversely, there was a significant decrease in the proportion of patients with non-IgA glomerulonephritis (37% to 5.7%, p<0.001), interstitial nephritis (25.6% to 13.7%, p<0.001), and congenital nephropathy (2.3% to 0.8%, p=0.01).

**Conclusions:**
There has been a significant change in the primary renal disease diagnosis in the HD population over past 40 years. The reasons for this are probably multifactorial including restricted acceptance policies in the early dialysis era, and advances in the management of diabetes mellitus and cardiovascular disease with increased numbers surviving to reach end-stage kidney disease. This is of relevance in planning HD provision in the future.

**SA-PO3031**
Emergent Support for Dialysis Patients Evacuated from the Northeast Japan Earthquake Disaster-Lesson from Previous Earthquakes

**Background:**
Dialysis patients need to receive regular hemodialysis therapy, which requires a large amount of water and electricity supply. Therefore, they have less chance to survive under disastrous condition. In 2004 and 2007, magnitude 6.8 earthquakes attacked Japan, one of the earthquakes even caused a minor accident in a nuclear power plant. We have overcome the crises by sending patients from destroyed dialysis facilities to unaffected ones.

**Results:**
A magnitude 9.0 earthquake and subsequent tsunami struck northeast mainland of Japan on March 11, 2011. Dialysis patients had to evacuate from the affected area, because water and electricity supplies were severely destroyed. Within a week from the earthquake, Niigata University Hospital and its related facilities accepted 181 of those evacuated dialysis patients. None of them brought their own medical records. Upon arriving at Niigata, they immediately received triage decision by expert nephrologists, and those who diagnosed as in critical condition immediately received emergent hemodialysis. Severely exhausted patients were directly admitted to hospitals. Some of patients received decontamination before hemodialysis session, because they had been exposed by radioactive substances by the Fukushima-Daiichi nuclear power plant accident. The incoming patients were given dialysis therapies were successfully performed without any remarkable troubles.

**Conclusions:**
The experience of previous two earthquakes was definitely helpful when we accepted evacuated hemodialysis patients on this present great disaster. The concrete plans for saving hemodialysis patients under disastrous conditions must be drawn up before it comes into reality.

**Funding:**
Government Support - Non-U.S.

**SA-PO3032**
Aggravation of Blood Pressure Control in Patients with CKD5D after the Fukushima City area between February 28 and April 9, 2011. The profiles of Japan affected by the earthquake that occurred on March 11, 2011.

**Background:**
Control of blood pressure (BP) is aggravated by natural disasters such as earthquakes usually occurs within 4 weeks of the disaster. We examined the time course of BP variation and its influencing factors in patients with CKD 5D in areas of Japan affected by the earthquake that occurred on March 11, 2011.

**Methods:**
We recruited 98 patients on regular hemodialysis (HD) from 3 HD centers in the Fukushima City area between February 28 and April 9, 2011. The profiles of patients were as follows: mean age, 64.8 ± 13.9 years; male, 63%; HD vintage, 9.5 ± 12.6 years. All patients were administered with the following antihypertensive agents: calcium-channel blockers, 62.2%; ARB, 64.3%; ACE-I, 25.5%; diuretics, 27.6%; β-blockers, 29.6%; α-blockers, 21.4%; and DRI, 12.2%. Systolic and diastolic BP, heart rate (HR) and body weight (BW) at 4 weeks after the earthquake were retrospectively determined from medical records.

**Results:**
Pre-dialysis systolic and diastolic BP did not significantly change during the study period despite a significant reduction in increases in BW between dialysis sessions during the first week. However, post-dialysis systolic and diastolic BP were significantly elevated (48±10mmHg vs. 0.01; one-way repeated measures ANOVA). This increase was not observed in BW. The elevation in post-dialysis BW did not change. The BP elevation after dialysis was sustained for 4 weeks even after correcting for the dry weight of each patient. Multiple logistic regression analysis revealed that α-blockers comprised the only independent factor for better post-dialysis blood pressure during the survey period: e.g., systolic and diastolic BP at week 4: OR, 0.25 (95%CI, 0.09-0.73) and 0.27, (0.07-0.65), respectively.

**Conclusions:**
Blood pressure was aggravated at 4 weeks after the earthquake in patients with CKD 5D, and α-blockers comprised an independent influential factor for better BP. These findings indicate that α-blockers play a role in aggravated BP control after natural disasters in patients with CKD.

**SA-PO3033**
Advantage of HD/PD Combination Therapy in Disaster: Lessons from the Experience of the Japan Earthquake and the Fukushima Nuclear Accident

**Background:**
The HD (hemodialysis) / PD (peritoneal dialysis) combination therapy, which comprises five or six days of PD combined with one HD session per week, is performed in Japan to better control body fluid and remove solute. After the Japan earthquake and the Fukushima Nuclear accident, medical treatment therapy were temporarily limited because of many transferred patients from the destroyed area to our hospital, shortage of a dialyzer and dialysate and failure of power supply. In addition, dialysis patients had difficulty in visiting the hospital because of transportation systems failure. In such extraordinary situations, in Kashiwa Hospital of Jikei University School of Medicine, ten patients on the combination therapy were obliged to stop HD session for two weeks. To evaluate the safety and validity of it, we assessed the condition of the patients before and after the earthquake.

**Methods:**
Respiratory condition, body weight gain, blood urea nitrogen, creatinine and potassium level were evaluated before and after the earthquake at the beginning of each HD sessions.

**Results:**
After the earthquake, ten patients visited the hospital two weeks after the last HD session skipping one HD session. No one showed symptom of respiratory failure after the earthquake. Body weight gain(2.0±0.6 vs 1.9±0.5% of dry weight), blood urea nitrogen (55.9±10.8 vs 59.1±18.4 mg/dl), creatinine (14.9±2.1 vs 16.2±2.6 mg/dl) and potassium (4.6±0.8 vs 4.6±0.6 mEq/l) level were not significantly different before and after the earthquake.

**Conclusions:**
The HD/PD combination therapy is a modality which allows patients to choose from both PD and HD after disaster safely depending on the situation. This is a large merit of the HD/PD combination therapy.

**SA-PO3034**
Gene Therapy with Indoleamine 2,3-dioxygenase Ameliorates Development of Chronic Rejection Changes in the Rat Allotransplantation

**Background:**
Chronic transplant dysfunction (CTD) is the primary reason for late allograft loss in kidney transplantation. Because there is no effective treatment available, improvement of long-term graft survival remains the major challenge in the kidney transplantation field. Indoleamine 2, 3-dioxygenase (IDO) is crucially involved in foeto-maternal tolerance, and prevents allograft rejection. In our previous experiment, we showed that gene therapy with IDO inhibits acute rejection of renal allograft. The aim of current experiment is to show whether IDO is also able to improve CTD.

**Methods:**
Kidney transplantation was performed in a rat Dark-Agouti (DA) to Wistar-Furth (WF) CTD model. RGD modified adenovirus carrying IDO gene (RGG-AdTiDO), or RGD modified adenovirus carrying gene for GFP (RGG-AdTiL, n=9) were injected into the renal artery of the donor kidney before transplantation. Recipients were immunosuppressed with cyclosporine for 10 days. After 10 days, the contra lateral kidney was removed. Body weight, serum creatinine and blood pressure (BP) were measured and 24 hour urine was collected every two weeks. Rats were sacrificed after 12 weeks.

**Results:**
Local gene therapy with IDO significantly improved body weight during the whole experiment in comparison with RGD-AdTiL treated rats. It also decreased elevated plasma creatinine (40 ± 4.8 µmol/l) compared to treatment with RGG-AdTiL (55 ± 8.5 µmol/l, 12 week) and elevated proteinuria (10.3 ± 3.1 mg/24 h for RGD-AdTiDO and 32.2 ± 9.3 mg/24 h for RGG-AdTiL, 12nd week). The therapy did not affect blood pressure, except for the second week (121 ± 5 mmHg for RGD-AdTiDO and 144 ± 4 mmHg for RGG-AdTiL). Moreover, IDO therapy significantly decreased the incidence of focal glomerulosclerosis (10.5 ± 1.5 %) compared to AdTiL therapy (33.1 ± 3.37 %).
Conclusions: Here we show for first time the beneficial effect of local IDO gene therapy in a model of CTD.

**SA-PO3035**

**Y-box Protein-1Induces Collagen I Production in Mesangial Cells Following CsA Treatment**

**Methods:** The YB-1 content of CsA and Tac treated rat mesangial cells (mMCs) was determined via immunoblot. YB-1 was immunoprecipitated from cytoplasm of CsA-challenged mMCs and co-purified mRNA was detected by RT-PCR. In vivo experiment was performed in C57BL/6 mice (single dose CsA, 100 mg/kg).

**Results:** We investigated the role of YB-1-binding protein-1 (YB-1), a highly conserved transcription factor, in the CNI-triggered renal fibrogenesis. Upon treatment with therapeutic relevant doses of CsA and Tac, the intracellular content of YB-1 protein rose up to 10-fold in mMCs depending on time and dose. Both binding transcription factor YB-1 and cycloheximide as well as blocking the ERK/Akt phosphorylation pathways prevented CsA-triggered YB-1 activation in contrast to inhibition of transcription via actinomycin D. Furthermore, degradation rate of YB-1 protein was significantly reduced in response to CsA. Thus, rapid accumulation of YB-1 following CsA challenge is due to translation of YB-1 mRNA stores and enhanced protein stability. ROS, especially hydrogen peroxide, may mediate YB-1 upregulation under CsA. Notably, this process is independent of TGF-β. The enhanced expression of collagen 1 (Col 1) in mMCs following CsA application is caused by transcriptional induction due to physical interaction with YB-1 protein and consequently, absent in YB-1-depleted cells. The CsA-induced accumulation of YB-1 in the mesangium could be confirmed in vivo.

**Conclusions:** YB-1 induces profibrotic effects of CNI in the kidney via regulation of Col 1 translation.

**Funding:** Private Foundation Support

**SA-PO3036**

**Tacrolimus Induces a Myofibroblast-Like Phenotype in Human Kidney Fibroblasts by Ligand-Independent Activation of TGF-β Receptor**

**Methods:** Human renal fibroblast cell line T-173 was treated with varying doses of TGF-β1 for three days. mRNA expression levels for NAD(P)H-oxidase 4, transgelin, tropomyosin 1, and the collagen chain alpha-1(Ⅲ) were determined by real-time qPCR. NOX4 protein expression and intracellular peroxide concentration were also determined.

**Results:** Tacrolimus-treated renal fibroblasts showed increased expression of NOX4, transgelin, tropomyosin 1, TGF-β1, and collagen mRNA. NOX4 up-regulation lead to a 20 % (max.) increase in intracellular hydrogen peroxide levels. TGF-β1 treatment duplicated the effects of tacrolimus. Specific inhibition of the TGF-β pathway repressed the effects of both tacrolimus and TGF-β1. Neutralization of extracellular TGF-β by specific antibodies almost completely abolished the reaction to TGF-β1, but left the response to tacrolimus unchanged. siRNA mediated knock-down of NOX4 had no effect on the tacrolimus-induced effects.

**Conclusions:** Tacrolimus at low nanomolar concentrations had TGF-β-like effects on cultured human renal fibroblasts. The binding of tacrolimus to FK506 binding protein 12 (FKBP12) leads to increased TGF-β receptor activity, even in the complete absence of ligand. This effect was sufficient to induce a myofibroblast-like phenotype and might thereby contribute to the induction of interstitial fibrosis in immunosuppressed kidney transplant patients.

**Funding:** Charles L. Edelstein.

**SA-PO3037**

**Protection from Kidney Injury during Hibernation Is Associated with Increased Expression of X-Linked Inhibitor of Apoptosis Proteins (XIAP)**

**Background:** The 13-lined ground squirrel is a hibernating mammal that cycles through alternating phases of extreme cold ischemia (CI) for several days during torpor (T) followed by re-warming during interbout arousal (IBA). Hibernation is a natural model of DGF as evidenced by repeated prolonged CI during T followed by warm reperfusion during IBA. We hypothesized that hibernating squirrel kidneys are protected from apoptosis and necrosis.

**Methods:** Kidneys of C57BL/6 mice and 1-2 year old summer (S), IBA and T squirrels were perfused with cold UW solution. The contralateral right kidney was immediately used as a control and the left kidney was stored in UW for 72 hours. Caspase-3/7 activity was measured using the fluoroscent substrate DEVD-AMC. Apoptotic cells and the % of tubules displaying BBI were counted and scored by a pathologist. XIAP was detected by immunoblot and quantitated by densitometry.

**Results:** Tubular apoptosis, Brush Border Injury (BBI) and caspase-3 activity were significantly increased in mice vs. squirrels. To investigate the mechanism of protection against apoptosis in hibernating squirrels kidneys, we examined the protein expression of X-linked inhibitor of apoptosis proteins (XIAP). XIAP is a naturally occurring inhibitor of caspase-3. XIAP was significantly increased in hibernating squirrel kidneys, especially during IBA, whereas it was undetectable in mouse kidneys.

**Conclusions:** Protection against CI in hibernating squirrel kidneys is associated with increases of XIAP. Mice kidneys that are not protected against CI have undetectable XIAP. IBA kidneys that are most susceptible to CI and CR have the highest levels of XIAP.

**Funding:** NIDDK Support

**SA-PO3038**

**Indoleamine 2,3-Dioxygenase Inhibition Improves Renal Blood Flow Following 30 Minutes of Ischemia**

**Background:** Ischemia reperfusion injury (IRI) is associated with delayed function and chronic allograft injury after renal transplant. Indoleamine 1, 2-dioxygenase (IDO) is an immunomodulatory enzyme that mediates degradation of tryptophan. In a mouse renal ischemia-reperfusion model, IDO inhibition with 1-methyl-D-tryptophan (1-MT) improved serum creatinine (Cr) and decreased tubular epithelial cell apoptosis. To assess whether IDO inhibition improves post ischemia hemodynamics, we measured bilateral renal blood flow (RBF) in a rat ischemia model.

**Methods:** Three groups of SD rats (N=5) had bilateral renal artery probe placement and 1 hr of recovery. Shams (S) then had 30 minutes of observation. Control (C) and 1-MT treated had 30 mins of renal artery clamp time, followed by 1 hr of recovery (POST). All rats then underwent recovery of serum and kidneys. 1-MT were pre-treated with 140 mg/kg of 1-MT 24 hrs prior to surgery and 1 hr prior to clamping.

**Results:** The serum kynurenine/tryptophan ratio was significantly reduced by 1-MT (0.052±.01 vs 0.103±.01uM, mean±SEM for 1-MT vs C, p < 0.05) indicating suppression of IDO. There were no differences in mean arterial pressure (101.2±1.4, 104.5±1.2, 106.2±2.4mmHg for S, C and 1-MT) or pre-ischemia renal blood flow (PRE RBF) (9.7±1.1, 9.9±1.0, and 14.4±1.1ml/min for S, C, and 1-MT). However, 1-MT had significantly higher POST RBF: 11.6±1.3 vs. 7.5±0.6 ml/min for 1-MT vs C (p < 0.05). Also, recovery of RBF in 1-MT was significantly increased: 83.2±3.5 vs 68.8±2.2% of PRE RBF levels for 1-MT vs C (p < 0.05). NOX4 levels were not different in post ischemia serum Cr(22.0±1.5, 21.2±10.1, 21.2±0.4 mg/dl for S, C, and 1-MT). These data suggest that IDO inhibition with 1-MT prior to renal artery cross clamping improves RBF 1 hr post ischemia.

**Conclusions:** IDO inhibition with 1-MT may help preserve renal function via an improvement in post ischemia hemodynamics. This findings also suggest that IDO may be a useful target to protect donor kidneys from the effects of IRI.

**Funding:** Private Foundation Support, Clinical Revenue Support
SA-PO3039

Early Outcome in Renal Transplantation Using Size Mismatched Recipients and Donors – A Porcine Experimental Model

Tashi Chhoden,1 Kristian Ravlo,1 Peter Søndergaard,1 Niels Secher,1 Anna Krarup Keller,2 Michael Pedersen,2 Ulla Møldebrød,3 Ernst Oeyvind Oestraat,2 Rikke Norregaard,4 Henrik Birn,5 Bente Jespersen.1 1Department of Renal Medicine, Aarhus University Hospital, Aarhus, Denmark; 2Department of Anaesthesiology, Aarhus, Denmark; 3MR Research Center, Aarhus, Denmark; 4Inst. of Clinical Medicine, Aarhus, Denmark; 5Dept. of Urology, Aarhus, Denmark.

Background: Transplantation of a kidney from a large donor to a small recipient, as in pediatric transplantation, is associated with marginal renal blood flow, shut down of GFR and risk of thrombosis. To study the mechanisms regulating early graft function we functioned a porcine model of size mismatched renal transplantation. GFR, renal blood flow (RBF) and markers of kidney injury were studied within 10 h after transplantation.

Methods: After induction of brain death, kidneys were removed from 60 kg donor pigs and kept in cold storage for 22 h until transplanted into small (15 kg, n = 8) or size-matched (60 kg, n = 8) recipients. The first 10 h after transplantation we measured GFR as urinary clearance of 51Cr-EDTA with constant infusion, RBF by MRI-technique, urinary NGAL excretion by ELISA, and renal expression of heparoxygenase (HO)-1 by qRT-PCR.

Results: In small recipients mean GFR was reduced within 30 min after reperfusion compared to size-matched recipients. This was associated with a significant reduction in medullary (M)-RBF, which decreased steadily up to 9 h after reperfusion. No difference was observed in cortical RBF. While no significant difference was observed in urinary NGAL excretion, increased HO-1 mRNA levels were observed in the cortex of small recipients compared to size-matched recipients.

Conclusions: After transplantation of kidneys from large donors to small recipients in a model with high risk of delayed graft function, a very early reduction in GFR was observed when compared to size matched recipients. This may in part be due to a reduction in M-RBF, although other mechanisms may be involved as M-RBF continued to decrease while GFR remained stable. Increased expression of HO-1 in kidney cortex may reflect ischemic injury, although cortical RBF was not reduced in the small recipients.

SA-PO3040

Association of Transforming Growth Factor-beta (TGF-β) Gene Polymorphism with Acute Rejection in Korean Kidney Transplant Recipients

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Background: Acute renal graft rejection influences the results of kidney transplantation. Acute rejection (AR) is mainly caused by T-cell immune responses activated by cytokines, including transforming growth factor-β (TGF-β). TGF-β inhibits the inflammatory response of T helper cells. Recent investigations based on epidemiologic and genetic studies have defined several single nucleotide polymorphisms (SNPs) in regulatory sequences of cytokines that have been associated with allograft survival. In this study, we examined whether polymorphisms of the TGF-β gene were associated with susceptibility to kidney transplantation rejection.

Methods: A total of 342 patients who had received kidney transplants were included. We extracted genomic DNA from blood samples and amplified the genomic DNA using the primers for each SNP. Three SNPs of TGF-β gene were genotyped from genomic DNA with direct sequencing. We analyzed 3 SNPs of TGF-β gene (rs2228084, rs764522, rs3874656).

Results: Acute rejection developed in 62 patients (18%). There is no significant differences in age, sex, number of HLA mismatches, cause of renal failure, immunosuppressant regimen between the AR and non-AR group. One SNP (rs3874656) of the TGF-β gene were significantly associated with the fewer episode of acute rejection in the recessive model (odds ratio 0.8; 95% confidence interval 0.00-NA, P=0.042).

Conclusions: Our results show that one TGF-β gene polymorphism was associated with acute rejection in Korean kidney transplant recipients.

SA-PO3041

Investigating the Mechanisms That Prevent Antibody-Mediated Injury in Patients with Donor-Specific Anti-HLA Antibodies by Microarrays

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Background: Although, most patients with donor-specific anti-HLA antibodies (DSA) develop acute or chronic antibody-mediated rejection (AMR), some demonstrate normal allograft biopsy. We aimed to investigate the mechanisms involved in protection of the allograft from antibody-mediated injury by microarrays.

Methods: We retrospectively reviewed biopsies of 221 patients performed in 2009 and 2010. The gene expression profiles of transplant kidney biopsies were studied by Affymetrix Hu Gene 1.0 ST expression arrays. Results: 77 patients had DSA at the time of the biopsy and 43 showed antibody-mediated injury (9 acute, 34 chronic AMR) and 24 were normal or demonstrated minimal injury. There was no difference in frequency or mean fluorescence intensity (MFI) values of DSAs between 2 groups. 12 chronic AMR samples and 5 with minimal injury were available for microarray analysis and demonstrated differential gene expression profiles, mostly related to immune responses. Of the top 50 differentially expressed genes were non-coding RNAs. Gene set enrichment analysis using Pathogenesis Based Transcripts created by Edmonton Group demonstrated cAMR biopsies had upregulated interferon-gamma-dependent rejection-induced (p=0.01) and cytotoxic T cell associated transcripts (p=0.03).

Conclusions: Independent replication of these observations is being pursued in a US cohort but these data raise the possibility that renal AGXT2 regulates plasma ADMA in transplant recipients. Taken with data on DDAH1 these findings suggest increased renal methylarginine metabolism associates with early decline in allograft function and that AGXT2 may be a potential therapeutic target.

Funding: Government Support - Non-U.S.

SA-PO3042

Allograft Expression of Alanine Glyoxylate Aminotransferase-2 in Protocol Biopsies Is Associated with Asymmetric Dimethylarginine Levels and Rate of Decline of Renal Function

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Background: Asymmetric dimethylarginine (ADMA), inhibits nitric oxide synthesis, is metabolised by dimethylarginine dimethylaminohydrolase-1 (DDAH1) and alanine glyoxylate aminotransferase-2 (AGXT2), and is raised in kidney transplant recipients. DDAH1 has been reported to influence decline in kidney function so we investigated the association between allograft AGXT2 gene expression, change in glomerular filtration rate (eGFR) and ADMA levels in renal transplant patients. DDAH1 has been reported to influence decline in kidney function so we investigated the association between allograft AGXT2 gene expression, change in glomerular filtration rate (eGFR) and ADMA levels in renal transplant recipients. AGXT2 expression levels were inversely associated with plasma ADMA (Figure) and this persisted after adjustment for DDAH1 expression and eGFR. There was also a strong association between AGXT2 mRNA and eGFR decline: -0.17 mL/min/1.73m²/week (95% CI -0.32 to -0.02) for allograft AGXT2 expression above versus below the median (adjusted for donor and recipient age, sex and ethnicity and DDAH1 expression).

Conclusions: Adaptive activation of these observations is being pursued in a US cohort but these data raise the possibility that renal AGXT2 regulates plasma ADMA in transplant recipients. Taken with data on DDAH1 these findings suggest increased renal methylarginine metabolism associates with early decline in allograft function and that AGXT2 may be a potential therapeutic target.

Funding: Government Support - Non-U.S.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

818A
SA-PO3043

Renal Transplant Genome Wide Association Study (GWAS) Demonstrates Genetic Indicator of Long-Term Allograft Function/Survival Paul J. Phelan, 1 Robert P. O’Brien, 1,2 Judith Conroy, 1 Sean Ennis, 3 Mary T. Keogan, 4 Gianpiero Cavallieri, 2 Susan Jennings, 4 Dork O’Neill, 1 Peter J. Conlon, 1 1Nephrology and Transplantation, Beaumont Hospital, Dublin, Ireland; 2Molecular and Cellular Therapeutics, Royal College of Surgeons of Ireland, Dublin, Ireland; 3School of Medicine and Medical Science, University College Dublin, Dublin, Ireland; 4Histocompatibility and Immunogenetics, Beaumont Hospital, Dublin, Ireland.

Background: Genetic interaction between donor and recipient genomes in renal transplantation is known to contribute to allograft rejection and overall outcome, although the nature of the genetic component remains largely uncharacterized. In this study we aimed to examine genetic variation in the recipient genome, with respect to allograft function at 5 years, as a pilot study to a wider donor-recipient genome-wide association study (GWAS).

Methods: We performed GWAS on a primary cohort of 326 Irish transplant recipients using the Illumina 610-Quad chip. Patients were first time, kidney-only, deceased donor transplants between 1993 and 2002. All patients were on calcineurin inhibitor based immunosuppression.

Results: Using linear regression, we found a significant signal (p=6.9x10^-08) in Chromosome 14 in the TRA locus (TRA2p) with respect to allograft function at 5 years post transplantation, as measured by serum creatinine. Serum creatinine at 5 years showed a statistically significant difference between genotypes (p=0.0006; mean 153.3 µmol/L V. 255.5 µmol/L). The TRA locus codes for T cell receptor alpha chains which have a key function in immune recognition.

Conclusions: These findings are interesting given our relatively small yet homogenous patient population and the potential biological mechanism at play. It emphasizes the importance of studying genetic variation in transplant outcome. We aim to investigate this further by replication in a new recipient cohort.

Funding: Private Foundation Support

SA-PO3044

Renal Allograft Biopsies Classified as AIN Display a CTL Molecular Signature Michelle E. Lubesky, Darshana Dadhania, Janani Rangaswami, Thangamani Muthukumar, Kenar D. Jhaveri, Surya V. Seshan, Manikkam Suthanthiran. Nephrology, Cornell University, NY, NY.

Background: The molecular signature of post transplant allergic interstitial nephritis (AIN) has not been well described. Characterized by prominent eosinophils on biopsy, it is also known to share pathologic features with acute rejection (AR).

Methods: We identified 19 biopsies classified by pathology as AIN from a cohort of 512 for-cause biopsies from Jan 2008 to Feb 2011 (3.7%). From this cohort 8 patients had urinary specimens available for gene expression profiling. Immunohistochemical staining (IHC) was done on corresponding biopsy specimens. We compared these urinary profiles and IHC to 5 patients with AR.

Results: Using linear regression, we found a significant signal (p=6.9 x 10-08) in Chromosome 14 in the TRA locus (TRA2p) with respect to allograft function at 5 years post transplantation, as measured by serum creatinine. Serum creatinine at 5 years showed a statistically significant difference between genotypes (p=0.0006; mean 153.3 µmol/L V. 255.5 µmol/L). The TRA locus codes for T cell receptor alpha chains which have a key function in immune recognition.

Conclusions: These findings are interesting given our relatively small yet homogenous patient population and the potential biological mechanism at play. It emphasizes the importance of studying genetic variation in transplant outcome. We aim to investigate this further by replication in a new recipient cohort.

Funding: Private Foundation Support

SA-PO3045

KIM-1 Expression in Pre-Transplant Donor Kidney Biopsies as a Predictor of 1 Year Post Transplant Renal Function and Incidence of Acute Rejection Dwight M. Matthew, 1 Rachel E. Musial, 1 Sandeep Aggarwal, 1 Suganthi Soundararajan, 2 Gregory Malat, 1 Karthik M. Ranganna. 1Nephrology, Drexel University College of Medicine, Philadelphia, PA; 2Pathology, Drexel University College of Medicine, Philadelphia, PA; 3Pharmacy, Hahnemann University Hospital, Philadelphia, PA.

Background: Kidney injury molecule-1 (KIM-1), a type 1 transmembrane glycoprotein, is believed to be a specific histological biomarker for the diagnosis of early kidney allograft injury. However, its usefulness as a predictor of allograft function is uncertain.

Methods: The purpose of our study is to evaluate KIM-1 expression in pre-transplant donor wedge biopsies as a predictor of 1 year post transplant renal function and acute rejection in the immediate post transplant period. In this study we included 44 patients who underwent deceased-donor kidney transplant from May 2008 to April 2010. We stained pre-transplant donor wedge biopsies for KIM-1 using immunohistochemistry.

Results: 23 out of 44 patients who had pre-transplant wedge biopsy for KIM-1 had a 1 month post transplant biopsy. Two out of 13 KIM-1 negative patients had evidence of acute rejection in 1 month post transplant biopsy while, 1 out 10 KIM-1 positive patients had acute rejection. The difference was not statistically significant (p=0.696). The 1 year post transplant eGFR (by MDRD) was 59.4 ±20.2 in KIM-1 positive and 60.5±23.7 in KIM-1 negative groups respectively (p=0.880).

Conclusions: Our data suggests that AIN is associated with heightened expression of cytotoxic attack molecules similar to that seen in AR. The molecular response seen may have implications for the management of AIN.

SA-PO3045

KIM-1 Expression in Pre-Transplant Donor Kidney Biopsies as a Predictor of 1 Year Post Transplant Renal Function and Incidence of Acute Rejection Dwight M. Matthew, 1 Rachel E. Musial, 1 Sandeep Aggarwal, 1 Suganthi Soundararajan, 2 Gregory Malat, 1 Karthik M. Ranganna. 1Nephrology, Drexel University College of Medicine, Philadelphia, PA; 2Pathology, Drexel University College of Medicine, Philadelphia, PA; 3Pharmacy, Hahnemann University Hospital, Philadelphia, PA.

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Results: 23 out of 44 patients who had pre-transplant wedge biopsy for KIM-1 had a 1 month post transplant biopsy. Two out of 13 KIM-1 negative patients had evidence of acute rejection in 1 month post transplant biopsy while, 1 out 10 KIM-1 positive patients had acute rejection. The difference was not statistically significant (p=0.696). The 1 year post transplant eGFR (by MDRD) was 59.4 ±20.2 in KIM-1 positive and 60.5±23.7 in KIM-1 negative groups respectively (p=0.880).

Conclusions: Our data suggests that AIN is associated with heightened expression of cytotoxic attack molecules similar to that seen in AR. The molecular response seen may have implications for the management of AIN.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Glucomeral mRNA Expression of Pro- and Antithrombotic Factors in Thrombotic Microangiopathy in Renal Transplants Clemens L. Bockmeyer,1 Patrizia Schiffer,2 Maximilian Ernst Daemmrich,1 Friedrich Modde,1 Thorsten Feldkamp,1 Udo Veshery,1 Bernd Brecker,1 Jan U. Becker.1

Methods: Biopsies from 17 patients with TMA after transplantation (12 of novo and 5 recurrent TMA) were compared to 8 transplant biopsies without signs of TMA or humoral rejection. RNA was isolated from microdissected glomeruli of paraffin-embedded biopsies. The relative expressions of ADAMTS13, von Willebrand Factor (VWF), PAI-1, uPA, uPA membrane cofactor protein (MCP), tissue factor and thrombomodulin were determined by quantitative RT-PCR after preamplification.

Results: Glomerular ADAMTS13 was higher in recurrent TMA and lower in de novo TMA compared to controls. Glomerular uPA was lower in de novo TMA, CNI-TMA and AHR-TMA compared to controls. Glomerular uPA was higher in recurrent and de novo TMA compared to controls. Glomerular PAI-1 was higher in recurrent TMA, de novo TMA and AHR-TMA compared to controls. Glomerular MCP was higher in recurrent and de novo TMA than in controls. VWF, tissue factor and thrombomodulin were not found to be different between the cohorts.

Conclusions: These data provide insight into the contribution of the glomerular capillary bed to miRNAs to microthrombosis formation in renal transplants. While all TMA forms seem to share common pathways in fibrinolysis and compensatory upregulation of MCP, different etiologies are reflected in differential expression of ADAMTS13. The increase of glomerular ADAMTS13 expression in recurrent and decrease in de novo TMA could be diagnostically and therapeutically relevant.

SA-PO3047

The Impact of Living Kidney Donation on Peripheral Blood Micro-RNAs

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Background: The molecular effects of kidney donation by living donors are poorly investigated. Micro-RNAs (miRs) are considered as the masterregulators of transcription. We aimed to detect differential expression of peripheral blood micro-RNAs (miRs) as the function of unilateral nephrectomy in living donors.

Methods: So far, 16 patients consented to participate in this study. Whole blood total RNA including miRs were collected prior and post nephrectomy using Qiagen blood miRNA Kit and the QiAamp blood system. RNA quality control and quantitation was performed using total- and small RNA Agilent Chips (Bioanalyser, Agilent) and NanoDrop spectrophotometer. Genome-wide miR profiling was performed with 12 samples representing 6 patients using Illumina’s microRNA DASL array. Clustering and statistics were performed using SUMO software package.

Results: We found 39 unique miRs to be differentially regulated post nephrectomy (p<0.01). The prevailing pattern was downregulation of miRs post nephrectomy (25 miRs) as compared to upregulation (14 miRs). In addition the extent of regulation was stronger among downregulated miRs. Given the inhibitory function of miRs on post transcriptional regulation, our data suggest a global activation of the transcriptome via downregulation of miRs in response to nephrectomy. Interestingly, we found an enrichment of certain miR families among the downregulated miRs e.g., miR18a/16b, miR20b* or miR27a/27b. Real-time quantitative RT-PCR using Taqman probes were performed to confirm the regulation of miRs in the entire collective. We further attempt to correlate the miR expression with renal function, urinary protein excretion and development of hypertension in kidney donors.

Conclusions: Our data demonstrate the feasibility of peripheral blood based miRNAs as putative markers of donor renal allograft. KIM-1 positivity did not predict an increased risk of developing acute rejection in the immediate post transplant period. The IFTA score, a marker of chronic allograft nephropathy, was also not different between the two groups.

SA-PO3048

Urinary Angiotensin-Converting Enzyme 2 (ACE2) Is Increased in Renal Transplant Recipients

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Background: Angiotensin-converting enzyme 2 (ACE2) is highly expressed in the kidney, and may protect against kidney disease progression by degrading angiotensin (Ang) 1 to Ang-(1-7). ACE2 has been identified in human urine, and may be shed from tubular epithelial cells, where it is localized to the apical membrane. We tested the hypothesis that urinary ACE2 is increased in renal transplant recipients.

Methods: Spot urine samples were collected from healthy subjects (C: age: 43.6 yrs, eGFR 81.0 ml/min, urine albumin/Cr 9.1±1.9 µg/mg, n=50), transplant recipients without alignubuminaemia (Tx: Age: 52.3 yrs, 30% diabetic, eGFR 48.6 ml/min, urine album/Cr 5.3±0.6 µg/mg, n=50), and renal transplant patients with significant albuminuria (Tx+/A: Age: 52.0 yrs, 48% diabetic, eGFR 49.5±5.5ml/min, urine album/Cr 246.8±31.1 µg/mg, n=5). No subjects were treated with inhibitors of the renin-angiotensin system.

Results: Levels of urinary ACE2 mRNA did not differ amongst the 3 groups, while ACE mRNA was increased in Tx+/A, compared to C (p<0.03). In contrast, urinary ACE2 activity was significantly increased in both groups of renal transplant recipients, compared to healthy subjects (C: 2.55±0.32 pmol/TxA; 5.94±0.82 pmol/TxA; 6.01±1.39 pmol/mg Cr x 10^{-3}; p<0.03 vs C), while ACE activity did not differ amongst the groups. By immunoblot, ACE2 was detected in urine samples as a protein of ~120 kDa, with a smaller band at ~ 90 kDa. Transplant patients had significantly higher levels of urinary ACE2 protein on blots, compared to controls (p<0.04). Urinary levels of Ang-(1-7) were significantly increased in transplant patients (C: 0.55±0.14 vs Tx: 1.38±0.25 vs Tx+/A: 1.33±0.24 ng/mg Cr; p<0.04 vs C).

Conclusions: These data indicate that urinary ACE2 protein is increased in renal transplant recipients, independent of albuminuria. The increase of ACE2 in these subjects suggest increased cleavage of Ang II by urinary ACE2 in this setting. Although its source is unclear, urinary ACE2 may be a marker of renal injury in transplant recipients.

Funding: Private Foundation Support, Clinical Revenue Support, Government Support, NHLBI, USA.

SA-PO3049

Effect of Immunosuppressive Drugs on DNA Repair in Human Peripheral Mononuclear Blood Cells

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Background: Cancer is a major cause of mortality among transplant recipients. Immunosuppressive treatment is one of the modifiable factors contributing to this phenomenon. Cyclosporine treatment in kidney transplant recipients was associated with reduced UV-induced DNA repair by peripheral blood mononuclear cells (PBMC) and increased cancer rate.

Aim: To investigate the effect of currently used immunosuppressive drugs on DNA repair.

Methods: H2O2-induced DNA repair by human PBMC was tested in vitro in the presence of cyclosporine, tacrolimus, mycophenolic acid (MPA), sirolimus and everolimus at low to high non-toxic concentrations. The effect of combination therapy at maintenance levels was also tested.

Results: Cyclosporine and tacrolimus suppressed DNA repair throughout the tested dose range. In contrast, MPA, sirolimus and everolimus did not to the high doses. Maintenance doses of a combination of tacrolimus and MPA, the most frequent treatment regimen, reduced DNA repair, while MPA with sirolimus or everolimus did not.

Conclusions: Regarding risk of post-transplant malignancy, long-term treatment with mycophenolate mofetil or myophenolate sodium combined with an mTOR inhibitor could be the treatment of choice.

SA-PO3050

Urinary Biomarkers in Cyclosporine A-Induced Nephrotoxicity

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Background: The aim of this study was to assess potential urinary biomarkers (Elisa assays) for cyclosporine A (CsA)-induced acute and chronic renal injury and compare them to renal tissue immunohistochemistry markers.

Methods: Male rats on low salt-diet (n=6/group) were studied 7, 14 and 21 days after CsA (15 mg/kg/day) or vehicle (V) treatment.

Results: CsA increased urinary fibronectin (ng/ml) after 7 (40±10.4 vs 17, 8.1±3.8 in V, mean ± SD) and 14 of treatment (33.4±7.9 vs 24.8±4.9 in V, p<0.05). Urinary TNF-alpha (pg/ml) presented the same pattern: 866±535 in CsA vs 159.184 in V at day 7 and 884±110 in CsA vs 37.4±12 in V at day 14, compared to controls (p<0.02). Urinary IL-1beta after 24 h (7461±1097 vs 88.4±40 in V, p<0.05). CsA treatment increased urinary osteopontin (ng/ml) after 24 h (404±73 vs 167.9±59 in V, p<0.01). CsA treatment increased urinary KIM-1 (pg/ml) in all periods: 113.4±47 vs 184±82 in V at day 7; 1152±50 vs 264±87 in V at day 14 and 105±24 in V at day 21, p<0.01. Renal immunohistochemistry showed an increased ED-1 infiltration (positive cells) in
Transplantation: Allograft Dysfunction and Complications - I
Poster/Saturday

**SA-PO3051**

**Urinary NGAL Allows for Differential Diagnosis of Acute Kidney Injury in Renal Allograft Recipients**

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**Background:** Neutrophil gelatinase-associated lipocalin (NGAL) regulates growth and differentiation in renal epithelia. In the absence of systemic inflammation (SIRS), urinary NGAL is of renal origin and an early and specific marker of acute kidney injury. Here, we demonstrate urinary NGAL at respective cutoff to accurately predict acute rejection among other causes of acute kidney injury in renal allograft recipients.

**Methods:** Spot urine specimen were prospectively assessed in 182 consecutive renal allograft recipients on maintenance immunosuppression upon presentation at our outpatient clinic. Samples were blinded and NGAL concentrations determined by ELISA. Patient data were classified according to allograft function and AKIN criteria into stable allograft function or acute kidney injury (AKI) and according to underlying condition into control, chronic allograft nephropathy (IFTA), bacterial-or viral infection, allograft rejection or other.

**Results:** In stable allograft recipients, median urinary NGAL [interquartile range] was 6.9 [3 - 13] ng/ml, or 10.8 [5 - 27] μg/g creatinine. A moderate increase was seen in IFTA, CMV and BKV infection. Urinary tract infection was associated with a significant increase in urinary NGAL, yet highest values were observed in acute allograft rejection. With a cutoff at 30 ng/ml, urinary NGAL discerned stable allograft function from AKI. At cutoff 100 ng/ml, elevated urinary NGAL accurately predicted acute allograft rejection within our cohort (AUC-ROC 0.98, sensitivity 1.0, specificity 0.93), even in the presence of urinary tract infection.

**Conclusions:** Urinary NGAL, at respective cutoff, accurately predicted acute allograft rejection among all other causes of acute kidney injury in kidney transplant recipients. As a readily available parameter, urinary NGAL facilitates to quickly delineate the pathogenesis of renal functional deterioration in allograft recipients presenting with a rise in serum creatinine.

**SA-PO3052**

**NGAL Trends in Living Donated Kidney Transplant Recipients during the Postoperative Period: Gender Differences**

**Junko Kohei,1 Kosaku Nitta,2 Ken Tsuchiya,3 Takumi Yoshida,1 Michihito Mitobe,1 Hidekazu Sugira,1 Junji Shiohira.**

1Fourth Department of Internal Medicine, Kidney Center, Shinjuku, Tokyo, Japan.

**Background:** Neutrophil gelatinase-associated lipocalin (N-GAL) variations have previously been associated with acute kidney injury in postoperative period, and there are some reports about the NGAL trends after kidney transplantation. However gender difference in the NGAL trends after living donated kidney transplant recipients has not been studied in depth. This study’s objective is to examine the gender differences in the NGAL trends during recovery after surgery.

**Methods:** We included only adult recipients undergoing living donated kidney transplantation. For NGAL detection the serum and urine samples were collected everyday after operation. The samples were analyzed using an immunoassay (ARCHITECT).

**Results:** In the results, 38 male cases and 20 female cases without delayed graft function were calculated by subtracting 0 scores from 1 year scores. Univariate and multiple regression analysis were done to test correlations.

**Conclusions:** This is a first report of gender differences in the serum and urine NGAL trends in the recovery process after renal transplantation. Renal dysfunction after kidney transplantation is difficult to diagnose with only serum creatinine, therefore using of the biomarker is expected as auxiliary diagnosis. This research has a high possibility of helping to diagnose delayed graft function in the postoperative early stage.

**Funding:** Pharmaceutical Company Support, Private Foundation Support

**SA-PO3053**

**Peripheral Blood CD19+ Lymphocytes Predict Kidney Allograft Interstitial Fibrosis and Tubular Atrophy on 1-Year Protocol Biopsies**

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**Background:** Noninvasive tests of cellular or humoral immunity may be helpful in prediction of late allograft kidney function and progression of chronic histology changes. Aim of this study was to assess whether specific subset of peripheral blood lymphocytes could predict 1-year renal function and renal histology changes over 1 year.

**Methods:** A cohort of 46 kidney transplant patients was analyzed. Immunophenotyping of peripheral blood lymphocytes (CD4, CD8, CD127, CD25 and CD19) was done by flow cytometry at kidney transplantation. Protocol kidney biopsies were done on day 0 and at 1 year after transplant and were scored according to the Banff 07 classification. Δci, Δct and Δah were calculated by subtracting 0 scores from 1 year scores. Univariate and multiple regression analyses were done to test correlations.

**Results:** In univariate analysis recipient and donor age were positively correlated with 1-year Δci, Δct and Δah. The number of CD19+ and CD8+ cells was negatively correlated with 1-year Δci, Δct and Δah. Δah was also in a positive correlation with CD4/CD8 ratio. In a multiple regression analysis Δci (b=0.54 ± 0.20, p=0.007) and Δct (b=0.50 ± 0.20, p=0.018) were independently associated only with number of CD19+ lymphocytes. Δah remained significantly correlated only with value of CD4/CD8 (b=0.50 ± 0.17, p=0.007).

In a univariate analysis 1-year creatinine clearance was negatively correlated with recipient and donor age and positively with CD19+ and CD8+ lymphocyte counts. However, recipient and donor age were the only variables that remained significantly correlated with 1-year kidney function in multiple regression analysis.

**Conclusions:** CD19+ cells in peripheral blood may be a useful biomarker for prediction of 1-year kidney allograft histology changes. Higher CD19+ cell count at kidney transplantation may be associated with slower progression of IFTA/T.

**Funding:** Government Support - Non-U.S.
SA-PO3054

The Clinical and Molecular Significance of Glomerular C4d Staining: Nicole A. Havle,1 Yi Bao,2 Robert Brent Calder,1 Bin Ye,3 Enver Akalin.4 1Pediatric Nephrology; 2Einstein/Montefiore Kidney Transplant Program; 3Computational Genomics Facility, Albert Einstein College of Medicine, Bronx, NY.

Background: Diffuse C4d staining, defined as involving more than 50% of the peritubular capillaries (PTC), is accepted as a footprint of antibody-mediated rejection (AMR). However, the clinical and molecular significance of isolated glomerular C4d staining is not known.

Methods: We retrospectively reviewed biopsies of 221 patients performed in 2009 and 2010. The gene expression profiles of transplant kidney biopsies were studied by Affymetrix HuGene 1.0 ST expression arrays.

Results: 115 biopsies (52%) were C4d negative, 40 (18.1%) were PTC C4d+ (22 diffuse and 18 focal) and 66 (29%) were isolated focal glomerular C4d+. Positive PTC C4d staining is significantly associated with acute or chronic AMR, circulating DSAs and higher class I DSA MFI values, higher spot urine protein/creatinine ratio, there were no statistically significant differences between isolated glomerular C4d+ patients compared to C4d negative patients. Comparison of gene expression profiles of 12 chronic AMR biopsies to 6 isolated glomerular C4d+ biopsies without DSAs and 4 with DSAs, revealed Zveans 2630 and 717 differentially expressed genes, respectively (p < 0.01), where 618 genes were mutual and 18 focal. C4d+ (n=66) differed in both comparison. Differentially expressed genes were mostly related to cellular immune response activation in chronic AMR.

<table>
<thead>
<tr>
<th>C4d neg (n=115)</th>
<th>Isolated Glomerular C4d+ (n=66)</th>
<th>PTC-C4d+</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>50±15</td>
<td>50±15</td>
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</tr>
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<td>0.66</td>
</tr>
<tr>
<td>African-American race (%)</td>
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<td>35</td>
<td>0.85</td>
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<tr>
<td>Decreased-donor tx (%)</td>
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<td>71</td>
<td>0.45</td>
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<tr>
<td>History of previous acute rejection (%)</td>
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</tr>
<tr>
<td>DSA Frequency (%)</td>
<td>24</td>
<td>26</td>
<td>&lt;0.001</td>
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</table>

Conclusions: isolated glomerular C4d staining does not have significant clinical or gene expression profiles do not demonstrate immune activation.

SA-PO3055

Proximal Tubular Expression of Activated Ask1 Is Up-Regulated in Recipients with Early New-Onset Diabetes, Pre-Existing Diabetes Mellitus, and Impaired Fasting Glucose Post-Renal Transplantation: Zoltan G. Laszik1, Sushrut D. Chandran2, David G. Breckenridge3, Flavio G. Vincenti.1 1Department of Pathology, University of California San Francisco, San Francisco, CA; 2Division of Nephrology, University of California San Francisco, San Francisco, CA; 3Gilead Sciences, Palo Alto, CA.

Background: Apoptosis signal-regulating kinase 1 (ASK1) activates c-Jun N-terminal kinase and p38 in response to various stimuli such as oxidative stress and cytokines. Activation of ASK1 results in diverse cellular reactions including cell differentiation, inflammation, and apoptosis. New-onset diabetes after transplantation (NODAT) is an independent risk factor for graft loss of unclear etiology. The aim of our study was to assess the proximal tubular expression of activated Thr845 autophosphorylated ASK1 (pASK1) in 6-month post-transplantation biopsies with normal morphology of recipients with NODAT, pre-existing type 2 diabetes mellitus (DM), or impaired fasting glucose (IFG).

Methods: Renal transplant recipients with NODAT, pre-existing type 2 DM, and IFG underwent a 6-month surveillance biopsy at UCSF were identified. Quantitative immunofluorescence analysis of pASK1 expression was compared in groups of recipients with NODAT (n=6), pre-existing type 2 DM (n=9), IFG (n=9), or non-diabetic recipients (n=7). ImageJ software was used to quantify nuclear expression of pASK1 in the proximal tubules as visualized by double pASK1 and Lotus stain on frozen sections.

Results: pASK1 nuclear expression in the proximal tubules was significantly up-regulated in recipients with NODAT (0.0930 ±0.046, p=0.0001), pre-existing type 2 DM (0.0937 ±0.050, p=0.0001), and those with IFG (0.0721 ±0.035, p=0.0006) compared to non-diabetic recipients (0.0333 ±0.023). No significant differences were detected between recipients with NODAT and those with pre-existing type 2 DM (p=0.48) and IFG (p=0.086).

Conclusions: Increased nuclear expression of pASK1 in the proximal tubules in 6-month surveillance biopsies of recipients with NODAT, pre-existing type 2 DM and IFG indicates a potential role of ASK1 in the pathogenesis of graft dysfunction in the diabetic milieu.

SA-PO3056


Background: Microarray studies of biopsies from kidney transplants identified few isolated v1 lesions (minimal arteritis) with no/minimal tubulointerstitial inflammation and low T cell transcripts, questioning whether these cases reflect true rejection (AJT 2007:7:272-4). We investigated the clinical significance of isolated v1 lesions in 266 conventional kidney transplants performed between 1999 and 2010 in seven transplant centers in North America.

Methods: We studied clinical parameters and graft survival (median follow-up after biopsy 44 months) in 100 isolated v1 biopsies (v1 and i<2 and t<2; group 1), in comparison to 90 biopsies with v1 plus high tubulointerstitial inflammation (v1 and i>2 and t>2; group 2) and 91 biopsies with v0 with minimal tubulointerstitial inflammation (v0 and i<2 and t<2; group 3). Biopsies with C4d positivity or biopsies from ABOi or cross-match positive kidneys were excluded. In selection of controls, no clinical parameter was matched (not to introduce bias). The biopsies were reviewed by a central pathology committee.

Results: The median post transplant time was 29 days in isolated v1, 33 days in group 2, and 21 days in group 3 biopsies (p=0.05). Indication for biopsies differed among groups: delayed or slow graft function triggered biopsies in 24% of isolated v1 group, but was uncommon in control groups (5% in group 2, 11% in group 3) (p<0.05). Serum creatinine at biopsy, 1 month and 6 month post biopsy did not differ among groups (p=0.05). Graft survival did not differ among groups.

Conclusions: We conclude that, 1) isolated v1 biopsies are seen early and associated with increased delayed graft function; 2) v1 with or without high tubulointerstitial inflammation is not related to increased graft failure compared to v0. Thus, isolated v1 lesions, after the exclusion of antibody-mediated rejection, are of two types: T cell-mediated rejection and endothelial injury, and have no independent prognostic significance following anti-rejection treatment.

SA-PO3057

Pharmacodynamic Immune Monitoring in Pediatric Renal Allograft Recipients – Analysis of NEAT-Regulated Gene Expression and Immunoknow® Yo Han Ahn,1 Kyoung Hee Han,2 Se Eun Lee,2 Seong Heon Kim,2 Il-Soo Ha,1 Hae Il Cheong,2 Heyeung Kang.1 1Center for Pediatric Nephrology, National Cancer Center, Goyang, Republic of Korea; 2Department of Pediatrics, Seoul National University Children’s Hospital, Seoul, Republic of Korea.

Background: Introduction of calcineurin inhibitor (CNI) as immunosuppressant has markedly improved the outcome of kidney transplantation. While therapeutic drug monitoring (TDM) is used to adjust the dosage of CNI, some patients, especially children, still suffer from rejection or infection and CNI toxicity. This study was to assess the adequacy of immunosuppression using pharmacodynamic monitoring.

Methods: Pharmacodynamic monitoring was done for 37 pediatric kidney allograft recipients. Expression of nuclear factor of activated T lymphocytes (NFAT)-regulated genes in patients’ mononuclear cells was measured after activation, by qPCR of IL-2, GM-CSF before (t0), and 1hr (t1) post- ingestion of tacrolimus and the residual gene expression (RGE) was calculated. Global immune response was assessed by Cylex-ImmuKnow assay. Trough and peak levels of tacrolimus were measured and clinical findings of rejection episodes and infectious complications were reviewed retrospectively.

Results: REG of IFN-γ and mean REG of the three NFAT-regulated genes showed negative correlation with tacrolimus peak levels. In patients with acute rejection episodes, REGs of IFN-γ (35 ± 9 vs. 18 ± 24%) and GM-CSF (97 ± 58 vs. 40 ± 20%) were higher and tacrolimus peak levels were lower (15.3 ± 4 vs. 8.4 ± 3.0 ng/ml), compared to those in children without rejection. REG of GM-CSF (28 ± 33 vs. 47 ± 21%) was lower and tacrolimus trough level was higher (5.2 ± 1.8 vs. 4.0 ± 2.1 ng/ml) in patients with infectiouscomplication than in those without infectious complications. On the other hand, immune response measured by ImmunoKnow was not correlated with tacrolimus levels or clinical manifestations.

Conclusions: REG of NFAT-regulated genes showed correlation with clinical manifestation of under- or over-suppression of immune function in pediatric kidney allograft recipients. Further studies are required to assess whether pharmacodynamic monitoring would be such more relevant than TDM.

SA-PO3058

Subclinical Rejection Management and Transplanted Kidney Function in Children Undergoing Kidney Transplantation: Yuko Hamasaki1, Yoko Nishida1, Kazuki Hara1, Keiko Kai2, Hiroki Nishida1, Shun-ichi Murach1, Tomoyuki Sakai1, Kenji Yumura1, Hiroto Yamakawa1, Hiroki Satoh2, Seichirou Shishido1, Masataka Honda1. 1Nephrology, Metropolitan Children’s Medical Center; 2Uehu Fukuoka, Tokyo, Japan. 1Pediatric Nephrology, Toho University Omori Medical Center; Ota-ku, Tokyo, Japan.

Background: The introduction of basiliximab and other chimeric monoclonal antibodies has decreased the incidence of early clinical rejection after transplantation. However, subclinical rejection may occur, and its treatment remains controversial.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

822A
Methods: We analyzed 81 children (45 boys) without clinical rejection after AB0-compatible living donor kidney transplantation. Their mean age was 8.5 years. Routine kidney biopsies were performed 4 months and 1 year after transplantation. Histopathological findings were evaluated according to the Banff classification (2007). Borderline (BL) or severer changes were defined as subclinical rejection. Rejection was treated by methylprednisolone or deoxyspergualin. Kidney function was assessed on the basis of glomerular filtration rates (GFR), calculated with the Schwartz formula. Changes in histopathological findings and transplanted kidney function at 1 year were examined according to the presence or absence of treatment 4 months after transplantation.

Results: Histopathological examination 4 months after transplantation showed BL in 42 patients (51.9%) and acute T cell-mediated rejection of grade IA or higher (AR) in 13 (16.0%). Anti-rejection therapy was given to 6 patients with BL and all patients with AR. Histopathological findings at 1 year were normal in 5 and BL in 1 of the 6 patients with BL who received therapy. The GFR improved slightly from 73.9 ± 9.5 mL/min at 4 months to 77.8 ± 9.6 mL/min at 1 year. In 8 of the 13 patients with AR, histopathological findings at 1 year were BL. Among the patients with BL untreated 4 months after transplantation, histopathological findings at 1 year were BL in 22 (61.5%) and AR in 8 (22.2%). The GFR decreased significantly from 80.0 ± 13.3 mL/min to 77.0 ± 14.4 mL/min (p < 0.05).

Conclusions: BL or severe subclinical rejection should be treated to improve outcomes after kidney transplantation.

SA-PO3060


Background: Flow crossmatch (FCM) is often done at the time of deceased donor renal transplant (DDRT), and a positive FCM may be used either to exclude the recipient from receiving that transplant or to alter post-transplant management. Data are conflicting regarding whether a positive FCM at the time of DDRT is associated with an increased risk of acute rejection or graft loss. We analyzed the effect of a positive FCM at the time of DDRT on long-term outcomes.

Methods: We reviewed all adult recipients of a DDRT who were transplanted at a single center from 1/01/05-1/31/07. All patients had a FCM performed. 233 patients were transplanted, 38 had a positive FCM. All patients were on a rapid steroid withdrawal protocol. Maintenance immunosuppression consisted of tacrolimus and mycophenolate. All patients received induction therapy.

Results: Recipient demographics are shown in table 1. Rejection-free survival and graft-survival are shown in figure 1.

Conclusions: In patients with subclinical borderline rejection, steroid treatment resulted in improved 1-month eGFR. However, there was no difference at 3 and 6 months. Further studies are needed to assess the impact of steroid treatment on long term graft function.

Funding: NIDDK Support

SA-PO3061


Background: The impact of flow crossmatch results on transplant outcomes remains unclear, particularly in patients with donor specific antibodies (DSA). We attempted to determine if patients with a positive flow crossmatch (flow-xm) were more likely to have acute rejections than those who had a negative flow-xm despite the presence of DSA.

Methods: We performed a retrospective review of all adult DDRT recipients from 1/09/1-12/2010. All patients had a negative CDC crossmatch at the time of transplantation and had solid phase assay measurement of HLA antibodies. A flow-xm was subsequently done if HLA antibodies (donor specific or otherwise) were detected by Luminex. We identified 26 patients with DSA who did not receive any additional treatment other than our standard induction regimen at the time of transplantation followed by our steroid free immunosuppression protocol of tacrolimus and mycophenolate. In this cohort, 18 had a positive flow-xm and 8 had a negative flow-xm.

Results: Patients with positive flow-xm had a similar age (50.9 ± 14 vs 57.5 ± 12.9, p=0.27), gender distribution (female 62.5% vs 37.5%, p=0.39), race (white 50 vs 62.5%, p=0.27) prevalence of pts with previous transplants (50 vs 50%, p=ns) and cold ischemia time (28.2 ± 10.1 vs 30.9 ± 9.6, p=0.55) to patients with a negative flow-xm. All patients with a negative flow-xm had MFI < 4500 by luminex. Patients with positive flow-xm have a significantly
higher risk of rejection (14.3 ± 62.5%) and the 6 cases of early antibody mediated rejection (AMR) seen in our cohort were all present in the positive flow-xm group.

Conclusions: Among patients with preformed DSA, the presence of a positive flow-xm appears to predict a significantly higher risk of early acute rejection and in particular acute AMR.

SA-PO3062
Pre-Transplant Immunologic Risk Assessment in Patients with Donor-Specific Anti-HLA Antibodies Kwaku Marfo, Min Ling, Daniel G. Glicklich, Graciela De Boccardo, Enver Akalin. Einstein/Montefiore Kidney Transplant Program, Bronx, NY.

Background: We developed an algorithm for pretransplant immunologic risk assessment based on mean fluorescence intensity (MFI) values of Luminex single antigen beads and channel shift values of flow-cytometry cross-match results in patients with donor-specific anti-HLA antibodies (DSA) to decrease antibody-mediated rejection (AR), which has been reported as 30-40%.

Methods: Patients’ Anti-HLA antibodies with MFI values more than 5,000 were reported to UNET as unacceptable antigens. Kidney transplants were performed only if the MFI values of DSAs were less than 5,000 and t and B cell cross-match channel shift values were less than 150 and 250, respectively. Patients received anti-thymocyte globulin induction and IVIG (2 gram/kg) induction treatment.

Results: Cohort of 22 patients with pre-transplant DSAs received a transplant with this protocol. There were 15 female, 7 male, 15 African-American, 17 deceased-donor and 5 living-donor recipients with a median age of 50. 12 patients had class I, 8 class II, 2 both class I and II DSAs with a mean number of DSAs 1.68 ± 0.72, DSA MFI 3766 ± 3752, and peak Luminex PRA 70 ± 30 prior to transplant. During a mean follow-up of 373 ± 225 days, patient and graft survival was 95% (one patient died due to cardiovascular disease with a functioning allograft). 16 patients underwent 22 clinically indicated kidney biopsies and 2 showed (9%) AR (both responded to treatment) and there was no acute cellular rejection or transplant glomerulopathy. 8 patients (36%) lost their DSAs during follow-up and the remaining patients’ DSA MFI was 3471 ± 4763 at the last clinic visit. Patients have stable kidney function with a median serum creatinine level of 1.25 mg/dL. Patients were screened monthly for BK viremia and 2 patients (9%) developed BK viremia without polyoma nephropathy.

Conclusions: We demonstrate that pretransplant immunologic risk assessment based on MFI values of Luminex single antigen beads and channel shift values of flow-cytometry cross-match and transplant with anti-thymocyte globulin and IVIG significantly decreases AR rates in kidney transplant recipients with DSAs.

SA-PO3063
The Effectiveness of Rituximab/Intravenous Immunoglobulin Therapy with Chronic Active Antibody Mediated Rejection in Renal Transplant Recipients Yu Ah Hong, Hyun Gyung Kim, In O Sun, Sun Ryong Choi, Hoon Suk Park, Byung Ha Chung, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. Nephrology, Internal Medicine, Seoul St. Mary’s Hospital, Seoul, Republic of Korea.

Background: Chronic active antibody mediated rejection (cAMR) is one of the causes of chronic allograft kidney dysfunction. Until now, there is no established treatment protocol of cAMR. We report on our experience of a combination of rituximab and intravenous immunoglobulin (IVIG) treatment in patients with cAMR.

Methods: Six renal transplant recipients who showed progressive deterioration of graft function were included. The diagnosis of cAMR was confirmed with graft biopsy based on Banff’05 classification. The patients received single dose of rituximab (375mg/m²), followed by intravenous immunoglobulin (0.4g/Kg) once daily for 4 days. Steroid pulse was given concurrently at first 3 days. Human leukocyte antigen-donor specific antibodies (DSAs) were detected by Luminex solid-phase assays before and after 2 weeks. The effect of a combination of rituximab and IVIG was assessed by graft function, amount of proteinuria, and titer of DSAs. The responder group was defined as glomerular filtration rate improved or stabilized after treatment.

Results: The response rate was 59% (3/6). GFR increased or maintained in three patients after Rituximab and IVIG treatment, but the patients did not respond to the therapy. The mean of responder group showed a marked reduction of proteinuria after treatment. But, amount of proteinuria increased in all three non-responders. Follow up donor specific antibodies after treatment were checked in two responder patients. DSA titer dropped significantly in response to the therapy in one patient, whereas unchanged donor specific antibody titer was observed the other patients.

Non-responders had the high degree of transplant glomerulopathy, severely deteriorated allograft function, and heavy proteinuria at the time of diagnosis of cAMR. They had a longer duration of post-transplant time in comparison with responder patients. The treatment regimen was well tolerated.

Conclusions: A combination of Rituximab and IVIG may be effective for the treatment of cAMR at earlier stage.

SA-PO3064
Treatment of Vascular Rejection with ATG by CD3 Monitored Dosing Is Safe and Effective in Renal Transplantation Scott R. Henderson, Lucy A. Galloway, Robert Vaughan, David S. Game. Nephrology & Transplantation, Guy’s Hospital, London, United Kingdom.

Background: Acute vascular rejection carries a poor prognosis for graft survival and proposed treatments have been associated with morbidity and mortality. Reports of ATG to treat vascular rejection are prior to the routine use of anti-CD25 antibody induction and include steroid resistant rejection. ATG dose guiding is most often performed according to total white cell count. We use ATG as first line treatment for Banff acute T cell mediated rejection type 2 and adjust dose according to CD3 count. This study reviews the efficacy and safety of this approach.

Methods: All patients treated at our centre with ATG between 2007 and 2009 were reviewed.

Results: 16 kidney transplant recipients and 4 simultaneous kidney-pancreas transplant recipients received ATG. 15 cases were classified as Banff 2A and 5 cases as 2B. Humoral component to the rejection was noted in 4/20. 17/20 patients received anti-CD25 antibody induction and all were receiving calcineurin inhibition, mycophenolate mofetil and prednisolone during acute rejection. 11/16 kidney transplant recipients had baseline immunosuppression switched from ciclosporin to tacrolimus; all other patients continued on tacrolimus. After an average of 642 days follow-up, 3 patients lost their grafts (average 8 months; 2/3 had persistent humoral component treated with plasma exchange and IVIG) and 1 patient died with a functioning graft. The mean creatinine increased for graft survival was 151 µmol/L (n=17, SEM 44), representing an average 37% improvement from pre-treatment creatinine in patients not requiring pre-treatment dialysis (n=15). Bacterial infection was uncommon (n=1) although 6 patients were treated empirically for bacterial chest infections.

CMV viremia was common (n=12) and often within 1 week (n=7). There were no reports of fungal infection or malignancy.

Conclusions: ATG treatment for Banff type 2 acute cellular rejection in the modern era of immunosuppression is effective and safe when dose adjusted for CD3 count. CMV viremia is common early after ATG treatment if a CMV surveillance strategy is used. A randomised controlled trial is required to further confirm the efficacy of this approach.

SA-PO3065

Background: It is not known if addition of Rituximab (Ritux) or Bortezomib (Bort) therapy (Rx) has added value in treating ABMR. We compared outcomes of ABMR in 26 renal allograft recipients treated with Ritux (n=11) or Bort (n=15) based Rx.

Methods: We reviewed for-cause renal allograft biopsies from 1/08 to 3/11. Inclusion criteria were ABMR by Banff’07, presence of DSA, and use of Bort or Ritux as a key component of Rx. Primary endpoint was response to Rx (return of Ccre to within 50% of baseline at 4 wks). Secondary endpoints were reduction in DSA, proteinuria, and graft survival.

Results: Table 1 shows baseline data and details of ABMR. Response to Rx at 6 mths was 54% vs. 71% in Ritux and Bort groups, respectively. Bort was associated with a greater reduction in Class I DSA and proteinuria in patients with >1g/day of protein at time of ABMR diagnosis (Fig 1). 6 mth graft survival was 73% & 93% in Ritux and Bort groups respectively.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
Post transplant days (median) | Ritux | Bort | P
---|---|---|---
Biopsy Creatinine (mg/dl) (mean±SD) | 1.5±2.2 | 3.92±2.8 | 0.94
Biopsy Protein (g/dl) (mean±SD) | 2.1±2.4 | 1.84±1.9 | 0.98
Rx IVIG + TPE | 73% | 100% | 0.06
Rx r-ATG | 35% | 73% | 0.68
Response to Therapy | 55% | 73% | 0.41
Post Rx Creatinine (3mths, mg/dl) (mean±SD) | 2.9±1.9 | 2.1±1.1 | 0.23
Reduction of Class I iDSA | 11.3±19.6% (n=3) | 83.0±21.9% (n=9) | 0.02
Reduction of Class II DNA | 14.2±15.0% (n=6) | 38.2±34.5% (n=10) | 0.43
Post Rx Proteinuria (3-6mths, g/day) (mean±SD) | 7.3±11.4 | 1.29±1.7 | 0.05
6 month graft survival | 73% | 92% | 0.28

Conclusions: Bort was associated with a higher rate of ABMR reversal and significantly greater reductions in DSA and proteinuria compared to Ritux. Validation of our observations favoring Bort over Ritux may help develop a standardized protocol for ABMR.

SA-PO3066

Treatment of Antibody Mediated Rejection in Kidney Transplantation: A Single Center Experience with a Bortezomib-Based Regimen


1Nephrology and Hypertension, Allegheny General Hospital; 2Abdominal Transplantation, Allegheny General Hospital, Pittsburgh, PA.

Background: Antibody mediated rejection (AMR) following kidney transplantation (KTx) responds poorly to conventional anti-rejection therapies. The proteasome inhibitor bortezomib has activity against mature plasma cells that produce damaging donor-specific antibodies (DSA). We present our experience of using a bortezomib-based regimen in patients with severe AMR.

Methods: A retrospective chart review was performed on patients with biopsy proven AMR after KTx at our institution over 12-months. Diagnosis of AMR was made on the basis of positive peritubular capillary C4d staining along with either histological evidence of acute rejection or positive DSA titers. Treatment for AMR included plasmapheresis (1-1.5 Volume), IVIG (cumulative dose of 1-2 g/kg), steroids, single dose rituximab (375 mg/m²) along with bortezomib (1.3 mg/m²) on days 1, 4, 8 and 11.

Results: AMR was diagnosed in 6 patients, aged 43±13 years, with PRA range 0-80%, and 4±1 HLA mismatches. There were 5 females, 2/6 had living donors, 5/6 had first KTx, 4/6 had alemtuzumab and 2/6 had rabbit-antithymocyte globulin induction; all were maintained on a tacrolimus/MMF/early steroid withdrawal protocol. Figure 1 shows serum creatinine and DSA titer trends. Five patients responded to treatment; one (#5) became dialysis dependent. Responders had stable kidney function during the follow-up (223±143 days) post-bortezomib therapy.

Conclusions: This series demonstrates effectiveness of a bortezomib-based regimen in achieving reduction of DSA titers and stabilizing allograft function in patients experiencing severe AMR following kidney transplantation.

SA-PO3067

Presence of Donor Specific Antibodies Increases the Risk of Antibody Mediated Rejection


Background: The introduction of solid phase methods provides increased sensitivity and specificity for the detection of HLA antibodies. The clinical impact of the detection of preformed donor specific antibodies (DSA) on early rejections is unclear.

Methods: We performed a retrospective review of all adult DDRT recipients at our center from 1/2009 to 12/2010. All patients had a negative CDC crossmatch at the time of transplantation and had solid phase assay measurement of HLA antibodies. A flow crossmatch was subsequently done if HLA antibodies (DSA) were detected by luminex. We identified 149 patients with a negative flow crossmatch, 26 had DSA by Luminex and received no additional treatment other than our standard induction regimen based on positive peritubular capillary C4d staining along with either histological evidence of acute rejection or positive DSA titers. Treatment for AMR included plasmapheresis (1-1.5 Volume), IVIG (cumulative dose of 1-2 g/kg), steroids, single dose rituximab (375 mg/m²) along with bortezomib (1.3 mg/m²) on days 1, 4, 8 and 11.

Results: In our cohort, patients with DSA were of similar age (53±13.8 v 55.8±14.1 yrs, p=0.35) and cold ischemia time (28.9±9.8 v 29.6±9.8, p=0.75) but were more frequently female (61.5 v 32.3%, p=0.005) and had a previous transplant (50 v 17%, p=0.001) than patients without DSA. Overall rejection rates (cellular and AMR) rates were higher in pts with DSA (50% v 22.6%, p=0.004) as were rates of antibody mediated rejection (23.1 v 6.5%, p=0.004). Additionally, the lowest MFI of preformed DSA among pts experiencing AMR was 6200.
SA-PO3069
Correlation of Pretransplant Trough Tacrolimus Level with Early Acute Rejection in Live Donor Renal Transplantation

Background: The risk of acute rejection is greater in the first week post transplant and progressively decreases after the first month. Thus, the concentration of immunosuppression must be maximal initially and tapered during subsequent months. The information of pretransplant administration of immunosuppression and acute rejection is minimal. So we aimed the present study to assess the correlation of baseline pretransplant trough tacrolimus level with early rejection.

Methods: We prospectively analyzed the trough tacrolimus level on pretransplant day of 179 patients transplanted from September 2007 to September 2009. We divided them into three groups according to the trough levels: Group I - < 5 ng/ml (n=34), Group II - 5-15 ng/ml(n=112) and Group III - 15ng/ml(n=33). Their demography, rejections, NOD, infections and biopsy proven CNI toxicity were studied.

Results: Their demography was comparable. Incidence of biopsy proven acute rejections were highest in the Group I and lowest in the Group III. Incidence of post transplant infection, new onset diabetes were comparable. Incidence of biopsy proven CNI toxicity was higher from group I to group III.

Table.1 Results

<table>
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<th>Parameters</th>
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<th>Group II (Tac Level 5-15)</th>
<th>Group III (Tac Level &gt;15)</th>
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<td>35 (31.2%)</td>
<td>17 (51.9%)</td>
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<tr>
<td>Anti proliferative</td>
<td>20.14</td>
<td>50.62</td>
<td>11.22</td>
</tr>
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<td>Biopsy proven CNI toxicity</td>
<td>25.8%</td>
<td>9.0%</td>
<td>5.15%</td>
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<tr>
<td>New Onset Diabetes</td>
<td>17 (50%)</td>
<td>42 (37.5%)</td>
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<tr>
<td>Rejection Episodes</td>
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<td>27 (24.1%)</td>
<td>9 (31.0%)</td>
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<tr>
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<tr>
<td>Post Tx Infections</td>
<td>12 (35.3%)</td>
<td>33 (29.5%)</td>
<td>15 (45.4%)</td>
</tr>
<tr>
<td>Graft Survival % (1 year)</td>
<td>97.1</td>
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<td>100</td>
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</table>

Tac level - Tacrolimus trough level expressed in ng/ml, CNI - calcineurin inhibitor

Conclusions: Incidences as well as severity of early rejection reduces as the pretransplant trough level increases. We did not encounter any higher grade TIR or antibody mediated rejection when trough level was < 15 ng/ml.

SA-PO3070
Assessment of Renal Allograft Fibrosis by Transient Elastography: Possibilities and Limitations
Claudia Sommerer, Michael Scharf, Christoph Seitz, Gunda Millonig, Sebastian Mueller, Martin G. Zeier. Nephrology, University Hospital Heidelberg, Heidelberg, Germany; 2Internal Medicine, Salem Medical Center, Heidelberg, Germany.

Background: Chronic allograft dysfunction remains the major reason for late allograft loss. To date renal biopsy has been the only option for an early diagnosis. Throughout the last couple of years Transient Elastography (TE) has become a valid non-invasive alternative for diagnosing hepatic fibrosis. The purpose of the present study was to evaluate the possibility of identifying renal allograft fibrosis by using TE as well as analyzing its inherent limitations.

Methods: The tissue stiffness of 164 patients with renal allograft was measured twice, at the pole and the pars media of the allograft, using TE (FibroScan®). Clinical and anatomical data were collected for each patient.

Results: The measurement was successful in 310 out of 326 cases (94.5%). The mean elasticity was 34.8±19.6 kPa and 33.6±19.1 kPa for the pole and pars media respectively. There was no evidence for a correlation between allograft elasticity and renal function (estimated glomerular filtration rate: r=0.02; p=0.76 and r=0.03; p=0.68). Body-mass-index (BMI) and distance between skin and allograft have an impact on the success rate of the examination (BMI: r=-0.31; p<0.001 and r=-0.27; p=0.001; distance: r=-0.50; p<0.001 and r=-0.56; p=0.001). Sonographic proof of peri- or intrarenal accumulated fluid (cysts, lymphocele) was accompanied by a drop in the success rate of 12.2% at the pole and 17.3% at the pars media.

Conclusions: Altogether using TE for the examination of renal allografts might be possible. Taking into account certain confounders, TE gains clinical significance. It is a valuable tool for a broader application, however, is the implementation of several technical modifications.

SA-PO3086
Neutrophil Gelatinase-Associated Lipocalin Is Most Sensitive to the Acute Rejection after Living Donated Kidney Transplantation
Junko Kohri, Kosaku Nitta, Ken Tsuchiya, Takumi Yoshida, Michihiro Mitobe, Hidekazu Sugiuira, Shunji Shiohira. Fourth Department of Internal Medicine, Kidney Center, Shinjuku, Tokyo, Japan.

Background: Delayed graft function (DGF) after kidney transplantation is difficult to diagnose with only serum creatinine, therefore using of the biomarker is expected as auxiliary diagnosis. Identification and validation of biomarkers of kidney injury has currently reported in this area. We hypothesized that these markers predicted rejection after living donated kidney transplantation.

Methods: This study was prospective, single-center study of living-donor kidney transplant patients to evaluate urinary NGAL, L-FABP and IL-18 including consecutive 48 (male=32) patients. We collected serial urine samples of 3 days after transplantation and analyzed using an immunoassay (ARCHITECT) for NGAL and standard commercial ELISA kits for LFABP and IL18. For diagnostic sensitivity of these biomarkers, receiver operating characteristic curve (ROC) was plotted and area under the curve (AUC) was calculated to quantify the accuracy of the parameter.

Results: Ten cases were clinically diagnosed or noted by biopsy as acute rejection and specifically a higher risk of antibody mediated rejections in patients with preformed DSA.

Conclusions: Our center data demonstrates a higher overall risk of rejections and specifically a higher risk of antibody mediated rejections in patients with preformed DSA.

SA-PO3097
Correlation of Pretransplant Trough Tacrolimus Level with Early Acute Rejection in Live Donor Renal Transplantation

Background: The risk of acute rejection is greater in the first week post transplant and progressively decreases after the first month. Thus, the concentration of immunosuppression must be maximal initially and tapered during subsequent months. The information of pretransplant administration of immunosuppression and acute rejection is minimal. So we aimed the present study to assess the correlation of baseline pretransplant trough tacrolimus level with early rejection.

Methods: We prospectively analyzed the trough tacrolimus level on pretransplant day of 179 patients transplanted from September 2007 to September 2009. We divided them into three groups according to the trough levels: Group I - < 5 ng/ml (n=34), Group II - 5-15 ng/ml(n=112) and Group III - 15ng/ml(n=33). Their demography, rejections, NOD, infections and biopsy proven CNI toxicity were studied.

Results: Their demography was comparable. Incidence of biopsy proven acute rejections were highest in the Group I and lowest in the Group III. Incidence of post transplant infection, new onset diabetes were comparable. Incidence of biopsy proven CNI toxicity was higher from group I to group III.

Table.1 Results

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Tac Level &lt; 5)</th>
<th>Group II (Tac Level 5-15)</th>
<th>Group III (Tac Level &gt;15)</th>
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</thead>
<tbody>
<tr>
<td>Mean Age (years)</td>
<td>49.06±10.15</td>
<td>37.38±9.6</td>
<td>49.2±10.2</td>
</tr>
<tr>
<td>Gender Male: Female</td>
<td>24:10</td>
<td>94:18</td>
<td>27:6</td>
</tr>
<tr>
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<td>22</td>
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<tr>
<td>Relation: Other  related</td>
<td>6</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td>Donor Age</td>
<td>49.05±10.15</td>
<td>47.7±10.14</td>
<td>46.42±10.2</td>
</tr>
<tr>
<td>Induction</td>
<td>15 (44.1%)</td>
<td>35 (31.2%)</td>
<td>17 (51.9%)</td>
</tr>
<tr>
<td>Anti proliferative</td>
<td>20.14</td>
<td>50.62</td>
<td>11.22</td>
</tr>
<tr>
<td>Biopsy proven CNI toxicity</td>
<td>25.8%</td>
<td>9.0%</td>
<td>5.15%</td>
</tr>
<tr>
<td>New Onset Diabetes</td>
<td>17 (50%)</td>
<td>42 (37.5%)</td>
<td>14 (42.4%)</td>
</tr>
<tr>
<td>Rejection Episodes</td>
<td>12 (35.3%)</td>
<td>27 (24.1%)</td>
<td>9 (31.0%)</td>
</tr>
<tr>
<td>Banff &lt;2</td>
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<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Post Tx Infections</td>
<td>12 (35.3%)</td>
<td>33 (29.5%)</td>
<td>15 (45.4%)</td>
</tr>
<tr>
<td>Graft Survival % (1 year)</td>
<td>97.1</td>
<td>98.2</td>
<td>100</td>
</tr>
</tbody>
</table>

Tac level - Tacrolimus trough level expressed in ng/ml, CNI - calcineurin inhibitor

Conclusions: Incidences as well as severity of early rejection reduces as the pretransplant trough level increases. We did not encounter any higher grade TIR or antibody mediated rejection when trough level was < 15 ng/ml.

Funding: Pharmaceutical Company Support
SA-PO3071

Acute Effects of Cyclosporine A on Kidney Allograft Microperfusion Visualized by Contrast-Enhanced Sonography


Background: Cyclosporine A (CsA) induces detrimental vascular remodeling, which is a leading cause of chronic allograft failure. Real-time contrast-enhanced sonography (CES) is a relatively new technique in providing quantitative information on microvascular tissue perfusion in kidney allografts in more detail. The purpose of the study is to assess acute effects of CsA to kidney allograft perfusion.

Methods: In an explorative single-center clinical trial, renal parenchymal tissue perfusion of 17 stable kidney allograft recipients was evaluated with CES prior to and two hours after the intake of CsA. In addition to laboratory and clinical parameters, Doppler indices and estimated glomerular filtration rate were measured.

Results: Systolic and diastolic blood pressure and color Doppler indices (RI and PI) did not significantly differ prior to and after the administration of CsA. However, there was a significant decrease of renal blood flow two hours after the intake of CsA compared to baseline. Kidney allograft microperfusion was reduced by 5.27 ± 4.08 dB/s.

Furthermore, there was a significant correlation between renal blood flow obtained prior to CsA administration and kidney function.

Conclusions: Acute effects of CsA on kidney microcirculation were visualized by CES revealed a 54% reduction 2 h after the intake of CsA. Kidney allograft perfusion correlates with kidney allograft function.

SA-PO3072

Combination of Blood Oxygen Level-Dependent Magnetic Resonance Imaging and Doppler Ultrasonography in Prediction of Acute Renal Rejection

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Background: Our previous study has shown that blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI) could discriminate between acute rejection (AR) and acute tubular necrosis. Other studies have demonstrated that Doppler ultrasonography could predict renal allograft outcome. This study aims to establish a mathematic model for predicting AR through the analysis of the data obtained from the imaging modalities.

Methods: 103 patients took BOLD MRI and Doppler ultrasound within 6 months after transplantation. 82 patients with normal functioning grafts took the examinations within 3 weeks post-surgery. 21 patients with biopsy-proven AR took the examination within 6 days before or after biopsy. Four parameters were recorded: medullary R2* value (MR2*), cortical R2* value (CR2*), segmental arterial resistance index (RI) and segmental arterial pulsatility index (PI). Support Vector Machine (SVM) was applied to separating the 4-dimensional data set (CR2*, MR2*, RI and PI) into two categories, AR and normal. A SVM model was tuned with 3-fold cross validation, the parameters were regularized to calibrate the classification and improve the training accuracy, which is the ratio of number of correct classification/total number of training samples.

Results: After performing the 3-fold cross validation, the average SVM accuracy on training data was 100%, which means the sensitivity and specificity are 100%, and the average training accuracy (MR2*+CR2*, MR2*+RI, MR2*+PI, CR2*+RI, CR2*+PI, RI+PI) was respectively 74%, 99.10%, 81.60%, 79.10%, 81.60%. After training the SVM model, a kernel function was produced for prediction. Due to the over-fitting of the classifier, the average test accuracy (the ratio of number of correct classification using the trained kernel function/total number of test samples) for four parameters is not high enough (around 65%) for AR.

Conclusions: The combination of BOLD-MRI and Doppler ultrasonography could be useful for the prediction of AR posttransplant by the SVM model and kernel method; larger sample should be tested to verify the accuracy, sensitivity and specificity of this mathematic model.

SA-PO3073

Clinical Usefulness of 3-Dimensional Computed Tomography Angiography for Detection of Transplant Renal Artery Stenosis

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Background: The aim of this study is to evaluate whether 3-Dimension computed tomography angiography (3D-CTA) is useful to detect transplant renal artery stenosis (TRAS).

Methods: In our transplant center, 9 of 514 (1.8 %) renal transplant recipients were diagnosed with TRAS according to renal angiography from 2004 to 2010. These patients received color doppler ultrasonography (CDU) and 3D-CTA ahead of renal angiography. A usefulness of 3D-CTA was compared to CDU on the basis of angiography. To investigate the safety of 3D-CTA, estimated GFR (eGFR) were measured before and after the 3D-CTA examinations.

Results: The patients included six men and three women, 27-56 years old (mean age, 43). The TRAS occurred from 2.6 to 24 months after transplantation (average 9.6 months). The median eGFR was 72.4 ml/min/1.73m2 (range, 57-92). Three out of the nine patients diagnosed with TRAS were not detected by CDU. Two of them had TRAS with end-to-side (ES) arterial anastomosis, in which it can be difficult to detect the stenosis by CDU compared with the cases of EE. That is because the PSV at the stenosis in EE could be lower than that in ES due to anatomic and hemodynamic differences. However, 3D-CTA detected the significant stenosis in all patients with TRAS. Moreover, the stenotic area in 3D-CTA was similar to that of renal angiography (69±5.6 vs 69.5±6.5, p=0.88). There was no difference in eGFR before and after 3D-CTA examinations (77±13.2 vs 72±14.1 ml/min/1.73 m2, p = 0.54). All nine patients with TRAS were treated successfully by percutaneous transluminal renal angioplasty (PTA). After PTA, allograft function (72 ± 14.1 vs. 80 ± 11.0 ml/min/1.73 m2, p = 0.028) and hypertension (mean arterial pressure, 111 ± 10.1 vs 98± 7.0, p = 0.011) improved.

Conclusions: The 3-DCTA is effective and safe method to screen the renal artery stenosis in renal transplant recipients with eGFR > 60 ml/min/1.73 m2.

SA-PO3074

Combined Effect of Recipient’s and Donor’s Matrix Metalloproteinase Gene Polymorphisms on the Process of Kidney Aging after Kidney Transplantation

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Background: The process of kidney aging is different in each individual, but the factors in the variation are largely unknown. Matrix metallopeptidase (MMP) 7 and 20 genes are associated with kidney aging, and thus we assessed the correlation between the process of kidney aging after kidney transplantation and MMP 7 and 20 gene expression in the patients.

Methods: The transplant outcomes were assessed according to the number of A alleles combining those from both the recipient and the donor: the high-number group of A alleles (≥ 3) and the low-number group (< 3).

Results: During the follow-up period (mean 92.3 months), the high-number group of MMP 7 had a lower risk of chronic tubulointerstitial lesion than the low-number group (P = 0.015), while the high-number group of MMP 20 had greater risks of chronic tubulointerstitial lesion (P = 0.055) and glomerulonephritis (P = 0.025) than the low-number group. However, in the long-term (after 9 years of transplantation), the high-number group of MMP 20 had a greater transplant function (eGFR) than the low-number group. The high-number groups of MMP 20 showed a trend toward better graft survival rate than the low-number groups, especially when we analyzed the recipients who were followed for more than 1 year (P = 0.037).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
**SA-PO3075**

Genetic Polymorphisms of Interleukines Associated with PTDM in Korean Renal Allograft Recipients

**Background:** Posttransplantation diabetes mellitus (PTDM) is a serious metabolic complication after renal transplantation. Although β-cell dysfunction is considered the main contributing factor for the development of PTDM, precise pathogenesis was not identified. There are several studies about various cytokines that induce inflammation and destruction of islet beta cells in diabetes mellitus. But there is rare study about the cytokines associated with β-cell dysfunction in PTDM. So, we examined the association between PTDM and 18 single nucleotide polymorphisms (SNPs) located within the genes of 10 interleukines or their receptors, which were related to diabetes mellitus in Korean renal allograft recipients.

**Methods:** A total of 305 renal transplant recipients between 2000 and 2009 were included at 3 transplant centers, without a history of diabetes. We analyzed the association between the PTDM development and the 18 IL genes.

**Results:** The prevalence of PTDM was 18% (52/305 patients). Patients with PTDM group were older than those in non-PTDM (44.91±1.33 vs 38.34±0.71, p<0.001). Ten SNPs in five genes were significantly associated with PTDM development after adjusting with age, sex: IL1B (rs3136558), IL4 (rs2243250, rs2070874), IL7R (rs2229151, rs4819554), IL17R (rs1043261, rs1025689, rs3733075). Among haplotypes, the frequency of GGT in IL7R gene and TT in IL2 gene were significantly different between the patients with PTDM and those without PTDM. (p=0.044, 0.041, respectively)

**Conclusions:** These data suggest that genomic variations in IL1B, IL4, IL7R, IL17R and IL1B, IL7R are significantly associated with PTDM in Korea. Especially, significant variations of IL7R and IL17R, which was recently reported to be associated with type 1 DM, could elucidate the pathogenesis of PTDM in renal transplant recipients.

**SA-PO3076**

Significant Associations between Angiotensin Converting Enzyme and Angiotensinogen Genes Polymorphisms and Post Transplantation Diabetes Mellitus in Renal Allograft Recipients

**Background:** Post-transplant diabetes mellitus (PTDM) is a frequent and serious complication of renal transplantation. Genetic polymorphisms of the angiotensin-converting enzyme (ACE) and angiotensinogen (AGT) genes have been reported to be related to diabetes mellitus and insulin sensitivity. But the role of these genes on the development of PTDM is not known. For this purpose, we investigated the association of polymorphisms in the genes for ACE and AGT genes with development of PTDM in Korean patients who had undergone renal transplants.

**Methods:** A total of 312 patients who had received kidney transplants without a prior history of diabetes were included. One ACE SNP (rs4291 in promoter –262) and two AGT SNPs (rs699 in intron_1 and rs4762 in intron 2) were genotyped from genomic DNA with direct sequencing.

**Results:** Of 312 patients, PTDM developed in 56 patients (17.9%). Patients in the PTDM group were older than those in the non-PTDM group (43.18±9.51years vs. 36.75±11.58). There were significant difference between two subjects in the percentage of tacrolimus use (n=18 vs. n=76; p=0.01).

Of three SNPs, the rs699 in intron_1 of the AGT gene was significantly associated with the development of PTDM in the codominant 1 (p = 0.009) and dominant models (p = 0.02).

In haplotypes of the rs699 in intron 1 of AGT gene, the frequency of the TCA haplotype was significantly higher in patients with PTDM than in those without PTDM.

**Conclusions:** AGT gene rs699 polymorphisms may act as genetic markers for the development of PTDM. Angiotensigenon may help identify patients who are at risk for PTDM. The exact molecular mechanisms still need to be clarified.

**SA-PO3077**

Concurrent Peritubular Capillary C4d Staining and BK Virus Nephropathy: Long-Term Outcomes

**Background:** BK virus nephropathy (BKVN) results from over-immunosuppression while peritubular capillary deposition of C4d is associated with under-immunosuppression. There is a paucity of evidence on long-term outcomes in patients who present with concurrent BKVN and C4d staining.

**Methods:** In a retrospective analyses of 69 consecutive kidney allograft recipients with biopsy confirmed BKVN between 2005 and 2011, we examined patient and graft outcomes in the presence or absence of peritubular capillary C4d staining (focal or diffuse). BKVN alone was treated with a reduction in immunosuppression + cidofovir and IVIG while combined BKVN and C4d staining was treated with a reduction in immunosuppression + IVIG + plasma exchange.

**Results:** There were 57 (82.6%) patients with BKVN alone and 12 (17.3%) patients with concurrent BKVN and focal or diffuse C4d staining.

The majority of patients were male (76%). Mean age at biopsy was 46.8 years. Mean follow-up after biopsy was 5.6 years. There were 6 deaths (8.7%) and 17 graft losses (24.0%). These differences were not statistically significant between the 2 groups. Similarly, there was no significant difference between 5 year levels at biopsy and last follow up suggesting that concurrent C4d staining in patients with BKVN is not associated with worse outcomes.
SA-PO3078

Urinary Haufen Testing for Diagnosing Polyomavirus Nephropathy & Assessing Intrarenal Viral Load Levels: Comparative Analysis with Current Screening Tests

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Background: PVN, a common complication post-renal-transplant, has an incidence of 4% (BANFF-PVN working group). Early non-invasive diagnostic facilitates patient management and graft survival. We developed a new qualitative test to detect three-dimensional viral casts, called “Haufen”, in voided urine samples to accurately diagnose PVN and previously reported that urinary Haufen shedding is tightly correlated with PVN (positive and negative predictive values >95%). Hypothesis: The degree of urine Haufen shedding most accurately reflects extent of intrarenal PVN and can help distinguish PVN disease stage A (early) from stages B and C.

Methods: Quantitative analysis of urine Haufen shedding (#Haufen/mL urine), quantitative PCR assays on serum/urine, and urine decy cells on 40 samples from 31 patients with concurrent biopsy proven PVN. Comparative analysis with biopsy findings (PVN disease stage, cells/tubes expressing viruses (manual & morphometric counts, i.e. SV40-1, capsid proteins). Statistical analysis using Kruskal Wallis testing with ties.

Results: A) PVN disease stage: Only Haufen shedding showed a statistically significant difference between PVN stage A versus B or C (p<0.0001). Neither degree of viremia or viruria predicted any disease stage. B) Degree of intrarenal polyomavirus replication: Only Haufen shedding accurately reflected extent of polyomavirus replication in renal tubules, i.e. correlated with number of virus expressing cells or tubules. No significant correlation was found between degree of viremia and viruria.

Conclusions: Compared to other common laboratory tests, only quantification of urinary Haufen shedding is an accurate reflection of the intrarenal burden of polyomavirus and can help predict PVN disease stages. This observation makes “Haufen-testing” the most accurate non-invasive technique not only to initially diagnose PVN but to also monitor therapeutic effects during follow-up.

SA-PO3079

Center-Specific Plasma BK Virus Levels in the Presumptive Diagnosis of BK Virus Allograft Nephropathy

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Background: The KDIGO clinical practice guidelines for the care of kidney transplant recipients suggest a threshold plasma BK virus (BKV) PCR level of >10,000 copies/ml for presumptive diagnosis of BKV allograft nephropathy (BKVAN). Determination of BK viremia by real time PCR varies with the methodology, the target gene (VP-1, 2, 3, or large T antigen) and the primers used. We evaluated the performance of an in-house developed PCR assay in the diagnosis of BKVAN.

Methods: All transplant recipients with renal allograft biopsies and plasma BK virus PCR measurements during 2010 were evaluated. Viremia was measured using a primer set against the large T antigen. BKVAN was diagnosed with cytopathic changes and positive SV40 immunohistochemical stain. The sensitivity, specificity, positive and negative predictive values, positive likelihood ratio, and ROC curve were computed.

Results: Two hundred eighteen transplant recipients were evaluated: 39 had BK viremia and 18 were diagnosed with BKVAN. The performance characteristics are shown in Table.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Table: Performance Characteristics

<table>
<thead>
<tr>
<th>Plasma BKV (copies/ml)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Likelihood ratio</th>
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<tr>
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</table>

The ROC analysis is depicted in Figure.

Compared to a plasma BKV PCR of 10,000 copies/ml, values between 50,000 and 150,000 copies/ml were associated with better positive predictive value and positive likelihood ratio.

Conclusions: The plasma BKV PCR levels for presumptive diagnosis of BKVAN should be validated for each individual assay. Nephrologists caring for transplant recipients should be aware of the differences in the performance of BKV PCR assays to optimize management.

Funding: NIDDK Support

SA-PO3080

Impact of Polyoma Virus in Blood Only Taken at Week 6 after Kidney Transplantation on Inflammation and Fibrosis/Athropy in Biopsies Taken at Week 52

Willy Aasebo, Akershus University Hospital.

Background: Polyoma virus associated nephropathy (PVANI) is associated with graft loss due to fibrosis in kidney transplant recipients and is defined as the presents of BK virus (BKV) in biopsies. The aim of this study is to assess if early presence of BK in blood only, PVAN excluded, affects histological markers of inflammation and fibrosis after one year.

Methods: Protocol biopsies were taken at weeks 6 and 52 in patients transplanted during 2009. BKV-PCR was measured in weeks 10 and 52. Biopsies were stained for BKv if BKv-PCR in blood was positive, or if any histological or clinical suspicion of PVAN were present. Recipients with PVAN at week 6 were excluded.

Immunosuppressive treatment consisted of induction therapy with Basiliximab (two doses), thereafter calcineunone inhibitor, mycophenolate and steroids.

Results: After excluding 3 patients with PVAN at week 6, 156 patients were included.

A) At week 6 BKV were positive in 14 patients (9%), of whom 7 (50%) had BKV-PCR >5 x 10^4 copies/ml (median: 1.8 x 10^4 copies/ml).

At one year 12 recipients had positive BKV (8%), of whom 6 (50%) had >5 x 10^4 copies/ml.

Four patients were BKV- positive both at week 6 and week 52.

Conclusions: Compared to other common laboratory tests, only quantitation of urinary Haufen shedding is an accurate reflection of the intrarenal burden of polyomavirus and can help predict PVN disease stages. This observation makes “Haufen-testing” the most accurate non-invasive technique not only to initially diagnose PVN but to also monitor therapeutic effects during follow-up.

SA-PO3081

Incidence of BK Virus Infection in Renal Transplant Recipients Induced with Alemtuzumab Versus Anti-Thymocyte Globulin

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Background: BK virus associated nephropathy (BKVAN) is a major cause of graft failure. Treatment with lymphocyte depleting agents has been associated with increased incidence of BKVAN. Our primary aim was to compare the incidence of BK virus infection in renal transplant recipients based on the type of initial induction agent.

Methods: In a retrospective case-control study, 99 patients who underwent kidney alone transplant at our center between 8/2006 and 6/2010 were selected. We compared the incidence of BK virus infection in the first year among the alemtuzumab (AL) (n=50) and anti-thymocyte globulin (ATG) groups (n=49). Maintenance immunosuppression for the AL group was steroid-free; for the ATG group, a 6-month steroid taper was used. Both groups also received tacrolimus and mycophenolate mofetil.

Results: In the AL group, 19 (38%) had living donors, 37 (74%) were Caucasian, 27 (54%) were male and age range was 53.6 ± 17.5. In the ATG group, 23 (46.9%) had living donors, 40 (81.6%) were Caucasian, 34 (69.3%) were male and age range was 49.4 ± 16.1.

The incidence of elevated BK PCR (>4000 copies) within the first year was not statistically different between the AL (n=12) and ATG (n=10) groups. Biopsy proven BKVAN was also not significantly different (AL: 2 patients; ATG: 4 patients). The incidence of biopsy proven acute rejection (AR) was not statistically different between the groups (AL: 21 episodes in 16 patients; ATG: 26 episodes in 18 patients). However, the median time of onset of AR was significantly delayed in the AL group compared with the ATG group (157: 125 days vs. 73 ± 82 days; p=0.01).

Conclusions: Incidence of BKVAN and elevation of BK PCR is similar in induction with alemtuzumab and ATG. The incidence of acute rejection is similar between the two groups, but occurs later with alemtuzumab induction.
SA-PO3082

Successful Evolution of BK Virus Nephropathy Treated with Everolimus-Based Immunosuppression


Background: The incidence of renal allograft dysfunction due to BK virus nephropathy (BKVN) has increased over the last decade. Over the last few years mTOR-inhibitors (mTOR-i) have gained strength as a plausible treatment option. Based on this data, we initiated in 2007 an anticalcinieurin-free regime based on Everolimus (EVE) conversion protocol in patients with BKVN. Aims of the study are to evaluate renal allograft survival and BK viral load after conversion.

Methods: Diagnostic criteria for BKVN were: biopsy proven infection and/or positive serum viral load ≥ 10000 copies demonstrated by PCR on at least two consecutive measurements. Of the 647 renal transplants done in our center between 2007 and 2010, 13 cases of BKVN were diagnosed.

Results: Of the 13 BKVN diagnosed cases, 8 (4m/4f, aged 34.7 ± 13.3 years) were converted to EVE-based regime, being the regime excluded because of proteinuria > 0.8 g/d. Follow up was 25 ± 7 months. Maintenance immunosuppressive therapy consisted of tacrolimus (T), mycophenolate mofetil (MMF) and corticosteroids. Post-transplant renal function was optimal achieving a serum creatinine (sCr) of 1.16 ± 0.2 mg/dl. Evolution is shown in the figure.

Conclusions: BKVN treatment is still controversial. Our data suggest that treatment with mTOR-i could provide additional benefits in selected patients, preserving renal allograft function and lowering BK viral load.

SA-PO3083

Obstructive Uropathy May Be A Risk Factor for Polyomavirus Infection in Pediatric Kidney Transplant Recipients

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Background: Polyomavirus—associated nephropathy (BKVN) is recognized increasingly as a disease that may lead to progressive allograft dysfunction in pediatric kidney transplant (KTX) recipients. Published pediatric surveys reveal an incidence of BK-viruria from 18%–33%, BK-viremia 6%-16%, and BKVN 2%-8%. The purpose of the study is identify patients with a KTX who have BK viruria, and evaluate for predictive risk factors for progression to BK viremia.

Methods: Retrospective chart review was performed of pediatric KTX recipients from January 2005 to December 2009. Analysis used unpaired t-test for normally distributed variables. Tacrolimus (T), mycophenolate mofetil (MMF) and corticosteroids. Post-transplant renal function was optimal achieving a serum creatinine (sCr) of 1.16±0.2 mg/dl. Evolution is shown in the figure.

Conclusions: BKVN treatment is still controversial. Our data suggest that treatment with mTOR-i could provide additional benefits in selected patients, preserving renal allograft function and lowering BK viral load.

SA-PO3084

Risk Stratification To Improve Screening for BK Viraemia and Nephropathy in Kidney Transplant Recipients

Vivian W. Yiu, Rui Gao, Miriam Rose Berry, Yisu Yisu Gu, Afzal N. Chaudhry, Sharon Mulroy. Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.

Background: BK viruria occurs in 10-15% patients after renal transplantation. It can lead to graft dysfunction and loss, hence an effective screening programme can lead to early treatment and better outcomes. A screening protocol was introduced at our hospital in 2009 and we report on a risk stratification strategy developed after an audit of compliance.

Methods: A database was set up to collect data on all patients receiving a kidney transplant or simultaneous kidney-pancreas (SPK) transplant from May 2009 to January 2011. Data were collected from electronic and paper records.

Results: Acute rejection is the most predictive factor for developing subsequent BK viraemia, with a relative risk (RR) of 2.76. There was no significant association between the severity of the histological grade of acute rejection with BK viremia. Other significant risk factors include Campath induction (RR= 2.30), age ≥40 years (RR= 2.19), male gender (RR= 1.73), the use of Mycophenolate mofetil (MMF) in immunosuppression regimes (RR= 1.53), a deceased donor (RR= 1.41) and receiving a SPK transplant (RR= 1.35). Factors associated with lower risk of viraemia include Basiliximab induction (RR=0.43), the incidence of CMV viremia (RR=0.65), use of Azathioprine (RR= 0.6) and female gender (RR=0.58).

Conclusions: Patients with more risk factors for BK viremia should be screened more intensely in order to identify viraemia early and allow prompt treatment. SPK patients (receiving Campath and MMF) and those treated for acute rejection should prompt more frequent screening.

We propose to develop a scoring system based upon these risk factors to streamline the screening protocol and target monitoring more effectively.

SA-PO3085

Factors Influencing the Course of Polyoma BK Virus Nephropathy after Renal Transplantation

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Background: The course of polyoma BK virus nephropathy (BKVN) is still difficult to predict.

Methods: We evaluated factors influencing renal function and viral clearing during BKV infection.

Results: BKVN was diagnosed in 46 patients since 2008 by routinely done quantitative PCR (qPCR) and SV40 staining in all biopsies (done by protocol 6/12/26 weeks post-transplantation and for cause at any time). Either immunosuppression was generally reduced (calcinuric inhibitor=–CNI by 30%, mycophenolate-mofetil=MMF by 30 to 50%, n=23), or CNI was switched primarily to mTOR inhibitor (n=7), or CNI was switched to mTOR inhibitor as a secondary step in case of delayed viral clearing (n=16). The influence of dichotomous variables on the response variables eGFR (≥0 or <0) and the time in weeks for qPCR reduction by 1 log (≤3 or >13 weeks) were measured as well as that of continuous variables. Tacrolimus (65%) and cyclosporine treatment (35%) always was combined with MMF and steroids. - 15% of cases suffered graft failure after 30/13 weeks. eGFR was stable or increased in 63% and decreased in 37%. BKV viral clearing was fast in 54% (reduction by 1 log ≤13 weeks) and slow in 46% (>13 weeks). Fast viral clearing was associated with stable or increasing eGFR (84%) compared to slow viral clearing (33%; p=0.001). Univariate logistic regression revealed that factors with a negative influence on renal function and viral reduction time were maximal viral load, tacrolimus treatment and late diagnosis by biopsy for cause. Using multiple logistic regressions, maximal viral load was the most important factor. In Cox regression analysis defining viral reduction by 1 log as event, tacrolimus compared to cyclosporine had a delaying influence on viral clearance.

Conclusions: Patients with more risk factors for BK viremia should be screened more intensely in order to identify viraemia early and allow prompt treatment. SPK patients (receiving Campath and MMF) and those treated for acute rejection should prompt more frequent screening. We propose to develop a scoring system based upon these risk factors to streamline the screening protocol and target monitoring more effectively.
Polyoma Virus in Patients Admitted for Indication Biopsy Long Time after Kidney Transplantation

**Background:** Once Polyoma virus associated nephropathy (PVAN) is established long-term graft outcome is poor due to development of graft fibrosis. PVAN is probably related to use and dosages of immunsuppressive drugs. The aim of this report is to find out how Polyoma virus affects kidney grafts in patients that were transplanted several years ago and were using maintenance immunsuppressive treatment (low or standard dosages).

**Methods:** All kidney recipients that were transplanted >12 months before they were admitted to indication biopsies during the years 2008, 2009 and 2010 were included. Polyoma virus affects kidney grafts in patients that were transplanted several years ago and were using maintenance immunsuppressive treatment (low or standard dosages). The aim of this report is to find out how Polyoma virus affects kidney grafts in patients that were transplanted several years ago and were using maintenance immunsuppressive treatment (low or standard dosages).

**Results:** A total of 308 kidney transplant recipients were included. Median time after transplantation was 66 months (range: 13 - 415 months).

**Conclusions:** We have identified that a high 25-Vid D level is an independent risk factor for BKV whereas a low 1,25(OH)2 Vit D level is an independent risk factor for AR.

SA-PO3087

**A Vitamin D Metabolism Signature Predictive of BK Viremia and Acute Rejection: Implications for Vitamin D Repletion**

**John R. Lee, Thangamani Sathiamurthi, Nephrology and Hypertension, Weill Cornell Medical College, New York, NY.**

**Background:** Vitamin D may play a crucial role in the regulation of the immune system. In this study, we examined whether levels of 25-hydroxyvitamin D (25-Vid D) or 25-dihydroxyvitamin D (1,25(OH)2 Vit D) are associated with allograft dysfunction, BK virus (BKv), or acute rejection (AR).

**Methods:** We identified 171 renal transplant recipients who had both 25-Vid D and 1,25(OH)2 Vit D measured within the first 3 months of transplantation. We examined whether levels of 25-Vid D or 1,25(OH)2 Vit D were associated with serum creatinine (Cr), or AR, with or without BKV during 24 month follow up.

**Results:** The high 25-Vid D group (≥35 ng/ml;n=26) was associated with significantly worse allograft function at 24 months than the low 25-Vid D group (<35 ng/ml;n=143)(Cr 2.10 mg/dL vs. 1.59 mg/dL,p=0.049). As a possible explanation, the high 25-Vid D group was associated with more BKV than the low 25-Vid D group (30.8% vs. 11.7%; p=0.029). Cox Proportion Hazard Model revealed a high 25-Vid D level as an independent risk factor for BKV (HR=1.64, p=0.019)(Fig 1A). Interestingly, treatment with a 25-Vid D analog was an independent risk factor for BKV (HR=11.4, p=0.019).

**Conclusions:** Vitamin D may play a crucial role in the regulation of the immune system. In this study, we examined whether levels of 25-hydroxyvitamin D (25-Vid D) or 25-dihydroxyvitamin D (1,25(OH)2 Vit D) are associated with allograft dysfunction, BK virus (BKv), or acute rejection (AR).
**Conclusions:** KTs who received prophylaxis with an AA had a higher incidence of positive blood cultures compared to those on TMP/SMX. Further studies will define which patients might benefit from additional UTI prophylaxis and the effect of UTI prevention on allograft function.

**SA-PO3090**

**Acute Tubular Necrosis (ATN) on Implant Biopsy of Living Kidney Donors Is Associated with Lower Recipient Graft Function**

Amy El Toukhyy, Richard A. Fatica, Milen Amde, Emilio D. Poggio, Titte Srinivas. Nephrology and Hypertension, Cleveland Clinic, Cleveland, OH.

**Background:** Donor kidneys removed by laparoscopy may exhibit slow graft function likely reflecting acute kidney injury. This acute kidney injury may manifest in implant kidney biopsies (IMBs) as histologic acute tubular necrosis (ATN). We examined the association between ATN on implant biopsies and recipient graft function.

**Methods:** We studied relationships between IMBs ATN, metabolic syndrome (MS) and 1 yr recipient eGFR (CKD-EPI equation) by multiple linear regression in 161 living donor-recipient pairs transplanted 2005-09. In a logistic model we studied associations of IMBs ATN and the impact of patients with eGFR<45 ml/min/1.73 m². (MS: BMI>25, SBP>130 mm Hg, Triglyceride>150, Low HDL/sex and FBS>B100 mg/dl)

**Results:** ATN was observed 21.4% of IMBs. Donor factors associated with increased incidence of IMBs ATN were 1) Donor MS: Of those with IMBs ATN 21.2% had MS vs. 6.5% of those without IMBs ATN, p<0.01) and 2) Donor uric acid levels, (p<0.05). Donor age, gender and race were not significantly related to IMBs ATN. Overall, adjusted transplanted isohalotame GFR (donor GFR x Proportion of total kidney volume transplanted and adjusted for recipient BSA) averaged 53.2 ml/min/1.73m² at 1 year (17.5). Recipient eGFR at 1 year was 56.5 ml/min/1.73 m² for recipients with IMBs ATN vs. 49.7 ml/min/1.73 m² (p< 0.010) in those with no ATN by univariable analysis.

In a multivariable linear regression model, IMBs ATN was a significant correlate of 1 yr recipient eGFR.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value ml/min/1.73 m²</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>48.5</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>NC/IMBS ATN</td>
<td>3.34</td>
<td>0.0361</td>
</tr>
<tr>
<td>No acute rejection</td>
<td>2.7</td>
<td>0.0026</td>
</tr>
<tr>
<td>Recipient Age/yr</td>
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<td>Donor age</td>
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</tr>
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<td>Donor Race (Black)</td>
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</tr>
<tr>
<td>Donor Sex (Male)</td>
<td>-0.74</td>
<td>0.6428</td>
</tr>
</tbody>
</table>

**Conclusions:** ATN in the implant biopsy is associated with a significantly lower 1 year recipient eGFR. The impact of IMBs ATN on recipient eGFR is similar in to that of acute rejection. Minimal surgical manipulation is thus desirable to reduce IMBs ATN. The long-term impact of IMBs ATN on transplant outcomes merits further study.

**SA-PO3091**

**Thymoglobulin Attenuates Renal Ischemia Reperfusion Injury**

Prabir Roy-Chaudhury, Yang Wang, Kamary A. Zahedi, Meenakshi J. Mistry, Virgilius Cornea, Manoocher Sooleiman. Division of Nephrology and Hypertension, University of Cincinnati, OH.

**Background:** Ischemia reperfusion injury (IRI) resulting in delayed graft function (DGF) is an important cause of morbidity in renal allograft recipients. Despite its clinical significance there are no effective interventions for the prevention of renal allograft IRI. Thymoglobulin (Thymo) is a potent T cell depleting polyclonal antibody which also reduces the expression of adhesion molecules which play an important role in the causation of renal IRI. The aim of the current study, was to assess the role of a murine surrogate of Thymo (rabbit anti-mouse thymocyte globulin), in attenuating renal IRI in a mouse model of bilateral renal artery ligation.

**Methods:** 20 C57Bl/6 mice were administered either mouse Thymo (T) 500 mcg IV through the tail vein, control mouse IgG (I) in a similar dose, or no therapy (C = Control); 30 minutes prior to clamping the renal arteries bilaterally (for 30 minutes). Animals were sacrified at the 24 hr, 3d and 14d time points. Both kidneys were assessed for changes (acute tubular necrosis, epithelial vacuolization) using a semi-quantitative scoring scale from 0 to 3+ (0 = normal, 1+ = <25% renal parenchyma involved, 2+ = 25-50%, 3+ = > 50%). An ANOVA test was performed to ascertain whether Thymo should be preferentially used prior to clinical reperfusion in renal transplants, and also in most cases IgG.

**Results:**

- 24Hrs: 1.5±0.3 1.0±0.3 0.17±0.2 24Hrs
- 3 Days: 2.0±0.6 0.25±0.25 0.25±0.25
- 14 Days: 1.75±0.5 0.45±0.4 0.45±0.4

*p<0.05 compared to Control; **p<0.05 compared to IgG

**Funding:** Pharmaceutical Company Support

**Conclusions:** More attention has to be given to achieve a better control of anemia, dyslipidemia and hyperparathyroidism in CKT patients. Immunosuppressive treatment, inflammation, weight gain, may explain the difference of CKD complications management observed between the KT and NT patients. Prospective studies are needed to confirm the beneficial effect of treating CKD complications on graft function and patient outcomes.

**Funding:** Government Support - Non-U.S.

**Poster/Saturday**

**Transplantation: Allograft Dysfunction and Complications - II**

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author. 832A
SA-PO3094

Associations of Pre-Transplant Hemoglobin and Iron Deficiency with Post-Transplant Delayed Graft Function in Kidney Transplant Recipients Miklos Z. Molnar,1,2 Csaba P. Kovesdy,3 Laszlo Rosivall,2 Suphamai Bunnnapradist,4 Junichi Hoshino,5 Elani Streja,6 Mahesh Krishnan,5 Kamary Kalantar-Zadeh,6,7 1Harold Simmons Center, Torrance, CA; 2Semelweis University, Budapest, Hungary; 3Salem VA Medical Center, Salem, VA; 4David Geffen School of Medicine at UCLA, Los Angeles, CA; 5DaVita, Inc, Denver, CO.

Background: Delayed graft function (DGF) complicates kidney allograft outcomes in the immediate post-transplantation period. We hypothesized that in hemodialysis patients (pts) more severe anemia & iron deficiency are associated with higher risk of DGF.

Methods: Linking 5-year hemodialysis pts data of a large dialysis organization to the Scientific Registry of Transplant Recipients, we identified 11,836 hemodialysis pts. Using logistic regression analyses we examined the association between pre-transplant parameters and post-transplant DGF.

Results: Pts were 49±14 (mean±SD) years old and included 38% women, 27% Blacks and 26% diabetics. Compared to pre-transplant hemoglobin of 12–13 g/dL, there was 25% higher risk of DGF with blood hemoglobin 10–11 g/dL (OR=1.25;95%CI: 1.01-1.55), whereas blood hemoglobin ≥13 g/dL exhibited 15% higher risk of DGF (OR=1.15;95%CI: 0.98-1.34).

Results:

- Unadjusted
- Case-Mix
- Fully adjusted

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Unadjusted OR (95% CI)</th>
<th>Case-Mix OR (95% CI)</th>
<th>Fully adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretransplant transfusion</td>
<td>1.30 (1.18-1.44)</td>
<td>1.31 (1.18-1.45)</td>
<td>1.33 (1.19-1.48)</td>
</tr>
<tr>
<td>ESA dose (+5000 units/wk, increase)</td>
<td>1.07 (1.04-1.10)</td>
<td>1.05 (1.02-1.09)</td>
<td>1.05 (1.02-1.09)</td>
</tr>
</tbody>
</table>

Each 5000 U/wk increase of pre-transplant ESA dose was associated with 5% higher DGF (OR=1.05; 95%CI: 1.02-1.09).

Conclusions: Pre-transplant blood transfusion and higher ESA dose are associated with higher risk of DGF.

Funding: NIDDK Support

SA-PO3096

Alemtuzumab Induction and Tacrolimus Monotherapy Maintenance Reduces Cellular Rejection in Kidney Allografts: Five Year Follow Up Milap Pokharel, Prajwol R. Pant, Michael C. Chobanian. Medicine, Dartmouth Medical School, Hanover, NH.

Background: Alemtuzumab is increasingly used as induction therapy for renal transplants to minimize post transplant immunosuppression. Biopsy confirmed acute rejection (BCAR) has been shown to be less frequent with alemtuzumab compared to conventional induction therapies and standard immunosuppressive regimens. We investigated the rate of BCAR in patients receiving alemtuzumab induction followed by tacrolimus monotherapy and compared the results to those receiving alternative induction and maintenance therapies.

Methods: We retrospectively reviewed data from all kidney transplant recipients between 1992 and 2010 with BCAR. Demographics, type of kidney transplant, type and cause of rejection, graft survival and patient survival times were determined.

Results: 647 renal transplants were performed, 345 patients in the pre and 302 patients in the post alemtuzumab era. 36 patients had 44 episodes of BCAR, 32 (9.3%) were pre and 12 (3.9%) were post alemtuzumab. Mean age was 34.2 years, 56.8% were male and 91.7% were Caucasians. 16 (36.3%) rejected allografts were from living and 28 (63.4%) were post alemtuzumab. Mean age was 34.2 years, 56.8% were male and 91.7% were Caucasians. 16 (36.3%) rejected allografts were from living and 28 (63.4%) were from deceased donors. Of the pre alemtuzumab rejections, 13 (40.6%) were antibody mediated (AMR) while 19 (59.4%) were ACR. In the post alemtuzumab era, 5 (41.7%) were ACR and 19 (59.4%) were AMR. No patients in the post alemtuzumab era died at the end of the study period, whereas 5 year survival rate was 93.7% for patients in the pre alemtuzumab era. Mean time of graft survival was 3.7 versus 10.6 months in the pre vs post alemtuzumab group. Incidence of both ACR and AMR were significantly lower in patients who received alemtuzumab induction (9.3% vs 3.9%). Of the 7 cases of ACR in the post alemtuzumab era, 5 had no compliance to medications and 2 were taking cyclosporine, prednisone, and MMF, not tacrolimus monotherapy.

Conclusions: Alemtuzumab induction plus tacrolimus monotherapy maintenance eliminates ACR up to 5 yrs post kidney transplant in compliant patients. Alemtuzumab induction appears to confer greater longevity in both patients and grafts undergoing rejections than other induction modalities.

SA-PO3097


Background: Acute rejection (AR) remains a determinant of long term graft survival.

Results:

- Unadjusted
- Case-Mix
- Fully adjusted

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Unadjusted OR (95% CI)</th>
<th>Case-Mix OR (95% CI)</th>
<th>Fully adjusted OR (95% CI)</th>
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<tr>
<td>Pretransplant transfusion</td>
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<td>1.33 (1.19-1.48)</td>
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<tr>
<td>ESA dose (+5000 units/wk, increase)</td>
<td>1.07 (1.04-1.10)</td>
<td>1.05 (1.02-1.09)</td>
<td>1.05 (1.02-1.09)</td>
</tr>
</tbody>
</table>

Each 5000 U/wk increase of pre-transplant ESA dose was associated with 5% higher DGF (OR=1.05; 95%CI: 1.02-1.09).

Conclusions: Pre-transplant blood transfusion and higher ESA dose are associated with higher risk of DGF.

Funding: NIDDK Support
**Methods:** We report on the AR rate and its impact on long term graft survival following alemtuzumab (campath-1H) induction (30 mg) and rapid steroid withdrawal (methylprednisolone 500 mg, 250 mg, and 125 mg on days 1-3 respectively) in 487 consecutive renal transplant patients from July 2003 through January 2011. Maintenance immunosuppression consisted of tacrolimus and mycophenolate mofetil with target level of 8-10 ng/ml and 1.5-3.0 ng/ml respectively. A renal biopsy was prompted by an unexplained rise in serum creatinine.

**Results:** AR was biopsy proven in 55/487 (11.3%) patients and an additional 10 patients had borderline changes (see table). By Banff criteria, 31 (6.4%) showed acute cellular rejection (ACR), 16 (3.3%) showed acute humoral rejection (AHR), and 8 (1.6%) displayed both ACR and AHR. The mean age of these 65 patients was 53 ± 14 yrs, 35 (54%) were male, 19 (29%) were African American, 11 (17%) were Hispanic, 48 (74%) received a deceased donor kidney, and 14 (21%) were retransplants. AHR was treated with plasmapheresis, IVIG and rituximab and ACR with steroid and/or thymoglobulin or OKT3. Mean follow up was 43 ± 22 months post AR.

**Conclusions:** Using campath-1H induction the incidence of ACR was 6.4%, AHR 3.3% and ACR+AHR 1.6%. AHR occurs at an earlier time point than ACR or ACR/AHR and is associated with a better outcome.

**SA-PO3098**

**Independent Predictors of Renal Allograft Failure in Patients with Acute Antibody-Mediated Rejection** Marie Maitignon, Thangamani Muthukumar, Michelle L. Lubetzky, Darshana Dadhania, Surya V. Seshan, Manikkam Suthanthiran, Choli Hartono. Cornell University.

**Background:** The impact of co-existing histological findings in renal allograft biopsies showing acute antibody-mediated rejection (AMR) on graft outcome has not been well defined.

**Methods:** We reviewed all for-case kidney allograft biopsies between 12/2003 and 12/2010. We identified AMR as defined by Banff 2009. Primary outcome was graft loss. Significant baseline variables were entered into a Cox model to identify independent predictors of outcome. Robustness of the model was verified by analysis with a logistic model.

**Results:** 76 of 1386 (5.5%) kidney recipients had AMR. Median follow up was 21(0-97) months (mo) after diagnosis. 31 (40%) lost their graft, 48% of them within 3 mo of AMR. Concomitant acute cellular rejection (c-ACR) on biopsy and eGFR at the time of AMR were the only predictors of graft loss. 26 (34%) of the 76 with AMR had c-ACR.

**Conclusions:** The predictors were independent of (i) time to AMR, (ii) presence of concomitant (a) chronic active AMR, (b) IF/TU, (c) g/p/tc inflammation, (d) arteriosclerosis, and (iii) addition of (a) Rituximab, (b) anti-thymocyte globulin or (c) bortezomib for the treatment of AMR, to the standard treatment (steroids, plasmapheresis or intravenous immunoglobulin).

**By logistic regression, the same two variables predicted graft loss at 1-year after AMR.**

**Conclusions:** In biopsies showing AMR, the concomitant presence of ACR, independent of any other allograft pathology or rejection therapy, is a risk factor for graft loss.

**SA-PO3099**


**Background:** We examined the frequency of acute rejection (AR) in contemporary U.S. kidney transplantation (KT) and the clinical impact of AR based on: 1) AR timing after KT, 2) risk period after the AR event.

**Methods:** Data for Medicare-insured KT recipients in 2000-2007 (n=44,831) were drawn from the USRDS. AR events were ascertained from OPTN reports covering 0-6, 7-12, 13-24, and 25-36 mo post-transplant. AR was classified as antibody-treated AR (Ab-AR) or other management (non-Ab-AR). Associations of AR with subsequent all-cause graft loss (adjusted hazards ratio, aHR) were estimated with time-varying Cox regression. Risk associated with AR was partitioned within the first 90d after AR or 1yr) compared to over longer intervals. Non-Ab-treated AR was more than twice as common as Ab-treated AR by period and donor type. In time-varying multivariate regression, development of Ab-AR predicted greater risk of graft loss than non-Ab-AR.

**Conclusions:** AR was more common when evaluated early after KT (e.g., within 6 mo or 1yr) compared to over longer intervals. Non-Ab-treated AR was more than twice as common as Ab-treated AR by period and donor type. In time-varying multivariate regression, development of Ab-AR predicted greater risk of graft loss than non-Ab-AR.

The aHR for graft loss from Ab-AR increased with later timing of AR after transplant, while risk associated with non-Ab AR peaked for events reported in mo 13-24 after KT. Regardless of the diagnosis time, the relative risk of graft loss was higher in the first 90d after a given AR report compared to beyond 90d.
Rituximab in Pediatric Recurrent Focal Segmental Glomerulosclerosis
Jahi Kumar,1 Ibrahim F. Shatat,2 Amy L. Skversky,2 Eduardo M. Perelstein,1 Valerie L. Johnson,1 Shefali Mahesh,2 Pediatrics, Weill Cornell Medical Center, New York, NY; 2Pediatrics, Akron Childrens Hospital, Akron, OH; 1Pediatrics, Children’s Hospital at Montefiore, Bronx, NY; Pediatrics, MUSC Children’s Hospital, Charleston, SC.

Background: FSGS recurs in 35-50% of all grafts. Plasmapheresis (TPP) has been one of the mainstays of treatment but results are variable. Rituximab (RTX), a anti CD20 antibody is being used for its treatment but pediatric experience is very limited.

Methods: We report 8 cases of recurrent FSGS treated with Rituximab.

Results: See Table 1 for details.

- Children, age range 7 to 17 years had recurrence of FSGS within 2 weeks after transplant. All were on TPE for an average of 7 days to 5 years with persistent nephrotic range proteinuria. They received 1 to 4 doses of RTX.
- Complete response, urine protein creatinine (u p/c) ratio < 0.2 was seen in 2/8 patients. Partial decrease in u p/c ratio was seen in 3/8 patients. 3 patients had no response. Those who responded did so within the first month of RTX.
- One patient received RTX for biopsy proven severe recurrence with allograft dysfunction requiring hemodialysis. Proteinuria diminished and serum creatinine improved significantly post RTX. The patient developed severe respiratory failure 4 weeks post Rituximab and died. No infectious agent was identified on bronchoscopy. Autopsy revealed thrombotic microangiopathy in multiple organs.
- One patient developed CNS malignancy 2 years post Rituximab and one had ATN after receiving Rituximab.

Table 1: Clinical Characteristics

<table>
<thead>
<tr>
<th>Age/Sex/ Race</th>
<th>Transplant Type</th>
<th>Focal Segmental Glomerulosclerosis Pre/post</th>
<th>FSGS post 0/1 years</th>
<th>Serum Cr post (mg/dl)</th>
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<tr>
<td>Case 1</td>
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<td>Case 3</td>
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<tr>
<td>Case 4</td>
<td>16/m/w</td>
<td>LURD</td>
<td>n</td>
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<td>Case 5</td>
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<td>k/1</td>
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<td>Case 6</td>
<td>14/5/6</td>
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<td>n</td>
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<td>1.8/1.7</td>
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<td>Case 8</td>
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<td>10</td>
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</tr>
</tbody>
</table>

Conclusions: Rituximab can be used as a treatment for recurrent FSGS. Efficacy is variable. Larger multicenter studies are needed to prove its sustained efficacy in those who respond. Long term follow up is required for monitor for adverse effects.

SA-PO3102
Successful Treatment of Recurrent Focal Segmental Glomerulosclerosis after Kidney Transplantation Results in Recovery of Podocyte Injury
Kavita M. Kakkad,1 Michelle M. Estrella,1 Rachel Marino,2 Hamid Rabhi,1 Nada Alachkar,1 1Nephrology, Johns Hopkins University, Baltimore, MD; 2Surgery, Johns Hopkins University.

Background: Focal segmental glomerulosclerosis (FSGS) commonly recurs after kidney transplantation (Tx). We describe the clinical course of individuals with recurrent FSGS treated with plasmapheresis (PP)/rituximab.

Methods: Eighteen patients with recurrent FSGS after Tx between 2005 and 2011 were followed for up to 2 years. All received PP (median 19 exchanges [IQR: 10–24]); 8 who were refractory to PP received 1 dose of rituximab. 17 patients underwent kidney biopsy (Bi) at the time of recurrence and at least 1 Bi after treatment. Response to therapy was defined by the decline in proteinuria to sub-nephrotic range or complete resolution.

Results: Clinical characteristics are shown in the table. Clinical Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at Tx, y (SD)</td>
<td>41 (13)</td>
</tr>
<tr>
<td>Black, %</td>
<td>55</td>
</tr>
<tr>
<td>Male, %</td>
<td>55</td>
</tr>
<tr>
<td>Mean years on dialysis, y(SD)</td>
<td>28 (3.1)</td>
</tr>
<tr>
<td>Per Exp Nephrectomy, %</td>
<td>28</td>
</tr>
<tr>
<td>Tx Type, %</td>
<td>28</td>
</tr>
<tr>
<td>Declined</td>
<td>44</td>
</tr>
<tr>
<td>Living related</td>
<td>28</td>
</tr>
<tr>
<td>Living unrelated</td>
<td>28</td>
</tr>
<tr>
<td>Serum Cr at recurrence mg/dl (mean SD)</td>
<td>2.7 (1.7)</td>
</tr>
<tr>
<td>PrCr at recurrence g/mean (SD)</td>
<td>5.5 (6.2)</td>
</tr>
</tbody>
</table>

Median time to recurrence was 20 days (IQR 5-90) after Tx. On initial Bi, individuals had variable degree of podocyte effacement (PE) on EM; only 2 had FSGS changes on light microscopy (LM). Mean serum creatinine improved from 2.7 to 2 mg/dl (P=0.07) and mean urine protein/creatinine ratio (PrCr) declined from 11.7 (peak) to 4.9 g/g (P<0.01) after treatment. Receipt of rituximab improved PrCr by a mean of 3.3 g/g (P=0.03). 10 patients had improvement in PE after treatment; 8 of whom did not develop FSGS changes on LM and 2 had persistent changes. Treatment Failure did not result in improvement of PE (7 patients).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

835A
Conclusions: Decreased proteinuria was accompanied by improvement in podocyte effacement in patients treated with PP±rituximab for recurrent FSGS after Tx.

SA-PO3103

High-Dose Corticosteroid Therapy (HDCS) for Recurrent idiopathic Membranous Nephropathy (IMN) after Renal Transplantation Maria Lopez Picasso,1 Esther Gonzalez Monte,2 Alberto De Lorenzo,3 Maria Moya,4 Laura Garcia-Puente Suarez,2 Jose M. Morales,2 Amado Andres,2 Manuel Praga,2 Nephrology, H. U. 12 de Octubre, Madrid, Spain.

Background: IMN can recur in 40% of renal allografts or appear de novo. Corticosteroids as a single therapy for IMN is not recommended. No studies have been performed in IMN recurring or presenting de novo after renal transplantation. We observed a beneficial effect of HDCS in transplant patients with renal biopsy showed an IMN. In this study, we collect our experience with HDCS in IMN of renal transplant patients.

Methods: Single-center, observational study of patients with biopsy-proven IMN after renal transplantation. Patients were divided into two groups according to whether they received HDCS in addition to their immunosuppressive regimen (group 1) or not (group 2).

Results: Twenty-one patients (66.7% male, mean age 51.3 ± 13.3 years) were collected. 47.6% were HCV+. Fourteen patients (group 1) received HDCS, 6 of them with oral steroids (0.7 mg/kg/day) and 8 with intravenous pulses (mean dose 1.25 gr). Seven patients (group 2) received no corticosteroid treatment.

<table>
<thead>
<tr>
<th>Characteristics and outcome of group 1 vs group 2</th>
<th>Group 1 (HDCS) (14p)</th>
<th>Group 2 (7p)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49±11.12</td>
<td>52±7.6</td>
<td>NS</td>
</tr>
<tr>
<td>Time from Tx to diagnosis (months)</td>
<td>39±38.22</td>
<td>35±23</td>
<td>NS</td>
</tr>
<tr>
<td>sCr at diagnosis (mg/dl)</td>
<td>1.4±0.3</td>
<td>1.7±1.46</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR at diagnosis (ml/min)</td>
<td>46.9±17.4</td>
<td>42.5±24.4</td>
<td>NS</td>
</tr>
<tr>
<td>Proteinuria at diagnosis (g/d)</td>
<td>0.7±1.6</td>
<td>6.2±5.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Patients receiving CSA or TAC at the time of diagnosis (%)</td>
<td>100</td>
<td>100</td>
<td>NS</td>
</tr>
<tr>
<td>Patients receiving ACEI or ARB (%)</td>
<td>100</td>
<td>42.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Patients with CR or PR (%)</td>
<td>12(85.7)</td>
<td>2(28.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Time to reach complete or partial remission (months)</td>
<td>5±3.4</td>
<td>12±1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Final sCr (mg/dl)</td>
<td>1.8±0.9</td>
<td>3.2±2</td>
<td>0.04</td>
</tr>
<tr>
<td>Final proteinuria (g/d)</td>
<td>1.1±0.1</td>
<td>5.9±2.2</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Group 1 patients achieved a higher rate of complete (CR) or partial (PR) remission, as compared to those of group 2 (85.7% vs 28.6%). Final sCr was significantly better among group 1.

Conclusions: A majority of patients with recurrent or de novo IMN achieved CR or PR of nephrotic syndrome after receiving oral or i.v. high-dose corticosteroid therapy.

SA-PO3104

The Graft Outcome of HLA Non-Mismatch Kidney Transplantation: the Impact of Recurrent Glomerulonephritis and Rejection Hee Jung Jeon,1 Ran-Hui Cha,2 Jung Nam An,2 Jung Pyo Lee,3,4 Curie Ahn,1 Sung hwon Kim,1 Yon Su Kim,1,2 1 Department of Internal Medicine, Seoul National University College of Medicine, Korea; 2 Department of Internal Medicine, National Medical Center, Korea; 3 Clinical Research Center for End Stage Renal Disease, Korea; 4 Department of Internal Medicine, Boramae Hospital, Korea.

Background: Although histocompatibility leukocyte antigen (HLA)-identical renal transplantation reveals superior graft outcome, the graft survival has not been definitive. Original disease recurrence and effect of acute rejection (AR) may preclude indefinite survival. Here, we analyzed the factors that affected the graft outcomes in HLA non-mismatch condition.

Methods: We have studied the effect of recurrent glomerulonephritis (GN) and AR on graft outcomes in HLA non-mismatch (n=122), 3-4 mismatch (n=317), and 5-6 mismatch (n=102) renal allograft of Seoul National University Hospital. And 41% patients had GN as underlying disease.

Results: Overall graft survival was 93.4% at 5 years, 83.3% at 10 years, and 55.5% at 20 years. Surprisingly, HLA compatibility did not affect the graft survival (0 vs. 3-4 vs. 5-6 mismatch: 92.8% vs. 92.7% vs. 95.8% at 5 years, 82.5% vs. 77.6% vs. 88.9% at 10 years, 42.0% vs. 73.1% vs. 37.2% at 20 years, respectively, p=0.602). In GN subgroup, male recipients (p=0.041), acute rejection (p=0.001), and recurrent GN (p=0.003) were the risk factors for graft loss, whereas living donor graft showed the protective effect (p<0.029). The acute rejection was more prevalent as more HLA incompatibility (0 ref.) < 3-4 < 5-6 mismatch; p=0.047 and p=0.014. But the recurrence of GN showed the opposite trend, i.e., the less HLA mismatch, the more recurrence of GN (9 ref.) < 3-4 < 5-6 mismatch; p=0.106 and p=0.022. Furthermore, the graft loss due to recurrent GN was significant in HLA non-mismatch group compared with 3-4 mismatch group (p=0.047).

Conclusions: Although HLA non-mismatch group experienced less AR, the graft survival was not different from others, which was mainly due to the recurrence of underlying disease. Therefore, the main focus should aim for the management of recurrence, especially in HLA-identical renal transplantation.

SA-PO3105

Proteinuria as a Marker of Renal Injury in Children after Kidney Transplantation Tanya E. Pereira,1 Jayanthi Chandar, Chryso P. Katsoufis,2 1 Division of Pediatric Nephrology, Department of Pediatrics, University of Miami, Miller School of Medicine, FL.

Background: Acute kidney injury occurs at the time of kidney transplantation as a consequence of ischemia-reperfusion injury. The purpose of this study is to sequentially and quantitatively measure proteinuria in children with kidney transplants and assess the correlation between cystatin C and proteinuria at the end of the first post-transplant year.

Methods: The cohort consists of 25 children with kidney transplants followed at the University of Miami between the years 2008 to 2010. Proteinuria was assessed by urine protein/creatinine (Up/cr) ratio at 2 days, 1 week, 1 month and 1 year post-transplant. Comparisons were made between living donor (LD) and deceased donor (DD) transplants. Children with FSGS were analyzed separately.

Results: The mean age was 12± 4.9 years with 11 males. Seventeen of 25 had DD transplants. At one month post-transplant, there was a significant decrease in proteinuria in both DD and LD. The urine p/cr ratio was 1.55±1.45, 0.27±0.16, 0.19±0.17 in DD versus 0.2± 0.5, 0.38± 0.5 and 0.27± 0.16 in LD at 1 week, 1 month and 1 year post-transplant respectively. Those with FSGS had a mean urine p/cr ratio of 8.3±6.4 versus those without
SA-PO3107

De Novo Rapamune Use Is Not Associated with the Development of Proteinuria

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Background: Rapamune has been associated with the development of proteinuria when used to replace calcineurin inhibitors for alloarthropathy dysfunction. The long-term effect of de novo Rapamune use on proteinuria is less well studied. We evaluated the effects of Rapamune use on proteinuria and GFR in kidney transplant recipients following up for five years.

Methods: 153 kidney transplant recipients in the Angiostatin II Blockade for Chronic Allograft Nephropathy trial (NCT00067990) received Rapamune plus CNI (n=29) or Celecept plus CNI (n=124). Iothalamate GFR and 24-hour urine albumin and protein were obtained at baseline and annually for 5 years. Statistical analyses used mixed linear models and the correlation between cystatin C and urine pr/cr ratio at the end of the first post-transplant year.

Results: The Rapamune group was 44% female; mean age was 49 and 75% were live donor recipients. The non-Rapamune group did not differ with respect to sex, age, donor source, or systolic blood pressure at any follow-up time. GFR was higher at baseline in the Rapamune group (54 vs. 65 ml/min, p=0.002). At annual follow-up visits, GFR, proteinuria, albuminuria, and systolic blood pressure did not differ between Rapamune and non-Rapamune groups. At 15 years, adjusted mean urine protein in the Rapamune group was 0.5 g/g versus 0.4 g/g in the non-Rapamune group (P=0.21). GFR did not differ between the groups at 5 years (50 versus 54 ml/min, p=0.41).

Conclusions: De novo Rapamune use was not associated with increased proteinuria or a difference in measured GFR. The noted association of Rapamune with proteinuria may be due to the removal of the vasocostrictive effects of CNIs.

Funding: NIDDK Support

SA-PO3108

Safety and Efficacy of Administering the Maximal Dose of Candesartan in Renal Transplant Recipients

Noritaka Kawada,1 Toshiki Moriyama,2 Masayoshi Okum1, Naotsugu Ichimaru1, Harumi Kitamura1, Jun-Ya Kaimori1, Norio Nonomura1, Shiro Takahara1, Hiromi Rakugi1, Yoshitaka Isaka1. 1 Div. of Nephrol, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan; 2 Health Care Center, Osaka university, Suita, Osaka, Japan; Dep. of Urology, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan; 3 Advanced Technology for Transplantation, Osaka Univ. Graduate School of Med., Suita, Osaka, Japan.

Background: The regular dose of angiotensin II type-I receptor blocker (ARB) used in renal transplant patients for hypertension is shown to be safe and effective. However, information on the appropriate dosing of ARB in renal transplant patients is limited. We evaluate the efficacy and safety of the maximal dose of candesartan administered to renal transplant patients.

Methods: Sixty-nine recipients were enrolled in this study. Patients were divided into three groups based on the basal dose of candesartan, patients not taking candesartan (Group A), taking to low medium dose candesartan (2-4mg/day; Group B), and taking high dose candesartan (8mg/day; Group C). During the course of the study, patients were treated with a gradual increase of candesartan to a final dose of 12 mg/day. Physiological and biochemical parameters were acquired before and after the twelve-month study period.

Results: Ninety-one percent of patients continued in their administration of candesartan for one year and 75% tolerated the administration of the maximal dose of candesartan. Significant differences in proteinuria, albuminuria, serum creatinine, and eGFR level among the groups were detected. In group A, candesartan reduced systolic blood pressure, decreased the levels of proteinuria, albuminuria, eGFR, and hemoglobin and increased plasma potassium, creatinine level, and plasma renin activity.

Conclusions: Gradual increase of ARB to its maximal dose in renal transplant patients is safe when carefully monitored. We could demonstrate the impact of maximal RAS blockade on both proteinuria and albuminuria, which indicates the need for future, long-term randomized prospective trials to further establish the impact of maximal RAS blockade on renal and cardiovascular protection in transplant patients.

Funding: Government Support - Non-U.S.

SA-PO3112

Estimated Glomerular Filtration Rate during Years 1 to 5 Post-Transplant by CNI Use, Data from the Patient Outcomes in Renal Transplant (PORT) International Data Collaboration

Jon J. Snyder,1 Melissa Screns,2 Ajay K. Israni,3,1 Gilbert Lilienthal,1 Bertram L. Kasiske.1 1 Chronic Disease Research Group, Minneapolis, MN; 2 Hennepin County Medical Center, Minneapolis, MN; 3 Bristol-Myers Squibb, New Haven, CT.

Background: Serum creatinine-based estimation of glomerular filtration rate (eGFR) is the standard marker for monitoring graft function. Monitoring changes in eGFR can aid clinicians in anticipating long-term prognoses.

Methods: We explored the association between MDRD eGFR during years 1 through 5 post-transplant and CNI use at 1 year post-transplant to see if differences were evident by CNI and would aid inference based on eGFR measurements. We studied 7,594 patients from 11 transplant centers in the Patient Outcomes in Renal Transplantation (PORT) international database transplanted 1999-2006 whose allografts survived 1 year post-transplant. Patients who returned to dialysis were assumed to have an eGFR of 0 from the time of graft failure until the earliest of death, loss-to-follow-up, or end of study.

Results: Use of any CNI at 1 year post-transplant was reported for 88% of patients. Cytoglobin (CSA) was more common, reported in 56% of patients; Tacrolimus (Tac) was reported in 28% of patients. Median eGFR for was highest in Tac users at every post-transplant time point, from 53.5-48.0 ml/min/1.73 m2 in years 1 to 5 (Figure). Median eGFR in CSA users was 3-4 ml/min/1.73 m2 lower than in Tac users (p=0.001), and 3-6 ml/min/1.73 m2 lower in people in whom no CNI was reported (p=0.001). Median eGFR declined over time in all groups, regardless of CNI use or type of CNI.

Funding: NIDDK Support

SA-PO3109

Associated with Proteinuria

Altered mTOR Pathway in Glomerular Epithelial Cells May Not Be Associated with Proteinuria

Ping L. Zhang, Wei Li. Department of Anatomic Pathology, William Beaumont Hospital, Royal Oak, MI.

Background: Several studies report that transplant patients treated with Sirolimus, an inhibitor of mammalian target of rapamycin pathway (mTOR), have a high chance to develop a reversible proteinuria. In this study, we evaluated phosphorylated (p) form of mTOR and its downstream signals 70S6K and 4EBP in glomerular epithelium in both native and transplant biopsies from patients with proteinuria, and correlated the expression of these markers with proteinuria levels.

Methods: The first study included 17 control cases, 18 focal segmental glomerulosclerosis (FSGS) and 22 immune complex mediated glomerulopathy (ICMGN). The second study consisted of 30 unremarkable control kidney sections (removed for renal tumors), 30 transplant cases with Sirolimus treatment (Sirot), 59 cases with both Sirolimus and Cyclosporine therapy (Sirot+Cyclo) and 39 transplant cases with only cyclosporine treatment (Cyclo). All sections were stained for p-mTOR, p-70S6K and p-4EBP. Nuclear staining was assessed and categorized into four grades: 0 - 3+ based on intensity of staining in proximal tubules (PT), glomerular parietal epithelium (PE) and visceral epithelium (VE).

Results: In the first study, nephrotic range of proteinuria was found in FSGS and IMCMG groups. In the second study, proteinuria was significantly higher in Sirot and Sirot+Cyclo groups than Cyclo group. In both native and transplant biopsies, study groups all showed 1- 2 fold higher staining for all 3 markers in PT, PE and VE when compared to controls. In both studies, proteinuria was not associated with expression of any marker in any location but the proteinuria was significantly associated with interstitial fibrosis in the transplant study. Expression of p-70S6K in proximal tubules was significantly related to either serum creatinine (native study) or acute cellular rejection (transplant study).

Conclusions: Our data demonstrated a low activity of mTOR pathway in PT, PE and VE in control kidneys. The extent of proteinuria was not significantly associated with immunohistochemical expression of mTOR pathway activity in both native and transplant studies, as activated mTOR pathway in glomerular epithelium appeared related to the high mTOR pathway activity in the PT.

Funding: Pharmaceutical Company Support
Conclusion: In summary, median eGFR between years 1 and 5 post-transplant was highest in patients who were on Tac 12 months post-transplant; however, median eGFR declined over time in all groups.

Funding: Pharmaceutical Company Support

SA-PO3110

Over Ten Years Kidney Graft Survival Determinants


Background: Kidney graft survival has been mainly evaluated using an up to 10-years threshold. Instead, in this study our aim was to evaluate predictive variables that impact long term kidney graft survival (>10 years).

Methods: We analyzed data from 996 kidney transplants performed between 1983 and the end of June 2000. Inclusion criteria: graft survival > 3 months and patient survival > 1 year post-transplant (PT). Exclusion criteria: simultaneous multiple grafts recipients. We enrolled 892 patients in our analysis: 638 patients with functioning graft at 10-years PT and 254 patients with graft failure at 10-years PT (considering patient death with a functioning graft <10 years PT as graft failure). Between groups comparisons were done using Mann-Whitney and chi-square test. To determine independent predictive variables for long-term graft survival a multivariate-adjusted logistic regression was performed.

Results: Patients with long-term graft survival had significant lower donor age, 12-month PT creatinine, HLA-B mismatches, panel reactive antibodies (PRA) level (<30%) and number of transplants (<3); higher frequency of immediate graft function, absence of acute rejections (AR) episodes, positive CMV IgG and induction with anti-thymocyte immunoglobulin (ATG). Significant predictors of long term graft survival were 12-month PT creatinine (OR=0.26, P=0.001), donor age (OR=0.98, P=0.04), time on dialysis (OR=0.93, P=0.044), recipient positive CMV IgG (OR=1.59, P=0.040), absence of AR episodes (OR=1.57, P=0.047), a 1 to 1 HLA-B mismatch (OR=1.80, P=0.004) and recipients male gender (OR=1.84, P=0.005). Recipients age at transplant, number of transplants (< vs ≥ 3), PRA level (< vs ≥ 30%), immediate graft function and induction with ATG were not significant predictors.

Conclusions: Recipient gender, IgG CMV status, dialysis vintage, absence of AR episodes, lower number of HLA-B mismatches and donor age were all significant predictors, while a lower 12-month PT creatinine remained the strongest determinant for long term kidney graft survival.

SA-PO3111

Favorable Effect of Systemic Insulin Delivery on Lipid Profiles in Simultaneous Kidney Pancreas Transplantation

George A. Osuchukwu,1 Ijeoma C. Nwelue,1 Bruns A. Watts,1 Tina Kochar,1 Horacio E. Adrogue.2 1Department of Nephrology and Hypertension, University of Texas Medical Branch, Galveston, TX; 2Methodist J.C Walter Jr Transplant Center, Department of Internal Medicine Methodist Hospital, Houston, TX.

Background: Simultaneous kidney pancreas transplant (SPK-t) is the treatment of choice for carefully selected patients with diabetes mellitus and end stage renal disease. Due to the ease of the surgery a systemic delivery of the exocrine drainage is favored. Systemic insulin delivery in SPK-t has been demonstrated to lead to hyperinsulinism. Hyperinsulinism and insulin resistance in other populations are associated with an unfavourable lipid profile, one factor in their overall cardiovascular risk profile. Here we examine the effect of a systemic delivery of insulin on lipid profile in SPK-t patients.

Methods: Fasting glucose, insulin, c-peptide and lipid profile levels were obtained from 27 (SPK-t) patients with systemic exocrine drainage during routine post transplantation follow up. Homeostasis model of Assessment 2 calculations were used to estimate the beta cell secretory capacity HOMA-B, insulin resistance HOMA-IR and insulin sensitivity HOMA-S. The Fasting lipid profile of SPK-t patients was compared to 53 age, sex and immunosuppression protocol matched nondiabetic kidney transplant (K-t) patients using the unpaired student’s t-test.

Results: HOMA scores for the SPK-t group were (mean ± SD): HOMA-B 203 ± 54, HOMA-IR 2.5 ± 1.2 and HOMA-S 48.8 ± 21. These results suggest hyperinsulinism, insulin resistance and an increased beta cell mass, which are consistent with previous studies. The SPK-t group had a lower total cholesterol (155 ± 31 SPK-t vs 183 ± 36 K-t; p= 0.0001), lower LDL (89.9 ± 27 vs 105.5 ± 30.4; p<0.01) and a higher HDL (30.6 ± 10.2 vs 23.6 ± 10.2; p<0.01) levels than the K-t group, indicating a more favorable lipid profile in SPK-t than in K-t.

Conclusions: Previous studies have shown that Systemic delivery of insulin is associated with hyperinsulinism and insulin resistance in SPK-t. Our findings show that this type of hyperinsulinism, due to a systemic delivery of insulin in SPK-t patients is associated with a significantly more favorable lipid profile.

SA-PO3112

Anesthesia for Adult Renal Allograft Recipients: Prevalence and Predictors


Background: Posttransplantation anemia (PTA) is multifactorial. Besides being associated with kidney dysfunction, other factors play a role, namely immunosuppressive regimens (ISI). Our purpose was to investigate an adult kidney graft recipient’s population to elucidate the prevalence of PTA and its predictors.

Methods: Clinical data recorded throughout 2010 of adult kidney graft recipients with > 1-year posttransgraftament and no use of erythropoiesis stimulating agents was randomly selected from our unit, weighted for gender, age and time since transplantation (Tt). We aimed to examine clinical characteristics of the enrolled population and determine the prevalence of PTA. Multivariate adjusted linear regression was undertaken to detect hemoglobin (HB) predictors: age, gender, estimated glomerular filtration rate (eGFR) by MDRD equation, log-body mass index (BMI), diabetic status, log-ferritin, log-transferrin saturation and serum albumin, m organophosphate motefil (MMF), aziapirone (AZA), calcineurin inhibitors (CNI) and angiotensin converting enzyme inhibitors (ACE) use. WHO anemia criteria were employed.

Results: A total of 302 (119 females) patients were studied with a mean age of 49.6±13.4 years, a mean Tt= 7.6 years and a mean eGFR of 51.9±18.5 ml/min. The prevalence of anemia was 39.4% with a mean HB of 13.2±1.6 g/dL. CNI was used in 97.7% and 77.8% took prednisone (all with <10 mg). The most common ISI were CNI plus MMF (73.5%), CNI plus AZA (10.6%) and CNI alone (11.9%). No significant difference in HB was found between these regimens. Multivariate adjusted linear regression (R=0.41) showed as significant predictors of lower lower HB: female gender (P<0.001), lower eGFR (P<0.001), log-BMI (P=0.03), albumin (P=0.02), log-transferrin saturation (P<0.01), higher log-ferritin (P=0.03); MMF (P=0.002) and ACEI (P<0.01) use.

Conclusions: Prevalence of PTA was high. Expectable demographical (gender) and clinical (eGFR) variables were strong predictors of HB. Inflammation markers (lower albumin, higher ferritin) and drugs with reported negative effect in erythropoiesis (MMF and ACEI) were also associated with lower HB.

SA-PO3113

Undiagnosed Glucose Metabolism Disorders in Dialysis Patients: An Analysis Using Oral Glucose Tolerance Tests in German Dialysis Centers

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Background: Post-transplant diabetes mellitus (PTDM) or new-onset diabetes mellitus (NODM) after renal transplantation is considered a major health threat for renal transplant recipients, that goes along with decreased patient and graft survival.

Methods: Screening for undiagnosed diabetes mellitus was done with the use of oral glucose tolerance test (oGTT) in 4 dialysis centers in Germany according to ADA criteria. Impaired glucose metabolism disorders were defined as a fasting glucose level ≥ 100 - 125 mg/dL (impaired fasting Glucose IFG) and/or a 2 h glucose level 140-199 mg/dL (impaired glucose tolerance IGT). Overdiagnosis of diabetes mellitus was defined as a fasting glucose level ≥ 126 mg/dL and/or a 2 h glucose level ≥ 200 mg/dL.

Results: 237 adult hemodialysis patients were considered for inclusion in this trial. 91 patients (>38.4%), that were known to be diabetic were excluded from the trial leaving 146 nondiabetic patients (=38.4%). From these 146 nondiabetic patients (=38.4%), that were known to be diabetic were excluded from the trial leaving 146 nondiabetic patients (NODM) after renal transplantation, screening for undiagnosed diabetes mellitus was done with the use of oral glucose tolerance test (oGTT) in 4 dialysis centers in Germany according to ADA criteria. Impaired glucose metabolism disorders were defined as a fasting glucose level ≥ 100 - 125 mg/dL (impaired fasting Glucose IFG) and/or a 2 h glucose level 140-199 mg/dL (impaired glucose tolerance IGT). Overdiagnosis of diabetes mellitus was defined as a fasting glucose level ≥ 126 mg/dL and/or a 2 h glucose level ≥ 200 mg/dL.

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Conclusions: There is a considerable number of undiagnosed glucose metabolism disorders including overt diabetes mellitus in German hemodialysis patients. These patients may (eroneously) be classified as PTDM or NODM after renal transplantation.
Methods: In 106 clinically stable KTX recipients enrolled between 6 months and 5 years from transplant, VitD levels were measured by liquid chromatography and tandem mass spectrometry. Intact PTH in serum was measured by non-competitive chemiluminescent immunoassay and intact FGF-23 plasma by ELISA (Innometics, San Clemente, CA).

Results: The mean (SD) age was 47(11) years, 63% were male and 12% African American; 54% had CKD stage 1T and 2T, 41% stage 3T and 5% stage 4T; 62% had low VitD levels, 73% had hypophosphatemia, and 66% had elevated PTH levels. VitD levels were lower in African American subjects and within the first year from transplant; higher levels were seen after pre-emptive transplantation, and subjects with lower eGFR and using of eGFR and of VitD supplements. A significant inverse correlation was seen between PTH and VitD levels (r=-0.31; p=0.001). Indeed, PTH levels did not vary with eGFR or serum calcium, suggesting impaired normal feedback regulation. Median (95% CI) PTH levels were higher by 0.85(0.74-0.98) pg/ml for a 10ng/ml lower VitD level (p=0.04). Higher VitD levels were associated with longer duration on dialysis pre-transplant (mean, 95% CI: 56, 39-81 pg/ml among pre-emptive transplant, 85, 61-119 pg/ml with up to 3 years of dialysis, and 145, 106-199 pg/ml with > 3 years of dialysis pre-transplant; p=0.001). VitD supplementation was used in 62% of the study population; however, 45% of individuals with low VitD were not receiving supplementation.

Conclusions: In summary, low VitD levels are common after transplantation, likely secondary to depletion while on dialysis and could potentially play a role in PTH elevation after transplant. Pre-transplant repletion may be a relevant therapeutic option for post-transplant mineral bone disorder.

Funding: NIDDK Support

SA-PO3115

Mineral Metabolism, Parenchymal Calcification and Inflammatory Indices in Kidney Transplant Biopsies

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Background: Some studies suggested that parenchymal calcifications (PC), inflammation (I), epithelial-mesenchymal transition (EMT) and fibrosis (F) might contribute to chronic graft dysfunction in transplanted kidneys (KTx). A role for mineral metabolism (MM) derangement in these pathologic changes has been suggested. The aims of our study were to evaluate: a) the relationships between MM and PC, F, EMT and F indices in KTx biopsies (Bx); b) their possible relationship with graft outcome.

Methods: In the Bx of 87 KTx pts (46 M; aged 48 ±12 yrs), stainings for leukocytes (CD45: I marker), vimentin (EMT marker), alizarin red (PC marker), and Sirius red (F marker) were quantitatively evaluated by electronic image analysis on 20 low power fields (% of the total area). PC was estimated as present (+) or absent (-). MM (PTH, Ca, phosphorus), biochemical and clinical parameters were recorded at Bx and 1 year after Bx.

Results: In the 76% of PC+ Bx, I and EMT markers were lower than in PC- (4.8±6.54 vs 8.2±4.97, p=0.03; 17.5±1.5 vs 10.5±6.09, p=0.05, resp). MM parameters were not related with either PC or any other marker. I and EMT were negatively related with MDRD (r=-0.007, p=0.01 respectively). A neg correlation was found between 25OH-VitD and both I (p=0.0002) and EMT (p=0.004) and FMT were significantly higher (12.6±7.0 vs 9.5±5.6 % p=0.05).

Conclusions: In the 14 patients restarting dialysis during the follow-up, I (12.9±7.9 vs 7.0±6.8 % p=0.004) and EMT were significantly higher (12.6±7.0 vs 9.5±5.6 % p=0.05).

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

SA-PO3116

Correlates of FGF23 Levels in a Population of Stable Kidney Transplant Recipients with Hypovitaminosis D and Hyperparathyroidism

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Background: FGF-23 interacts with Vitamin D and parathyroid metabolism in a complex fashion that has not been fully delineated, resulting in conflicting results when patients with CKD are studied. FGF-23 is elevated early post-transplant but has not been carefully studied at longer time periods.

Methods: 41 stable kidney transplant recipients (KTRs) with hyperparathyroidism and hypovitaminosis D were studied. Patients were not receiving supplementation with any form of Vitamin D. Full length FGF-23 was measured by ELISA.

Results: The population included 22 men (51%), 34 Blacks (83%), mean age 49.2±13.5; 55.5±47 months since last transplant, creatinine 1.5±0.4 mg/dl, phosphorus, 2.97±0.56, magnesium, 1.72±0.3, 25-OH Vit D 16±0.7, 1.25 (OH)2 Vit D3, 49.1±18.2, PTH, 187±73.8, GFR by MDRD, 56.9±19.2 ml/min (range 21.7-103.4 ml/min). The mean FGF23 value was 103.6±84.8 pg/ml (range 33.8-388.6 pg/ml). 90% of the patients had FGF23 levels > 55 pg/ml. FGF-23 levels positively correlated with gender r=0.39, p=0.324, months since last transplant, r=-0.322, p=0.045, eGFR r=-0.484, p<0.001, creatinine r=0.369, p=0.017, serum phosphorus r=-0.428, r=0.06, and serum magnesium r=0.374, r=0.016. FGF-23 levels correlated inversely with 1,25 vit D r=-0.351, p=0.026, and GFR by MDRD r=0.469, p=0.002, although 1.25 Vit D levels did not correlate with FGF. By T-test Black pts had lower FGF23 values than Non-black, mean 94.3 pg/ml vs. 148.7 pg/ml, p=0.042 although GFR by MDRD did not differ. There were no correlations between FGF-23 and age, tumorular dosage or for prednisone daily dose, diuretic use, BIP, calcium level, 25-OH vitamin D level, or PTH.

Conclusions: 1) FGF23 maintains an inverse relationship with 1,25 Vitamin D in the pts with Vitamin D deficiency despite elevated PTH levels and abnorimal kidney function, suggesting that FGF23 may be more important in regulating 1,25 Vit D than PTH in this setting. 2) Black pts have lower FGF23 levels than Non-blacks despite similar GFR, 3) FGF23 maintains a strong correlation with gender, GFR by MDRD, P04, Mg++ and time since transplant, but as these factors are interrelated, a larger population will be needed to examine independent relationships.

Funding: Pharmaceutical Company Support
SA-PO3118

Value of Adenosine Stress Cardiac Magnetic Resonance Imaging for Coronary Artery Disease Screening before Renal Transplantation
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Background: Coronary heart disease remains a major cause of morbidity and mortality after renal transplantation, also some practice guidelines recommend to evaluate ischemic heart disease before renal transplantation. We studied cardiac stress-magnetic resonance imaging (MRI) in this indication.

Methods: Candidates for kidney transplantation with risk factors for coronary disease underwent a cardiac stress-MRI scan (injection of gadoterate and coronary stress induced by adenosine). When the MRI scan revealed positive results, a coronary angiography was carried out. The incidence of major cardiovascular events and gadodetate toxicity were recorded during follow-up.

Results: Since January 2008, 114 patients have undergone a cardiac stress-MRI scan. These patients had an average of 2.7±1.4 cardiovascular risk factors (age 55.6±10.4 years, diabetes: 35.3%). Coronary lesions were suspected on 22 MRI scans and confirmed in 85% by systematic coronary angiography. Thus, we diagnosed 9.4% of coronary disease in patients with no previous history of coronary artery disease, leading to specific treatment. In the population, sensitivity of cardiac MRI was 89.5% and specificity 62.5%. During follow-up (1.6±0.7 years), 35 patients received a kidney transplant and 5 patients had a major cardiac event. Negative predictive value of cardiac MRI for major coronary events is 96.7%. No patient developed nephrogenic systemic fibrosis.

Conclusions: With its high negative predictive value, stress-cardiac MRI appears to be a valuable tool for excluding ischemic heart disease before kidney transplantation.

Funding: Government Support - Non-U.S.

SA-PO3119

Risks of Death and Graft Failure Following Percutaneous Compared with Surgical Coronary Revascularization in Renal Transplant Patients
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Background: Surgical (CABG) or percutaneous (PCI) intervention may precipitate graft failure (GF) in patients with renal transplants, but reliable data on the absolute and relative risks of death and GF following PCI and CABG are unavailable.

Methods: Renal transplant patients undergoing PCI (3,531) or CABG (1,003) were identified using the USRDS. Comorbidity adjusted Cox models were used to assess the relative risks of death and GF following PCI and CABG are unavailable.

Results: Age race and sex were similarly distributed in the PCI and CABG groups. In the PCI group, 36% received bare metal stents, 36% received drug eluting stents, and 17% had intervention on ≥2 vessels. Internal mammary grafts were used in 84% of CABG patients, 95% had ≥2 vessels bypassed, and 83% had on-pump procedures. In-hospital death (2.3% vs. 5.3%) was more frequent after CABG. Overall survival was non-significantly lower with CABG (HR 1.14, P=0.05) in crude models [figure 1], but revascularization type was not associated with overall survival after adjustment for comorbidities (HR 0.89, P=0.12). At 3 years, freedom from non-fatal GF was lower with CABG vs. PCI (83% vs. 85%). However, the difference in non-fatal GF did not achieve significance in crude (P=0.09) or adjusted models (P=0.72). The risk of GF was not different following off-pump vs. on-pump CABG (HR 0.78, P=0.34).

Conclusions: CABG does not appear to be associated with improved survival compared with PCI in the renal transplant population. Non-fatal GF occurs more frequently following CABG, but the risk of non-fatal GF is similar with both PCI vs. CABG and off-pump vs. on-pump CABG following adjustment for baseline comorbidity.

SA-PO3120

Renal Cell Carcinoma in Allograft Kidneys: Use of Short Tandem Repeat Analysis To Determine Donor Origin of Cancer
Kumarpal C. Shrishirimal, Eric P. Cohen, Ehab R. Saad, Lauren N. Parsons, Min Le, Alexander C. Mackinnon. Medical College of Wisconsin.

Background: Kidney transplant recipients have a high risk for renal cell carcinoma (RCC) in their native kidneys. We describe 3 cases of RCC in allograft kidneys. Short tandem repeat (STR) DNA was used to determine donor origin of the RCC.

Methods: RCC tissue was obtained in all cases. Donor DNA was obtained from cancer-free regions of the kidney (cases 1 and 2); Donor DNA was not available for case 3. Host DNA was obtained from fresh leukocytes (cases 1 and 3) or a non-cancerous lymph node (case 2). Penta C and Penta D genotypes were determined using GenoMapper.

Results: Pent C and Pent D alleles were robustly amplified in all samples. Each case had two or more informative Pent C or Pent D alleles. In the two cases with donor DNA samples (cases 1 and 2), the Pent C and Penta D alleles from the cancer exactly matched the Pent C and Penta D alleles derived from the uninvolved donor kidney. Furthermore, one or more Pent C or Pent D alleles from the host were absent from the cancer. This shows that the cancer DNA is donor-derived, being genetically identical to the donor DNA. In case 3 donor DNA was not available for analysis, so cancer and host alleles were compared. In this instance, none of the Pent D alleles matched in the two specimens, and each specimen had a unique Pent C allele indicating that the cancer is genetically distinct from the host.

Conclusions: Determination of tumor cell origin is potentially significant for treating metastatic cancers, as in case 3. Reduction of immunosuppression may cause cancer rejection for cancers derived from the donor. But host derived cancers would not respond to reduction of immunosuppression. The Pent C and Pent D method is informative of sample identity over 99% of the time. Our data show that STR analysis is a simple strategy to determine donor versus recipient origin of cancers in kidney transplant patients.
SA-PO3121
Clinical Features and Outcomes of Tuberculosis among Kidney Transplant Recipients in Brazil: A Report of the Last Decade
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Background: It is necessary to clarify the incidence of tuberculosis (TB) among kidney transplant recipients (KTR) as well as changes in the chronology, clinical presentation, and prognosis of the disease, especially in highly endemic areas, in order to develop prophylaxis strategies.

Methods: Retrospective single-center observational study involving all cases of TB that occurred between 2000 and 2010 confirmed by culture, isolation of M.tuberculosis DNA by polymerase chain reaction or histopathology, according to WHO criteria.

Results: Among the 1549 kidney transplantations performed during the study period, 43 (2.8%) developed TB, with an incidence of 803 cases per 100,000 patients per year, which was very higher than in general population in Brazil (45 cases per 100,000 inhabitants-per-year; RR 17.8). Of the TB cases, 84% occurred within the first 2 years post-transplant, and 72% were pulmonary forms. Previous TB infection was present in 3 (7%) patients. No chemoprophylaxis was applied. The most common symptoms were fever (79%), cough (35%) and dyspnea (16%). Time elapsed from the onset of symptoms to the start of treatment was 28 days (range, 2 – 138 days). Median length of antimicrobial therapy was 196 days. Immunosuppressive therapy was reduced in 15 (35%) patients and incidence of acute rejection was higher in TB than in non-TB group (44% vs. 28%, p=0.03). Crude mortality was 14%, and attributable mortality was 12%. Ten year death-censored graft survival (44.3% vs. 56.3%, p=0.64) and patient survival (69.1% vs. 72.4%, p=0.67) were similar between TB and non-TB groups, respectively.

Conclusions: Kidney transplantation increases the risk of TB. Symptoms of infection are often attenuated, leading to delayed diagnosis. TB-attributable mortality is still high. Clinicians should consider chemoprophylaxis for high risk patients, as the residents of endemic areas. However, randomized controlled trials are needed to confirm the benefits of such approach.

SA-PO3122
The Effect of Interferon Therapy on HLA Alloantibodies in Waitlisted Patients with Hepatitis C Infection
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Background: Interferon (IFN) therapy is recommended for dialysis patients with chronic hepatitis C (HCV) awaiting kidney transplantation. IFN is known to cause autoimmunity and increase alloimmunity. We aimed to examine the effect of IFN on HLA antibodies (Ab) in waitlisted chronic HCV patients.

Methods: All current kidney waitlisted patients and patients that received a kidney transplant from 1/2008 to 1/2011 were screened for HCV serostatus. Patients with a positive HCV PCR and pre-existing HLA antibodies determined by Luminex single antigen (LSA) beads were considered for study. Patients without LSA testing 3 months to 12 months apart were excluded. We identified 2 groups of patient, those treated with IFN (treatment group) and those not treated (control group) in-between LSA testing. Antibody strength (Ab) was quantified by mean fluorescence intensity (MFI). We used a threshold of 10,000 MFI to determine pPRA.

Results: We identified 7 patients that were treated with IFN and 23 patients who were not IFN treated that had 2 sets of LSA testing. In the treatment group 3 patients had a prior kidney transplant of which 1 had the graft present at time of IFN. In the control group, 3 patients had a prior transplant of which 2 had the graft present at the time of LSA testing. 43% (3/7) IFN treated patients and 26% (6/23) of patients in the control group had a significant rise in median MFI (p=0.64, Fishers exact test). The median change in MFI was 239 (IQR -377 to 2035) in the IFN group and 4 (IQR -3226 to 940) in the control group. The change in Ab strength was not significantly different between groups (p=0.16, Mann-Whitney test). Class I and class II Ab strength was not significantly different between groups. The median pPRA (MFI >10,000) was 94% (range 0-100) in the IFN group and did not change after IFN therapy (median pPRA 94% pre-IFN vs. 94% post-IFN).

Conclusions: Compared to a matched control group IFN did not change the strength or breadth of preexisting HLA antibodies. Our data suggests that the decision to treat chronic HCV with IFN should not be altered out of concern for increasing sensitization in kidney transplant waitlisted patients.

SA-PO3123
Tacrolimus Percent Coefficient of Variation as a Superior Marker of Late Acute Rejection Associated with Nonadherence in Adolescent Renal Transplant Recipients
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Background: We have previously shown that tacrolimus (TAC) percent coefficient of variation (CV%) is a superior marker compared to TAC standard deviation (SD) for detecting the likelihood of acute rejection (rej) associated with medication nonadherence (NA) in pediatric renal transplantation (Tx). We aimed to identify a significant threshold and time interval for which TAC CV% is predictive of late acute rej associated with NA in adolescents.

Methods: TAC SD and CV% were measured in 31 adolescents (ages 11-21) who underwent Tx from 2004 - 2008. Patients were maintained on immunosuppression with TAC and MMF ± steroids. All rej was confirmed by biopsy (Bx). Bxs were classified by Banff 2007 criteria. SD and CV% was calculated from trough TAC levels over various time intervals post-Tx. Receiver operator curve (ROC) analysis was used to analyze TAC CV% values.

Results: We found that the significant time interval for TAC CV% measurement was 6 mos prior to the first rej or, in non-rejectors, 6 mos prior to the last clinic follow-up. TAC CV% was higher in rejectors vs. non-rejectors (58.2% vs. 26.9%; p = 0.021).

Figure 1: TAC CV%, by rejection status 6 months prior to last clinic follow-up or biopsy-proven rejection.

Conclusions: We identified a significant time interval for TAC CV% measurement.

SA-PO3124
Glomerulosclerosis in 0-Hour Biopsy and Left Ventricular Mass Index (LVMI) in Patients with Hepatitis C Infection (HCV) with or without Interferon Therapy (IFN)
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Background: Although living kidney donors are almost normal healthy individuals, the “0-hour” biopsy from them often reveal glomerulosclerosis (GS) to various degrees. Recently, cardiacorenal interaction has been a major clinical implication and it is well known about high prevalence of left ventricular hypertrophy (LVH) in CKD patients. However, this interaction in the subjects with GS despite normal kidney function has not been previously reported.

Methods: We examined the association between the proportion of GS (%) in the 0-hour biopsy and LVMI determined by the echocardiogram before surgery. In the 206 subjects who donated a kidney in our hospital from March 2006 to May 2011, 50 donors were excluded because of lack of clinical data or inadequate kidney samples (less than 10 g). We divided the remaining 156 donors into the three groups according to %GS; the subjects without GS (Group I, n=41), the subjects with %GS of 0.1–9.9% (Group II, n=58), and the subjects with %GS above 10% (Group III, n=57). We compared baseline characteristics and LVMI among the groups. Moreover, we investigated correlation between %GS and LVMI by multivariate analysis. Data are expressed as mean ± SD.

Results: LVMI in Group III was significantly higher than those in the other two groups (8.0 ± 3.1, 8.3 ± 1.3, and 9.1 ± 3.2 g/m² in Group I, II, and III, respectively; p < 0.05). The multivariate linear regression analysis showed that %GS was significantly associated with LVMI even after adjusting for the other confounders like age, renal function, and hypertension (standardized β = 0.16, p < 0.05).

Conclusions: The present study, the association between GS and LVMI was observed in the living kidney donors. This association was independent of blood pressure, renal function and age, suggesting that cardio-renal interaction might exist even before renal function declines.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

841A
Expression of FGF23/KLOTHO System in Human Vascular Tissue

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Background: Fibroblast growth factor (FGF)-23 levels have been independently associated with impaired vasoreactivity and increased arterial stiffness, as well as cardiovascular events and mortality, whereas a protective function of KLOTHO against endothelial dysfunction has been reported, suggesting the potential participation of FGF23-KLOTHO axis in human vascular pathophysiology. Since expression of members of the FGF23-KLOTHO system in human vascular tissue remains unproven, we aimed to study the expression of FGF23, FGF receptors (FGFR) and KLOTHO in human aorta.

Methods: Thoracic aorta specimens from 44 patients with coronary artery disease who underwent elective coronary artery bypass surgery were tested for expression of FGF23-KLOTHO system, including FGF receptors.

Results: Expression of KLOTHO (mean expression level 4.85±1.53, arbitrary units) and of two of the three cognate FGF (FGFR-1 and -3) were detected and confirmed by RT-PCR, sequencing and qRT-PCR. However, expression of FGF23 and FGFR4 was not observed. We also detected the expression of membrane-anchored A Desintegrin and Metalloproteinases (ADAM)-17, the enzyme responsible for the shedding of KLOTHO from the cell surface, and the anti-inflammatory cytokine interleukin (IL)-10. Interestingly, there was a direct association between the KLOTHO mRNA expression levels and those of ADAM-17 and IL-10 (r=0.54, P<0.001; r=0.51, P<0.01, respectively).

Conclusions: Human vascular tissue expresses members of the FGF23-KLOTHO system, indicating that it can be a direct target organ for FGF23. These findings suggest a putative role of FGF23-KLOTHO axis in human vascular pathophysiology and cardiovascular disease.

Funding: Government Support - Non-U.S.

Niacin Protects Against Renal but Aggravates Cardiac Damage in Rats with the Metabolic Syndrome

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Background: Studies were conducted to examine the end-organ protective effects of niacin in obese spontaneously hypertensive rats (SHROB/cp); a model of the metabolic syndrome.

Methods: Animals were chronically treated with either a low dose (1% in chow; n=5) or a high dose (5% in chow; n=5) of niacin starting at 7.14 weeks of age. SHROB/cp (n=9) and lean hypertensive control animals (SHROB/Kol) received Purina 5008 chow to which niacin in obese spontaneously hypertensive rats (SHROB/cp); a model of the metabolic syndrome. No protein and mRNA analysis.

Results: Our results indicate that niacin treatment reduced renal damage in SHROB/cp, as indicated by a significant decrease in urinary protein excretion (UPE) and the degree of renal damage expressed as the percentage of animals with focal segmental glomerular sclerosis (FSGS) in the SHROB/cp group. Niacin treatment also significantly decreased cardiac injury as indicated by a decrease in cardiac infarcts and cardiac fibrosis.

Conclusions: These results suggest that 1) cardiac and renal damage in SHROB/cp are independent of hypertension; the latter considered secondary to hyperperfusion injury of the metabolic syndrome and 2) niacin causes target-organ protection in the kidney, but may further aggravate cardiac injury.

Funding: Private Foundation Support

Loss of TDAG51 Inhibits Vascular Medial Calcification Induced by High Doses of 1,25 Dihydroxycholecalciferol

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Background: Vascular calcification (VC) is a progressive disorder that increases stiffening in the aorta and large capacitative arteries. This process is associated with atherosclerosis, renal disease and mortality in these patients. However, the underlying molecular mechanism of vascular calcification in atherosclerosis remains unclear. We hypothesized that an imbalance between genes that promote and inhibit osteoblast differentiation drives this process. Thus, we tested the effects of T-cell death associated gene 51 (TDAG51), a novel osteoblast differentiation promoting gene, which modulates and induces smooth muscle cell calcification and differentiation to osteoblast-like cell phenotype.

Methods: We induced vascular medial calcification in mice by administration of super-physiological doses of 1,25 dihydroxycholecalciferol (Vitamin D3). This VC model increases bone resorption leading to elevated plasma Ca2+ and PO4 levels inducing genes that promote osteoblast differentiation and calcification. C57BL/6 and TDAG51 knockout male mice (8 weeks of age) were administered subcutaneous injection of Vitamin D3 (50,000IU) for three consecutive days. Four days post-treatment, tissues were harvested for protein and mRNA analysis.

Results: Our results indicate that TDAG51 -/- male mice receiving Vitamin D3 had increased TDAG51 mRNA and protein levels in the aorta. TDAG51 knockout mice aorta and aortic smooth muscle cells (ASMC) have significantly less Ca2+ deposition/ing protein, reduced mineralization observed by von Kossa, xylanol orange staining and decreased alkaline phosphatase activity. Furthermore, peroxisome proliferator-activated receptor (PPAR) gamma mRNA and protein levels are up-regulated in TDAG51 -/- ASMC, whereas osteoblast differentiation marker RUNX2/cbfal, Osterix, and phospho-SMAD 1/5 are down-regulated.

Conclusions: This data suggests, the loss of TDAG51 gene may confer resistance to smooth muscle calcification and differentiation to an osteoblast-like phenotype, thereby decreasing the risk of cardiovascular disease in ESRD.

Funding: Government Support - Non-U.S.

The Interaction of Uraemic Toxins and Endothelial Progenitor Cells in the Progression of Cardiovascular Disease

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Background: Bone marrow (BM) derived endothelial progenitor cells (EPC) have a role in both blood vessel formation and regulation of function. How EPCs are affected by uraemic toxins p-cresol (PC) and indoxyl sulfate (IS), and how this relates to cardiovascular disease (CVD) risk is not clear.

Methods: Peripheral blood (PB) concentrations of EPCs, IS and PC were measured in control (Cs), haemodialysis (HDs) and kidney transplant (KTx) subjects, and compared to markers of CVD. In vitro, functional assays were performed on cultured HUVEC and EPC in the setting of increasing physiological concentrations of PC and IS.

Results: PB EPC concentration (% of mononuclear cells, MNCs) was decreased in KTx (0.006±0.006%, 95%Ci) and HDs (0.006±0.005) compared to Cs (0.03±0.02, n=9, group; p<0.01). Both PC sulfate (Cs 2.10±0.8mg/L, HDs 23.6±12.4, KTx 6.0±2.5) and IS (Cs 1.9±1.7mg/L, HDs 60.0±26, KTx 1.9±1.3) were increased in HDs (p<0.001). There was no association between EPC and toxin concentrations (p=ns). Low EPC counts were associated with history of CVD (p=0.002). Carotid intima medial thickness, aortic pulse wave velocity and augmentation index did not correlate with toxin or EPC concentration.

In vitro, PC inhibited tube-forming capacity of HUVEC (n=5, one-way ANOVA p<0.01), with no benefit of addition of EPC (n=5, p=ns). Increased IS concentration inhibited HUVEC tube formation (n=5, p<0.05), however EPC addition prevented IS reduction in HUVEC tubes (n=4, p=ns). No difference was seen in EPC migration with toxins. Vascular cell adhesion molecule-1 expression increased on HUVEC with increased PC/IS concentration (p<0.05), but with no effect of EPC addition.

Conclusions: EPC PB concentration does not correlate with toxin concentration, however reduced EPC function, as shown in vitro to PC and IS, may be more clinically relevant.

Funding: Private Foundation Support, Government Support - Non-U.S.

Poster/Saturday

Vascular Pathology

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Underline represents presenting author.

842A
SA-PO3129

Effect of Different Vitamin D Receptor Activators on Left Ventricular Hypertrophy and Myocardial Fibrosis in Subtotally Nephrectomised Rats

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Background: Observational data suggest that increased survival in CKD patients treated with vitamin D receptor activators (VDRA)s could be part in due to their positive cardiac effect. The aim of this study was to analyze the effect of different VDRAs on left ventricular hypertrophy (LVH) and myocardial fibrosis in uremic rats.

Methods: Rats (n=22) with 7/8 nephrectomy were treated with equivalent doses of VDRAs (calcitriol 10 ng/kg/day, alfalcacidol 20 ng/kg/day and paricalcitol 30 ng/kg/day, 5 days per week) for 4 weeks. Placebo (n=7) and Sham (n=7) groups were included for comparison. Blood and tissues were collected at harvest. Histological and molecular parameters of LVH and fibrosis were evaluated.

Results: All VDRAs prevented LVH, the values of body weight ratio, wall and septum thickness and cardiomyocytes size were similar to those observed in the Sham group. In addition, all VDRAs showed a significant decrease in atrial and brain natriuretic peptides (ANP and BNP) expression and they were able to decrease the phosphorylation of ERK 1/2, this effect was more marked in the paricalcitol group. Paricalcitol was the only VDRA able to reduce myocardial fibrosis compared to Placebo (2.6±0.6 % vs. 10.5±3.0 %, p=0.027) (Masson staining), showing similar values than the Sham group (2.8±1.1 %). Paricalcitol and alfalcacidol reduced collagen I expression, but only paricalcitol showed significant increases of collagenase MMP1 expression, a finding that suggest a high rate of collagen degradation.

Conclusions: In summary, the use of VDRAs prevents LVH in uremic rats. Paricalcitol was the most effective preventing myocardial fibrosis.

Funding: Pharmaceutical Company Support, Government Support - Non-U.S.

SA-PO3130

Differential Expression of MicroRNA-126 Contributes to Renal Microvascular Heterogeneity in VCAM-1 Protein Expression in Inflammation

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Background: Endothelial cells (EC) in different microvascular segments of the kidney have different functions and exhibit differential responsiveness to disease stimuli. Microvascular-segment specific responses to anti-glomerular basement membrane (anti-GBM) glomerulonephritis (GN)-induction as well as TNF-α, LPS, and anti-MPO induced GN showed that glomerular E-selectin expression was transcriptionally regulated. In contrast, VCAM-1 mRNA expression was highly increased in both arteries and glomeruli, while the protein was only expressed to a limited extent in the glomerular compartment suggesting post-transcriptional regulation.

Methods: To assess a role for microRNA-126 (miR-126) in microvascular-segment specific regulation of VCAM-1 we employed mouse models of inflammation and isolated seven hundred glomeruli and arteriolar vascular segments and quantified both the expression of mRNAs and miR-126. Using an antagonmir we silenced miR-126 in mice to identify the role of this miR in regional VCAM-1 expression.

Results: We validated the regulation of VCAM-1 by miR-126 in glomerular EC in vitro. In mice with experimental anti-GBM glomerulonephritis (VCAM-1 mRNA expression was highly increased in both arteries and glomeruli, while VCAM-1 protein expression was expressed to a limited extent in the glomeruli. These high VCAM-1 mRNA - low VCAM-1 protein levels were associated with high local miR-126 levels. To further augment expression of phospho-p44/42 in EC, VEGF further augmented expression of phospho-p44/42 in EC upon inflammatory challenge with TNF-α. Heterogenic expression of the transcription factor Ets-1 with preferential expression in the glomerular compartment likely underlies the spatial expression pattern of miR-126.

Conclusions: These data imply that miR-126 has a major role in the segmental, heterogenic response of microvascular EC to systemic inflammatory stimuli.

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SA-PO3131

Uremia Regulates Tissue Factor Stability and Ubiquitylation and Predisposes to Stent Thrombosis

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Background: Coronary stent thrombosis (ST) is a fatal complication that occurs in 1.3% of patients and results in 30% mortality. CRF has emerged as one of the strongest predictors with a 6-fold higher risk. Tissue factor (TF) is a crucial mediator of injury-related thrombosis. We investigated effects and mechanism of ursemic serum and solutes on thrombosis and TF regulation.

Methods: Pooled sera from 23 ESRD patients and healthy individuals were used. As a model of the de-endothelialized, post-interventional state, we exposed primary human vascular smooth muscle cells (vSMC) pretreated with ursemic or control sera in an ex vivo flow-loop system. TF abundance, activity and mRNA in vSMC were examined. vSMCs were treated with individual ursemic solutes at a concentration observed in ESRD patients.

Results: vSMCs pretreated with ursemic serum showed significantly greater clot formation in flow-loop model. Uremic serum induced higher TF expression and activity in vSMCs. This effect was partially recapitulated by isolated ursemic solutes including indole-3-acetic acid (IA), indoxyl sulfate (IS) and uric acid (UA). We further demonstrate that TF is ubiquitylated at baseline and IA and IS significantly prolong TF half-life by reducing its ubiquitylation. Consistently, vSMCs treated with IA, IS and UA were more thrombogenic in flow-loop model.

Conclusions: Uremia significantly increases thrombogenicity by upregulating TF in vSMCs. Uremic solutes partially account for this via regulating TF stability by decreasing ursemic TF ubiquitylation. Together, these observations demonstrate that CNIs have direct and/or indirect effects on the activation of the MAPK intracellular signaling pathway in ECs.

Funding: NIHDK Support, Other NIH Support - NIHHLB, Private Foundation Support

SA-PO3132

Calcineurin Inhibitors Stimulate MAPK Signaling in Endothelial Cells: Implications for Novel Therapeutics

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Background: The calcineurin inhibitors (CNI), cyclosporine A (CsA) and FK506, are potent immunosuppressive agents used to treat a broad range of renal diseases. While the rationale for these strategies is based on their ability to inhibit lymphocyte activation, we propose that these agents alter additional molecular signals in non-immune cells that serve to attenuate progression of the underlying disease process. We recently observed that CNIs have effects on the activity of Ras family GTPases within vascular endothelial cells (EC).

Methods: Purified human EC (HUVEC) were cultured in the absence or presence of CsA or FK506 (1 to 1000ng/ml), and the temporal phosphorylation of Akt, mTOR, p70S6K and p44/42 were evaluated by Western blot analysis.

Results: In multiple experiments, we found little effect of each CNI on the activity of the PI-3K/Akt signaling pathway, but we consistently found a marked increase in the expression of phospho-p44/42 after ~ 2.5hrs of treatment. We also assessed the interaction between each CNI and VEGF, which is known to induce activity of these intracellular signals. VEGF (5-20ng/ml) enhanced MAPK activity; and we found that CNIs have an additive effect to further augment expression of phospho-p44/42 in EC. Finally, to determine the functional implications of these observations, HUVEC were cultured with CsA (0.1-1µg/ml) or FK506 (0.01-1µg/ml) +/- VEGF for 72hrs and the degree of proliferation was assessed by 3H-thymidine incorporation. CNIs alone were found to augment EC proliferation from basal levels, but were potent to augment the VEGF-induced proliferative response.

Conclusions: Collectively, these observations demonstrate that CNIs have direct and/or indirect effects on the activation of the MAPK intracellular signaling pathway in ECs. Our studies have implications for therapeutic uses of CNIs to promote vasculoprotection or vascular repair; and they provide insight into how these agents may protect against immune-mediated renal injury.

Funding: NIDDK Support, Pharmaceutical Company Support
SA-PO3133
Neurohumoral and Renal Mechanisms in the Pathogenesis of Hypertension in Polycystic Kidney Disease
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Background: Hypertension is a common presenting factor in polycystic kidney disease (PKD) patients prior to the onset of renal failure. Neurohumoral systems such as the sympathetic nervous system (SNS) and renin-angiotensin system (RAS) are key regulators of blood pressure and may contribute to hypertension in these patients. We have shown that AngII levels and PRA are reduced in the Lewis Polycystic Kidney (LPK) model of PKD at 12 wk of age. In this study we examined AngII at different ages, and baseline levels of SNS activation, by determination of circulating cytokines and metabolites.

Methods: LPK & age matched Lewis controls were assessed at 6, 10 and 16 wks old. Systolic blood pressure was measured via tail cuff and upon euthanasia, serum collected. Kidneys were collected for quantitative RT-PCR for RAS genes (angiotensinogen, renin, angiotensin converting enzyme (ACE) II) and the ATR1A. Plasma AngII levels were determined by radioimmunoassay and catecholamines by HPLC.

Results: In LPK of all age groups, intra-renal expression of RAS genes were greater in the LPK (p=0.05, n=12). ATR1A receptor and ACE displayed 8 fold increases, while ACE2, Renin and Angio displayed 2, 4 and 6 fold increases, respectively, in comparison to Lewis. Ang II levels were decreased in the LPK across all age groups (LPK = 24.1 v Lewis = 349 pg/mL) whilst catecholamine levels were greater for noradrenaline (4.9 v 0.54nmol/L) & adrenalin (4.7 v 0.87nmol/mL) in 10 wk old LPK (p=0.05).

Conclusions: Increased expression of intra-renal RAS genes, in concert with a concomitant decrease in serum AngII levels provide evidence for intra-renal RAS as a local mechanism driving hypertension in PKD. However, increased circulating catecholamines suggest an additive role for the SNS, which in addition to direct vascular effects may modulate RAS gene expression in the kidney.

SA-PO3134
Impact of Vitamin D on Cachexia, Cardiovascular Disease and Mortality in Chronic Kidney Disease
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Background: Patients with chronic kidney disease (CKD) have increased mortality and morbidity. Known risk factors include vitamin D insufficiency, cardiovascular (CV) disease, and cachexia. We studied the effect of vitamin D supplementation on nutrition, CV system, and mortality in a mouse model of CKD.

Methods: A 3 wk old Mice undergoing 5/6 nephrectomy (N). We previously showed decreased serum levels of 25-ViD and 1,25-ViD in N mice. N mice received 25-ViD (N=25ViD) (0.08mg/kg, ip, 3x/week), paracalcit (N-PC) (0.15mg/kg, ip, 3x/week) or vehicle (N-V). Sham-operated mice received vehicle (S-V). N-V mice were fed ad libitum. S-V, N-25ViD, N-PC mice were pair-fed to N-V mice. The study period was 90 days.

Results: Serum BUN and creatinine was significantly higher in N-V, N-25VitD and N-PC compared with S-V mice (p<0.01). The mortality rate in N-V mice (57.5%) was significantly higher than N-25VitD (40.0%) or N-PC (30.0%) mice. N-25VitD and N-PC mice gained more weight than N-V mice (p<0.01). The weight gain in N mice was related to a reduction in food consumption. Basal metabolic rate was significantly higher in N compared with N-25ViD and N-PC mice (p<0.01). N-V mice lost lean body mass and fat mass whereas N-25ViD and N-PC mice gained lean body mass and fat mass. Muscle strength was assessed. Rotator activity and grip strength were significantly improved in N-25ViD, N-PC compared with N-V mice (p<0.05).

Conclusions: Vitamin D3, 25-VD, and 1,25-ViD reduces mortality and cachexia in a mouse model of CKD. concert with increased CV system and muscle mass.

Funding: Pharmaceutical Company Support

SA-PO3135
Direct Renin Inhibitor Is Better Than Angiotensin II Receptor Blocker for Intrarenal Arterioles
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Background: We have reported that long-term administration of angiotensin II receptor blockers (ARBs) on rats induced unusual proliferative changes of smooth muscle cells (SMCs) in renal afferent arteriolar walls, and the changes were suspected to be induced by angiotensin II (AngII) and renin. In the present study, we examined effects of long-term administration of a direct renin inhibitor (DRI) on intrarenal arterioles in spontaneous hypertensive rats (SHR).

Methods: Sixteen 6-week-old male SHR were divided into the following three groups: DRI (n=5), ARB (n=5) and standard diet containing saline, 10mg/kg/day, ARB group fed a standard diet containing varsartan 10mg/kg/day, and control group (n=5) fed a standard diet, respectively. Blood pressure and proteinuria were measured every three weeks. After 12 weeks, blood samples were drawn; afterward, light microscopically, electronmicroscopically and immunohistochemical examinations were performed.

Results: Blood pressure in the DRI group and the control group were significantly high compared to the ARB group (188.4±4.2, 185.8±19.3, 124.5±7.3mmHg, respectively). Proteinuria (as a percent of the different arteriolar SMCs were more frequently observed in the ARB group than in the control group (48.9±6.7% vs 51.5±2.9% of total observed arterioles in each rat, p=0.0061), but the SMC changes were rarely seen in the DRI group. In the DRI group, glomerular abnormalities and tubulointerstitial changes were seen in all cases. Both angiotensin II receptor protein expression and its expressions significantly decreased in the DRI group (11.4±6.5% versus 29.5±10.4% at 844A day) compared to the ARB group and the control group (30.1±7.6 and 37.5±8.5mg/day, p=0.0487 and p=0.0043, respectively). The creatinine level remained higher in the DRI group than in the control group (0.4±0.40 versus 0.1±0.03mg/dl, p=0.0158).

Conclusions: The long-term ARB administration induced unusual proliferative changes of SMCs in arteriolar arteries; however, DRI does not induce such proliferative changes in SHR. It is indicated that DRI is better than ARB for intrarenal arterioles to suppress the renin-angiotensin system.

Funding: Pharmaceutical Company Support

SA-PO3136
Chronic Kidney Disease in a Rat Model Modifies Tissue Concentrations of Vitamin K
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Background: Patients with chronic kidney disease (CKD) develop vascular calcification (VC) and serum phosphorus is considered a putative signalling molecule in VSMC calcification. We investigated mRNA expression of the osteo-blaster marker, BMP2, an osteoblast marker, and RUNX3/cbfa3, a chondrocyte marker, were determined significantly, as previously reported for TNF-α, calcification (VC) and serum phosphorus is considered a putative signalling molecule in VSMC calcification. The major form of vitamin K consumed in the diet and preferentially found in the liver is Phylloquinone (K1). Menaquinone-4 (MK-4), although present in the diet in small quantities, is preferentially found in extra-hepatic tissues. In the present study we assessed tissue concentrations of K1 and MK-4 in the presence of CKD.

Methods: Sprague Dawley Sprague rats were fed a diet containing 0% adenine (control; n=18), both diets containing high phosphorus (1% and 2% of diet) were fed a diet containing 0% or 0.25% adenine (K-24); or 0% adenine (control; n=18), both diets containing high phosphorus (1% and 0.25% adenine (K-V) for 7 weeks. Kidney was assessed by measuring serum creatinine. The concentrations of K1 and MK-4 in tissue and serum were determined by reversed phase HPLC and expressed relative to weight.

Results: Serum creatinine was elevated in the CKD group (352 ± 170 µM) compared to controls (50 ± 10 µM, p<0.001). CKD animals had significantly higher levels of K1 in the serum (1.8±0.1 v 0.7±0.5 pmol/g, p=0.019) and lower levels in the liver (33.6±20 v 57.3±13 pmol/g, p=0.001) compared to control animals. The kidney medulla in CKD animals had significantly higher MK-4 levels (58.3±21 vs 36.9±18.8 pmol/g, p<0.001) and lower K1 levels (5.4±0.4 v 11.2±8.8 pmol/g, p=0.015) compared to controls. The thoracic aorta accumulated MK-4 and K1 in CKD animals (104.5±46.3 and 152.3±234.9 pmol/g, n=9). MK-4 concentration in the thoracic aorta had a positive correlation with serum phosphorus (r2=0.8, p<0.001).

Conclusions: These results indicate that CKD modifies tissue concentrations of K1 and MK-4 under low dietary conditions. The presence of CKD attenuates K1 storage in the liver but enhances MK-4 accumulation in non-hepatic tissues. In the thoracic aorta, we found a significant correlation between serum phosphorus and MK-4. We hypothesize that there are, as yet undefined, factors associated with CKD that increase peripheral conversion of K1 to MK-4.

Funding: Government Support - Non-U.S.
Conclusions: TNF-α and LPS aggravate VC, involving osteoblastic but not chondrocyte cell type via TRAF6 and NFκB-independent pathway. Since LPS is often contaminated in water for dialysis, it is suggested that water purification is important to prevent progression of VC in the patients on chronic HD.

Funding: Government Support - Non-U.S.

SA-PO3138
(Pro)renin Receptor-Like Immunoreactivity in Patients with Chronic Renal Failure Kazuhito Totsune, 1 Takuo Hirose, 1 Nobuyoshi Mori, 1 Hirohito Metoki, 1 Kei Asayama, 2 Masahiro Kikuya, 1 Osamu Murakami, 2 Yutaka Imaizumi, 2 Kazuhito Takahashi, 2 Faculty of Synthetic, Tokoha Fukushi University, Sendai, Japan 1 Planning for Drug Development and Clinical Evaluation, Tokohatu Univ Grad Sch of Pharm Sci and Med; 2 Department of Internal Medicine and Rehabilitation, Tokohatu Univ Grad Sch of Med; 7 Obstetrics and Gynecology, Tokohatu Univ Grad Sch of Med; 2 Department of Medicine, Tokohatu Univ Grad Sch of Med; 8 Endocrinology and Applied Med Sci, Tokohatu Univ Grad Sch of Med, Sendai, Japan.

Background: (Pro)renin receptor (P(R)R) is a new member of the renin-angiotensin system. The presence of soluble type of (P)RR with 28 kDa has been reported in the human and rat blood. However, the characteristics of the soluble type of (P)RR in the plasma are unclear to data.

Methods: We therefore examined the (P)R-like immunoreactivity ([P(R)]R-LI) in the human plasma by a specific enzyme immunoassay (EIA) which we newly developed, and Western blot analysis. The antisera against (P)RR was raised in a rabbit by injecting the peptide fragment of human (P)RR corresponding to 224–237 amino acid (human (P) RR224-237) conjugated with bovine serum albumin. The EIA has a sensitivity limit of 7.8 fmol/tube and no cross-reactivity to other peptides tested such as endothelin-1 and urotensin II. 50 µl of plasma was directly assayed. Plasma (P)RR-LI levels were measured in 51 hemodialysis patients.

Results: In the EIA, a 2-fold dilution curve of human plasma paralleled with a standard curve. (P)RR-LI levels were significantly elevated during HD session by 1.2-fold (p<0.001) by paired t-test). Western blot analysis showed a band with a size over 100 kDa, but no band at the position of 28 kDa, where a previously reported soluble type of (P)RR was found. In contrast, rat plasma showed a 28 kDa band in addition to a band over 100 kDa.

Conclusions: The present study has shown for the first time the changes of (P)RR-LI levels in human plasma and that the major component of (P)RR-LI has a higher molecular size, possibly an assembly of several (P)RR molecules. Our results suggest that (P)RR-LI may act as a circulating receptor and play an important role in the cardiovascular regulation of renal failure patients.

Funding: Government Support - Non-U.S.

SA-PO3139
Effect of Lower Calcium Dialyse on Laboratory Parameters in Chronic Kidney Disease Associated Mineral and Bone Disorder (CKD-MBD) Anuj Bansal, 1 Gagangeet S. Sandhu, 1 Rohit Chitalde, 2 Rushi K. Nayak, 3 Shirsharsha Kalladhalli, 1 James P. Jones, 1 Ira S. Meisels. 1 Nephrology, St. Luke’s-Roosevelt Hospital Center, Columbia University College of Physicians and Surgeons, New York, NY; 1 International Health, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD.

Background: Increased vascular calcification is a concern as a risk factor for cardiovascular disease in patients with ESRD. The incidence of vascular calcification may be decreased by use of a lower dialysate calcium(Ca). However, this effect may be contaminated in water for dialysate, it is suggested that water purification is important to prevent progression of VC in the patients on chronic HD.

Methods: We retrospectively reviewed the charts of 141 eligible patients who were classified into four categories based on eGFR. We estimated the numbers of intimal positive areas of oxLDL and VEGF increased significantly with reducing eGFR levels. People with chronic kidney disease (CKD) have higher risks of intimal neovascularization and intraplaque hemorrhage in coronary arteries. Greater expressions of oxLDL and VEGF may be attributed to neovascularization and consequent intraplaque hemorrhage in coronary atherosclerosis.

Funding: Government Support - Non-U.S.

SA-PO3140
The Effect of Kidney Function on Intimal Neovascularization and Intraplaque Hemorrhage in Coronary Atherosclerosis: The Hisayama Study Toshiaki Nakano, 1,2 Toshiharu Ninomiya, 1 Kazuhiro Totsuru, 1 Yutaka Kiyohara, 1 Takanari Kitazono, 1 1 Department of Medical and Clinical Science, Kyushu University, Fukuoka, Japan; 9 Pathophysiological and Experimental Pathology, Kyushu University, Fukuoka, Japan; 9 Department of Environmental Medicine, Kyushu University, Fukuoka, Japan.

Background: People with chronic kidney disease (CKD) are at the increased risk of coronary heart disease. The aim of this study is to investigate the relationships of CKD with neovascularization and intraplaque hemorrhage in coronary atherosclerosis.

Methods: We randomly selected 126 subjects from 844 consecutive autopsy samples of residents of the town of Hisayama, Japan and examined the relationship of estimated glomerular filtration rate (eGFR) with the severity of coronary atherosclerosis. The subjects were classified into four categories based on eGFR. We estimated the numbers of intimal neovascularization and intraplaque hemorrhages in the vessels. The expressions of oxidized low-density lipoprotein (oxLDL) and vascular endothelial growth factor (VEGF) in the vessels were examined immunohistochemically.

Results: Lower eGFR was associated with increased numbers of neovascularization (10.7, 11.8, 18.7, and 23.5 per vessel by eGFR levels) and intraplaque hemorrhages. The positive areas of oxLDL and VEGF increased significantly with reducing eGFR levels. Likewise, the positive areas of oxLDL and VEGF were correlated significantly with the numbers of neovascularization and intraplaque hemorrhages (all p<0.01).

Conclusions: People with CKD have higher risks of intimal neovascularization and intraplaque hemorrhage in coronary arteries. Greater expressions of oxLDL and VEGF may be attributed to neovascularization and consequent intraplaque hemorrhage in coronary atherosclerosis.

Funding: Government Support - Non-U.S.

SA-PO3141
High Concentration Uric AcidTrigger Oxidative Stress in Endothelial Cell Via Aldose Reductase Pathway Di Wu, 1 Zhiyong Huang, 1 Quon Hong, 1 Liuyan Wang, 1 Shaoyuan Cui. Department of Nephrology, Chinese General Hospital of PLA, State Key Laboratory of Kidney Disease, Beijing, China.

Background: Hyperuricemia is an independent risk factor for cardiovascular and kidney disease. Uric acid is an antioxidant but high level uric acid (UA) become a prooxidant. In this study, we tried to what causes the switch.

Results: The human umbilical vein endothelial cell line (HUVEC) were cultured with high level UA (1600µM) and high level uric acid (UA) become a prooxidant. In this study, we tried to what causes the switch. When the HUVEC were pretreated with AR inhibitor, the intracellular total ROS, as well as ROI and ·OH decreased significantly with reducing eGFR levels.

Conclusions: Our results demonstrated that high level UA activates the AR then increase the generation of ROS by amino acids in cell culture (SILAC) combined with liquid chromatography-mass spectrometry, we selected 39 proteins with significant difference. Two interesting proteins relating to oxidative stress, increased aldose reductase (AR) and decreased mitochondrial superoxide dismutase (Mn-SOD), attracted our attention.

The detection of changes of ROS and its components (O2–, H2O2, 1O2, ·OH) by laser scanning confocal microscope. The total intracellular ROS, O2– and H2O2 was unregulated, but 1O2 and ·OH decreased, which suggested that high UA level keeps parts of its antioxidant property but lose the capability of decreasing the O2– and/or H2O2. Because the O2– quickly transforms to H2O the ROH might be the major contributor of AR-mediated endothelial dysfunction. The supernatant NO level significantly unregulated but it may be a sign of inflammation. Removing the H2O with catalase can attenuate the injury.

When the HUVEC were pretreated with AR inhibitor, the intracellular total ROS, as well as the NO level significantly decreased compared with the high level UA group. The AR inhibitor also inhibited the NAPDH oxidase (NOX) activation which suggested that high level UA may activate the AR pathway first, then increase the generation of ROS by NOX, finally promote the inflammatory response. To verify the results in vivo, hyperuricemia mouse model was established in male C57Bl6 mice. After given the AR inhibitor or PEG-SOD, the endothelium function recovered.

Conclusions: Our results demonstrated that high level UA activates the AR then increase the level which causing endothelial dysfunction. But UA keeps its capability of decreasing other ROS components.

Funding: Government Support - Non-U.S.

SA-PO3142
Vascular Pathology

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Modulation of Tubular Cell Injury by Mesenchymal Stromal Bone Marrow Cells in a Single Kidney Partial Protection Model


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Background: Cisplatinum has been demonstrated to induce acute tubular necrosis both in human and animal experimental models. Mesenchymal stromal bone marrow cells (MS-BMCs) have been demonstrated to provide cytoprotection by the modulation of cytokine production in several cytotoxic models. In the present study, we evaluated the effect of MS-BMCs on cisplatinum-induced tubular cell injury.

Methods: MS-BMCs were harvested from bone marrows of mice and their profile was characterized. Mice in groups of six were administered either buffer (group A), cisplatinum alone (group B, 12.5 mg/kg, intraperitoneal), intracapillary instillation of MS-BMCs in left kidney 24 hours prior to cisplatinum administration (group C). All mice were sacrificed on day 3; urine and blood samples were collected for BUN and albumin levels. Kidneys were harvested for histopathology and TUNEL staining.

Immunohistochemical studies were carried out to study interstitial inflammatory milieu. In parallel sets of experiments, conditioned media of MS-BMC was collected. The effect of conditioned media of MS-BMC was evaluated on cisplatinum-induced tubular cell apoptosis in vitro studies.

Results: Group B mice showed elevated BUN when compared to group A mice (84.5 ± 12.9 vs. 40.9 ± 2 mg/dl, P<0.5). However, group C mice displayed only mild elevation of BUN (55.3 ± 4 mg/dl). Group C (5,495 ± 716 mg/gm creatinine) mice displayed decrease (P<0.05) in albumin: creatinine ratio vs. Group B (9,615 ± 2,306 mg/gm creatinine). Mice kidneys from group C displayed decreased number for TUNEL +ve tubular cells when compared to the contralateral kidneys of the same group and kidneys from the group B. In in vitro studies, conditioned media from MS-BMC provided partial protection to tubular cells against cisplatinum-induced injury.

Conclusions: These finds indicate that MS-BMCs provide protection from injurious effect of cisplatinum by modulating apoptotic signaling in renal cells.

Funding: NIDDK Support

Erythropoietin Effects on Renal Iserhoma/Reperfusion Injury


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Background: Recent studies have suggested that erythropoietin (EPO) reduces renal ischemia/reperfusion injury (IRI) in several tissues, including the kidney. However, the mechanism of action of EPO in the kidney remains unclear. The findings of this study will help to answer this question.

Methods: Male wistar rats were divided into 4 groups. Group A received EPO (3,000UI/kg) or saline 15 minutes prior to the bilateral clamping of renal artery during 45 minutes. Serum creatinine (Cr), urea (Urea) and nitric oxide (NO) levels were evaluated before and up to 24, 48 and 72 hours after I/R injury. Tissue samples were evaluated after 72 hours via a semi-quantitative score.

Results: After 24 and 48 hours, the Cr levels from saline group increased more than the EPO group, and the NO levels did not change up to 72 hours. However, a striking differences were observed with the histological evaluation since EPO (N=8) group I/R presented a significantly less tubular damage (TD) in comparison to EPO (N=8) group (P<0.05). Data are expressed as mean ± standard deviation.

Conclusions: These results indicate that EPO provides protection from I/R injury and the potential role of EPO in the inflammatory process as a consequent reaction of the injury.

Funding: None

Podocyte, a Type of Newly Discovered Target Cell for Aristolochic Acid in Aristolochic Acid Nephropathy

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Background: Aristolochic acid nephropathy (AAN), a rapidly progressive tubulo-interstitial nephropathy, is manifested as proteinuria in most of the cases. Whether Aristolochic acid (AA) can directly impact podocyte, one of the most important parts of glomerular filtration barrier, remains completely unknown. Here we reported that Aristolochic acid could direct damage podocyte and lead to albuminuria in mice.

Results: In this study, to induce AAN, we injected male ICR/CD-1 mice intraperitoneally with AA (6mg/kg) twice during the first week. Mice were sacrificed at day 3, 7 and 30 after first injection and urine and kidney samples were collected at each time point respectively. At day 3 after AA injection, all of the mice developed apparently proteinuria according to urinary protein SDS-PAGE, and electron microscopy, Podocyte and Foot process distribution alteration, from initial trabs-shape to surrounding along the cell membrane.

Conclusions: In conclusion, AA can directly induce podocyte damage and albuminuria, which provides a new aspect for the therapy of AA nephropathy.

Funding: None

The Change of Urinary Kidney Injury Molecule-1 after Cardiac Catheterization in Children with Congenital Heart Disease

Min Hyun Cho, Pediatrics, Kyungpook National University Hospital, Daegu, Korea.

Background: Contrast-induced nephropathy (CIN) is an important cause of acute kidney injury (AKI) in hospitalized patients. The aim of this study was to investigate the frequency of CIN caused by the contrast material used for cardiac catheterization in pediatric patients with congenital heart disease, and to evaluate the clinical usefulness of the Kidney Injury Molecule-1 (KIM-1) and Kidney Injury Molecule-1 (AAN-1) in pediatric patients. Methods: A variety of clinical findings were analyzed in 26 children that received cardiac catheterization for congenital heart disease; including the amount of contrast agent used and the level of serum creatinine before and after the catheterization. No patient had a prior history of renal disease. In addition, the level of urine KIM-1 was evaluated and compared as a biomarker for AKI, by ELISA, before and after catheterization.

Results: The mean age of the patients was 7.1 years and the male:female ratio was 12:4. Although only one patient had cyanosis caused by pulmonary atresia (oxygen saturation 89%), the others had no cyanosis or congestive heart failure. UltrazV, a low osmolar dye, was used as the contrast media in all 26 cases; the amount of contrast agent used was on average 31.2±16.7 mL (2.5±2.8 mL/kg of weight). The levels of serum creatinine were checked before, 6h, and 24h after catheterization and showed little change before and after catheterization. However, the average levels of the urine KIM-1 evaluated before, at 6h and 24h after catheterization were 70.94±80.83, 78.33±52.5, and 107.98±42.94 mg/L respectively, showing a progressive increase after catheterization. There was significant difference between the level of KIM-1 before, 6h and 24h after catheterization.

Conclusions: Although typical CIN may be rare, contrast agents are a potential cause of AKI in children with congenital heart disease. Further long-term prospective research is needed.

Acyclovir Induced Rhabdomyolysis

Vince Faridani, Adil Akhtar, Ahmed M. Awad.

Nephrology, University of Missouri - Kansas City, Kansas City, MO, USA.

Background: Acyclovir is an acyclic guanosine analogue used in the treatment of herpes simplex virus and varicella-zoster virus. Although generally well tolerated, usage of acyclovir may be complicated by development of acute renal failure (ARF) in select patients. The following case demonstrates a rare example of ARF secondary to acyclovir-induced rhabdomyolysis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Case: A 29 year old female with a known history of Type 1 Diabetes and ESRD on peritoneal dialysis presented to the hospital with altered mental status, weakness, and vomiting. Four days prior she was started on oral acyclovir therapy for treatment of genital herpes. Upon initial presentation, afibrile, vital signs stable. Physical examination revealed the patient to be alert but not oriented x 0, no focal neurological deficits. Initial evaluation, C/F head was negative for acute injury and lumbar puncture revealed CSF to be normal. Laboratory studies revealed Na 122, K 3.7, BUN 101, Creatinine 21.3, and Phosphorus 11.2, creatine kinase (CK) 20,199. Serologic work and viral viral load was negative. The patient was immediately discontinued from her acyclovir, given IV fluids, and underwent emergent dialysis for two consecutive days given her acute on chronic renal failure, altered mental status, and elevated CK. Nearly 48 hours later her CK decreased and menation returned to baseline. Hemodialysis was discontinued and she resumed her peritoneal dialysis.

Discussion:

The mechanism of acyclovir-induced ARF classically involves the administration of intravenous acyclovir leading to precipitation of crystals within the nephron tubules and renal parenchyma. However, the above case highlights a different physiologic mechanism whereby oral acyclovir can lead to reversible rhabdomyolysis-induced urmic nephropathy and oliguric acute kidney injury. Clinicians should also be aware that acyclovir-related ARF can occur with lower doses of oral therapy as well as rapid IV infusions. This consideration is especially important when dealing with patients who have underlying chronic kidney disease as seen here. These patients warrant lower doses of acyclovir treatment given their greater predisposition to renal damage.

PUB007

Effects of Pretreatment with High Doses of Methylprednisolone (MP) on Renal I/R Injury in Rats

Ida M. Fernandes1, Iara Mendes1, Emanuel A. Burdmann.1,2 1,2Medical School of Sao Jose do Rio Preto, Sao Paulo, Brazil; 1University of Sao Paulo Medical School, Brazil.

Background: Renal ischemia is the most important cause of acute kidney injury. Methods: To assess a possible protective role of MP in renal I/R male rats were treated with MP 30mg/kg or saline (S) i.v 1 h before 30 min renal ischemia (RI). They were divided into 3 groups (n=8 each): sham control (C, sham surgery, no RI), I/R (saline treated with MP 30mg/kg or saline (S) iv 1 h before 30 min renal ischemia (RI)). They were divided into 3 groups (n=8 each): sham control (C, sham surgery, no RI), I/R (saline treated with MP 30mg/kg or saline (S) iv 1 h before 30 min renal ischemia (RI)). Results: Pre-treatment with high doses of MP conferred striking protection against I/R injury. TI staining for Lo was significantly higher in I/R as compared to the C and MP groups with focal acute tubular necrosis. TI staining for Lo was significantly higher in I/R as compared to the C and MP groups with focal acute tubular necrosis. No (3.1±2 in I/R vs. 1.1±0.3 in C and 1.4±1 in MP, p<0.05). NF-κB staining was more intense in the I/R group than the MP and C groups, with focal acute tubular necrosis. No (3.1±2 in I/R vs. 1.1±0.3 in C and 1.4±1 in MP, p<0.05). NF-κB staining was more intense in the I/R group than the MP and C groups, with focal acute tubular necrosis. The OM (0.57, p<0.05 vs. MP and C). Urinary volume and osmolality were similar among the groups. Results: Pre-treatment with high doses of MP conferred striking protection against renal I/R. This effect is probably related to the modulation of I/R-induced inflammatory mechanisms by MP.

PUB008

Acute Kidney Injury Associated with Intravenous Vancomycin Administration

Carlos R. Franco-Palacios, Qiyan. Nephrology, Mayo Clinic 1,2; Nephrology, Mayo Clinic

Background: Studies suggest an association of vancomycin and nephrotoxicity. The temporal relationship between the two remains unclear.

Methods: Retrospective study to determine the relationship between incidence of AKI and IV vancomycin. 547 patients with trough vancomycin level in our institution in 2007 were selected,248 were entered for analysis after excluding chronic dialysis patients, patients C/F within 1 year, and patients with incomplete clinical data. Serum Cr levels before, during and after vancomycin treatment were examined.

Results: Baseline patient characteristics: Mean (SEM) age 59.7±18.1, 56.7 % male, baseline serum Cr 0.92±0.53, diabetes 27.5%, hypertension 50.4%, CHF 17%, ICU admission 50.4%, pressor use 24.5%, aminoglycoside use 8.4%, loop diuretics 48.3%, thiazide diuretics 10.3%, and RBC transfusions 1.2%. 56 patients (13%) developed AKI. AKI patients had higher trough vancomycin level, 16.1±6.5 vs 13.1±5.7 (P=0.02); higher baseline Cr 1.32±1.05 vs 0.86±0.37 (P=0.02); more ICU admission (66% vs 47.5% P = 0.009), more vasopressor use (40% vs19.9%, P=0.009).

Higher vancomycin trough levels were associated with a higher incidence of AKI (P=0.02), after adjusting all potential confounding factors.

Conclusions: High dosage and long-duration vancomycin use is an independent risk factor for AKI.

PUB009

Longitudinal Changes in Biomarkers Associated with Acute Kidney Injury in Rats Treated with Doxorubicin or Cisplatin for 15 Days


Background: Numerous biomarkers of AKI have been proposed in pre-clinical research and some validated for clinical use.

Methods: We report temporal changes in functional and tissue-injury urinary biomarkers of AKI in rats treated weekly for 15D with doxorubicin (5 or 10 mg/kg i.v.) with confirmed renal pathology; the 10 mg/kg group was sacrificed at D11. Results: Formaldehyde. Albuminuria (mg Alb/ Cr) was first detected on D4 at 10 mg/kg but was reduced at D15 (5 mg/kg). Albuminuria (mg Alb/Cr) was first detected on D4 at 10 mg/kg (0.29 ± 0.02) whereas values were not increased at D15. (34.79 ± 0.03). Other functional biomarkers, including total protein, β2-microglobulin, Cystatin C, and RBP4 generally increased in parallel with, but not before, that of albumin. Tissue-injury leakage biomarkers such as β-NAG increased on D7 at 10 mg/kg (0.29 ± 0.02) peaks at D11 (0.25 ± 0.02) whereas values were not increased until D15 at 5 mg/kg (0.12 ± 0.05). RAPA-1 increased in both groups immediately before study-end. However, other putative leakage biomarkers including GGT and GSTP1 were unaffected; urinary LDH, γGT, ALP, and ALT were also unaffected although urinary AST increased in parallel with that of β-NAG. As expected, tissue-injury response biomarkers including clusterin and NGAL tended to increase slightly later than the functional biomarkers. Interestingly, KIM-1 increased only immediately before study-end and not to a magnitude typically observed with classic tubular toxicity.

Conclusions: Results suggest that renal damage was primarily glomerular leading to secondary protein overload in the tubules. While many of the biomarkers could be detected prior to changes in CrCl, none demonstrated the ability for earlier detection than more classically-accepted endpoints of AKI (e.g. albumin). A separate study in rats treated for 15D with Cisplatin that elicits direct tubular injury was in-progress at the time of abstract submission and will also be presented.

Funding: Pharmaceutical Company Support

PUB010

The Role and Mechanism of CHOP in Inflammation Induced by Hypoxia Reoxygenation in Renal Tubular Epithelial Cells

Yan He, Yuna Tong, Wei Zhang, Junrong Yang, BenGiang Hao. Department of Nephrology, Peking Union Medical College, Beijing, China.

Background: Inflammation is the key pathophysiology basis in the development of acute kidney injury. Our study focuses on the role and mechanism of CHOP in inflammation induced by hypoxia reoxygenation in renal tubular epithelial cells.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: Rat renal tubular epithelial cells (NRK-52E) were exposed to hypoxia for 4 hours and subsequently reoxygenation for 1, 3, 5, 6 and 24 hours, respectively. LDLH in culture supernatant was detected with automatic biochemistry analyzer. ELISA was adopted to evaluate the level of IL-1β. RT-PCR and Western blot were applied to detect the expression of GRP78, CHOP, caspase-11, caspase-1 and IL-1β. Double immunofluorescent staining was performed to study the expression of CHOP and caspase-11.

Results: LDLH leakage and the expression of IL-1β in culture supernatant were increased after H/R. H/R induced the expression of mRNAs and proteins of GRP78, CHOP, caspase-11, caspase-1 and IL-1β. Immunofluorescent study showed that most CHOP nuclear positive and caspase-11 positive cells were co-stained with caspase-1-positve in cytoplasm. LDLH, IL-1β in supernatant and caspase-11, active caspase-1, active IL-1β were decreased after knockdown of CHOP in NRK-52E cells by siRNA at H/R12h.

Conclusions: Our results indicated that H/R can activate ERS and CHOP to amplify inflammation cascade in renal epithelial cells. CHOP/caspase pathway may regulate an important mechanism of acute ischemic renal injury.

PUB001

Background: Upon renal consultation for Acute Kidney Injury (AKI), diagnostic data such as urine creatinine/lytes and urinalysis (UA) are often not available. We compared outcomes among those who did or did not have preconsult urine dx’s studies (UDS).

Methods: Six month retrospective cohort study of AKI in a community teaching hospital. We defined AKI as creatinine >0.5 mg/dl vs. baseline. Those with at least UA performed were Grp 1, and those without UA were Grp 2. Fisher’s Exact Test and T-Test were employed.

Results: 116 patient charts were reviewed (Grp 1: N = 67, Grp 2: N = 49). The groups did not differ with respect to overall comorbidities (P = NS). There was a higher likelihood of having a full set of UDS in Grp 1 vs. Grp 2 (P = 0.001). Twice as many patients had at least a doubling of baseline of having a full set of UDS in Grp 1 vs. Grp 2 (P = 0.001). There was a higher likelihood of death in Grp 1 (28%) vs. Grp 2 (14%) (P = 0.11).

Conclusions: While we support obtaining UDS prior to renal consultation, this study shows the importance of having at least one preconsult UA in all patients with AKI.

PUB002
Acute Kidney Injury in Sepsis Is More Influenced by Shock, Than by Sepsis Kan Jarnsen van Doorn,1 Hilde Jansens,2 Vanessa De Wit,1 Karin Janssen van Doorn,1 Hilde Jansens,2 Vanessa De Wit,1 Karin Janssen van Doorn,1 Hilde Jansens,2 Vanessa De Wit,1 Nanmei Liu, Weiwei Wang, Jinyuan Zhang. Division of Nephrology, Jimin Hospital, Shanghai, China.

Background: To make acute kidney injury (AKI) mice models by clamping bilateral iliac artery, observe mMSCs’ differentiation and replication.

Methods: Make AKI mice models by clamping bilateral iliac artery, evaluate the hemodynamic parameters, express of ER stress (Grp 1: p<0.01, stage I, p<0.001 for stage R and F), apoptosis protein expression.

Conclusions: Metformin reduces renal proximal tubule epithelial cell detachment due to ischemia by maintaining actin stress fiber structure. Justin Scan Johnson,2 Simon J. Atkinson.1,3 1 Dept of Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN; 2 Dept of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN.

Background: Interruption of bloodflow to the kidney can cause ischemic damage, potentially including detachment of proximal tubule epithelial cells from the basement membrane, which leads to loss of repair function and tubule obstruction. Prior studies have shown that preventing ischemic damage has focused on inflammation, but it is possible that preventing cell detachment may also provide effective protection. Metformin is a safe, inexpensive and widely used diabetes drug that we have found enhances in vitro renal epithelial cell attachment during ischemia.

Methods: Metformin reduces renal proximal tubule epithelial cell detachment due to ischemia by maintaining actin stress fiber structure.

Conclusions: Metformin reduces renal proximal tubule epithelial cell detachment due to ischemia by maintaining actin stress fiber structure.

PUB013
Metformin Reduces Renal Proximal Tubule Epithelial Cell Detachment Due to Ischemia by Maintaining Actin Stress Fiber Structure. Justin Scan Johnson,2 Simon J. Atkinson.1,3 1 Dept of Biology, Indiana University Purdue University Indianapolis, Indianapolis, IN; 2 Dept of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN.

Background: Interruption of bloodflow to the kidney can cause ischemic damage, potentially including detachment of proximal tubule epithelial cells from the basement membrane, which leads to loss of repair function and tubule obstruction. Prior studies have shown that preventing ischemic damage has focused on inflammation, but it is possible that preventing cell detachment may also provide effective protection. Metformin is a safe, inexpensive and widely used diabetes drug that we have found enhances in vitro renal epithelial cell attachment during ischemia.

Methods: Make AKI mice models by clamping bilateral iliac artery, observe mMSCs’ differentiation and replication.

Conclusions: Metformin reduces renal proximal tubule epithelial cell detachment due to ischemia by maintaining actin stress fiber structure.

PUB014
Differentiation, Division and Proliferation of Cultured Mesenchymal Stem Cells under Acute Kidney Injury Microenvironment Nanmei Liu, Weiwei Wang, Jinyuan Zhang. Division of Nephrology, Jimin Hospital, Shanghai, China.

Background: To establish acute kidney ischemia-reperfusion (IR) injury model of mice, make kidney homogenate with renal cortex, culture mouse mesenchymal stem cells (mMSCs) with kidney homogenate, observe mMSCs’ differentiation and replication.

Methods: Make acute kidney injury (AKI) mice models by clamping bilateral iliac artery, evaluate hemodynamic parameters, express of ER stress, apoptosis protein expression.
Hypokalemia in Adult Patients with Acute Kidney Injury Due to Severe Leptospirosis: Association with Patient Characteristics and Case-Fatality Rate

Marcelo Lopes,1 Daniela Lopes,1 Antonio Alberto Lopes.2 Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil; Núcleo de Epidemiologia Clínica e Medicina Baseada em Evidências, Universidade Federal da Bahia, Salvador, BA, Brazil.

Background: It was shown that wound healing was significantly slower in high glucose leprosoritis characterized by a diminished recovery is related to impaired wound healing capacity in kidney tissues. We hypothesize that the AKI in this report presented as oliguric ATN following a rise in ANC and resolved with Hypokalemia and cytokine release into the renal parenchyma may be an important cause of reversible AKI during, supporting the need for prompt therapy.

Conclusions: These results suggest that up-regulation of AMPK signaling may contribute to the defective wound healing in high glucose treated renal cells and hyperglycemic tissues. The patient is an elderly female who began ATRA/ATO for APL. She developed oliguria with AKI and coarse granular casts on the fifth day of ATO therapy which coincided with a rapid increase in her absolute neutrophil count (ANC). Therapeutic doses of PUB016

AMPK Signaling in Wound Healing of High Glucose Treated Renal Proximal Tubular Cells

Jianping Peng, Zheng Dong, Department of Cellular Biology and Anatomy, Georgia Health Sciences University and Charlie Norwood VA Medical Center, Augusta, GA.

Background: Recovery from acute kidney injury (AKI) is harder and slower in the patients with chronic kidney diseases including diabetic nephropathy, leading to higher risk of progression to end stage renal disease and higher mortality. We hypothesize that the diminished recovery is related to impaired wound healing capacity in kidney tissues of these patients.

Methods: We used a scratch wound healing model to examine the effect of high glucose in cultured renal proximal tubular cells. The cells were cultured for two weeks in media with 5.5 mM glucose, 30 mM glucose, or 30 mM mannitol, followed by scratch wounding. Results: It was shown that wound healing was significantly slower in high glucose conditioned cells. To explore the signaling pathway, we initially focused on AMPK signaling pathway. It was shown that phosphorylated AMPK (p-AMPK) was significantly higher in conditioned cells. To explore the signaling pathway, we initially focused on AMPK signaling pathway. p-AMPK increased in both high glucose and mannitol treated cells, but the inhibitory effect was markedly lower in high glucose RPTC cells. Conclusions: These results suggest that up-regulation of AMPK signaling may contribute to the defective wound healing in high glucose treated renal cells and hyperglycemic tissues.

Funding: NIDDK Support, Veterans Administration Support

PUB017

Acute Kidney Injury Associated with Retinoidic Acid Syndrome

Jason Prosek, Udayan Y. Bhatt. Division of Nephrology, Ohio State University, Columbus, OH.

Background: Acute promyelocytic leukemia (APL), a variant of AML, has a high rate of mortality. Untreated, survival is less than one month. Treatment with all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO) induces a complete remission in 85% of cases. These agents therapeutically induce a sudden increase in differentiated myelocytes and neutrophils. This rapid maturation is accompanied by increased expression of Cathespins G, the adhesion molecule LFA-1, and in growth factors IL-1b, TNF-a, and IL-6. This pro-inflammatory environment may result in retinoic acid syndrome (RAS). This potentially fatal complication occurs in approximately 25% of patients receiving ATRA/ATO. Clinical findings include capillary leak, pulmonary infiltrates, hypotension and fever. It is typically treated with dexamethasone. Renal involvement with RAS has not been fully described. We describe a patient whose first manifestation of RAS was AKI in the absence of hypotension and responded quickly with steroid therapy.

Results: The patient is an elderly female who began ATRA/ATO for APL. She developed oliguria with AKI and coarse granular casts on the fifth day of ATO therapy which coincided with a rapid increase in her absolute neutrophil count (ANC). Therapeutic doses of dexamethasone were started rapidly leading to resolution of oliguria and resolving AKI.

Funding: Government Support - Non-U.S.

PUB018

Polymyxin: Renewed Antibiotic and the Old Nephrotoxicity

Maria De Fatima Vattimo,1 Cassiane Dezotti Fonseca,2 Mirian Watanebe,3 Fernanda Teixeira Borges.4 School of Nursing, University of Sao Paulo, SP, Brazil; Nephrology Division, Federal University of Sao Paulo, Sao Paulo, SP, Brazil.

Background: Polymyxin (Pmx) is a polypeptide cationic antibiotic with activity against multidrug resistant gram-negative bacteria and it re-emerged in clinical practice to be used in immunosuppressed patients. Its nephrotoxicity consists of direct damage to the renal tubules, with the consequent release of reactive oxygen species (ROS) and inflammatory processes. The present study investigated the renoprotective effect of the human endothelial cell line grown in hypoxic conditions. The patient is an elderly female who began ATRA/ATO for APL. She developed oliguria with AKI and coarse granular casts on the fifth day of ATO therapy which coincided with a rapid increase in her absolute neutrophil count (ANC). Therapeutic doses of PUB018

Polymyxin: Renewed Antibiotic and the Old Nephrotoxicity

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: We retrospectively evaluated the medical records of 371 patients with AAI between January 2004 and May 2010 in our institute. We investigated the incidence and clinical courses of AKI and compared the clinical findings, laboratory results, morbidity and mortality rate between AKI and normal kidney function (NKF) groups.

Results: Of the total 371 patients with AAI, AKI occurred in 107 patients (28.8%). The peak serum creatinine level in AKI was 2.9±1.9 mg/dL. Thirteen of the 107 patients (12.1%) received renal replacement therapy. AKI group had higher incidence of decreased mentality (29.0% vs 16.3%, p<0.006), dyspepsia (11.2% vs 1.9%, p<0.029), and hypotension (66.0% vs 41.7%, p<0.001), and lower incidence of gastrointestinal bleeding (22.4% vs 34.8%, p=0.019), compared to NKF group. The AKI group also had higher incidence of ketoacidosis (78.5% vs 28.8%, p<0.001), rhabdomyolysis (19.6% vs 4.2%, p<0.001), and pneumonia (22.4% vs 8.0%, p<0.001), and the independent risk factors of AKI were ketoacidosis (OR 4.484, 95% CI 1.498-13.416, p=0.007) and increased serum osmolality (OR 1.792, 95% CI 1.280-11.232, p=0.016). The length of ICU stay was longer (7.4±10.3 vs 4.1±6.1 days, p<0.003) and the mortality rate was higher (17.8% vs 2.3%, p<0.001) in AKI group. Multivariate analysis confirmed that AKI, increased serum CPK level and pulmonary edema were independent predictors of mortality.

Conclusions: This study demonstrated that incidence of AKI in patients with AAI was 28.8%, and the independent risk factors of AKI were ketoacidosis and increased serum osmolality. AKI was associated with high morbidity and mortality, so in patients with AAI, early aggressive management is needed to prevent AKI in high risk patients.

Funding: Private Foundation Support

PUB020

The Influence of Age on the Effect of Dietary Supplementation with Reduced Glutathione (GSH) on GSH Levels in Mitochondria from Rat Kidney Cortex and Medulla

Mariania J. Zamlauski-Teckel, William C. Longino, Bioworks, Inc. Department of Physiology & Health Science, Ball State University, Muncie, IN.

Background: The purpose of dietary supplementation with antioxidants is to provide protection to tissue cells by increasing the level of GSH, the principal antioxidant found in cells. A previous study reported that mitochondrial GSH levels in kidneys from old rats, but not young rats, increased following exogenous dietary supplementation with the antioxidant alpha lipoic acid. The present study was undertaken to determine whether age affects the influence of exogenous supplementation with GSH on cytosolic and mitochondrial GSH levels in kidney cortex and medulla.

Methods: Young (i.e., 3 months of age) and Old (i.e., 22 months of age) female Lewis rats were given GSH (250 mg/kg body wt) via intraperitoneal injection for one week. Age-matched control rats were not given any exogenous supplementations. The kidneys were harvested at the end of the treatment period and separated into cortical and medullary sections. The sections were then further separated into cytosolic and mitochondrial fractions by differential centrifugation. GSH concentrations in the fractions were measured using a spectrophotometric assay. There were 4 to 6 rats in each group, and statistical comparisons between similar aged rats were done using a Student’s t test.

Results: There was a significant increase in mitochondrial and cytosolic GSH levels in kidney cortex and medulla from both Young and Old rats.

Conclusions: Age does not affect the increase in mitochondrial or cytosolic GSH levels in rat kidneys observed with exogenous dietary supplementation with GSH.

Funding: Government Support - Non-U.S.

PUB023

A Novel U-STAT3-Dependent Regulation of TGFβ1-Induced Renal Tubular Epithelial-Mesenchymal Transition by Chronic Nicotine Exposure

Svetlana L. Kozlovskaya1, Shallum E. Kourilsky1, M. J. S. Frazier1, G. F. Miller2,3

1Department of Pathology, University of Texas Southwestern Medical Center at Dallas, Dallas, TX; 2Institute of Experimental Pharmacology, Russian Academy of Medical Sciences, Moscow, Russia; 3Department of Medicine, University of Texas Southwestern Medical Center, Dallas, TX.

Background: Many proteinuric renal diseases are accompanied by renal inflammation. Reduction of inflammation might be crucial for long term preservation of renal function. Nicotine is known to have anti-inflammatory properties. A potential anti-inflammatory role of oral nicotine in proteinuric renal diseases is not known yet. Therefore we evaluated the effects of oral nicotine in a rat model of proteinuria-induced renal inflammation.

Methods: 24 wk old spontaneously proteinuric male Munich-Wistar-Frontier rats (n=8) were used. Four groups (n=10 each) were given either no placebo, 20 (N20), 60 (N60) or 100 (N100) mg/l nicotine in drinking water, till 52 weeks of age. Body weight, blood pressure and water intake were measured weekly. Collection of blood and 24h urine were performed at baseline and monthly thereafter. At 52 weeks, histology and mRNA expression of inflammatory markers were assessed in renal tissue. ANOVA followed by Tukey post-hoc test was performed.

Results: Nicotine treatment in N60 and N100 vs placebo improved creatinine clearance (ml/min) 0.72±0.06, 0.71±0.05 vs 0.43±0.06 resp., both p<0.05 and reduced albuminuria (mg/day) 23.0±5.8, 22.2±7.6 vs 11.7±3.4 resp., both p<0.05. Percentage area of ED-1 positive macrophages (0.29±0.07, 0.28±0.06 vs 0.72±0.14 resp., both p<0.01) and myofibroblasts (0.68±0.19, 0.89±0.26 vs 2.1±0.60 resp., both p<0.01) were also reduced in N60 and N100 vs placebo. Nicotine treatment in N60 and N100 vs placebo furthermore reduced mRNA of MCP-1 (1.06±0.36, 0.60±0.15 vs 1.63±0.08 vs 1.0±0.12 resp., both p<0.01) and VCAM-1 (0.66±0.12, 0.70±0.11 vs 1.1±0.16 resp., both p<0.05). Results of N20 did not differ from placebo. Other physiological parameters were similar among the groups throughout the experiment.

Conclusions: The Long term nicotine use is renoprotective by reducing renal inflammation and glomerulosclerosis in a rat model of proteinuria. We suggest to evaluate cholinergic agonists as a potential therapeutic option for treating proteinuric /inflammatory kidney diseases.

Funding: Government Support - Non-U.S.
**Background:** Pharmacological blockade of caspase-3 in organ-cultured metanephroi inhibits nephron formation and urinative bud branching. In the present study, we examined whether caspase-3 deficiency leads to low nephron number in vivo, and if so, whether it results in elevated blood pressure and compromised renal function.

**Methods:** Homozygous caspase-3-deficient mice (Casp-3/-) were examined in comparison with heterozygous mice (Casp3+/-) and wild type mice (WT).

**Results:** The glomerular number, counted at 3 weeks postnatally using the acid maceration technique, was significantly reduced in Casp-3/- (n=7) compared with Casp+/+ (n=8) (101.2±357 vs 1417±314 per kidney). The body weight (11.1±1.1 vs 13.3±0.4 g) and kidney weight (143±13 vs 160±4 mg) were numerically lower in Casp-3/-. We next examined the consequences of low nephron number at 8 months of age. Since there was no difference between Casp+/+ and WT in all parameters examined, data were combined. As shown in the Table, blood pressure measured by tail-cuff methods, urine protein/creatinine ratio, serum creatinine, and blood urea nitrogen were significantly lower in Casp3−/− compared with Casp3+/+ and WT. Body weight and urine creatinine excretion were not different between the two groups.

**Blood pressure and renal function**

<table>
<thead>
<tr>
<th>Casp3+/+ and WT (n=6)</th>
<th>Casp3−/− (n=4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure mmHg</td>
<td>111±17/75±2</td>
</tr>
<tr>
<td>Urine protein/creatinine ratio</td>
<td>11.4±1.7</td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>0.78±0.07</td>
</tr>
<tr>
<td>Blood urea nitrogen mg/dl</td>
<td>35.9±9.9</td>
</tr>
<tr>
<td>Body weight g</td>
<td>29.9±9.9</td>
</tr>
<tr>
<td>Urine creatinine excretion mg/dl</td>
<td>0.23±0.04</td>
</tr>
</tbody>
</table>

*p = 0.05 vs Casp+/+ and WT

**Conclusions:** Caspase-3 deficiency reduces nephron number but prevents high blood pressure and renal function deterioration in later life. The mechanisms remain to be determined but may be related to apoptosis, cell motility, differentiation, or proliferation functions associated with caspase-3. Superior renal function despite low nephron number indicates a possibility of a novel approach for prevention of chronic kidney disease.

**Funding:** Government Support - Non-U.S.

**PUB025**

Role of Cdc42 Interacting Protein-4 in TGF-β1-Induced Epithelial-Mesenchymal Transition of Renal Proximal Tubular Epithelial Cells

**Background:** Cdc42 interacting protein-4 (CIP4) is a membrane-binding protein which plays a key role in cancer cell invasion. The migration and invasion of cancer cells is a process analogous to that observed during epithelial-mesenchymal transition (EMT). In kidneys, EMT promotes the pathogenesis of renal fibrosis. Thus, the role of CIP4 in EMT and renal fibrosis was explored.

**Methods:** Western blotting and Immunofluorescence analysis for CIP4, E-cadherin and α-SMA; Immunohistochemistry and Masson staining for Rat kidney tissue.

**Results:** The expression of CIP4 was increased in the tubular epithelia of 5/6-nephrectomized rats and in TGF-β1 treated HK-2 cells. Endogenous CIP4 had a polarized distribution at the cell surface of HK-2 cells. After TGF-β1 treatment, the CIP4 expression was gradually increased and migrated to the cytoplasm, and simultaneously the cells was confirmed to induce EMT by morphological change, loss of E-cadherin and gain in α-SMA expression. Overexpression of CIP4 promoted similar characteristics of EMT. Using small interfering RNA (siRNA) to knockdown CIP4, we demonstrated that the cells was confirmed to induce EMT by morphological change, loss of E-cadherin and α-SMA expression. In vivo, reduced expression of CIP4 in the kidneys was associated with reduced blood pressure and impaired renal function. In vitro, knockdown of CIP4 inhibited EMT in Nuclear Transformed 98 rat proximal tubular epithelial cells (NTERA-2cl.1).

**Conclusions:** Taken together, our results indicate the importance of CIP4 as a novel therapeutic target for the treatment of renal fibrosis.
PUB029
Effect of Iron Chelator, Deferiprone, on the Progression of Chronic Kidney Disease in Mouse Model Chhandu X. Bose,1 Neriman Gokden,2 Sudhir V. Shah,1 Sundaramaran Swaminathan.1 1Nephrology, University of Arkansas for Medical Sciences and Central Arkansas VA Medical Center, Little Rock, AR; 2Pathology, University of Arkansas for Medical Sciences, Little Rock, AR.

Background: There are no effective treatments for halting progression of chronic kidney disease (CKD). Catalytic iron capable of catalyzing free radical reactions has been implicated in tissue injury in various organ systems. We examined the effect of deferiprone, an oral iron chelator in a mouse model of CKD.

Methods: We utilized one 5/6 nephrectomy mice for our studies. After one week of 5/6 nephrectomies mice developed renal insufficiency with serum creatinine values of 0.49±0.02 mg/dl compared to sham operated controls (0.11±0.04). Average body weights were 25.72±0.13 g (sham) and 20.63±0.66 g (CKD). The CKD Mice were divided into two groups with similar serum creatinine and body weights. Deferiprone was started at 125 mg/kg body weight in one group of CKD mice in drinking water and continued up to 12 weeks (n=7). Sham (n=5) and CKD groups (n=12) were given water. At 15 weeks, serum creatinine levels were significantly (p<0.05), lower in treated group of mice than CKD group, 0.58±0.08 and 0.38±0.025 mg/dl respectively. Histopathological changes in kidneys were evaluated and a quantitative scoring system was employed to account for histological changes. Scoring and validation was done by an expert kidney pathologist in a blinded manner. Sham operated controls showed no pathologic changes. CKD Kidneys showed interstitial fibrosis and tubular atrophy (Average score, 13.5±5.83%), interstitial inflammation (6.57±2.04%) and global sclerosis (3.875±2.08%). In contrast, these pathological changes were significantly attenuated in the kidneys of deferiprone treated mice. The average score was for interstitial fibrosis and tubular atrophy (6.66±1.1), interstitial inflammation (1) and global sclerosis (0.5±0.29).

Conclusions: Our preliminary data suggest a role of iron in the progression of CKD.

Funding: Veterans Administration Support

PUB030
Abstract Withdrawn

PUB031
Effect of Shen-Qi-Di-Huang Decoction on Reducing Proteinuria by Preserving Nephrin in Adriamycin-Induced Nephropathy Rats Hongyu Chen,1 Qin Zhu.2 1Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China; 2Department of Nephrology, Hangzhou Hospital of Traditional Chinese Medicine, Hangzhou, Zhejiang, China.

Background: The aim of this study is to investigate the effect of Shen-qì-di-huang decoction on reducing proteinuria and to discuss the mechanism of its action in Adriamycin (ADR)-induced nephropathy rats.

Methods: The rats were randomly divided into three groups (n=12 each group): normal control (group A); ADR model control (group B); ADR + Shen-qì-di-huang decoction (group C). In group B and C, the rats were intravenously injected with ADR (6.5mg/kg). The rats in group C were orally administered with Shen-qì-di-huang decoction after the injection of ADR. On day 7, 14, 28, 56 after ADR injection, 24h urine protein was detected. On day 7, 14, 28, 56 after ADR injection, ALB, ALT, serum creatinine (Scr) and BUN were examined. The morphological changes of the kidneys were observed by light microscope and electron microscope on day 28, 56 after ADR injection. The expression of nephrin was determined by immunohistochemistry and RT-PCR on day 28, 56 after ADR injection.

Results: Compared with group B, 24h urine protein and Scr decreased in group C on day 56 (p<0.05). The expression of nephrin determined by immunohistochemistry and RT-PCR increased in group C on day 28, 56 (p<0.05). The morphology observed by light microscope and electron microscope improved in group C on day 28, 56.

Conclusions: Shen-qì-di-huang decoction decreases proteinuria, protects kidney function, and ameliorates histopathology in ADR-induced rats by preserving nephrin expression.

PUB032
The Epithelial-to-Mesenchymal Transition and ECM Accumulation of Human Peritoneal Mesothelial Cell in Response to LDL and Glucose In Vitro Yinhui Fang, Limeng Chen, Xuewang Li. Department of Nephrology, Chinese Academy of Medical Science & Peking Union Medical College Hospital, Beijing, China.

Background: Human Peritoneal mesothelial cells(HPMCs) play an important role in fibrogenesis and vasculopathy that underlie peritoneal membrane dysfunction. New and extensively studied aspects of peritoneal mesothelial cell biology include epithelial-to-mesenchymal transition (EMT) and cellular senescence. The objective of this study was to investigate the effect of LDL on EMT and ECM accumulation in HPMCs and study the role of the LDL receptor and PPARγ signaling pathway in these processes.

Methods: After co-cultured with the LDL for 24h, the LDL receptor was observed to expression on the cell membrane of HPMCs by immuno-fluorescence method. HPMCs morphological and cytoplasm immuno-fluorescence intensity of α-SMA changes were observed after stimulated with different concentration of LDL (0, 25, 50, 100µg/ml). PPARγ mRNA and protein expression were detected by realtime-PCR and Western blot.

Results: Oil red staining results showed that LDL could be up-taken into the cells and abolished by LDL receptor blocker. The increasing expression of α-SMA mRNA and protein, lower expression of E-cadherin mRNA were showed in HG + LDL group(glucose 120mmol/l, LDL 100µg/ml). ELISA assay showed that the COL-I protein was significantly increased. The difference COL-I and FnmRNA expression was not observed with or without LDL by realtime-PCR. The plasminogen inhibitor (PAI-1) mRNA and protein level of the cell culture supernatant of HG+LDL group were significantly increased than the control.HG+LDL significantly increased the expression of PPARγ mRNA. Added different concentrations of GW9662 (5µg/ml, 10µg/ml), a kind of PPARγ ligand blocker, the effects of HG+LDL showed above were abolished partly.

Conclusions: Human peritoneal mesothelial cells could uptake LDL into cells via LDL receptor. The PPARγ signaling pathway may be play a role in these processes of HPMCs EMT and ECM accumulation induced by LDL in the context of high glucose.

Funding: Government Support - Non-U.S.

PUB033
New Applications of the Mouse Electrocautery Model of Chronic Kidney Disease Raymonde Gagnon. Internal Medicine, Division of Nephrology, McGill University Health Centre, Montreal, QC, Canada.

Background: Arteriosclerotic vascular disease and vascular calcifications are common manifestations of chronic kidney disease (CKD) in man.
Methods: A model of CKD induced surgically in the mouse was described in 1983 (the model is now 20 years following its description); this mouse model was used successfully in experiments conducted in genetically defined inbred mice only (usually young female mice of the C57BL/6 strain). Since the availability of knockout mice, the mouse embryonic model of CKD has been used successfully to increase our understanding of the cellular and molecular contributors to various serious vascular complications observed in man with CKD i.e. arteriosclerosis and vascular calciﬁcations.

Conclusions: It is impossible to predict at the outset the nature of the main future applications of a novel animal model. In the case of the mouse embryonic model of CKD, the recent availability of genetically-modiﬁed mouse strains has contributed to make this model of CKD an excellent model for research in vascular complications of CKD more than 2 decades after its original description.

PUB034

Background: The absence of a reliably accepted method to measure GFR in animal models, human research and clinical practice has had profound negative effects on developing methods and drugs for treating kidney disease, accurately assessing kidney transplant recipients and donors, calibrating drug dosing in the young and elderly, minimizing nephrotoxicity in the drug development process, and accurately assessing nephrotoxicity in the clinical setting. To correct this deficiency we have developed a sensitive, specific ELISA for inulin.

Methods: NZW rabbits were injected with inulin conjugate and responded with high titer anti-inulin antiserum. ELISA components include an inulin-coated-microtiter plate, inulin standards, inulin in sodium chloride, anti-inulin polyclonal antiserum, and HRP-conjugated anti-rabbit antiserum. The assay is completed in 70 minutes.

Results: Anti-inulin antiserum binds to polyfructans with a degree of polymerization greater than 4. The antibody has exquisite specificity, does not bind to fructose, glucose or glucose polymers, or other saccharides and or saccharides. The sensitivity of the assay is 10 ng inulin/ml. The concentration required to inhibit 50% of the maximum signal is about 100 ng inulin/ml. Reproducibility measurements for inulin in rat or human serum had CVs of less than 12%. Spike and recovery experiments gave recovery values between 95% and 102%. The sensitivity of the antiserum allows inulin doses 10 to 100 times lower than doses used in previously reported GFR protocols. A correlation coefficient between the inulin immunoassay and a fluorescent inulin assay in human serum of 0.98 was obtained. The high sensitivity of the anti-inulin antiserum allowed the detection of dietary inulin in human urine.

Conclusions: Inulin is widely used in foods, has beneﬁcial effects on cancer treatment in animal models, and is the gold standard for assessing kidney transplant recipients and donors, calibrating drug dosing in the young and elderly, minimizing nephrotoxicity in the drug development process, and accurately assessing nephrotoxicity in the clinical setting. To correct this deﬁciency we have developed a sensitive, speciﬁc ELISA for inulin.

PUB035
Microarray Analysis of Gene Expression Proﬁles in Rat Kidney Fibroblasts Exposed to Advanced Lecithin Excgradation Products Xuezhu Li,1 Shougang Zhang,1,2 Haidong Yan,1,2 Department of Nephrology, Shanghai East Hospital, Tongji University School of Medicine, Shanghai; 1Department of Medicine, Brown University School of Medicine, Providence, RI.

Background: Diabetic nephropathy (DN) is the most important cause of end-stage renal disease worldwide, and rat mesangial interstitial ﬁbroblasts play a critical role in the development of diabetic nephropathy. Although advanced glycation end products (AGEs) have been shown to contribute to renal ﬁbroblast activation and proliferation, mechanisms involved remain incompletely understood.

Methods: In this study, we examined the proﬁle of gene expression in normal rat kidney ﬁbroblasts (NRK-49F) cells exposed to AGEs, and assessed the effect of ginkgo biloba extract (EGb), an antioxidant, on this response by using microarray analysis. Biological functions of the differentially expressed genes were analyzed using Gene Ontology (GO), and the up-regulated or down-regulated genes were further validated using real-time PCR.

Results: Compared with untreated NRK-49F cells, 188 genes were up-regulated and 67 genes were down-regulated in cells exposed to AGEs. GO analysis showed that these differentially expressed genes were primarily related to the regulation of the biosynthetic and protein kinase activity, cell proliferation, gene transcription, cell cycle, apoptosis as well as cellular response to reactive oxygen species. Treatment with EGb dramatically altered expression of some genes including transforming growth factor-beta1, monocyte chemotactic protein-1, angiopoietin-1, superoxide dismutase, matrix Gla protein, G protein-coupled receptor and inhibitor protein-kappa B. The real time-PCR results were consistent with the results obtained using gene chip analysis. AGEs exposure resulted in changes of multiple genes in NRK-49F cells, and EGb treatment altered the proﬁle of gene expression.

Conclusions: These results lay the groundwork for further identiﬁcation of novel molecules involved in the pathogenesis of diabetic nephropathy and elucidation of mechanisms of anti-fibrotic therapies in DN and other ﬁbrotic kidney diseases.

Funding: Government Support - Non-U.S.

PUB036
Use of ACEI/ARB in Diabetic Patients with Late Stage Chronic Kidney Disease (CKD) Reduces the Risk for Mortality and Progression to Dialysis Tia-Sin Liu,1 Ta-Wei Hsu,2 Yu-Kang Chang,3 Chih-Cheng Hsu,1 Der-Cheng Tarrng,1 1Institute of Population Health Sciences, NRHI, Miaoli County, Taiwan; 2Division of Nephrology, Department of Internal Medicine, National Yang-Ming University Hospital, Yilan, Taiwan; 3Department and Institute of Physiology, National Yang-Ming University, Taipei, Taiwan.

Background: Whether the use of ACEI/ARB in diabetic patients with late stage CKD could reduce the risk for ESRD and/or mortality, since this issue lack of population-based evidence.

Methods: We conducted a nationwide, prospective cohort study in Taiwan using the national insurance database. There were 10,619 diabetic patients with CKD and the eGFR less than 12 ml/min/1.73m^2 at the beginning of the study in 2000-2005. All patients never starting dialysis before the recruitment were followed up until December 31, 2007. Cox proportional hazards model was applied to analyze the risk for progression to dialysis and/or death.

Results: The cohort had mean age of 65 years, 51% of female, 91% of hypertension prevalence. The median follow-up period was 4 years. In the first 2 years of follow up, 60% and more of the patients progressed to ESRD necessitating dialysis therapy. After adjustment for age, gender and hypertension, ACEI/ARB nonuser had higher risk for progression to dialysis and/or death than ACEI/ARB user with a hazard ratio of 1.26 (95% CI: 1.21-1.31, P<0.001).

The risk of ESRD or death in late stage CKD patients with diabetes

<table>
<thead>
<tr>
<th>number</th>
<th>incidence case</th>
<th>incidence rate</th>
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<th>adjusted HR</th>
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<tbody>
<tr>
<td>Age</td>
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<tr>
<td>20-44</td>
<td>408</td>
<td>400</td>
<td>147.1</td>
<td>1.13 (1.03-1.26)*</td>
</tr>
<tr>
<td>45-64</td>
<td>4,663</td>
<td>4,595</td>
<td>132.4</td>
<td>1.19 (1.07-1.31)*</td>
</tr>
<tr>
<td>75 and</td>
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<td>2,056</td>
<td>105.2</td>
<td>0.85 (0.81-0.89)*</td>
</tr>
<tr>
<td>male</td>
<td>5,243</td>
<td>5,114</td>
<td>128.5</td>
<td>1.13 (1.09-1.18)*</td>
</tr>
<tr>
<td>female</td>
<td>5,376</td>
<td>5,233</td>
<td>108.5</td>
<td>1.11 (1.06-1.15)*</td>
</tr>
<tr>
<td>Hypertension prevalence</td>
<td>9,681</td>
<td>9,476</td>
<td>123.6</td>
<td>1.37 (1.28-1.47)*</td>
</tr>
<tr>
<td>none</td>
<td>938</td>
<td>871</td>
<td>76.4</td>
<td>1.38 (1.29-1.49)*</td>
</tr>
<tr>
<td>ACEI/ARB during the following</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonuser</td>
<td>4,784</td>
<td>4,730</td>
<td>134.2</td>
<td>2.12 (1.16-2.59)*</td>
</tr>
<tr>
<td>user</td>
<td>5,835</td>
<td>5,877</td>
<td>106.4</td>
<td>1.26 (1.21-1.31)*</td>
</tr>
</tbody>
</table>

per 1000 person years, * p<0.05, ref:reference

Conclusions: Diabetic patients with late stage CKD treated with ACEI/ARB could decrease the risk for progression to dialysis and/or all-cause mortality.

PUB037

Background: Chronic oral adenine (ADE) promotes interstitial (INT) inflammation due to tubular obstruction, being used as a model of CKD. We investigated whether ADE-induced CKD: 1. still present after ADE is discontinued. 2. responds to treatment with L, known to ameliorate CKD progression.

Methods: Adult male Munich-Wistar rats received ADE in the chow, at 0.75% for 1 week, then at 0.53% for 2 weeks. Two weeks after ADE was ceased, tail-cuff pressure (TCP, mmHg), albuminuria (ALB, mg/d), serum creatinine (Scr, mg/dl), glomerular volume (Vs, X 10 µm^2), % glomerular luminal area (%Lum), tubulointerstitial macrophage density (MØ, cells/mm^2), and % cortical INT (%INT) were measured in 5 rats, as prevented treatment controls (Group ADE0). The remaining rats were divided into groups ADE+V, untreated, ADE+L, receiving oral L, 10 mg/kg/d, and followed for an additional 1 week. In groups ADE+L, matched normal controls (C) were also studied. Results (Mean±SE, *p<0.05 vs. C, **p<0.01 vs. ADE)...

Conclusions: Diabetic patients with late stage CKD treated with ACEI/ARB could decrease the risk for progression to dialysis and/or all-cause mortality.
Results:  

<table>
<thead>
<tr>
<th>Diet</th>
<th>CON</th>
<th>CKD1/2</th>
<th>CKD5/6</th>
<th>P</th>
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<tr>
<td></td>
<td>55±10</td>
<td>53±11</td>
<td>55±11</td>
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</tr>
<tr>
<td>RD</td>
<td>40±11</td>
<td>39±11</td>
<td>39±11</td>
<td>NS</td>
</tr>
<tr>
<td>F10</td>
<td>43±11</td>
<td>39±11</td>
<td>39±11</td>
<td></td>
</tr>
<tr>
<td>F0</td>
<td>36±11</td>
<td>39±11</td>
<td>39±11</td>
<td></td>
</tr>
<tr>
<td>NS</td>
<td>0±1</td>
<td>0±1</td>
<td>0±1</td>
<td></td>
</tr>
</tbody>
</table>

These results confirmed that 10% fructose could stimulate while 60% fructose down-regulate pancreas islet insulin in vitro secretion in CKD rats. Extensive collagen formation was observed in CKD 5/6 and F0 group.

Conclusions: Our results suggest that overfeeding with fructose may tremendous influence on insulin in vitro secretion in very advanced CKD but clinical meaning of this finding need further study.

Funding: Other NIH Support - Nicolaus Copernicus University grants number 02/2010 and 03/2010

PUB040

Abstract Withdrawn

PUB041

HIV Enhances Tubular Cell Angiotensinogen Expression and Angiotensin II Production through Phosphorylation of p66ShcA

Divya Salhan,1 Himanshu Vashistha,2 Sabrina Rehman,3 Mohammad Husain,1 Ashwani Malhotra,1 Leonard G. Maggs,2 Pravin C. Singh.1 1Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; 2Medicine, Ochsner Clinic, New Orleans, LA.

Background: Renin angiotensin system (RAS) has been demonstrated to play an important role in the development of HIV-associated nephropathy (HIVAN). Moreover, modalities which either inhibit the production or block the effect of Ang II have been demonstrated to retard the progression of HIVAN. We recently demonstrated that HIV also enhances phosphorylation of p66ShcA in kidney cells (J BC 284:16648-58, 2009). We hypothesized that HIV would enhance renal cell angiotensin (Ang) II production through phosphorylation of p66ShcA.

Methods: To have an in vitro model of tubular cell HIV infection, human tubular cells (HK2) were transduced with either vector only or HIV (NL4-3) constructs. Vector/HK2s and HIV/HK2s were incubated in serum-free media (SFM) for 24 hours and lysates were prepared for protein electrophoresis. Western studies were conducted for protein expression of phospho-p66ShcA, total p66ShcA, and angiotensinogen (Agt). Ang II ELISA was carried out on cells prepared under similar conditions. To establish a causal relationship between Agt and p66ShcA, EV/HK2s and HIV/HK2s were transduced with mu36-p66ShcA and then evaluated for Ang expression. Similarly, p66ShcA silenced tubular cells (siRNA-p66ShcA/ HK2s) were assayed for Agt expression.

Results: HIV/HK2s displayed 2.5 fold increased (P<0.01) expression of Agt when compared with EV/HK2s. Similarly, HIV/HK2 showed 3-fold increased (P<0.01) Agt II production when compared to EV/HK2s. HIV/HK2s also displayed increased (P<0.01) expression of phospho-p66ShcA vs EV/HK2s. On the other hand, mu36-p66ShcA transduced HK2s showed diminished expression of Agt. Similarly, siRNA-p66ShcA/HK2 showed diminished Agt expression.

Conclusions: These findings indicated HIV-induced phosphorylation of p66ShcA promotes RAS activation in tubular cells. The present study provides an insight into the RAS activation in HIVAN patients.

Funding: NIDDK Support

PUB042

HIV-Associated Nephropathy: Role of Angiotensin Type 2 Receptor (AT,R) Divya Salhan,1 Rungrawees Thanaratwich,2 Subani Maheshwari,1 Madhuri Adabala,1 Mohammad Husain,1 Ashwani Malhotra,1 Ghoucha Ding,2 Praveen N. Chander,1 Pravin C. Singh.11 Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY; 2Medicine, Renmin Hospital of Wuhan University, Wuhan, Hubei, China; 3Pathology, New York Medical College, Valhalla, NY.

Background: Activation of AT,R and AT,R contributes to opposite outcomes in several kidney disease models. AT,R has been demonstrated to play a role in the progression of HIV-associated nephropathy (HIVAN). We evaluated the role of AT,R in a mouse model of HIVAN (Tg26).

Methods: Age and sex matched control (FVB/N) and Tg26 mice aged 4, 8, and 16 weeks (n=4) were studied for renal tissue expression of AT1R and AT2R (Protocol A). In protocol B, 4 weeks old Tg26 mice were treated with saline, telmisartan (TEL, TAT1 blocker), PD123319 (PD, AT,R blocker), or TEL + PD for 2 weeks. Renal tissues was evaluated or renal molecular and biomarkers.

Results: Renal tissue expression of AT2R was lower in Tg26 mice when compared with control mice. Eight weeks old Tg26 mice displayed HIVAN phenotype in the form of glomerulosclerosis and formation of microcysts. TEL-receiving Tg26 (TRG) displayed less advanced glomerular and tubular lesions when compared with saline-receiving Tg26 (SRTG). Although renal tissues of SRTGs displayed diminished expression of both AT,R and
Novel Experimental Model of CKD with Uremia in Mice: Involvement of Inflammatory Mechanisms

**Alexander Santana,** Humberto Deléll, Cleonice Silva, Sergey Catanozi, Sabrina Degaspari, Cristofo ro Scavone, Paula Lima, Irene L. Noronha, Kim Solez, Nephrology, Univ Sao Paulo, Brazil; Lipídis, Univ Sao Paulo, Brazil; Pharmacology, Univ Sao Paulo, Brazil; Pathology, Univ Alberta, Canada.

**Background:** Dietary adenine leads to the accumulation of 2.8-dihydroxyadenine in the kidney and causes progressive renal dysfunction that resembles stage CKD with uricemic manifestations. We studied the role of inflammatory mechanisms using thalidomide (Thalid) as an anti-inflammatory drug.

**Methods:** CKD was induced in C57/BL-6 mice (n=48) by adenine-containing diet for 6 weeks. Mice were divided into 3 groups: Control, receiving normal diet, Adenine, receiving adenine to develop CKD, and Adenine+Thalid, Adenine mice treated with Adenine+Thalid. The following parameters were analyzed: biochemical, histological, and IL-1β, TNF-α, IL-6 serum and kidney mRNA levels (real time PCR).

**Results:** Adenine-fed mice developed CKD and resulted in significantly higher serum and creatinine levels compared with controls. Histological analyses showed dilated tubules, loss of tubular epithelial cells, crystalline deposits in tubular lumens, cortical scarring, and macrophage infiltration (Mac-2+) in the renal tissue of Adenine mice. A significant increase of serum and relative mRNA levels of IL-1β, TNF-α and IL-6 was observed in mice with CKD. Thalidomide treatment significantly reduced all these parameters.

**Conclusions:** These data indicate that development of CKD is associated with increased oxidative stress. Thalidomide supplementation could prevent oxidative, ameliorate renal damage and reduce proteinuria. Thus, Thalidomide holds a potential therapeutic treatment for CKD.

**Funding:** Government Support - Non-U.S.

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**PUB045**

**Is Decrease Rate in ANCA Titer a Predictor of the Inappororortum Infection?**

**Ooniishi Takahiro, Nephrology, Yamada Red Cross Hospital, Ise, Mie, Japan.**

**Background:** Without therapy renal vasculitis in ANCA vasculitides will usually progress end-stage renal disease. The toxicity of high corticosteroid doses and immunosuppressive agent has been accepted to achieve this aim. Consequently elderly patients have a higher rate of severe infection and infective death. We found that some patients did not had immunosuppression treatment occurred in course, their ANCA titer was rapidly decreased. We hypothesized that ANCA titer is used to the incident of infectious disease.

**Methods:** We evaluated 10 (male:11 female:9 mean age:73.8) MPO-ANCA-associated vasculitides presenting with rapidly progressive glomerulonephritis undergoing corticosteroid and immunosuppressive agent therapy in 2008-2010. We measured ANCA titers before treatment and after 2 months later.

**Results:** We calculated the ANCA decrease rate. We defined rapidly decrease ANCA titer group (RD) (RD>80%) at that more than 80% reduction in ANCA titers between before and after 2 months. And we examined in RD-ANCA group (n=10) and non-RD group (n=10) about Creatinine, hemogulobin,infectious disease event and death.

**Results:** RD group are no difference in age, ANCA titer, aluminab,and Hb. However significant difference in Cr (4.25mg/dl vs 2.38mg/dl, p<0.05) between RD group and NRD group. 3 patients had pneumonia due to cytomegalovirus and 2 patients died. They were all RD group.

**Conclusions:** In our study, Rapidly decrease ANCA titer might be related to opportuny infection disease. Their renal function were decreased before immunosuppressive therapy. We suggest that ANCA titer is not only RPGN activity but also immune compromised condition.

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**PUB044**

**Intra-Renal Angiotensin System Activation, Oxidative Stress, and Impaired Nrf2 Activity in the Progression of Focal Glomerulosclerosis**

**Tadashi Sato,1 Sergio Catanozi,2 Sabrina Degaspari,** Universidad del Orinoco, Venezuela; 1Nephrology, Univ Sao Paulo, Brazil; 3Universitario, Universidad del Zulia and Instituto de Investigaciones Biomédicas, Maracaibo, Venezuela.

**Background:** The Imai rat is a model of spontaneous focal glomerulosclerosis (FGS) which leads to heavy proteinuria, hyperlipidemia and progressive renal failure. Treatment with AT1 blockers (ARB) ameliorates proteinuria, hyperlipidemia, and nephropathy in this model. We hypothesized that progressive nephropathy in the Imai rat is accompanied by oxidative stress, inflammation and impaired activity of the nuclear factor-erythroid 2-related factor 2 (Nrf2), the master regulator of genes encoding antioxidant enzymes activations and that amelioration of nephropathy with AT1 receptor blockade in this model may be associated with the reversal of these abnormalities.

**Methods:** Ten-week-old Imai rats were randomized to the ARB-treated (losartan, 10 mg/kg/day) or vehicle-treated groups. Sprague-Dawley rats served as controls.

**Results:** At 34 weeks of age Imai rats showed heavy proteinuria, glomerulosclerosis and tubulointerstitial inflammation, increased number of angiotensin II expressing cells and AT1 and AT2 receptor expression, up regulation of NADPH oxidase and inflammatory mediators, activation of nuclear factor kappa B (NF-kB) and reduction of Nrf2 activity and expression of its downstream gene products in the renal cortex. ARB therapy prevented nephropathy, suppressed oxidative stress and inflammation and restored Nrf2 activation and expression of the antioxidant enzymes.

**Conclusions:** These data indicate that development of CKD is associated with increased oxidative stress. Antioxidant GSPE supplementation can prevent oxidative, ameliorate renal damage and reduce proteinuria. Thus, GSPE holds a potential therapeutic treatment for CKD.

**Funding:** Government Support - Non-U.S.

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**PUB047**

**Erythropoetin Inhibited Complement 3-Mediated Renal Tubular Epithelial to Mesenchymal Transition**

**Jian-Xin Wan, Feng-Xia Zhang, Nephrology, First Affiliated Hospital of Fujian Medical University, Fuzhou, China.**

**Background:** Our previous research had suggested that complement 3 (C3) involved in renal tubular epithelial to mesenchymal transition (EMT). Sun showed erythropoietin (EPO) could decrease renal fibrosis in mice with ureteral obstruction through inhibiting TGF-β1 mediated EMT. This study should investigate EPO inhibit C3-mediated renal tubular EMT.

**Methods:** HKE-2 cells were cultured respectively as follows: Control group, 10U/ml EPO group, 3ng/ml TGF-β1 group, 3ng/ml TGF-β1 + 10U/ml EPO group, 0.1µm C3 group, 0.1µm C3 + 10U/ml EPO group. Expressions of α-SMA, E-cadherin and C3 mRNA and protein of HK-2 cells was respectively detected by RT-PCR, western blot and cell immunofluorescence. And then model of UUO rats was established. Experimental animals were randomly divided into four groups: Control group (sham operation group), TGF-β1 group, EPO group (100U/kg EPO) and UUO rats treated with EPO (1000U/kg EPO). EPO was injected in intraperitoneal every other day from 3th to 14th day after established UUO. All experimental rats were executed after anesthesia in
the 14th day after established UUO. Morphological changes of renal tissue by HE stain, collagen deposition by Masson trichrome stain, expressions of α-SMA, E-cadherin and C3 by immunohistochemical stain were observed.

**Results:** The expression of α-SMA mRNA and protein had increased, the expression of E-cadherin mRNA and protein had decreased, the expression of complement 3 had increased after HK-2 cells stimulating with C3a or TGFβ1. After HK-2 cells stimulating with C3a or TGFβ1 combining with EPO, the expressions of α-SMA, E-cadherin and C3 mRNA and protein had occurred conspicuous changing (P<0.05). The expressions of α-SMA of UUO rats was significantly increased, and the expression of E-cadherin of UUO rats was significantly reduced. Simultaneously expressions of C3 of UUO rats was significantly reduced. After UUO rats treated with EPO, the expressions of α-SMA was significantly reduced, and the expression of E-cadherin was significantly increased. and the expressions of C3 was significantly increased.

**Conclusions:** EPO can inhibit C3-mediated renal tubular epithelial to mesenchymal transition.

**PUB048**

**Generation of a Renal Fibroblast Cell Line To Study Modulation of Mineralocorticoid Receptor**

**Hong Wang, Cheng-Kon Shih, Steven S. Pullen.**

Cardiometabolic Diseases Research, Boehringer Ingelheim Pharmaceuticals, Ridgefield, CT.

**Background:** The renal protection provided by mineralocorticoid receptor (MR) antagonists has been ascribed to the blockage pro-inflammatory and pro-fibrotic genes in non-epithelial cells in the kidney. The NRK-49F rat cell line is derived from kidney tubular fibroblasts and thus represents a renal non-epithelial cell type which is potentially suitable for in vitro studies. However, these cells were not responsive to aldosterone at physiological concentrations in preliminary studies, suggesting a lack of MR expression which was confirmed by RT-PCR quantification.

**Methods:** Cloned NRK49F cell lines stably expressing rat MR were generated for cellular studies. Expression was confirmed by RT-PCR quantification. A panel of genes previously described to be regulated by MR including PAI-1, Col1a1, Col4a1, OPN, and ORM-1 were quantified to characterize the response of the stable cell lines to aldosterone. Subsequently, the effect of the MR antagonist Spironolactone on the response of the cells was determined.

**Results:** Of the genes studied, ORM-1 was the most highly regulated in response to aldosterone. Using cell lines that express different levels of MR, it was demonstrated that the level of ORM-1 induction by aldosterone correlated with MR expression. Aldosterone induced ORM-1 expression was demonstrated to be inhibited by spironolactone in a dose responsive manner.

**Conclusions:** An aldosterone responsive, stably transfected rat kidney fibroblast cell line was generated and characterized. This cell line will be useful for further studies to investigate the effect of MR antagonism in fibroblast cells.

Funding: Pharmaceutical Company Support

**PUB049**

**Prolonged Hypertension and Renal Injury in Old Age of Neonatally Overfed Rats**

**Hyung Eun Yim, Kee Hwan Yoo, Seoong Woo Nam, In Sun Bae, Joou Won Lee.**

Pediatrics, Korea University Medical Center, Seoul, Republic of Korea.

**Background:** Neonatal growth plays a key role in "developmentally programmed" adult diseases. The objective was to evaluate the long-term influence of early postnatal overnutrition on the renal pathophysiological changes in aging rats.

**Methods:** Three or 10 male pups per mother were assigned to either the small litter (SL) or normal litter (NL) control groups during the first 21 days of life. The effects of early postnatal overnutrition on body weight, blood pressure, blood glucose, and renal changes were determined at 12 months.

**Results:** Pups in the SL group weighed more than controls between 4 days and 6 months of age (P<0.05). However, there was no difference of body weights between the two groups at 12 months. In the SL group, at 12 months of age, systolic blood pressure levels were higher than those of the controls (P<0.05). The numbers of ED-1 positive two groups at 12 months. In the SL group, at 12 months of age, systolic blood pressure was significantly reduced, and the expression of E-cadherin was significantly increased. and the expressions of C3 was significantly increased.

**Conclusions:** EPO can inhibit C3-mediated renal tubular epithelial to mesenchymal transition.

**PUB050**

**Cell Surface Biliverdin Reductase Help To Sustain E-Cadherin Expression**

**Level in Proximal Renal Tubular Cell**

**Rui Zeng, Guoping Zheng, So Ra Lee, Jianlin Zhang, Tim Tzu-Ting Hsu, Thian Kui Tan, Ye Zhao, David A.F. Loebel, Isabelle Rubera, Michel Tsao, Ya Wang, Qiao Cao, Yiping Wang, Patrick P. Tam, David C. Harris.**

**Centre for Transplantation and Renal Research, Westmead Millennium Institute, University of Sydney, Australia; 3University’s Children’s Medical Research Institute, Australia; 4University of Nice-Sophia Antipolis, France.**

**Background:** Mineralocorticoid receptor (MR) antagonists has been ascribed to the blockage pro-inflammatory and pro-fibrotic genes in non-epithelial cells in the kidney. The NRK-49F rat cell line is derived from kidney tubular fibroblasts and thus represents a renal non-epithelial cell type which is potentially suitable for in vitro studies. However, these cells were not responsive to aldosterone at physiological concentrations in preliminary studies, suggesting a lack of MR expression which was confirmed by RT-PCR quantification.

**Methods:** Cloned NRK49F cell lines stably expressing rat MR were generated for cellular studies. Expression was confirmed by RT-PCR quantification. A panel of genes previously described to be regulated by MR including PAI-1, Col1a1, Col4a1, OPN, and ORM-1 were quantified to characterize the response of the stable cell lines to aldosterone. Subsequently, the effect of the MR antagonist Spironolactone on the response of the cells was determined.

**Results:** Of the genes studied, ORM-1 was the most highly regulated in response to aldosterone. Using cell lines that express different levels of MR, it was demonstrated that the level of ORM-1 induction by aldosterone correlated with MR expression. Aldosterone induced ORM-1 expression was demonstrated to be inhibited by spironolactone in a dose responsive manner.

**Conclusions:** An aldosterone responsive, stably transfected rat kidney fibroblast cell line was generated and characterized. This cell line will be useful for further studies to investigate the effect of MR antagonism in fibroblast cells.

Funding: Pharmaceutical Company Support

**PUB051**

**Conditional Knockout of E-Cadherin in Renal Proximal Tubular Epithelial Cells Aggravates Kidney Fibrosis in Murine Model of Unilateral Ureteral Obstruction (UUO)**

**Guoping Zheng, So Ra Lee, Jianlin Zhang, Tim Tzu-Ting Hsu, Thian Kui Tan, Ye Zhao, David A.F. Loebel, Isabelle Rubera, Michel Tsao, Ya Wang, Qiao Cao, Yiping Wang, Patrick P. Tam, David C. Harris.**

**Centre for Transplantation and Renal Research, Westmead Millennium Institute, University of Sydney, Australia; 3University’s Children’s Medical Research Institute, Australia; 4University of Nice-Sophia Antipolis, France.**

**Background:** E-Cadherin is a transmembrane glycoprotein that plays a crucial role in maintaining the integrity of epithelial tissues by mediating cell-cell adhesion. In kidney disease, E-Cadherin expression is downregulated, leading to epithelial-to-mesenchymal transition (EMT). EMT is a complex process that involves the loss of epithelial markers such as E-Cadherin and the acquisition of mesenchymal markers, and it is associated with the development of kidney fibrosis.

**Methods:** Conditional knockout of E-cadherin in proximal tubular epithelial cells (PTEC) was generated using a novel conditional knockout of E-cadherin in murine proximal tubular epithelial cells (PTEC).

**Results:** Conditional deletion of E-cadherin in proximal tubule does not directly cause kidney fibrosis, but aggravates kidney fibrosis in mouse UUO model, likely through a mechanism that involves upregulation of fibrosis-related genes such as COL1A1 and COL4A1. E-cadherin conditional knockout mice compared to control.

**Conclusions:** Conditional depletion of E-cadherin in proximal tubular epithelial cells does not directly cause kidney fibrosis, but aggravates kidney fibrosis in mouse UUO model, likely through increased level of α3 integrin and EMT in PTEC upon stimulation.

**Funding:** Government Support - Non-U.S.

**PUB052**

**Uric Acid Induces Renal Inflammation Via Activating Tubular NF-kB Signaling Pathway**

**Yang Zhou, Chunsun Dai, Junwei Yang.**

**Center for Kidney Disease, 2nd Affiliated Hospital, Nanjing Medical University, Nanjing, Jiangsu, China.**

**Background:** Renal Inflammation is a pathologic feature of hyperuricemia in clinical settings. However, the underlying mechanisms remain unknown.

**Results:** Here, we found a significantly increased T cells and macrophages infiltration in kidneys from mice with hyperuricemia. This increased inflammatory cell infiltration was accompanied by upregulated kidney TNF-α, MCP-1 and RANTES expression. Immunohistochemical staining showed that the induction of RANTES was primarily localized to tubular epithelial cells, suggesting that tubular cells may play a role in uric acid-induced renal inflammation. Evidences of uric acid-induced NF-kB signaling activation were obtained in vivo. In cultured rat renal tubular epithelial cells (NRK-52E), uric acid treatment could induce TNF-α, MCP-1 and RANTES mRNA as well as RANTES protein.
expression. Moreover, uric acid-induced TNF-α, MCP-1 and RANTES expression were largely abolished by a specific NF-kB signaling inhibitor, SN-50.

Conclusions: Taken together, these results suggest that tubular cell NF-kB signaling activation may play an important role in uric acid-induced renal inflammation.

Funding: Government Support - Non-U.S.

PUB053
Diabetes and High Protein Concentrations Alter Nrf2 Phosphorylation in Renal Tubule Cells
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Nephrology, University of Louisville, KY.

Background: Renal tubule cell exposure to high glucose and protein concentrations in diabetes increases formation of reactive oxygen species (ROS). A cellular mechanism to alleviate oxidative stress is activation of the transcription factor, nuclear factor erythroid- derived 2-related factor 2 (Nrf2). Nrf2 expression increases in glomeruli of diabetic patients and diabetic rat kidneys, suggesting a compensatory response to oxidative stress, yet the effect of diabetes on Nrf2 in tubules is not clear. The goal of this study was to define Nrf2 regulation in cortical renal tubules of diabetic mice and proximal tubule cells exposed to high protein concentrations.

Methods: Cortical tubules were isolated from 3 and 7 month old OVE26 diabetic and FVB non-diabetic mice and Nrf2 expression analyzed by immunoblotting (IB). Phospho-Ser40 Nrf2 (P-Nrf2) was analyzed by immunohistochemistry of mouse kidney sections fractionated to determine total and P-Nrf2 in the cytosol and nuclei by IB. For in vitro studies, proximal tubules cells (HK2) were treated with 1 mg/ml human serum albumin (HSA) for various time points and cell extracts fractionated to determine total and P-Nrf2 in the cytosol and nuclei by IB.

Results: Total Nrf2 expression was not different between isolated tubules of 3 and 7 month old diabetic and non-diabetic mice. Nuclear P-Nrf2 was decreased in cortical tubules of 7 month old mice compared to 3 month old mice within the same group. Furthermore, cortical tubules of 7 month old diabetic mice had less nuclear P-Nrf2 compared to age-matched non-diabetic mice. HSA increased P-Nrf2 and Nrf2 nuclear import in HK2 cells, suggesting activation.

Conclusions: Together, decreased P-Nrf2 in cortical tubules of older mice with unaltered Nrf2 protein expression suggests dysregulation of this compensatory mechanism with age. Additional attenuation of this system in the older diabetic mice may be a mechanism to exacerbate oxidative stress. The ability of HK2 cells to activate Nrf2 following HSA exposure alone, suggests that additional factors in the diabetic milieu may be responsible for decreased nuclear Nrf2 localization in tubules of older diabetic mice.

Funding: NIDDK Support

PUB054
Diabetic Nephropathy in FVB/NJ Akita Mice: Temporal Pattern of Kidney Injury and Urinary Nephrin Excretion
Jae-Hyung Chang,1 Susan B. Gurley,2 Michelle T. Barati,3 Robert F. Spurzem,4 Duke University, Durham, NC; 5Duke University and Durham VAMC, Durham, NC.

Background: Glomerular podocytes are terminally differentiated cells with little potential for proliferation. As a result, a sufficient loss of podocytes may lead to instability of the glomerular tuft and glomerulosclerosis. Increasing evidence suggests that a decrease in the number of glomerular podocytes is a characteristic feature of both animals and humans with diabetic kidney disease. Early detection of podocyte injury before podocyte loss might permit intensification of medical therapies to delay or prevent diabetic nephropathy (DN). In this regard, the podocyte protein nephrin can be detected in urine of both animals and humans with diabetic nephropathy and is correlated with the urinary albumin excretion rate. The goal of this study was to examine the role of urinary nephrin excretion as an early marker of diabetic kidney injury.

Results: In 4-week old male Akita mice, the onset of hyperglycemia was accompanied by increased podocyte apoptosis and enhanced excretion of nephrin in urine before the development of albuminuria. After 4 weeks of age, Akita mice developed albuminuria, which increased progressively over time. By 20 weeks of age, Akita mice developed a 10-fold increase in albuminuria and a decrease in the number of glomerular podocytes. Urinary nephrin excretion was also significantly increased at 16 and 20 weeks of age and correlated with the urinary albumin excretion rate.

Conclusions: In summary, enhanced urinary nephrin excretion was associated with podocyte apoptosis in normoalbuminuric 4-week old diabetic mice as well as increased albuminuria in older diabetic animals. These data suggest that urinary nephrin excretion may be a useful marker of early glomerular injury in diabetes mellitus.

Funding: NIDDK Support

PUB055
Astragalus Modulates Advanced Glycation End Products-Induced Microinflammation of Macrophage
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Background: Microinflammation induced by advanced glycation end products (AGEs)-activated macrophages is a cardinal mechanism in diabetic nephropathy (DN). Advanced effector from immunosuppressant limit immunosuppressive therapy apply for microinflammation in DN. Astragalus is an herb and possess bidirectional immunomodulation regulation properties in many diseases. So we evaluate the effects and mechanism of astagalus on macrophage secretion of the inflammatory cytokines, IL-1β and TNF-α, in AGEs and normal vitro environment to determine the value of astragalus treatment in DN.

Methods: The viability of AIA-1 murine macrophages on treatment with various concentrations of astagalus or AGEs was evaluated by MTT method. The cells were treated with astagalus and/or AGEs, and scavenger receptor for advanced glycation end products (RAGE) antibody, and scavenger receptor A (SR-A) antibody. The cells were pretreated with RAGE or SR-A antibody for 1h before astagalus addition and were pretreated with astagalus for 1h before AGEs addition. The protein secretion of TNF-α and IL-1β was measured by ELISA and mRNA expression by RT-PCR. The activity of nuclear factor (NF)κB was assayed using EMSA. The expression of Phospho-P38 mitogen-activated protein kinase (MAPK) was assessed by western blotting.

Results: We not found astagalus cytotoxicity in macrophage. Astragalus increased the protein secretion and mRNA expression of IL-1β and TNF-α and suppressed the AGEs-stimulated expression of IL-1β and TNF-α in macrophage. All the effects were seemed to occur via activation or inhibition NF-kB and Phospho-P38 MAPK pathway. Additionally, RAGE or SR-A antibody did not affect the secretion of IL-1β and TNF-α induced by astagalus in macrophage significantly.

Conclusions: Base on these data, we suggested that astragalus may decrease AGEs-stimulated microinflammation in macrophage while maintaining macrophage benefit of body protection. Astragalus may be efficacious and promising remedy in the microinflammation treatment of DN. The effects of astragalus on macrophage may associate multi-targets but the special receptor.

PUB056
Chondrogenic Phenotypic Change Contribute to the Irreversible Progression of the Diabetic Nephropathy
Seiji Kishi, Hideharu Abe, Tatsuya Tominaga, Kojiro Nagai, Toshio Doi. Nephrology, Univ of Tokushima, Tokushima, Japan.

Background: We reported that BMP4 -Smad1 signaling pathway played a critical role for mesangial expansion and phenotypic alteration in diabetic nephropathy (DN) and Sox9 was also involved in the glomerulosclerosis. We hypothesized that MCs acquire a chondrocyte-like phenotype which was mediated by BMP signaling pathway and SOX9 during glomerulosclerosis progression.

Methods: Cell Culture, Alcian blue staining or immunocytochemistry, Transient transfection of MCs, Western blotting, Histological Examination, Animals; #transgenic mouse expressing inducible nitric oxide synthase under control of insulin promoter (#INOS Tgm) &BMP4 knock-in transgenic mice (BMP4 Tgm)

Results: MCs showed chondrogenic potential in a micromass culture and BMP4 induced the expressions of chondrocyte markers (SOX9 and COL2) in MCs. AGEs induced the expression of chondrocyte markers downstream of BMP4-Smad1 signaling pathway in MCs. In addition, hypoxia also induced the expression of BMP4, SOX9, and chondrocyte markers. Overexpression of SOX9 caused ectopic expression of proteoglycans and COL2 in MCs. Furthermore, overexpression of Smad1 induced expressions of chondrocyte markers. Dorosomorphin inhibited these inductions. By using INOS Tgm which exhibited severe DN, glomerular expressions of HIF1α,BMP4, and chondrocyte markers were observed.SOX9 was partially colocalized with HIF-1 and BMP4.

BMP4 Tgm showed not only similar pathological lesions to DN, but also the induction of chondrocyte markers in the sclerotic lesions.

Conclusions: Chondrogenic potential of MCs provide new aspects in the progression of glomerular injury. BMP4-Smad1 signaling and SOX9 are candidate regulators of phenotypic change in DN. Chondrocytes are the only cells found in cartilage, an avascular and hypoxic tissue. Therefore, it makes sense that this transdifferentiation of MCs is an adaptation to chronic pathological hypoxia but cartilage related ECM production caused irreversible structural change. HIF-1, which are known to be upstream molecules of SOX9, also have important influence on the phenotypic change of MC in DN.

Funding: Government Support - Non-U.S.

PUB057
Reduced Beta Cell Mass and Function by High Glucose Feeding in Rats
Anil K. Mandal,5812 Linda M. Hiebert.

People with high normal blood glucose double the risk of diabetes (DM). We asked, does overeating reduce beta cell (BC) mass or function, causing DM? We designed an experiment to answer the question.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

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Publication Only
Methods: Wistar rats 250 g treated with 25% glucose (G) in water for 2 weeks (wk.) (G3), tap water (W) for 2 wk. (G4), and N insulin (I) 2.4 units s.c. daily for 2 wk. (G5), then W for 2 wk. (GW). Control given W for 2 and 4 wk. (C). Samples for G and cretinine (c) drawn prior to treating, and at 2 wk. for groups GW and GIW. Samples for G, c and I drawn from abdominal aorta at end of experiment (2 wk. for C, G, I, GW, and GIW). Isolated from peripheral blood for immunoblotting and immunostaining procedures, IHC staining run on Bond II immunostainer using guinea pig antitwist insulin primary antibody (1:150) and biotinylated rabbit anti-insulin secondary antibody (1:100; both DAKO). 5 slides (C, G, GW, GIW, 2 sections/sample) imaged on Olympus BX51 microscope using Retiga 4000R color camera and Velocity software. IHC stain area measured and lighter stained BC cell using intensity segmentation. BC areas and measured results pasted to a spreadsheet where the % of BC area to whole IHC area computed. Also computed mean, and standard deviation of each of the 5 ratios (BH/BC, BH/HC, BH/HC mean, and BH/HC standard deviation), I measured by ELISA (Merckodia). Mean ± SEM of data in all groups analyzed.

Results: G in group G (290.52 ± 23.22 mg/dL) was higher than C (196.9 ± 29.1 mg/dL, p=0.023) and GW (203.3 ± 20.5 mg/dL, p=0.014). IHC/BC ratio mean ± SD in G (5.83 ± 1.03) and GW (6.29 ± 0.84) was higher than C (3.74 ± 0.77). IHC/BC ratio in GW (4.26 ± 0.84) was lower than GW (0.85 ± 0.18). Significant reduction of BC mass was noted in all treated groups compared to C. (1.01 ± 0.27) was higher in both G (2.89 ± 0.72) and GIW (3.95 ± 0.84) groups, compared to C but two wk. W lowered I to control levels (GW 0.24 ± 0.04, GIW 0.41 ± 0.17). No difference across levels among groups.

Conclusions: G feeding reduces BC mass. Study highlights that overeating may shrink BC mass and cause DM in humans.

Funding: Private Foundation Support

PUB058
Effectiveness and Safety of Bicarbonate in the Prevention of Contrast Induced Nephropathy in Chronic Nephropathic Diabetic Patients Undergoing to Interventional Radiology of the Lower Limbs
Filippo Mariano,1 Luca Monge,2 Valter Verna,2 Giancarlo Rizzuto,2 1Department of Diabetic Rats
Oxidative Stress in the Kidneys of the Fetus and Pre-Adolescent Offspring of Diabetic Mothers
Benedict, 1 Gloria Valencia, 2 Jacob Aranda, 2 Kay Beharry.
Brooklyn, NY; 2Department of Pediatrics. Division of Neonatology, SUNY of Neonatology, University of California Irvine Medical Center, Orange, CA; Children's Hospital, Alexandria, Egypt.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
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PUB060
High Glucose Induces Human Monomeric Endothelial-to-Mesenchymal Transition via RhoA Activation
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Background: Emerging evidence suggests that endothelial-to-mesenchymal transition (EndMT) contributes to kidney fibrosis including STZ-induced diabetic nephropathy (DN). Researches including us have showed that hyperglycemia plays an important role in the development and progression of DN. Our aim is to investigate whether high glucose induces EndMT in human monomeric endothelial cells (hGECs).

Methods: Primary human glomerular endothelial cells (hGECs) were exposed for 24 hours to culture medium containing: (1) no additions (control), (2) high glucose medium 1 (HG, 15mM), (3) high glucose medium 2 (HG2, 30mM), (4) high glucose medium 2 with ROCK1 inhibitor Y27632 (HG2+ ROCK1 inhibitor Y27632 (HG2+Y27632), (5) mannitol control. Immunofluorescent staining was performed to detect the expression of CD31, fibroblast specific protein-1 (FSP-1), and α-smooth muscle actin (α-SMA) in hGECs of different groups. Phenotypic changes of hGECs were observed using phase contrast microscope. Protein and mRNA expression of FSP-1, α-SMA and CD31 were measured by Western blotting and real-time PCR respectively. RhoA activity was detected by pull-down assay.

Results: It was showed hGECs changed their phenotype from cobble-like to fibroblast-like cells in high glucose, which did not happen in mannitol control groups. It also showed that α-SMA with CD31 double positive percentage was significantly increased in HG groups with a dose dependent manner when comparing with control group and mannitol groups. However when pre-incubated with ROCK1 inhibitor Y27632 HG2+Y27632 in high glucose, α-SMA with CD31 double positive stained cells did not increase (P<0.05, n=3). Real-time PCR for Western blotting results indicated FSP-1, α-SMA and CD31 were significantly increased in HG group compared with control group and mannitol group, but not in Y27632 group (P<0.05, n=3). RhoA activity was significantly increased in HG group compared with control group (P<0.01, n=3).

Conclusions: Our results indicate that high glucose may contribute to the EndMT of glomerular endothelial cells through RhoA signaling pathway.

Funding: Government Support - Non-U.S.

PUB061
Value of the Neutrophil to Lymphocyte Ratio as a Predictive Tool of Worsening Renal Function in Diabetic Population
Jennifer Ross, Morton J. Kleiner, Suzanne E. El Sayegh. Nephrology, Staten Island University Hospital, Staten Island, NY.

Background: The role of inflammation and inflammatory cytokines in diabetic nephropathy was studied over the last few years. Neutrophil to lymphocyte ratio rather than other white cell parameters was found to be a useful inflammatory marker which predicts adverse outcomes in acute and chronic kidney conditions. Nevertheless, the use of Neutrophil to lymphocyte ratio (NLR) as an inflammatory marker for diabetic nephropathy has not been elucidated.

Methods: A338 diabetic patients were followed at our clinic between 2007 and 2009. The following white cell parameters were totally measured: neutrophil, lymphocyte, monocyte and NLR, serum levels (creatinine, BUN, hemoglobin, albumin, and those proteins), urinary albumin creatinine ratio and GFR. Demographic variables (race, gender, age and BMI), presence of risk factors (e.g. hypertension, smoking, CAD, CHF) and medication were retrieved from the last year's follow-up index. We arranged our patients into tertiles according to the initial NLR. Dropping of GFR ≥ 12 over a 3 year follow-up period with the last GFR ≤ 60 was considered a positive primary endpoint.

Results: The lowest NLR tertile had fewer patients (2.7%) with primary outcome (i.e. worsening renal functions) compared to the middle and highest NLR tertile, which had more patients with primary outcomes (8.7% and 11.5% respectively) with a significant P value (0.01). Higher prevalence of stage ≥ 3 of CKD was found in higher two NLR tertiles(32% and 25%) versus 12% in the lowest NLR tertile group.

Conclusions: NLR as an inflammatory marker predicted the worsening of the renal function in the diabetic population. Further studies over a longer follow-up period are needed to confirm this result
Urine pH as a Predictor of Developing Type 2 Diabetes
Majed Siramamed,
Morton J. Kleiner, Suzanne E. El Sayegh.
Majed Siramamed, Morton J. Kleiner, Suzanne E. El Sayegh.
Type 2 diabetes mellitus (T2DM) remains a major public health concern.
Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Urine pH is a potential marker of insulin resistance. Further studies are necessary to determine whether urine pH is a true predictor of diabetes mellitus. If validated, urine pH has the potential to become one of the screening tools for diabetes in the primary care setting.

Renal Function in Diabetic eNOS Knockout Mice Assessed by Dynamic 99mTc-MAG3 SPECT Imaging
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Endothelial dysfunction is a hallmark of diabetic vascular complications. Recent studies have shown that deficiency of endothelial nitric oxide synthase (eNOS) significantly advances diabetic renal injury in mice. The finding indicates a crucial role of eNOS in the pathogenesis of diabetic kidney disease. However, the underlying mechanisms are incompletely understood. In this study, we assessed renal function in diabetic eNOS knockout mice using 99mTc-MAG3 SPECT imaging.
Methods: Diabetes was induced in C57BL6 strain enos knockout (enosKO) and wild type males (n=5 per group) at the age of 8 wks by multiple low-dose STZ injections (50 mg/kg, i.p., 5 days). Citrate buffer-injected enosKO and wild type males (n=5 per group) were used as non-diabetic controls. Renal function was assessed by planar dynamic mode 99mTc-MAG3 NanoSPECT imaging (~37 MBq, retroorbital injection, 30min scan) at 6 and 14 wks post STZ injection. Time-activity curves (TACs) of each kidney were recorded over the duration of the scans. Renal perfusion was assessed by the peak activity, time-to-peak (TTP), and the slope of the first inflex (RBF).
Results: At 6 wks post STZ injection, the perfusion parameters (peak activity, TTP, and RBF) showed no differences among the four groups of mice. However, all diabetic enosKO mice showed a remarkable delay in renal 99mTc-MAG3 clearance, indicating impaired tubular function, while other groups of mice did not. At 14 wks post STZ injection, significant differences were also not observed in the perfusion parameters among the four groups of mice. Surprisingly, delayed 99mTc-MAG3 clearance was not observed in diabetic enosKO mice at this time point.
Conclusions: In STZ-enosKO (C57BL6) model, eNOS deficiency causes acute tubular dysfunction in the setting of diabetes. However, this disorder is restored in the following period. Significant changes in renal perfusion are not observed by 99mTc-MAG3 scintigraphy in STZ-enosKO KO mice up to 14 wks post STZ injection. Further follow-up study is currently underway.
Funding: NIDDK Support

A Murine Model of Diet-Induced Insulin Resistance and Metabolic Syndrome
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Background: BTBR is an inbred mouse strain that is susceptible to development of diabetes and advanced complications including diabetic nephropathy (DN). A10 (2010; 21:1531), particularly in the presence of the leptin-deficiency mutation ob/ob. Wild-type BTBR mice are not hyperglycemic on Chow diet but have insulin resistance (high insulin levels). We sought to determine whether dietary modification could induce diabetes and DN in the presence of an intact leptin leptin receptor axis. At 4 wks of age, female BTBR mice were divided into four groups (n=10 per group): Chow diet, high-fat (45% fat, 45% carbohydrate, 10% protein) diet, high-fat diet (45% fat, 45% carbohydrate, 10% protein) plus 0.15% chow diet high-fat diet) high in fat (45% body weight for 8 weeks. No significant differences were found in fasting glucoses between all four groups. After 16 weeks of dietary intervention, there was no significant increase in fasting glucose. Body weights were modestly increased in DD and DDC mice at 16 weeks. Histologically, a significantly increased glomerular influx of Mac-2 positive cells (monocytes) was detected in both DD and DDC compared to Chow group (1.1 and 1.0 vs. 0.6 Mac-2 positive cells per glom respectively, P<0.05, Fig.3). In all groups, there was no significant glomerular hypertrophy, mesangial expansion, glomerular cell activation or proliferation. Increased proteinuria in DD and DDC diet groups did not achieve significance. These results characterize early inflammatory changes of glomeruli in diet-induced insulin resistance and metabolic syndrome, which may be a first step towards development of structurally advanced DN.

ACE2 Deficiency Is Not Sufficient To Aggravate Kidney Damage in Diabetic Mice on a C57BL Genetic Background Resistant to Kidney Disease
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Background: Angiotensin II overactivity is believed to be a major factor in diabetic kidney injury. ACE2 is an enzyme highly expressed in the kidney that dissipates Ang II and its expression has been shown to be altered in diabetic mouse models. We reasoned that ACE2 deficiency especially in the context of diabetes could lead to local Ang II overactivity due to a decreased Ang II degradation thus causing increased kidney damage.
Methods: We compared male WT and ACE2 KO mice (ace2+/+ and ace2−/−, respectively) on C57BL/6J background which were rendered diabetic using low (5x40 mg/kg at week 0 and 7 of diabetes) and high-dose STZ (2x150 mg/kg).
Results: ACE2 deficiency alone was not associated with albuminuria or any gross pathological kidney changes except for a mild mesangial expansion in glomeruli from ace2−/− mice as compared to WT (0.49±0.05 vs. 0.24±0.07, respectively, P<0.05). After low-dose STZ, mesangial score was about equally increased in ace2+/+ or ace2−/− mice (0.74±0.08 vs. 0.78±0.10, respectively). Both WT and ace2−/− mice with high dose STZ did not cause albuminuria in either of the groups despite severe hyperglycemia, we used high dose STZ. High dose STZ was associated with a significant increase in albumin/creatinine ratio (ACR) in both groups. Initially, ACR was higher in ace2−/− than in ace2+/+ mice but the trend was abolished later on. Mesangial score in STZ-treated ace2+/+ mice was higher than in ace2−/− mice (0.89±0.29 vs. 0.56±0.13, respectively) but the difference was not statistically significant. Other indicators of kidney injury, such as glomerular fibronectin and MCP-1 staining as well as collagen expression were equally increased in diabetic ace2−/− and ace2+/+ mice.
Conclusions: Genetic ACE2 ablation in itself does not accelerate STZ-induced diabetic kidney injury in mice on the C57BL background and further demonstrates the resistance of this strain to chronic kidney injury. Studies in more injury-prone genetic backgrounds are needed to better understand the role of ACE2 in diabetic kidney disease.
Funding: NIDDK Support

Urineary ACE as a Biological Marker of ACE2 Activity
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Daniel Batlle.
Division of Nephrology & Hypertension, Northwestern University, Chicago, IL.
Background: Urine is an easily accessible biological fluid that, as it is formed, stays in a direct contact to kidney apical membranes from which proteins are instantly shed. We have shown that ACE2-related carboxypeptidase (ACE2) is excreted in the urine in large quantities particularly in diabetic mice. Here we describe a method of distinguishing between ACE2-deficient and ACE2-replete mice using a simple, single-step approach that detects urinary enzymatic activity of a protein such as ACE2 which is expressed abundantly in the apical membranes of kidney distal convoluted tubule. The results obtained by measuring urinary ACE2 activity, were confronted with the results validate the method we used WT mice carrying two alleles of the ace2 gene (ace2−/−). The results obtained by measuring urinary ACE2 activity, were confronted with the results.
Methods: The procedure is based on a cleavage of a ACE2-specific fluorogenic substrate, Mca-APK-Dnp. Four ul. of urine sample was used in two reaction wells (2 ul/ per well) where one of wells constituted a blank containing a specific ACE2 inhibitor, MUN-4760 (10M).
Results: Using purified recombinant mouse ACE2 it was established that the linear detection range of the assay is 0.005 to 2.14 (Fig.1, 2), but the difference was not statistically significant. Other indicators of kidney injury, such as glomerular fibronectin and MCP-1 staining as well as collagen expression were equally increased in diabetic ace2−/− and ace2+/+ mice.
Conclusions: Measuring urinary enzymatic activity of proteins such as ACE2 which normally are expressed in kidney apical membranes provides a convenient tool for identification of mice with deficiency of the enzyme in question or its over-expression.
Using sensitive assays to measure urinary enzyme activity can be useful for non-invasive phenotyping and determining effectiveness of time-restricted kidney-specific gene knock-out or over-expression attempts.

**Funding:** NIDDK Support

PUB067

Enhancement of Beta-2 Microglobulin Clearance by Shaking Hemodialyzer: Numerical and Experimental Studies

Jeong Chul Kim,1,2 Francisco Garzotto,1 Mauro Neri,1,2 Massimo de Cal,1 Dinna N. Cruz,1,2 Alessandra Brendolan,1 Claudio Ronco,1,2 Federico Nalessio.1 1Department of Nephrology, San Bortolo Hospital, Vicenza, Italy; 2International Renal Research Institute Vicenza, Italy.

**Background:** Clearances of middle-molecular-weight solutes in hemodialysis are limited by blood-membrane interaction such as protein gel layer and concentration polarization. To increase the clearances of middle molecules, shaking hemodialyzer can be useful because it increases wall shear stress at the surfaces of dialysis membrane and reduces resistances layers. We numerically predicted the effects of hemodialyzer shaking on hemodynamics inside hollow fiber and experimentally measured clearances of solutes at different shaking conditions.

**Methods:** Using a numerical package we analyzed hemodynamics of single hollow fiber according to the shaking profiles (longitudinal, transverse and rotational directions). To validate numerical model we developed a dialyzer shaking instrument and measured clearances of urea and beta-2 microglobulin in blood side and dialysate side and calculated mass balance errors.

**Results:** Numerical results showed that transverse shaking is optimal for clinical application because it provide uniform enhancement of effective blood flow rate and wall shear stress during the hemodialyzer shaking. These hemodynamic parameters were linearly proportional to the vibration amplitude or frequency. In experimental results, the effects of hemodialyzer shaking were negligible in area diffusion while beta-2 microglobulin clearance increased with shaking hemodialyzer. However, the linear relationship between shaking parameters and beta-2 microglobulin clearance was not observed.

**Conclusions:** Shaking hemodialyzer could improve middle-molecular-weight solute clearances during hemodialysis.

PUB068

Profilig Cardiovascular Phenotype by Continuous Measurement Using FIR Median-Hybrid Preprocessing

Scott Wilson, Gavin J. Becker. Royal Melbourne Hospital, Australia.

**Results:** The prediction and avoidance of significant haemodynamic instability during haemodialysis is a critical issue in clinical monitoring. A 'normal' response to HD and subsequently pathologial variants, is not described. It is likely that particularly complex patterns are missed by routine arm-cuff measurement. Haemodynamic parameters would be ideally captured by continuous recording and trend-based analysis; however monitoring typical HD creates a massive data set, typically yielding over 16,000 samples per session. Standard hardware and software can find usefully manipulating such a data load difficult. Separately, the sensitivity of research devices can create signal distortion and measurement artifacts when used in the clinical HD setting, obscuring true trend signals. Preprocessing is an attractive solution to these problems. Using real patient plethysmography data (Blood Pressure, Heart Rate, Peripheral Resistance) collected during HD treatments we have experimented with single and bidirectional, linear and nonlinear algorithms to reduce artifact whilst preserving the edges and direction of the underlying trends and subtrends. Linear temporal filters perform poorly through delay and sustained signal distortions depending upon the length of the observational window. Increasing filter complexity requires predefined assumptions about the character and quality of the underlying dataset. Single filter processes are inelastic and behave inconsistently across different haemodynamic parameters and individual patients. They do not alleviate the computational load of a large dataset.

We report the design and first known application of a 5 window Heart-Rate Dependent Finite-Impulse-Response Median Hybrid Filter (FIRMHF) algorithm to cardiovascular monitoring. This filter reduces measurement artifact and preserves signal edge to create a robust time-series estimate of haemodynamic variables. No predefined tolerance thresholds or assumptions about the original dataset are required. The character of the physiologic trend is retained for analysis whilst the data-point burden is condensed to approximately 15% of the original continuous sample.

**Funding:** Private Foundation Support

PUB069

Relationship between Fluid Overload and Serum Sodium Concentration in Hemodialysis Patients

Fansan Zhu,1 Li Liu,2 Jochen G. Raimann,1 Peter Kotanko,1 Stephani Thijssen,1 Nathan W. Levin.1 Renal Research Institute, New York, NY; 2Department of Nephrology, Peking University, Beijing, China.

**Background:** It has been proposed that fluid overload may be the cause of low serum sodium concentration in hemodialysis patients. The aim of this study was to investigate the relationship of changes in serum sodium and fluid status in chronic HD patients.

**Methods:** A prospective study of HD patients whose dry weight was determined and the effect of changes in dry weight on serum sodium was observed. Changes in dry weight were determined by the patient and documented in the hospital records. Serum sodium was measured at the same time as dry weight. The following measures were studied: the difference between pre and post HD dry weight (DWE, DWP), and the difference between pre and post HD serum sodium (DSN).

**Results:** Using a linear regression method, we found that changes in dry weight were significantly correlated with changes in serum sodium (R = 0.762, p < 0.001). The relationship was also linear for the differences in serum sodium and dry weight (R2 = 0.578, p < 0.001). These results suggest that changes in dry weight are a significant factor in the changes in serum sodium.

**Conclusions:** Our findings suggest that changes in dry weight are a significant factor in the changes in serum sodium. This finding can be used to adjust dry weight and serum sodium levels more accurately, improving patient care.
Background: This open-label, self-control study was aimed to evaluate the safety and efficacy of Sevelamer Hydrochloride in treating Chinese maintenance hemodialysis (MHD) patients with hyperphosphatemia.

Methods: Phosphate binders were discontinued during a two-week washout period. Patients whose serum phosphorus was more than 1.78 mmol/L after two-week washout period were eligible for treatment. The dose could be adjusted every two weeks as necessary.

Results: 111 of the 138 patients fulfilled the whole 14-week study. Mean serum phosphorus and calcium-phosphorus increased after the second washout period, but the levels were maintained. At the end of 10-week sevelamer hydrochloride treatment, mean serum phosphorus was 5.79 ± 1.50 mmol/L vs. 5.47 ± 1.40 mmol/L, p < 0.01. Of the 138 patients involved, 214 cases in 106 patients (52-year-old Caucasian female with history of DM-2, hypertension (HTN), Roux-en-Y gastric bypass (RYGB) 2 years ago, CKD stage 1-2, attributed to HTN and DM, was admitted with worsening renal function. She had no symptoms of uremia. Serum creatinine was normal. Trace proteinuria was noted. Renal chemistry showed BUN 66 mg/dl, creatinine CR of 4.5 mg/dl, eGFR 10.9 ml/min/1.73m², K+ 3.6 mmol/L and bicarbonate of 18 mmol/L. Urine analysis showed 27 RBCs/hpf and TP/Cr ratio was 0.7. Serological workup and renal ultrasound was normal. Hemodialysis (HD) was initiated for chemical exchange and improvement of platelet function prior to kidney biopsy. Biopsy showed calcium oxalate deposits within the tubules, early diabetic nephropathy and mild arterial thickening. Immunofluorescence was negative. Plasma oxalate level was 17 uM (ref ≤ 1.8 mmol/L), urine oxalate of 44.6 mg/mg creatinine (ref 7.7-40.5 mg/mg creat), oxalic acid of 53.2 mg/l (ref 1.4-9 mg/l). Her 24 h fecal fat was 8 g/kg. She was diagnosed with oxalate nephropathy secondary to RYGB. Patient was continued on HD and started on low oxalate and fat diet and calcium carbonate, as an oxalate binder. She was later switched to home HD six times a week to maintain a plasma oxalate level less than 30 uM and referred for a kidney transplant.

Conclusions: Hyperoxaluria and oxalate nephropathy are an uncommon complication after RYGB. RYGB results in fat malabsorption thereby leading to more calcium binding to free fatty acids. This results in an increased amount of oxalate, not bound to calcium, but free and readily absorbed causing increased calcium oxalate in the serum and hyperoxaluria. Patients may benefit from low oxalate diet, low fat diet and calcium supplement, but often times, patients may progress to becoming HD dependent. Patients, who have undergone RYGB, especially in the setting of CKD, as in our patient, may be at an increased risk of worsening renal function due to oxalate deposition. They should be closely monitored for oxalate nephropathy and counseled to follow strict dietary regimen. While those contemplating RYGB procedures may need to be screened and informed of this unfortunate complication.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only Underline represents presenting author.

[861A]
Results: Laboratory tests revealed: serum intact PTH, 816.0 ± 160.4 pg/ml (247.0 to 1256.0); serum ALP, 413 ± 150 IU/L; adjusted serum Ca, 10.1 ± 2.2 mg/dl; and serum P, 6.2 ± 2.1 mg/dl. Bone histomorphometric measurements were: OB/BV (osteoid volume), 9.96 ± 5.92%; ES/BS (erosed surface), 23.50 ± 9.09%; Bv/TV (bone volume), 1.39 ± 1.92%; mineral apposition rate (MAR), 0.82 ± 0.28 µm/day; and BFR/BS (bone surface-related bone formation rate), 0.02 ± 0.02 (mm/mm/year). Three patients had osteitis fibrosa, two showed mixed bone disease (ostitis fibrosa plus osteomalacia), and one only had mild changes.

Conclusions: Since the report by Pei et al. in 1995, diabetes mellitus has been considered a risk factor for bone disease overall. However, less than 10% of patients with diabetes mellitus have bone disease. In our study, bone mineral density was measured by dual-energy X-ray absorptiometry. Our data showed that bone mineral density in diabetic patients was significantly lower than in the control group. This suggests that diabetes mellitus may be a risk factor for bone disease in the general population. Further studies are needed to confirm this finding.

Funding: Private Foundation Support

PUB077
Uremic Toxins Exacerbate Bone Mechanical Property in Chronic Kidney Disease
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Background: Chronic kidney disease (CKD) patients are associated with greater fracture incidence and increased risk of bone loss, while the reason remains obscure. Bone quality is a prescriptive factor of bone strength. Bone quality is consisted of several components including bone chemical composition. We previously revealed that cortical bones from CKD rats showed decreased mechanical property and altered chemical composition, and moreover changes of these parameters were dependent on kidney function. Thus we conducted an in vitro study to elucidate whether uremic toxins affect bone fragility.

Methods: CKD rats were divided into two groups; those administered oral charcoal absorbent which decreases the circulating levels of uremic toxins (CKD-AST) and those receiving control vehicle (CKD-V). The control group underwent sham operation.

Results: Storage modules in the CKD-V group were significantly less than those in the Control group, indicating decreased mechanical strength in the uremic cortical bone. However, the levels were maintained in the CKD-AST group. The raman spectroscopic analysis revealed that mineral matrix ratio, physiological collagen crosslinks and the ratio of carboxymethyl lysine (CML) were increased while crystallinity was decreased in the CKD-V group. These parameters were comparable between the CKD-AST and Control groups. Serum parameters were compared between the CKD-AST and the CKD-V groups except the indoxyl sulfate level, which is surrogate marker of uremic toxins, was significantly higher in the CKD-V group. Multiple regression analysis revealed that the physiological collagen crosslink and the ratio of CML were independently associated with storage modulus.

Conclusions: In conclusion, accumulated uremic toxins are quite likely candidate for those deteriorate bone mechanical property through changing the chemical composition in CKD.

PUB078
Bone Turnover Markers in Haemodialysis Patients with Chronic Liver Disease
Guillaume Jean, Charles Chazot. Hemodialyse, NÉPHROCARE, Tassin, France.

Background: In chronic kidney disease (CKD) patients, bone turnover (BT) diagnosis remains challenging for nephrologists. Because it is an invasive procedure, bone biopsy cannot be performed as a routine examination. Furthermore, it is well recognized that the serum level of parathyroid hormone (PTH) is not a reliable bone turnover marker (BTM). The Kidney Disease: Improving Global Outcomes (KDIGO) foundation recommends regular sampling of BTMs such as total alkaline phosphatases (t-ALP) and bone-specific alkaline phosphatase (b-ALP) in the case of patients with liver diseases (LDs). Bone-collagen peptides such as beta-Cross-Laps (CTX) are not recommended by the KDIGO. The goal of this study was to determine the bone turnover index in chronic hemodialysis patients with liver disease.

Methods: Between March 2004 and March 2011, all HD prevalent patients in our dialysis center with biochemical marker of chronic liver disease (AST, ALT, GGT or bilirubin > 25% at least 2 times) were included. Bone biopsy was not performed in this study. The demographics data and the exact data about their pains, such as the region, degree and existence of bone deformity were collected. The demographic data and blood chemistry data were compared using t-test and Chi-square test for the pain on lumber supine, knee joint and shoulder joint.

Results: Ninety one patients (31%) had more than moderate bone and joint pain. The most frequently injured joints were lumber supine (94%), knee joint (66%) and shoulder joint (48%). The significant factors for lumber supine pain were the past history of lumbago and high level of serum parathyroid hormone. The significant factors for knee joint pain were sex, age and high level of serum parathyroid hormone. The significant factors for shoulder pain were pressure. In conclusion, the protection of secondary hyperparathyroidism and dialysis related amylodosis is essential to reduce dialysis patients of bone and joint pains.

PUB080
Oral Calcitriol Versus Intravenous Vitamin D Analogues in the Treatment of Secondary Hyperparathyroidism
Sandep Aggarwal, Ellie Kelepouris, Irfan Ahmed, Ami Patel. Nephrology, Drexel University College of Medicine, Philadelphia, PA.

Background: With the move toward bundled payment for dialysis to include injectable medications, alternative agents are being considered to save cost without impairing outcome in the treatment of secondary hyperparathyroidism.

Methods: We conducted a retrospective study of a single inner city dialysis unit where a cohort of dialysis patients with Medicare insurance were converted from intravenous vitamin D analogues (paricalcitol or doxercalciferol) to oral calcitriol. We investigated the differences in calcium, phosphorus, and intact parathyroid hormone (iPTH) levels before and after implementation of calcitriol therapy. A total of 52 dialysis patients were analyzed for serum calcium, phosphorus, and iPTH levels prior to calcitriol initiation and 8-12 weeks post calcitriol initiation. Equivalent doses of oral calcitriol were calculated using KDOQI guidelines.

Results: There was no significant difference in calcium and phosphorus levels detected after switching to calcitriol. The oral calcitriol dosage was significantly lower compared to the calculated equivalent doses of doxercalciferol or paricalcitol. The intact PTH levels were higher after introduction of calcitriol but did not reach statistical significance. Preliminary data suggested significant patient cost reduction with conversion to calcitriol. The incidence of hypercalcemia(>10.2 mg/dl) was 7% in intravenous group and 9% in oral group.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Trends in Intravenous Vitamin D Use among Dialysis Patients in the United States (1999-2008)

Anne C. Beaubrun, Abhijit V. Kshirsagar, Lily Wang, M. Alan Brookhart. University of North Carolina, Chapel Hill, NC.

Background: Injectable vitamin D agents are commonly used to manage renal osteodystrophy in the dialysis population. Yet, there is little data documenting the temporal variability and patterns of use of these agents. We sought to describe patient- and facility-level utilization patterns of vitamin D formulations (calcitriol, paricalcitol, doxercalciferol) in the US hemodialysis population.

Methods: We studied adult patients in the United States Renal Data System (USRDS) between January 1999 and December 2008 who initiated dialysis as a primary payer and who initiated dialysis at least 90 days prior to the month being investigated. Monthly percentages of patients treated with any vitamin D and each type of formulation were tabulated.

Results: We identified 140,790 dialysis patients in January 1999 and the number of eligible patients steadily increased to 214,113 in December 2008. Between 1999 and 2008, vitamin D use steadily increased with the exception of a slight decrease in use in the latter half of 1999. The use of calcitriol has declined since 1999, going from being administered to 44% of patients in January 1999 to 1.1% in December 2008. Paricalcitol is now the overwhelmingly preferred formulation. Doxercalciferol use in the dialysis cohort began in 2002, steadily increased and has begun to slightly decline since 2007. As of 2008, approximately 80% of the USRDS population used any vitamin D formulation.

Conclusions: Vitamin D use has increased and parallels the rise in use of paricalcitol and doxercalciferol. Furthermore, there is variation in formulation choice. Given the known pharmacologic differences in the vitamin D formulations, future research should focus on identifying the reasons for differences in vitamin D use, and, whether the variation in vitamin D use differentially affects patient outcomes.

Switching Haemodialysis Patients from Oral to Intravenous Vitamin D May Not Improve Adherence to Prescribed Medication

Alison Brown, Claire Jackson, James Shawcross, George Hartley. Renal Unit, Freeman Hospital, Newcastle upon Tyne, Tyne and Wear, United Kingdom; Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne, Tyne and Wear, United Kingdom.

Background: Achievement of recommended calcium, phosphate and parathyroid hormone (PTH) levels in haemodialysis (HD) patients remains a significant challenge. Our 266 HD patients are reviewed by renal dieticians who prescribe and adjust phosphate binders, and clinicians who adjust Vitamin D (Vit D) dose; medication is then dispensed in primary care. Our unit results in the UK Renal Registry compare well with the rest of the UK and Europe. However, compliance with prescribed medication remains variable. We change patients from oral self-administered Vit D to intravenous (IV) given on HD when control or compliance is poor. We describe the results of audit of bone chemistry and compliance with oral and IV Vit D.

Methods: Our local protocol based on KDOQI and UK Renal Association recommendations targets these predialysis levels: PTH 120-540 ng/l, Adjusted calcium 2.20-2.50 mmol/l, Phosphate 1.10-1.70 mmol/l.

We reviewed predialysis serum PTH, adjusted calcium and phosphate levels, and Vitamin D analogue prescription in all published HD patients during December 2010.

Results: Complete data was available for 222 of 260 patients. Only 10.8% had results within target for all parameters.

Of 138 patients receiving Vit D, 55% had levels within target range for PTH, 48% for calcium and 44% for phosphate.

Of 84 patients not prescribed Vit D, 55% had levels within target for PTH, 45% for calcium and 45% for phosphate.

36% of all patients were taking oral Vit D doses different to that recorded in hospital notes.

Only 70% of IV Vit D doses were administered as prescribed.

Conclusions: Although achieved bone chemistry compares well with other UK renal units, 36% of oral and 30% of IV doses of Vit D differed from the recommended dose in our HD patients; giving poorly controlled or compliant patients IV Vit D did not significantly improve compliance with medication.

Our study highlights the importance of documenting Vit D medication accurately to ensure alterations are appropriate. Future introduction of electronic and patient-held records may help to achieve this.

Long-Term Skeletal Health and Integrity in Renal Transplant Patients – Are we Measuring the Right Parameters?

Nihil Chitalia, Sharon Frame, Ana Sofia Rocha, David Goldsmith. Nephrology and Transplantation, Guy’s and St. Thomas’ Hospital NHS trust, London, United Kingdom; Renal Medicine, St. George’s Hospital NHS trust, London, United Kingdom.

Background: As the success of kidney engraftment improves, there is a progressively larger cohort of renal transplant patients surviving a long time. We established a specialized multidisciplinary clinic to focus on best management of these patients’ multiple medical issues. It is well-known that bone and mineral metabolism (CKD-MBD) parameters are abnormal after renal transplantation, but long-term data are scarce, particularly with reference to bone mineral density as measured by DEXA scans.

Methods: We took 151 well, ambulant renal transplant patients who were transplanted for more than 10 years and examined their CKD-MBD parameters and DEXA scans in detail.

Results: The findings are summarized in Table 1.

Table 1: Demographic, biochemical and bone-mineral density categories on DEXA scan in ambulant transplant recipients on long term follow-up (n=151)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean±SD or percentages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>54±12</td>
</tr>
<tr>
<td>Gender (% Males)</td>
<td>62</td>
</tr>
<tr>
<td>Months post transplant (median(IQR))</td>
<td>207(126)</td>
</tr>
<tr>
<td>eGFR (ml/min/1.73m2)</td>
<td>49±20</td>
</tr>
<tr>
<td>Haemoglobin (gm%)</td>
<td>12.8±1.5</td>
</tr>
<tr>
<td>Serum Calcium (mmol/l)</td>
<td>2.40±0.15</td>
</tr>
<tr>
<td>Serum Phosphate (mmol/l)</td>
<td>1.04±0.23</td>
</tr>
<tr>
<td>Serum Alkaline Phosphatase (μmol/l)</td>
<td>773±30</td>
</tr>
<tr>
<td>Serum PTH ymol/L</td>
<td>94±67</td>
</tr>
<tr>
<td>Serum 25 HydroxyVitamin D (mmol/L)</td>
<td>48±31</td>
</tr>
<tr>
<td>Vitamin D Deficient &lt;37.5 μmol/L</td>
<td>59(39.1%)</td>
</tr>
<tr>
<td>Vitamin D Insufficient 37.5-75 μmol/L</td>
<td>58(38.4%)</td>
</tr>
<tr>
<td>Vitamin D sufficient &gt;75 μmol/L</td>
<td>34(22.5%)</td>
</tr>
<tr>
<td>% on Steroids</td>
<td>77(51.9%)</td>
</tr>
<tr>
<td>% on Bisphosphonates</td>
<td>17(11.9%)</td>
</tr>
<tr>
<td>% on Vitamin D or Calcium supplements</td>
<td>26(17%)</td>
</tr>
<tr>
<td>BMD Category</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>65(43%)</td>
</tr>
<tr>
<td>Osteopenia</td>
<td>65(43%)</td>
</tr>
<tr>
<td>Osteoporosis</td>
<td>20(14%)</td>
</tr>
</tbody>
</table>

Diagnosis of osteoporosis based on the National Osteoporosis foundation guidelines 2008.

On bivariate correlations between bone biochemistry and T-scores on DEXA scanning, serum calcium showed a negative correlation with T-score at neck of femur (NOF) (r=-0.296, p=0.002), whereas serum PTH showed a positive correlation with T-score at NOF (r=0.324, p=0.01). Serum 25 hydroxy vitamin D did not show a significant correlation with T-scores at any sites on DEXA scanning.

Conclusions: In a selected ambulant population of long-term renal transplant survivors, there were only 8% of patients with normal serum calcium, phosphate, PTH and 25(OH)D ≥75nmol/L. Vitamin D deficiency and abnormal BMD indicative of osteopenia or osteoporosis is common in transplant recipients but did not seem to correlate with the usual biochemical parameters typically measured in patients on clinic visits. There is a need for further research into novel biomarkers and longitudinal study data.

Effectiveness of a Mg-Based Phosphate (P) Binder on the Development of Vascular Calcifications (VC) in Uremic Rats

Patrick C. D’Haese. Laboratory of Pathophysiology, University of Antwerp, Antwerp, Belgium; Fresenius Medical Care, Bad Homburg, Germany.

Background: As the success of kidney engraftment improves, there is a progressively larger cohort of renal transplant patients surviving a long time. We established a specialized multidisciplinary clinic to focus on best management of these patients’ multiple medical issues. It is well-known that bone and mineral metabolism (CKD-MBD) parameters are abnormal after renal transplantation, but long-term data are scarce, particularly with reference to bone mineral density as measured by DEXA scans. We compared the effect of 2 doses of the Mg-based P-binder Calmg (2.3/2.3 Ca-acetate and 1.3 Mg-carbonate, Oxsaven®) to that of selenamide carbonate (sev) on the development of VC in rats with CRF.

Conclusions: Although achieved bone chemistry compares well with other UK renal units, 36% of oral and 30% of IV doses of Vit D differed from the recommended dose in our HD patients; giving poorly controlled or compliant patients IV Vit D did not significantly improve compliance with medication.

Our study highlights the importance of documenting Vit D medication accurately to ensure alterations are appropriate. Future introduction of electronic and patient-held records may help to achieve this.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Methods: 56 male rats were divided in 4 groups: Vehicle (Veh), 375mg/kg CaMg, 750mg/kg CaMg, and sev (750mg/kg sev). CRF was induced by feeding 0.75% adenine (ADE)-2.5% protein diet for 4 weeks. After 1 wk of CRF, rats were gavaged with P-binders or Veh (7d/wk) until sacrifice at wk 6.

Results: Renal function was sign. impaired after 4 wks of AD-treatment and in all groups treated with CaMg, sev also developed severe hyperphosphatemia which was well controlled in the groups receiving P-binders particularly those receiving CaMg even at the lowest dose (CNax Serum P: Veh: 20.3 - CaMg375: 11.5 - CaMg750: 9.9 - sev: 16.3mg/dl). AUC0-6wks of serum Ca did not differ sign. between groups. Serum Mg AUC0-6wks dose-dependently increased in treated groups (Veh: 6.0 - CaMg: 20.8 - CaMg750: 27.16 - wk/l). Induction of CRF went along with a sign. increase in serum PTH. Treatment with CaMg dose-dependently prevented this increase whilst sev did not. The data showed that both CaMg and sev sign. reduced the area% calcification in the aorta. Results were confirmed on Von Kossa stained sections, by sev and showed a clear trend for CaMg, indicating a possibly different mechanism compared to Veh. Sev had no sign. effect on aortic calcifications although a clear reducing trend was seen. In calcifications, aortic media and aortas were sign. reduced by sev and showed a clear trend for CaMg, indicating a possibly different mechanism of calcification in these arteries. Results were confirmed on Von Kossa stained sections, showing that both CaMg and sev sign. reduced the area% calcification in the aorta.

Conclusions: Treatment with the Mg-based P-binder CaMg effectively controlled serum P and PTH levels resulting in reduced aortic calcifications in uraemic rats. Funding: Pharmaceutical Company Support

PUB087

Prevalence and Factors Associated with Vitamin D Insufficiency/Deficiency in Dialysis Patients: Results of the French National Observatory for Mineral and Bone Metabolism

Guillaume Jean,1 Hubert Roth,2 Tilman B. Druke,3 Gerard M. London,4 Thierry P. Hannedouche,5 Jean-Louis Bouched,5 Denis Fouque,6 NephroCare, Tassin, France; 2CRNH, Grenoble, France; 3INSERM, Amiens, France; 4Hôpital Manhes, Fleury Mergos, France; 5Hôpital Civil, Strasbourg, France; 6CTMR Saint Augustin, Bordeaux, France; 7Hôpital E. Herriot, Lyon, France.

Background: Vitamin D insufficiency/deficiency is very common in dialysis patients and has been associated with a less favourable survival rate.

The aim of this study was to analyse the prevalence and the factors associated with vitamin D deficiency in patients on maintenance dialysis.

Methods: Using October 2010 baseline data from a national, prospective observational study evaluating the management of mineral metabolism abnormalities in patients who were on haemodialysis for less than 12 months and not taking nutritional vitamin D, we have compared those with a serum 25-hydroxy vitamin D (25(D) > 75 mmol/l with those ≤75 mmol/l.

Results: Of 3,808 patients, 2,380 (62.5%) were tested for serum 25-D, mean age was 68.3 ± 15.5 years and 37.6% were female. Average serum 25-D was 64.3 ± 42.4 mmol/l. Among the subset of patients who were not treated with nutritional vitamin D (n = 928), serum 25-D was associated with the following factors.

Table 1

<table>
<thead>
<tr>
<th>Patients not treated with nutritional vitamin D</th>
<th>25-D &gt; 75 mmol/l n= 672</th>
<th>25-D ≤ 75 mmol/l n= 256</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.6 ± 15.5</td>
<td>67.6 ± 16.3</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.7 ± 6.0</td>
<td>24.5 ± 5.8</td>
</tr>
<tr>
<td>Serum albumin g/l</td>
<td>1.60 ± 0.58</td>
<td>1.56 ± 0.51</td>
</tr>
<tr>
<td>PTH pg/ml</td>
<td>236 [120-416]</td>
<td>193 [93-337] **</td>
</tr>
<tr>
<td>Albumin g/l</td>
<td>34.7 ± 5.1</td>
<td>36.3 ± 5.4 ***</td>
</tr>
<tr>
<td>Hemoglobin g/l</td>
<td>111 ± 15</td>
<td>113 ± 19 *</td>
</tr>
<tr>
<td>Diabetes</td>
<td>37±4%</td>
<td>31±3%</td>
</tr>
<tr>
<td>Vasc. Calculations</td>
<td>40±0%</td>
<td>34±8% **</td>
</tr>
</tbody>
</table>

* Median [Q1-Q3]; ** P < 0.05; *** P < 0.01; **** P < 0.001, Chi² or t-test.

No other clinical, biochemical or therapeutic factor was significantly associated with vitamin D deficiency.

Conclusions: Vitamin D insufficiency/deficiency frequently occurs in patients on maintenance dialysis. Factors associated with this insufficiency include larger BMI, low K/UV, albumin, haemoglobin and high PTH.

Project Photo-Graph 3 (SVCARB099910)

Funding: Pharmaceutical Company Support

PUB088

Prevalence and Factors Associated with Vitamin D Insufficiency/Deficiency in Non Dialysed Chronic Kidney Disease Patients: Results of the French National Observatory for Mineral and Bone Metabolism

Guillaume Jean,1 Hubert Roth,2 Tilman B. Druke,3 Gerard M. London,4 Thierry P. Hannedouche,5 Jean-Louis Bouched,5 Denis Fouque,6 NephroCare, Tassin, France; 2CRNH, Rhone Alps, Grenoble, France; 3INSERM, Amiens, France; 4Hôpital Manhes, Fleury Mergos, France; 5Hôpital Civil, Strasbourg, France; 6CTMR Saint Augustin, Bordeaux, France; 7Hôpital E. Herriot, Lyon, France.

Background: Vitamin D insufficiency/deficiency is frequently observed in CKD patients and has been associated with a more rapid CKD progression and reduced survival rate. We report the prevalence and the factors associated with vitamin D deficiency in CKD stage 4-5.

Methods: Using October 2010 baseline data from a national, prospective observational study evaluating the management of mineral metabolism abnormalities in CKD patients with eGFR ≤ 60 mL/min/1.73m² and not taking nutritional vitamin D, we have compared those with serum 25-hydroxy vitamin D (25(D) ≤ 75 mmol/l with those >75 mmol/l.

Results: Among a total of 876 patients, 585 (67%) were tested for serum 25-D, mean eGFR 21 ± 7 mL/min/1.73m² and 41% were female. Average serum 25-D was 62.9 ± 40.4 mmol/l. 67.2% had a serum 25-D ≤ 75 mmol/l. Among the subset of patients who were not treated with nutritional vitamin D (n=200), serum 25-D was associated with the following factors.

Table 2

<table>
<thead>
<tr>
<th>Patients not treated with nutritional vitamin D</th>
<th>25-D &gt; 75 mmol/l</th>
<th>25-D ≤ 75 mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68.3 ± 15.5</td>
<td>67.6 ± 16.3</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>25.7 ± 6.0</td>
<td>24.5 ± 5.8</td>
</tr>
<tr>
<td>Serum albumin g/l</td>
<td>1.60 ± 0.58</td>
<td>1.56 ± 0.51</td>
</tr>
<tr>
<td>PTH pg/ml</td>
<td>236 [120-416]</td>
<td>193 [93-337] **</td>
</tr>
<tr>
<td>Albumin g/l</td>
<td>34.7 ± 5.1</td>
<td>36.3 ± 5.4 ***</td>
</tr>
<tr>
<td>Hemoglobin g/l</td>
<td>111 ± 15</td>
<td>113 ± 19 *</td>
</tr>
<tr>
<td>Diabetes</td>
<td>37±4%</td>
<td>31±3%</td>
</tr>
<tr>
<td>Vasc. Calculations</td>
<td>40±0%</td>
<td>34±8% **</td>
</tr>
</tbody>
</table>

* Median [Q1-Q3]; ** P < 0.05; *** P < 0.01; **** P < 0.001, Chi² or t-test.

No other clinical, biochemical or therapeutic factor was significantly associated with vitamin D deficiency.

Conclusions: Vitamin D insufficiency/deficiency is frequently observed in CKD patients and has been associated with a more rapid CKD progression and reduced survival rate.

Project Photo-Graph 3 (SVCARB099910)

Funding: Pharmaceutical Company Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

864A
Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Sepsis in a Large Managed Care Organization

**Methods:** We identified 211 individuals with sepsis and 211 matched controls. The mean age: 77.7 year, mean plasma albumin value was 34.8 gr/L . CRP mean 437 (351) mg/l. The prevalence of elderly treated patients (pts) on hemodialysis (HD) has been increasing steadily. The Phosphocalcic disorders (PCD) have not been studied in this population. Six months, prospective, non randomized open-study was conducted in a single center.

**Results:** At month 6, 25(OH)D was>30µg/l in 82.8% of group2 pts and in 56.5% of group1 pts (p<0.01). No significant epidemic of hypercalcemia nor toxic 25(OH)D levels were observed.

**Conclusions:** Cholecalciferol is more effective than ergocalciferol in normalizing 25(OH)D in hd. Modalities of long term supplementation remain to be established.

**PUB092**

Efficacy and Safety of Paricalcitol Dosing Regimens for Subjects on Hemodialysis in China

**Methods:** In this randomized, multi-center, single-blind study 216 subjects received dosing regimens based on EU (iPTH/80) or US (0.04 µg/kg) packet insert (PI) of paricalcitol over 12 weeks. Primary efficacy analysis evaluated non-inferiority of EU compared to US PI in proportion of subjects achieving at least 2 consecutive ≥30% decreases from baseline iPTH. Secondary analyses evaluated the proportion achieving KDOQI (150-300pg/ml) and KDIGO (130-585 pg/ml) recommended iPTH ranges incidence of hypercalcemia (at least 2 consecutive Ca≤11.0mg/dL) and changes from baseline iPTH to each post-baseline visit.

**Results:** A higher proportion (88.6%) of subjects initiated at iPTH/80 achieved at least 2 consecutive ≥30% decreases in baseline iPTH than subjects receiving 0.04µg/kg (55.9%). A comparable treatment proportion with both regimens achieved KDOQI (17.6% EU and 19.4% US) and KDIGO (59.3% EU and 54.6% US) recommended ranges. Repeated measures analysis showed significantly greater mean reduction from baseline iPTH (p<0.001) in subjects receiving iPTH/80 compared to subjects receiving 0.04µg/kg. Overall, treatment with iPTH/80 and 0.04µg/kg dosing presented a low incidence of hypercalcemia (at least 2 consecutive Ca≤11.0mg/dL).

**Conclusions:** Paricalcitol dosing of iPTH/80 (EU) demonstrated superiority over 0.04µg/kg (US) in achieving ≥30% reductions from baseline iPTH levels. However, both regimens were comparable in achieving KDOQI and KDIGO recommended iPTH ranges with low risk of hypercalcemia. Paricalcitol represents a potentially beneficial treatment option for subjects with SHPT on HD in China.

**Funding:** Pharmaceutical Company Support
Ergocalciferol (ergo) Therapy in Calciod Deficient Hemodialysis (HD) Patients on Therapeutic Doses of Paricalcitol Does Not Decrease Cytokine Release in Peripheral Blood Mononuclear Cells

Vidya M. Raj Krishnamurthy,1 Srinivasan Beddu,1,2 Tom H. Greene,1,2 Guo Wei,1 Yuxia He,1 Huan Li,1,2 Alfred K. Cheung,1,3 Christi M. Terry.1 1VA; 2Univ Utah.

Background: Vitamin D possesses immuno-regulatory activities such as inhibiting nuclear factor-κB activity, increasing IL-10 production and decreasing TNF-α production. It is unclear whether additional therapy with ergo in those with calcidiol deficiency would inhibit cytokine release by peripheral blood mononuclear cells (PBMC) and thus have beneficial anti-inflammatory effects in this population.

Methods: We conducted a randomized double blind cross-over trial of ergo vs placebo in 24 HD patients treated with therapeutic doses of paricalcitol with IPT range between 150-600 pg/ml but with plasma 25(OH) vitamin D levels <30 ng/ml and high sensitive C-reactive protein (hs-CRP) >3mg/L. 24 HD patients were randomly assigned to ergo 50,000 U/wk vs. placebo for 12 weeks, followed by 4 wk washout and cross-over for 12 weeks. Blood was collected at baseline and wks 12, 16 and 26. PBMC were obtained using standard Ficoll-Paque isolation then incubated for 24 h plus minus 10 ng/ml LPS, in media with 10% patient serum. Cytokine release to media was tested (ELISA).

Results: The test population demographics were 59 ± 13 yrs, 42% men, 80% Caucasian, 67% diabetic and the average duration of ESRD was 3.7±4.6 years. Ergo treatment significantly increased plasma 25(OH) vitamin D levels (p<0.001). Table 1 shows the difference between pre and post ergo vs placebo treatment in LPS-induced release of IL-6 from PBMC.

Conclusions: Ergo treatment of HD patients increases 25(OH) vitamin D levels but had no significant effect on PBMC response to LPS treatment in vitro. These results suggest that ergo treatment will not decrease inflammation in this patient population.

Effect of Cholecalciferol Supplementation on Physical Function and Health Related Quality of Life in 25 Hydroxy Vitamin D Insufficient Hemodialysis Population

Wilmer Samson,1 Sharad Sathyan,2 Anne M. Kenny.1 1Nephrology, University of Connecticut School of Medicine, Farmington, Connecticut; 2Nephrology, Cornell School of Medicine, New York, NY.

Background: To determine the effects of Vitamin D supplementation on physical performance and health-related quality of life in a hemodialysis population.

Methods: Subjects were followed for 4 weeks without treatment (as a control period), then were supplemented with 3000 IU cholecalciferol (vitamin D3) for 8 weeks. The following tests were performed at 4 weeks, baseline, 4 and 8 weeks post-supplementation: 25-hydroxyvitamin D (25OHD); parathyroid hormone (PTH); physical assessment including 6 minute walk, handgrip strength and Short Physical Performance Battery (SPPB); health questionnaires including cognitive screen, activities and instrumental activities of daily living, SF-36 and Geriatric Depression Scale.

Results: 380 subjects (mean age 64 ± 16 years; 18 men; 12 women; 57% Caucasian) had 25 OHD levels of 19.9 ± 7.7 ng/dl. Twenty-four subjects (80%) had 25 OHD less than 30 ng/dl. Sixty-five percent of participants reported general health to be fair or poor. While 25 OHD increased significantly to 26.8 ± 8 ng/dl after supplementation, there were no changes in physical performance or health related quality of life.

Conclusions: Our data confirms that 25 OHD insufficiency is widely prevalent in the ESRD population. Further work will be required to address the appropriate dose of cholecalciferol to obtain sufficient 25OHD levels and whether physical performance or quality of life improve with supplementation in ESRD patients.

Cinacalcet Hydrochloride Prohibits Bone Mineral Density Loss in Daily Patients

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Background: In dialysis patients, bone mineral density(BMD) lossing is closely related not only to the progress of chronic kidney disease-mineral bone disorder(CKD-MBD) but also to the patient mortality. In this study we evaluated whether Cinacalcet hydrochloride (CH) is effective in keeping BMD or not.

Methods: We selected 106 patients from the consecutive 152 chronic hemodialysis patients for the current study. The inclusion criterion of the patients was that their %BMD was more than 80%. 106 patients were divided into the following 3 groups by the medication for secondary hyperparathyroidism (2HPT).

Group A: intravenous vitamin D administration (i.v. VD) + CH

Group B: i.v. VD

Group C: oral VD administration

We compared the changes in %BMD, intact-PTH (iPTH) and bone alkaline phosphate (BAP) among 3 groups 6 months and 18 months after the administration of CH.

Results: i-PTH when CH before started in group A was 450 ± 3266 pg/mL, then it was significantly reduced after CH started. %BMD was significantly reduced Group B and C but did not change in Group A. The mean values in iPTH and BAP 6 months and 18 months of each group were not significantly different (Table 1).

Cinacalcet Hydrochloride Prohibits Bone Mineral Density Loss in Daily Patients

Rui Toledo Barros,1 Rosa M. Moyses,1 Roberto Zatz.1 1Nephrology, University of Sao Paulo, Sao Paulo, Brazil.

Background: The frequency of hypovitaminosis D in CKD patients is very high, although causal mechanisms are still unclear. In this study, we analyzed the frequency and predictors of hypovitaminosis D in diabetic nephropathy (DN) patients.

Methods: Data on 56 DN patients with proteinuria and class III CKD was collected from 2005-07. Mann-Whitney and chi-square tests were used; linear and logistic regression models were built for 25OHD vitamin D levels, but a significant portion of patients remained below 35 ng/dl, a level required to benefit many health outcomes including physical performance, suggesting a need for higher doses of cholecalciferol. We found no significant change in physical performance or quality of life. Further work will be required to address the appropriate dose of cholecalciferol to obtain sufficient 25OHD levels and whether physical performance or quality of life improve with supplementation in ESRD patients.

Funding: Private Foundation Support

Hypovitaminosis D in Macroalbuminuric Diabetic Nephropathy

Silvia M. Titon,1 María Silvia Queiroz,2 Maria Alice Muniz Domingos,1 Vanda Jargetti,1 Rui Toledo Barros,1 Rosa M. Moyes,3 Roberto Zatz.2 2Nephrology Division, Hospital das clinicas, Sao Paulo University Medical School, Sao Paulo, Brazil; 3Nephrology Division, Hospital das clinicas, Sao Paulo University Medical School, Sao Paulo, Brazil.

Background: The frequency of hypovitaminosis D in CKD patients is very high, although causal mechanisms are still unclear. In this study, we analyzed the frequency and predictors of hypovitaminosis D in diabetic nephropathy (DN) patients.

Methods: Data on 56 DN patients with proteinuria and class III CKD was collected from 2005-07. Mann-Whitney and chi-square tests were used; linear and logistic regression models were built for 25OHD vitamin D levels, but a significant portion of patients remained below 35 ng/dl, a level required to benefit many health outcomes including physical performance, suggesting a need for higher doses of cholecalciferol. We found no significant change in physical performance or quality of life. Further work will be required to address the appropriate dose of cholecalciferol to obtain sufficient 25OHD levels and whether physical performance or quality of life improve with supplementation in ESRD patients.
**Conclusions:** Hypovitaminosis D is extremely frequent in macroalbuminuric DN patients. While 25(OH)d3 proteinuria and systemic inflammation were importantly associated to the risk of low 25vitD in our study.

**Funding:** Government Support - Non-U.S.

**PUB097**

**Oral Cholecalciferol Therapy in Prevalent Hemodialysis Patients: A Randomized Placebo Controlled Pilot Study**

Karen To, Azim S. Gangji, Egerton, 3

**Background:** Vitamin D is a prohormone that is activated in the liver and kidney. Besides the kidney, other tissues express 1α-hydroxylase. This local autocrine/paracrine activity is important for health maintenance and relies on adequate 25(OH)D levels.

**Methods:** We conducted a randomized controlled pilot study of 20 prevalent adult in-center hemodialysis (HD) participants when assigned, using concealed randomization, to receive 50,000 IU of D3 or identical-appearing placebo weekly for 12 weeks. Health care providers, participants, and research staff were masked to allocation. The primary outcome was the change in 25(OH)D between baseline and week 12. We measured other laboratory outcomes at the same time points, and calcium and phosphorus every two weeks. We assessed BP as the mean of pre- and post-dialysis and collected information on medication use. Analysis was done according to the intention-to-treat principle. Unpaired t tests were used to assess differences between mean baseline and week 12 blood work results. Chi-squared tests were used to compare categorical variables between groups.

**Results:** Mean serum 25(OH)D levels of both groups were similar at baseline [14.7±6.1 (D3) versus 13.2±1.8 ng/ml (placebo); p=0.63], consistent with insufficiency. The mean change (week 12 minus baseline) in 25(OH)D levels were 25.1±14.3 and 12.3±7.9 ng/ml in the D3 and placebo groups, respectively (p=0.001). There were no differences in the change in calcitriol, PTH, ALP, CRP, ESR, ferritin, Alb levels between treatment groups. There were no differences between treatment groups in the mean change of BP, or in haemoglobin/ESR ratio. There were no episodes of hypercalcemia (≥11 mg/dL). Episodes of hyperphosphatemia (≥9.3 mg/dL) were comparable between groups (n=2 for D3 vs n=3 for placebo; p=0.99).

**Conclusions:** Weekly D3 was effective in correcting the vitamin D status in HD participants.

**Funding:** Private Foundation Support

**PUB098**

**Vitamin D Deficiency in Renal Transplantation**

Kristin Vibeke Veijtery,1 David C. Wheeler,1 Mark Harber,2 Anne B. Dawney,3 Francis Lam,1 Martyn Egerton,1 Alyyje Karau,1 John Cunningham,1 1UCL Centre for Nephrology

**Background:** Animal studies have demonstrated a reduction in ischaemia-reperfusion (IR) injury following systemic administration of vitamin D. Vitamin D deficiency is endemic renal patients. We postulated that patients deficient in 25-hydroxyvitamin D (25(OH)D) and/or 1,25-dihydroxyvitamin (1,25(OH)2D) might have poorer cardiac and endothelial function, greater IR injury at transplantation, and increased fibrosis on protocol biopsies.

**Methods:** 103 patients transplanted from 2008-2011 had protocol biopsies at 6(5)/65 weeks(56) and had stored serum from the time of transplantation. The population was 53.40% White, 22.33% Black, 14.56% Asian, 2.91% Mixed and 6.80% Other. None received supplemental vitamin D. Serum 25OHD and 1,25OH2D were quantified by mass spectrometry (25OH D2/D3 by LC-MS/MS (Inter-assay CV <10%); 1,25OH2D by enzymimmonoassay (IDS)). Fibrosis on protocol biopsies was quantified using a validated index of chronic damage (ICD) (Mean inter-observer difference 1.0(CI 0.94-1.06)).

**Results:** In our cohort, 20(19.42%) were deficient (<25nmol/l), 57(55.34%) insufficient (25-75nmol/l), and 26(25.24%) replete (>75nmol/l) in vitamin D. 60% of Asian patients were deficient. In 97(94.17%), D2 was below the assay range. 62.7% of recipients were replete (≥100nmol/l) in 1,25-dihydroxyvitamin D. 80% of Asian patients were deficient. 25-hydroxyvitamin D levels did not predict 1,25-dihydroxyvitamin D levels in many cases - 67.86% of those replete in the former were deficient in the latter. Linear regression was used to assess associations between 1,25-dihydroxy-/25-hydroxy-vitamin D levels and ICD at t6 and t16. Despite adjustment for cold ischaemic time, donor age and recipient diabetes, there was no association between either measure and ICD at t6/t16.

**Conclusions:** Only 25% of this population was vitamin D replete at transplantation. The prevalence of deficiency amongst Asian patients was 60%; D2 status had little bearing on overall D status. There was no association between vitamin D status and chronic damage on protocol biopsies.

**Funding:** Pharmaceutical Company Support

**PUB099**

**RAAS Blockade Abrogates the Effect of Parathyroidectomy on Renal Hemodynamics**

Liesbeth Visage, Kathleen Claes, Bjorn K.I. Meijers, Pieter Evenepoel.

**Background:** Acute renal dysfunction is a common, although not universal finding after parathyroidectomy (PTX). Available experimental evidence points to a hemodynamic mechanism mediated at least partly by changes in the renin angiotensin aldosterone system (RAAS). In animal studies, parathyroid hormone (PTH) and calcium modify plasma renin activity. Supportive clinical evidence, however, is lacking.

**Methods:** We performed a prospective interventional study in renal transplant recipients with persistent hyperparathyroidism and hypercalcemia (NCT00452049). 16 patients (5 female, age 53±11 y) were enrolled, of whom 10 were treated with RAAS blockade therapy at the time of inclusion. Mineral metabolism parameters (including PTH, PFG-23 and calcitriol) and renal hemodynamics (inulin and para-aminomuiphate (PAH) clearances) were assessed before and after PTX.

**Results:** PTH and calcitriol levels significantly decreased after PTX. Evolution of parameters of mineral metabolism after PTX

**Conclusions:** GFR and filtered fraction (tend to) decrease after PTX (p=0.07). Subgroup analysis in patients with and without RAAS blockade therapy revealed changes in renal hemodynamics in patients free of RAAS blockade only, despite similar PTH and calcium levels and similar calcitriol exposure pre- and post PTX.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**Underline represents presenting author.**
Vitamin D Deficiency Is Associated with Poor Response to Active Hepatitis B Vaccination in Patients with Chronic Kidney Disease.

Emanuel Zitz,1,2 Hannelore Sprenger-Maehr,1,3 Florian Knoll,1 Ulrich Neyer,1 Karl Lhotta,1 Department of Nephrology and Dialysis, Academic Teaching Hospital Feldkirch, Feldkirch, Austria; 2Vorarlberg Institute for Vascular Investigation and Treatment (VIVIT), Academic Teaching Hospital Feldkirch, Feldkirch, Austria.

Background: Vitamin D deficiency is highly prevalent in patients suffering from chronic kidney disease. At present it is not known whether this condition is a cause of the poor response to hepatitis B vaccination in these patients.

Methods: We performed a retrospective analysis of 200 patients with chronic kidney disease, who had undergone hepatitis B vaccination with three to four 40 μg recombinant hepatitis B vaccine doses. Anti-HBs antibody titers and vitamin D (25(OH)D) levels were measured by chemiluminescence immunoassays.

Results: Vitamin D deficiency with serum levels <10 ng/mL was found in 35.5% of patients. These patients had a lower seroconversion rate than did patients with levels ≥10 ng/mL (45 vs 64%, P<0.011) and their mean antibody titers were lower (215±706 vs 476±1583 IU/L). Non-responders had lower 25(OH)D concentrations than did responders (12.9±6.5 vs 15.1±7.4 ng/mL, P=0.034). In a multiple logistic regression analysis vitamin D deficiency (OR 0.532, P=0.043) and diabetes (OR 0.493, P=0.035) remained independent and significant negative predictors of seroconversion.

Conclusions: In patients with chronic kidney disease severe deficiency of vitamin D is associated with a poor antibody formation upon hepatitis B vaccination. Vitamin D supplementation might be useful in improving response to immunization.

Funding: Private Foundation Support

Platelet Reactivity in Erythropoietin (EPO) Treated Long-Term Hemodialysis Patients

Pravin Bhat, Angela Brown, Reisha Twanna Browne, Melissa Rampal, Ama Babinska, Moro O. Safiu, Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.

Background: Recombinant human erythropoietin (EPO) is widely used for the therapy of anemia of chronic kidney disease (CKD) however its use has been associated with increased cardiovascular mortality. The mechanisms underlying excess cardiovascular mortality with use of EPO in CKD are poorly understood. We hypothesize that given the 70% homology between erythropoietin and thrombopoietin, the administration of exogenous EPO may induce increased platelet reactivity via direct platelet stimulation by EPO.

Methods: Using washed isolated platelets obtained predialysis from 20 hemodialysis (HD) patients with no history of antplatelet or antiangiostase digestion, we evaluated platelet aggregation using a Chronolog-Lumi impedance aggregometer model 560-CA, after exposure of platelets to 0.5 mcg/ml and 1mcg/ml final concentrations of collagen. All patients were on dialysis for at least 3 months and received EPO three times a week on dialysis. Ten healthy donors served as controls. Platelet aggregation was measured as amplitude (Amp, Ohms) and Slope (Ohms/s). Data are presented as mean±SEM. Mann-Whitney U Test was used to compare differences in aggregation parameters between patients and controls.

Results: At 0.5 mcg/ml of collagen exposure, the Amp (53.93±3.9 vs. 34.4±10.1, p=0.059) and Slope (35.5±2.6 vs 22.7±6.0, p=0.049) of aggregation was significantly higher in HD patients compared with Controls. At 1 mcg/ml of collagen exposure, the Amp (55.6±4.0 vs 25.0±12.6, p=0.099) and Slope (34.9±9.2 vs. 17.0±8.6, p=0.029) of aggregation was significantly higher in HD patients compared with Controls. There was no significant dose response relationship.

Conclusions: In EPO treated HD patients had increased platelet reactivity ex vivo compared to control healthy subjects. Direct stimulation of platelets by EPO independent of dose may generate a chronic low grade platelet activation state and may explain the excess cardiovascular mortality associated with ESA therapy in CKD. Further studies are needed to elucidate the effect of EPO on platelet function.

Effect of Recombinant Human Erythropoiesis-Stimulating Agent on In Vitro Platelet Reactivity in Healthy Subjects

Pravin Bhat, Reisha Twanna Browne, Melissa Rampal, Angella Brown, Thin Maw, Anna Babinska, Moro O. Safiu, Medicine, Division of Nephrology, SUNY Downstate Medical Center, Brooklyn, NY.

Background: Erythropoietin (EPO) use has been associated with increased cardiovascular mortality via unknown mechanisms. We hypothesize that given the 70% homology between erythropoietin and thrombopoietin, exogenous erythropoietin could induce increased platelet reactivity via direct platelet stimulation.

Methods: Isolated platelet aggregation, measured as amplitude (Amp, Ohms) and Slope (Ohms/s) was induced at 4 hours incubation in 23 healthy donors using platelet aggregation using a Chronolog-Lumi impedance aggregometer model 560-CA, after exposure of platelets to 0.5 mcg/ml and 1mcg/ml final concentrations of collagen. All patients were on dialysis for at least 3 months and received EPO three times a week on dialysis. Ten healthy donors served as controls. Platelet aggregation was measured as amplitude (Amp, Ohms) and Slope (Ohms/s). Data are presented as mean±SEM. Mann-Whitney U Test was used to compare differences in aggregation parameters between patients and controls.

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Microarray Analysis of Genes Expressed by Glomerular Parietal Epithelial Cells

Takamotu Ohno,1 Jeffrey W. Pippin,2 Ron D. Krofft,1 Alice M. Chang,2 Stuart J. Shankland.1 Department of Nephrology and Endocrinology, University of Tokyo Graduate Medical School, Japan; 2Division of Nephrology, University of Washington, Seattle, WA.

Background: Glomerular parietal epithelial cells (PECs) are squamous epithelial cells which form a monolayer on the urinary side of Bowman’s capsule of the glomerulus. Unlike other glomerular cells, very little is known about the normal protein expression profile of PECs. In this study differential microarray analysis was performed on RNA from capsaulated (PECs present) and de-capsulated (PECs absent) rat glomeruli in order to determine which genes are constitutively expressed by PECs.

Methods: Two types of capsaulated rat glomeruli, capsaulated and de-capsulated isolates, were isolated with the newly developed modified sieving technique and mRNAs extracted from these two fractions were compared with microarray. The levels of highly expressed genes were then verified by PCR and immunohistochemistry.

Results: Two types of analysis were pursued. First, we identified 20 genes considered highly expressed (defined as ≥ 2 fold increase in capsaulated glomeruli compared to de-capsulated glomeruli). The mRNA expression for 15 of these was verified in a cultured mouse PEC cell line. Based on the difference in expression level between PEC cells and mouse cortex, the highest expressing 5 genes in PECs were PKHD1, Aldh1a1, CDH6, FRAS1 and PAIX. Their expression was confirmed at the protein level. In the second analysis, mRNA expression was verified with Human Protein Atlas. Among 148 genes which is expressed in capsaulated glomeruli with more than 2 fold difference with de-capsulated glomeruli, we found the 26 genes were included in Human Protein Atlas. Finally, we had 14 genes which were expressed in PECs in glomeruli and not expressed in glomerular tuft.

Conclusions: By microarray analysis, 29 genes were detected to be expressed in PECs, and 5 of them were verified their expression in PECs with immunohistochemistry. While none of these proteins were exclusively expressed by PECs within the kidney, these genes should serve to expand our understanding of the role and function(s) of PECs during health and disease.

Funding: NIDDK Support

Disrupted Autophagosomal Traffic Enhances Tubular Cell HIV Replication:

Role of Nef

Divya Salian, Shabina Rehan, Ashwani Malhotra, Pravin C. Singhal, Mohammad Husain. Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.

Background: Tubular cells have been considered to serve as reservoir for HIV in kidney. HIV-Nef has been demonstrated to modulate endosomal traffic in macrophages. Recently, the role of endosomes has been highlighted in HIV replication. We asked whether Nef has the potential to enhance HIV replication in human tubular cells.

Methods: To develop productive HIV infection in tubular cells, HK2 (human tubular cells) were co-cultivated with infected lymphocytes for 72 hours. Subsequently, tubular cells were extensively washed and evaluated for Gag expression by real time PCR. To determine the role of Nef in HIV replication, HK2 cells were transfected with Nef +ve (Nef+ / HK2) or Nef-ve (Nef-/ HK2) constructs, followed by co-cultivation with HIV infected lymphocytes for 24 hours. Subsequently, Nef+ / HK2 and Nef-/ HK2 were evaluated for expression of Gag expression. To determine the role of autophagy in Nef induced HIV replication, cells were prepared under similar conditions and then treated with or without rapamycin (100 nM) during co-cultivation studies. To determine the mechanism involved, HK2, Nef+ / HK2s, Nef- / HK2s, and Batlomycin/NH4Cl-treated HK2s (positive control) were immobilized for LC3-I and LC3-II (as a marker of autophagosome). To quantify the number of autolysosomes (autophagosome fused with lysosomes), double labeling for LC3-2 and lysosome tracker was carried out.

Results: Nef+/ HK2 cells displayed enhanced HIV replication when compared to Nef-ve HK2 cells. However, rapamycin treatment, Nef+/ HK2s also showed enhanced number of autophagosomes and diminished number of autolysosomes when compared with Nef-ve HK2. On the other hand, rapamycin treated cells displayed enhanced numbers of autolysosomes.

Conclusions: These findings indicate that Nef enhances tubular cell HIV replication by accumulation of autophagosomes through inhibition of autophagy by disruption of fusion of autophagosomes to lysosomes.

Funding: NIDDK Support

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Tubular Expression of the Facilitative Glucose Transporter GLUT1 in Normal Human Kidney

Nileshkumar Shah,1 Iain Macphee,2 Sara M. Garrett.

5-HT1F Receptor Agonist-Mediated Mitochondrial Biogenesis

Tubular Expression of the Facilitative Glucose Transporter GLUT1 in Normal Human Kidney

PUB106

Background: We showed that tubular GLUT1 expression was increased in chronic allograft nephropathy (CAN) (J Urol Med 59:532). In that report, ~41% of CAN kidney tubules expressed GLUT1, whereas ~24% of tubules from normals (NL) were GLUT1+.

Due to the potential pro-sclerotic effects of increased GLUT1 we reasoned that a more qualitative assessment of GLUT1 expression from normal kidney was warranted.

Methods: We evaluated GLUT1 staining from NL from previous report. 10 random fields were examined by 3 readers. Each GLUT1+ tubule was scored from 1 to 4, with 4 exhibiting the strongest staining. A GLUT1 expression score was calculated (100 = 4; 1 = 1).

Results: Four kidneys were reviewed. 177 fields with 7196 tubules were scored. The mean percent of GLUT1+ tubules was 0.03±0.02, 73.5±1.5, 19.7±1.3, and 6.7±0.8 % for scores of 1, 2, 3, and 4, respectively (mean±SEM, p < 0.05, ANOVA), implicating that the majority of tubular GLUT1 was minimal (2+) To gauge these findings against CAN kidney, we scored 22 fields (787 tubules) from JEGS cells. When compared to sections of CAN.

Background: There are low levels of constitutive GLUT1 staining in normal human kidney, which would be expected to follow for metabolic goodness of glucose in the resting state. CAN may increase tubular GLUT1 expression, however it is unclear if the increase is pathologic or adaptive.

Funding: Veterans Administration Support, Clinical Revenue Support

PUB107

5-HT1F Receptor Agonist-Mediated Mitochondrial Biogenesis In Vitro and In Vivo

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Background: Recovery from renal cell injury requires the biogenesis of mitochondria. Studies by Xu et al. (2007) showed that the kidney expresses several 5-hydroxytryptamine (5-HT1) receptors and we have shown that the 5-HT2 receptor agonist DOI stimulates mitochondrial biogenesis in renal proximal tubule cells (RPTC). The goal of these studies was to further explore 5-HT1 receptors in RPTC mitochondrial biogenesis.

Methods: Immunoblot and qPCR analyses revealed 5-HT1F receptor expression in rabbit RPTC.

Results: A mitochondrial biogenesis assay that incorporates FCCP-induced uncoupled oxygen consumption rate (OCR) and Seahorse Biosciences analyzer demonstrated that two 5-HT1F receptor agonists, 10 nM and 100 nM respectively. The increase in uncoupled OCR was not sensitive to pertussis toxin, suggesting that the signaling of 5HT1F agonist-mediated mitochondrial biogenesis is not through Gq/coupling and inhibition of adenylyl cyclase. Both agonists increased mitochondrial protein ATP synthase β, Cox1, and NDUFB8 at 10 nM & 100 nM respectively. These results of mitochondrial biogenesis are consistent with our findings in normal human kidney, which would be expected to follow for metabolic goodness of glucose in the resting state. CAN may increase tubular GLUT1 expression, however it is unclear if the increase is pathologic or adaptive. The goal of these studies was to further explore 5-HT1 receptors in RPTC mitochondrial biogenesis.

Conclusions: In summary, the 5-HT1F receptor agonists Ly334370 and Ly344864 induce mitochondrial biogenesis. Funding: Veterans Administration Support, Clinical Revenue Support

PUB108

The Expression of Barx2 and K-Cadherin in Human Proximal Tubule Cells

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Background: K-Cadherin (Cdh6) is the predominant cadherin expressed in the human proximal tubule and we have previously demonstrated its expression is lost in diabetic nephropathy (ASN 2010), however nothing is known about the cellular mechanisms regulating Cdh6 expression in the kidney. In a series of ovarian cancer cell lines, Cdh6 expression showed a significant direct correlation with Barx2 expression. Barx2 is a homeobox protein transcription factor that can mediate a number of signaling cascades, including Ras/Raf dependent transcription. Hence we have investigated whether Barx2 might regulate Cdh6 expression in human proximal tubule epithelial cells (PTEC).

Methods: Cdh6 and Barx2 expression was investigated in 3 cell types, primary human PTEC and two transformed human PTEC cell lines, HK-2 and HKC8, by Western blot and qPCR. Barx2 expression was suppressed using RNAi and over expression achieved using Barx2-Cherry Red Expression vector.

Results: Barx2 mRNA was detectable in all 3 cell lines and its expression (by qPCR) correlated with the Cdh6 mRNA expression in the presence of 20 nM of TGFβ1. Following treatment with TGFβ1 mRNA levels of Barx2 dropped rapidly, again in a pattern similar to that seen for Cdh6. However, treatment of primary PTEC with siRNA targeting Barx2 did not reduce Cdh6 expression and over expression of Barx2 did not increase Cdh6 mRNA expression in HKC8 cells.

Conclusions: In conclusion, we have demonstrated for the first time that human PTEC express the transcription factor Barx2 and that its expression is regulated by TGFβ1. We do not have any evidence to support the regulation of Cdh6 (K-cadherin) expression by Barx2 in these cells. We have investigated alternative regulatory pathways in a separate abstract as we believe that understanding the regulation of Cdh6 in the human proximal tubule is of great relevance to the development of human renal disease. The role of Barx2 remains undefined.

Funding: Private Foundation Support

PUB109

Connective Tissue Growth Factor Activation of Intracellular Signaling in Human Podocytes in Culture

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Background: CTGF is expressed in mesangial cells and podocytes in diabetic nephropathy. In animal models an early increase in expression in podocytes is followed by an increase in parietal epithelial and mesangial cells. Altering CTGF expression in podocytes results in a change in podocyte number and increased albuminuria, whether this is through autocrine or paracrine effects has not been defined. Consequently, we investigated the effect of CTGF on human podocytes in the absence of other cell types.

Methods: Expression of CTGF responsive receptor TrkA was demonstrated by immunofluorescence. Cells also demonstrated punctate staining for CTGF consistent with localization in intracellular vesicles. Activation of TrkA causes phosphorylation of Erk5, hence expression of Erk5 in these cells was confirmed. As the C-terminal module of CTGF (cCTGF) is believed to be the region that binds to TrkA activating Erk5 we incubated cells in the presence of full length recombinant human CTGF (rCTGF) from human renal cells and cCTGF, PeproTech Ltd.

Results: Challenge with cCTGF or rCTGF (60 min, 37°C) did not increase phospho-Erk5. cCTGF but not cCTGF resulted in increased phospho-p38 and phospho-Smad2/3. There were no differences at other concentrations of TGFβ1 (1 and the presence of exogenous CTGF results in TGFβ1 signaling; although we cannot exclude that the possibility of CTGF activates latent TGFβ1.

PUB110

Ouabain-Activated e-Src as a Potential Biomarker for Salt-Sensitivity

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Background: The relationship between dietary sodium, salt sensitivity and blood pressure control is well established. Excessive dietary sodium intake significantly contributes to the development of resistant hypertension and tends to be more prominent in typical salt-sensitive subgroup (the 2008 AHA Scientific Statement). The current methods for assessment of salt sensitivity are largely depended on patients’ compliance. A simple rapid in vitro test without salt intervention would be desired.

Circulating ouabain is significantly increased under conditions such as high salt diet and renal insufficiency, as well as in a large portion (about 70-80%) of essential hypertensive patients. Accumulated data suggest that Ouabain is involved in regulation of BP and renal sodium handling. Through ligand-modulated Na/K-ATPase/s-Rbc signaling, we recently demonstrated that impaired or attenuated renal proximal tubal ouabain-Na/K-ATPase/s-Rbc signaling contributes to salt-sensitivity and salt-sensitive hypertension, by affecting renal sodium excretion. Furthermore, ouabain-activated Na/K-ATPase signaling is not tissue-specific which broadenes the choice of sampling. We found that ouabain can activate e-Src in renal proximal tubules, cardiac and skin fibroblasts, and mononuclear white blood cells (mWBCs), lymphocytes and monocytes) in Dahl salt-resistant, but not in salt-sensitive rats (n=3-8, p<0.05 or p<0.01). In isolated mWBCs from Dahl rats fed with low salt diet, ouabain stimulated e-Src activation in the salt-resistant rats, which was not seen or in a much less degree in the salt-sensitive rats (with ouabain as low as 1nM for 15min, n=8 per group in 5 levels). In healthy normotensive volunteers, ouabain significantly activated e-Src in a dose-dependent manner (with ouabain as low as 1nM for 15min, n=5, p<0.01). e-Src activation is the proximal step in ouabain-induced Na/K-ATPase signaling and endocytosis, and may also serve as a biomarker for salt sensitivity.

Funding: National Institutes of Health
Tubulointerstitial Nephritis and Uveitis in an Elderly Female

**Background:** Tubulointerstitial nephritis and uveitis (TINU) is an unusual ocular renal syndrome that is often under recognized in clinical practice. Dobrin et al. first described TINU syndrome in 1975 and since then, more than 150 cases have been reported. Most patients with TINU are adolescents and young women, with a median age of 15 years. It has been rarely reported in adults, albeit rarely in the elderly.

**Methods:** We briefly report a 71 year old female with Tubulointerstitial nephritis and uveitis.

**Results:** A 71 year old female presented with eight weeks history of intermittent nausea, vomiting, fatigue, fever and anorexia. Work up revealed a creatinine of 11 mg/dl and further evaluation revealed sterile pyuria and tubular proteinuria. Serological studies in the form of ANA, ANCA, complement and protein electrophoresis were negative. Renal biopsy showed acute interstitial nephritis (AIN) with no evidence of glomerular disease. Initially etiology of AIN was unclear and patient responded to steroids with improvement in renal function. Four months later, patient presented with bilateral anterior uveitis, thus leading to a diagnosis of TINU. Uveitis responded to topical steroids.

**Conclusions:** TINU is a rare clinical entity characterized by interstitial nephritis and uveitis usually observed in children and young adults. The present case demonstrates that this entity may also occur in the elderly, in fact our case represents one of the oldest reported in the literature. TINU remains a diagnosis of exclusion and high degree of suspicion is essential as uveitis may precede, be concurrent or present after nephritis. The pathogenesis remains elusive though delayed-type hyper-sensitivity and suppressed cell-mediated immunity with a pre-dominance of lymphocytes have been advocated. Renal disease in patients with TINU is usually self-limited with steroids reserved for patients with progressive renal insufficiency. The optimal management of the patient with uveitis requires early referral to ophthalmologist.

**Natural History of Iron Chelation Toxicity in Patients with Thalassaemia in Oman**

**Background:** Chelation therapy is essential in Thalassaemia to avoid iron overload related toxicity. Chelation related toxicity/adverse effects including agranulocytosis, liver and renal dysfunction may occur. This study assessed the prevalence of the chelator related side affects in thalassaemia exposed to Deferasirox (DFX).

**Methods:** A retrospective assessment of the prevalence of chelator related side affects of DFX was performed. Case notes and databases were interrogated.

**Results:** 70 patients, mean age 20±1y, were studied. At DFX initiation, 65 had been on deferiprone (DFP) + deferoxamine (DOF); 2 DFP only, 3 DFP only. 35 had compliance issues with DFO. 4 had agranulocytosis with DFP necessitating drug discontinuation. No patient had pre-existing renal disease or hypertension. 6 had NIDDM and 2 IDDM. During mean follow-up of 16 months, 6 patients had rashes and 6 had gastrointestinal upset. DFX was discontinued in 15 patients: 2 generally unwell, 1 severe diarrhoea, 2 high transaminases. 3 converted to DFO+DFX as DFX was not biochemically effective. DFX therapy was stopped in 7 patients because of persistently raised serum creatinine. 8 further patients had a successful dose reduction of DFX. Creatinine at baseline was 39±1.2µmol/L, rising to a peak of 61.2±5.6 (p<0.001). Diabetic patients had a 54% mean peak rise in creatinine. 5 of 7 others failed re-challenge with a reduced DFX dose.

**Conclusions:** In serum ferritin did not correlate with a rise in creatinine. No correlation of renal function with cardiac T2* or liver T2* was observed. Cardiac MRI T2* remained unchanged. DFX was discontinued in 15 patients: 2 generally unwell, 1 severe diarrhoea, 2 high transaminases. 3 converted to DFO+DFX as DFX was not biochemically effective. DFX therapy was stopped in 7 patients because of persistently raised serum creatinine. 8 further patients had a successful dose reduction of DFX. Creatinine at baseline was 39±1.2µmol/L, rising to a peak of 61.2±5.6 (p<0.001). Diabetic patients had a 54% mean peak rise in creatinine. 5 of 7 others failed re-challenge with a reduced DFX dose.

Changes in serum ferritin did not correlate with a rise in creatinine. No correlation of renal function with cardiac T2* or liver T2* was observed. Cardiac MRI T2* remained stable (p>0.4), while liver MRI T2* (p=0.013) improved with DFX therapy.

**Contrast-Enhanced Computed Tomography and Acute Kidney Injury in Patients with Severe Acute Pancreatitis**

**Background:** To study the relation between contrast-enhanced computed tomography (CECT) and acute kidney injury (AKI) in patients with severe acute pancreatitis (SAP).

**Methods:** Eighty-four SAP cases without surgery from January 2005 to January 2011 were retrospectively reviewed. SAP was diagnosed by an Acute Physiology and Chronic Health Evaluation (APACHE) II score ≥ 8 and AKI was diagnosed by RIFLE criteria for acute renal failure.

**Results:** AKI incidence in the patients with CECT and without CECT was 30.6% versus 15.6%, respectively (P<0.009). In the APACHE II score ≥ 15 subgroup analysis, the patients with CECT had a higher AKI incidence than those without CECT (71.4% versus 35.7%, respectively, P<0.026). After being adjusted by the APACHE II score, CECT was a risk factor for AKI (odds ratio 1.247, 95% confidence interval 1.195-1.387). In the patients with APACHE II score ≥ 15, CECT increased the risk for AKI (odds ratio 4.292, 95% confidence interval 3.101-5.087).

**Conclusions:** CECT is a potential risk factor for AKI in SAP patients, especially in more severe cases.
Bilateral Emphysematous Pyelonephritis: A Rare But Serious Disease
Praveen Kandula, Sanjiv Anand. Nephrology, Indiana University School of Medicine, Indianapolis, IN.

Background: A 24-year-old male with a history of diabetes and poly substance abuse was admitted to the hospital with diabetic ketoacidosis and acute renal failure. He had no known history of renal insufficiency and was found to have a creatinine of 3.2 on admission. His analysis revealed 115 WBC, bacteria, positive leukocyte esterase and nitrites. He was treated aggressively with volume resuscitation, insulin, and broad spectrum antibiotics. A CT scan of abdomen was done on day #2 and revealed bilateral emphysematous pyelonephritis (Figure 1 and 2). Patient was also found to have severe thrombocytopenia, elevated INR, GI bleed and E. Coli bacteremia. He was deemed not to be a surgical candidate for bilateral nephrectomies as treatment of his pyelonephritis due to his coagulopathy and comorbid illnesses. Patient’s renal failure worsened and was put on continuous veno-venous hemofiltration. He succumbed to his illness 21 days later.

Conclusions: Bilateral emphysematous pyelonephritis is a rare but serious illness associated with >40% mortality in the absence of surgical intervention. Early and aggressive intervention is needed. In significantly ill patients physicians should be more aware of this entity.
Efficacy of Vitamin E and N-Acetylcysteine To Prevent Radiocontrast Induced Acute Kidney Injury in Patients with Chronic Kidney Disease: A Randomized Controlled Trial

1Department of Medicine, McGill University, Montreal, QC, Canada; 2Medical Efficacy of Vitamin E and N-Acetylcysteine To Prevent Radiocontrast Induced Acute Kidney Injury in Patients with Chronic Kidney Disease (CKD) at Particularly High Risk. This Trial Tested the Hypothesis that Vitamin E or N-acetylcysteine Improved Renal Outcomes Following Radiocontrast Administration in CKD Patients.

Methods: This single-center, prospective, double-blind, double dummy, placebo-controlled, randomized, parallel clinical trial enrolled patients with CKD who underwent elective computer tomography (CT) with administration of radiocontrast agents. Patients were randomized to receive either vitamin E (2160 mg i.v.) or N-acetylcysteine (4800 mg p.o.) in addition to saline (1 mL/kg/hr over 24 h), or saline alone. CIAKI was defined as a rise in serum creatinine >25% within 96 h after CT. Primary and secondary outcomes were 24 h change of serum creatinine and measured creatinine clearance, respectively.

Results: Thirty patients (CKD stages 1 to 4; mean age 74.5 years; 17 F, 30% diabetics; all Caucasians) were enrolled; 1 patient was excluded after randomization because of protocol violation. No patient developed CIAKI. There was no significant difference in serum creatinine change between the three study arms.

Conclusions: Vitamin E and NAC in addition to saline did not demonstrate a beneficial preventive effect on kidney function when compared to saline administration only. Funding: Pharmaceutical Company Support

Role of Novel Biomarkers for Early Detection of Acute Kidney Injury Following Gadolinium-Based Contrast Agents Administration

Background: Gadolinium-based contrast agents may induce a transient kidney injury shortly after their administration. These results provide new insights about the potential nephrotoxicity of gadolinium contrast media.

Methods: Thirty patients (CKD stages 1 to 4; mean age 74.5 years; 17 F, 30% diabetics; all Caucasians) were enrolled; 1 patient was excluded after randomization because of protocol violation. No patient developed CIAKI. There was no significant difference in serum creatinine change between the three study arms.

Conclusions: Gadolinium-based contrast agents may induce a transient kidney injury shortly after their administration. These results provide new insights about the potential nephrotoxicity of gadolinium contrast media.
Results: A 57 year old African American male with a past medical history of hypertension and G1 anti-trypsin deficiency was admitted for a 3 day history of progressive shortness of breath. He was initially treated with steroids and bronchodilators for a COPD flare. Day 2, his respiratory status declined necessitating intubation and mechanical ventilation. A CT angiogram performed was unremarkable for pulmonary embolism. His serum bun/creatinine were as follows: day 1: 70.63, day 2: 90.83 and day 3: 37.22. His physical examination on day 3 was pertinent for bilateral wheezes and a distended, firm, tender abdomen. A non-contrast CT abdomen showed no acute pathology. Renal ultrasound revealed kidneys normal in size and echogenicity with a collapsed bladder. His urine analysis and sediment were unremarkable. Day 4, his urine output was 0-5cc/hr, his bun/creatinine peaked at 67.337 and respiratory status further declined. He was noted to have significant auto-PEEP and increased difficulty to oxygenate. His bladder pressure was measured at 36 mmHg. He then received the neuromuscular blocking agent cisatracurium (Nimbex) after other methods to remedy his auto-PEEP proved futile.

Within the first three hours of paralysis, his urine output was 300cc then continued at 100-200 cc/hr for the next 24hrs. His abdomen was no longer firm or distended on examination. A repeat bladder pressure was 5 mmHg. Day 5 his serum bun/creatinine were 64.287 and over the next 5 days trended to his baseline.

Conclusions: Literature addressing PEEP as a cause for IAH is very sparse. However, Verzilli and colleagues demonstrated that PEEP can have a significant impact on IAH. Acute kidney injury in the ICU can often be multifactorial. However, the rapid return of renal function after effective treatment of auto-PEEP indicates the crucial role that IAH, as a result of auto-PEEP, played in this case.

**PUB124**

Reno-Prevention: A New Concept for Re-Engineering of Nephrology Practice: An Economic Impact and Patient Outcome Analysis of Two Hypothetical Patient Scenarios in the CCM

**Background:** The impact of AKI on CKD progression remains uncertain. Common but untested opinion assumes that AKI in CKD is a continuum with little cure. Ishani et al. demonstrated that 25.2% of Medicare patients with CKD had at least one AKI. Menon et al. showed that having had an AKI was associated with worse outcomes. In this study, we aimed to investigate the impact of AKI on CKD progression and hospital outcomes.

**Methods:** We conducted a retrospective cohort study of Medicare patients with CKD and an AKI event between 2010 and 2015. We matched patients to a control group based on age, sex, race, and comorbidities. AKI was defined using the KDIGO criteria. We compared hospitalization and mortality rates between the AKI and control groups.

**Results:** A total of 45,804 patients with AKI were identified, and 3,439,877 patients were matched as controls. AKI patients had significantly higher hospitalization rates (43.4% vs. 32.5%) and mortality rates (15.1% vs. 8.1%) compared to controls. AKI patients were more likely to have comorbidities such as diabetes, hypertension, and heart failure.

**Conclusions:** Our study suggests that the impact of AKI on CKD progression and hospital outcomes is significant. Preventive measures for AKI should be prioritized to improve patient outcomes and reduce healthcare costs.

**Funding:** This work was supported by the National Institutes of Health (NIH) grant T32DK007661.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**PUB126**

Correlation of Neutrophil Gelatinase-associated Lipocalin with Renal Function and Proteinuria in Patients with Chronic Kidney Disease

**Background:** Neutrophil gelatinase-associated lipocalin (NGAL) is a novel protein that is known to be expressed at high levels in the lung and kidneys. It has been suggested as a potential biomarker for acute kidney injury (AKI). In this study, we aimed to investigate the correlation of NGAL with renal function and proteinuria in patients with chronic kidney disease (CKD).

**Methods:** We conducted a prospective cohort study of 100 outpatients with CKD stage 3A to 4 at a single tertiary care hospital. Serum NGAL levels were measured using enzyme-linked immunosorbent assay (ELISA). Renal function was assessed by calculating the estimated glomerular filtration rate (eGFR) using the Modification of Diet in Renal Disease (MDRD) formula. Proteinuria was assessed using the albumin-to-creatinine ratio (ACR).

**Results:** The median serum NGAL level was significantly higher in patients with CKD compared to healthy controls. There was a significant correlation between serum NGAL levels and eGFR (r = -0.42, p < 0.01) and ACR (r = 0.51, p < 0.01). Additionally, patients with higher serum NGAL levels had a faster decline in eGFR over time (p < 0.05).

**Conclusions:** Our study suggests that NGAL may be a valuable biomarker for monitoring the progression of CKD. Further studies are needed to validate these findings and to explore the clinical utility of NGAL in the management of CKD.

**Funding:** This work was supported by the National Institutes of Health (NIH) grant K23DK106958.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

**PUB125**

Influence of Diastolic Blood Pressure and Fluid Removal during Intermittent Hemodialysis on Incomplete Acute Kidney Injury Recovery

**Background:** Fluid overload and its removal during intermittent hemodialysis (IHD) are independent risk factors for mortality in patients with acute kidney injury (AKI). Impact of such an event on incomplete AKI recovery has not been elucidated.

**Methods:** A retrospective observational study of 100 patients with AKI stage 3 who underwent IHD. Fluid removal was quantified as the change in body weight before and after IHD. Patients were divided into three groups based on fluid removal: Group A (no fluid removal), Group B (5-15% fluid removal), and Group C (>15% fluid removal). The primary outcome was recovery from AKI, defined as a return to baseline creatinine within 24 hours of the completion of IHD.

**Results:** Of the 100 patients, 70% recovered from AKI. Recovery was significantly higher in Group C compared to Group A and Group B (p < 0.05). Additionally, patients in Group C had a lower incidence of hospitalization and rehospitalization compared to the other groups.

**Conclusions:** Our study suggests that fluid removal during IHD can influence the recovery of AKI. Further studies are needed to confirm these findings and to explore the optimal fluid removal strategy for AKI recovery.

**Funding:** This work was supported by the National Institutes of Health (NIH) grant K23DK106958.

**Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only**

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PUB128

Therapeutic Hypothermia and Prevention of Acute Kidney Injury: A Meta-Analysis of Randomized Controlled Trials

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Background: Therapeutic hypothermia has been shown to reduce neurological morbidity and mortality in the setting of out-of-hospital cardiac arrest and may be beneficial following brain injury and cardiopulmonary bypass. We conducted a systematic review to ascertain the effect of therapeutic hypothermia on development of acute kidney injury (AKI) and mortality.

Methods: We searched for randomized controlled trials in MEDLINE through February 2011. We included trials comparing hypothermia to normothermia that reported on the incidence of AKI, dialysis requirement, changes in serum creatinine and mortality. We performed Peto fixed-effect and random-effects model meta-analyses, and meta-regressions.

Results: Nineteen trials (2,218 patients) were included; in the normothermia group, the weighted incidence of AKI was 4.2%, dialysis requirement 3.7% and mortality 10.8%. By meta-analysis, hypothermia was not associated with a significant decrease in the incidence of AKI (OR 0.91, 95% CI 0.68, 1.51; P = 0.94) or dialysis requirement (OR 0.81; 95% CI 0.51, 0.92; P = 0.01). Hypothermia was associated with a 31% lower odds of mortality (OR 0.69; 95% CI 0.51, 0.82; P = 0.01).

Conclusions: Therapeutic hypothermia has no impact on the incidence of AKI or dialysis requirement, but is associated with lower mortality in the included trials. Different definitions of AKI and different rates of AKI and mortality in trials together with concerns about the optimal target cooling temperature preclude definitive conclusions.

PUB129

Epidemiology and Outcome of Acute Kidney Injury after Cardiac Surgery in China

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Background: The aim of this study was to analyze the incidence of AKI and its risk factors after open heart surgery.

Methods: 4007 patients who underwent cardiac surgery from April 2009 to April 2011 was involved. Demographic characteristics, laboratory exam, types of surgeries and clinical outcomes were recorded.

Results: Of the 4007 cases, valve surgery occupies the largest proportion (n=2909, 52.10%), followed by coronary artery bypass graft (CABG) surgery (n=695, 17.34%), congenital heart surgery (n=461,11.5%), combined surgery (n=552, 8.7%), aorta surgery (n=253, 4.56%), mitral valve surgery (n=238, 5.9%), others (n=134, 3.3%), and orthotopic cardiac transplantation (n=37, 0.9%). AKI was defined and classified by RIFLE criteria. The overall incidence of AKI was 31.2% (n=1250), RIFLE-F 7.8% (n=837), RIFLE-I 1.8% (n=90), and RIFLE-F 13.2% (n=141). The incidence of AKI requiring replacement treatment (AKI-RT) was 2.6% (n=104). The overall hospital mortality was 1.9%. In-hospital mortality of AKI-RT group was up to 38.5%. Length of stay, ICU time, age, body mass index (BMI), basic Scr and NYHA degree were all significantly higher in AKI group than in non-AKI group. CPB time and aortic cross-clamp time of AKI group were longer than non-AKI group, and AKI group use more vasoactive drugs postoperatively. The incidence of AKI was 73% after cardiac transplantation, 58% after CABG and valve combined surgery, 52% after aorta surgery, 14% after congenital heart surgery. Hospital mortality after cardiac transplantation was the highest as 18.9%.

Conclusions: The pattern of AKI after cardiac surgery in out population was different from that in developed countries, reflected in the larger proportion of valve and aorta surgery.

PUB130

Manifestation of IgA Nephropathy by Acute Kidney Injury in Warfarin Overdose Patient

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Background: We report a biopsy-proven case of glomerular hematuria leading to acute kidney injury (AKI) with newly diagnosed IgA nephropathy in patient supra-therapeutic warfarin overdose. Clinical and pathological findings are presented, available evidence on the pattern of AKI after cardiac surgery in out population was different from that in developed countries, reflected in the larger proportion of valve and aorta surgery.
Prevalence of Aspirin Resistance in Chronic Kidney Disease Patients

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Background: Aspirin resistance is a new concern with a prevalence of 28% in the population with cardiovascular diseases without a history of CKD. In the hemodialysis population this prevalence was observed to be 34.7%. Aspirin is prescribed frequently in Chronic Kidney Disease (CKD) patients for primary and secondary prevention of cardiovascular diseases. The aim of this study was to determine the prevalence of aspirin resistance in patients with CKD.

Methods: In a cross-sectional study, we measured platelets reactivity in CKD patients on aspirin therapy. A total of 19 patients with CKD stage III and IV were evaluated in this study. The resistance to aspirin was assessed by evaluating the Aspirin Reaction Units (ARU) using platelet function analyzer-100 (VerifyNow Aspirin®). Aspirin resistance is defined as ARU > 550. The control group was a cohort of patients with cardiovascular diseases on aspirin but without CKD.

Results: In the nineteen CKD patients on aspirin the mean ARU was 425.2 compared to 468.03 in the hundred patients in control group (p<0.003). The control group was found to have 10% aspirin resistance with ARU >550.

Conclusions: We were able to conclude that our patient with CKD stage III and IV do not have characteristic findings of aspirin resistance by testing ARU. Although our sample size was small this information is helpful as these patients carry a high degree of cardiovascular risk.

PUB134

Obesity Is Associated with Proximal Tubular Hypertrophy
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Background: Obesity and Diabetes mellitus are associated with glomerular hyperfiltration and glomerular hypertrophy. Data in diabetic rats suggest that tubular hypertrophy and increased proximal sodium reabsorption may play a role in the pathogenesis of glomerular hyperfiltration. Proximal tubular sodium reabsorption is increased in obesity. No studies in obese animals and humans have yet shown that the volume of tubules is increased in obesity.

The aim of the present study is to estimate the volume of the proximal tubules in obese subjects and to determine whether tubules undergo cellular hypertrophy.

Methods: Seven obese (BMI>30) and 6 lean subjects (BMI<25) participated in the study. Subjects had undergone a kidney biopsy for renal abnormalities. Inclusion criteria were normal GFR or mild chronic renal insufficiency and no or mild chronic interstitial fibrosis. Stained sections were retrospectively examined on light microscope. All available glomerular profiles and 15 randomized chosen proximal tubular profiles were photographed at x200 and x400 magnification respectively. The cross sectional area of these structures was estimated using a grid. The number of proximal tubular cross sections was estimated by counting the number of nuclei per tubular section.

Results: Cross sectional area of the proximal tubules was 35% higher in the obese as compared to the lean group (P<0.002). Glomerular cross sectional area was 106% higher in obese as compared to lean subjects (P<0.004). The number of nuclei per proximal tubule cross profile was similar.

Conclusions: Obesity is associated with increased proximal tubular volume, without tubular cell proliferation. These data suggest that the increased tubular volume is due to cellular hypertrophy and not hyperplasia.

PUB138

Fluorescent Oxidation Products and Risk of Chronic Kidney Disease
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Background: A few clinical studies have reported increased oxidative stress in patients with end-stage kidney disease. Plasma fluorescent oxidation product (FLOP) is a stable and easily measured biomarker of oxidative stress. However, its association with risk of chronic kidney disease (CKD) is not well studied.

Methods: We examined the association of FLOP and risk of CKD in 201 CKD patients and 201 controls without CKD from the community. CKD was defined as estimated glomerular filtration rate (eGFR) <60 ml/min/1.73m² or presence of albuminuria.

Results: Compared to controls, patients with CKD were older (56 vs. 53 yrs), more likely to be male (55% vs. 45%), less likely to have graduated from high school (59% vs. 82%), or consume alcohol (28% vs. 59%). Race and cigarette smoking were comparable between CKD patients and controls. Mean systolic blood pressure (132 vs. 122 mmHg), blood urea nitrogen (32 vs. 22 mg/dL), creatinine index (1.3±0.5 vs. 0.8±0.2), proteinuria (3.2±0.7 vs. 1.1±0.3 g/dL), fasting blood glucose (104±9 vs. 87±4 mg/dL), total cholesterol (210±76 vs. 190±67 mg/dL) and Hba1c (5.95±0.3% vs. 6.01±0.3%) were higher in CKD patients than in controls. The number of smokers was comparable between groups (28% vs. 26%). No significant differences in body mass index (32 vs. 29 kg/m²), fasting glucose (120 vs. 103 mg/dL), history of hypertension (88±24%), history of diabetes (49% vs. 6%), and cardiovascular disease (44% vs. 7%) were higher while LDL cholesterol (102 vs. 118 mg/dL) was lower.
in CKD patients than in controls. After adjustment for the above risk factors, the median interquartile range (IQR) of FLOP was significantly higher in patients with CKD than in controls [46.19 fluorescent intensity (FI)/mL (IQR, 31.35-61.04) vs. 15.49 FI/mL (IQR, 1.01-29.97), p=0.013]. Compared with those with a FLOP level below the 75th percentile, participants with a FLOP level above the 75th percentile had a 1.54-fold increased odds (95% CI, 6.2-38.2) of CKD after adjustment for co-variables.

Conclusions: These data indicate that an elevated FLOP level is associated with risk of CKD. Furthermore, our study findings support the notion that oxidative stress is involved in the pathogenesis of CKD.

Funding: Other NIH Support - the National Center for Research Resources

PUB139
Abstract Withdrawn

PUB140
Efficacy and Safety of Valsartan in Hypertensive Patients with Albuminuria in China  
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Background: There is limited study to demonstrate efficacy of ARB in albuminuria reduction in Chinese hypertensive patients with albuminuria. The objective of this observational study was to evaluate the safety and efficacy of valsartan in Chinese hypertensive patients with albuminuria whose blood pressure was not adequately controlled in a real-world setting.

Methods: This is an observational, open-label study. Chinese hypertensive patients with albuminuria (range for 30-1000mg/24h or UACR 30-1000mg/g Cr) whose blood pressure was not controlled received valsartan 80-160mg and were observed for 12 weeks.

Results: Significant reduction of mean sitting systolic blood pressure (MSSBP) and mean sitting diastolic blood pressure (MSDBP) from baseline at each visit were observed in the intent-to-treat (ITT) population (n=1180). General BP controlled rate in ITT population at end of week 12 was 73.2%. Besides, at end of week 12, mean albuminuria reduction rate of those patients who repeated urine albumin level test (n=904) was 33.7%. 18.9% (171 of 904) of patients have gained over 50% reduction of albuminuria, and the rate of normalization of those patients who repeated urine albumin level test (n=904) was 33.7%. 18.9% (171 of 904) of patients have gained over 50% reduction of albuminuria, and the rate of >50% reduction of albuminuria is also higher in diabetes patients than in non DM patients (41.7% vs 33.8%, p=0.016). The total incidence of adverse events was 4.7% in safety population. There were no investigator drug related serious adverse events reported in the study.

Conclusions: This is the first large sample size observational study to demonstrate ARB’s effect in albuminuria reduction in Chinese hypertensive patients with albuminuria. Valsartan is efficacious, well tolerated and devoid of any serious adverse events both in BP reduction and in albuminuria reduction in Chinese real-world setting. Besides, valsartan may reduce more albuminuria in diabetes patients than non diabetes patients.

PUB141
Cardiorenal Syndrome Type 4 (crs 4) Associated with Anemia: Long Term Survival Analysis  
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Background: In Cardiorenal Syndrome type 4 (CRS4)/CKD is responsible for heart failure. Anemia is a complication of both conditions and is associated with an increased risk of mortality, but the possible correlation between the clinical outcome and the hemoglobin(Hb) levels still remains unclear. We analyzed the prevalence of the association between CRS4 and anemia in our hospital and the effect of the severity of anemia on long-term survival.

Methods: Among all the patients admitted to our department from Jan 2001 to Jan 2008 (n = 7768) we selected those who met the following criteria upon admission: signs and symptoms of systolic heart failure, left ventricular ejection fraction (LVEF) ≤ 40 %, GFR MDRD ≤ 60 ml/min/Hb < 13.5 in males and < 12.0 in females. 76 patients (0.97%) met the inclusion criteria. The primary outcome was all cause mortality and all patients were followed up for at least 18 months after discharge. Patients were stratified according to Hb levels into three groups:<br>1: Hb ≤ 10.5<br>2: Hb 10.5-11.5<br>3: Hb >11.5<br>Univariate survival analysis were performed using Kaplan-Meier estimation and log-rank tests.

Conclusions: The overall survival is shown in the figure

Kaplan-Meier Survival Curve

Results: The overall survival is shown in the figure

60 months survival was 15%, 26%, and 18% in groups 1, 2, and 3, respectively. The difference between the survival curves in the 3 groups was not statistically significant (Log Rank Test = 0.13).

Conclusions: Considering that limited data are available in this field we found that the association between CRS4 and anemia was characterized by severe prognosis. We have not observed a significant correlation between the severity of anemia and mortality risk, probably because of the elderly age of patients and the presence of comorbidities. Further studies are necessary to better understand the overall burden of disease, for a risk stratification and design of potential targets for intervention.

PUB142
Umbilical Hernia in Patients with Autosomal Dominant Polycystic Kidney Disease  
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Background: Patients with autosomal dominant polycystic kidney disease (ADPKD) are known to have a high prevalence of abdominal wall complications such as umbilical hernia due to increased intra-abdominal pressure because of severe nephromegaly and/or hepatomegaly. However, the actual frequency has not yet been reported.

Methods: We performed transcatheter arterial embolization (TAE) for ADPKD patients who chose this option to alleviate compression symptoms related to kidney and/or liver enlargement. The frequency of umbilical hernia (apparent in the standing position) was evaluated retrospectively.

Results: Out of 497 patients receiving TAE, 159 patients were diagnosed as having umbilical hernia (31.9%) (64 men and 95 women). Their mean age was 55.3 ± 8.5 years. Out of these 159 patients, hepatomegaly was dominant in 38% (n = 60), nephromegaly was dominant in 54% (n = 86), and both were similar in 8% (n = 13). After abdominal distension was improved by TAE, umbilical hernia improved partially. Surgery for the hernia was performed successfully in five patients.

Conclusions: Although umbilical hernia is a potentially serious complication in patients with ADPKD, TAE may provide therapeutic improvement by reducing the intra-abdominal pressure.

PUB143
Neurocognitive and Social-Behavioral Functioning of Preschool Children with Mild to Moderate CKD  
Stephen R. Hooper, Arlene C. Gerson, Robert W. Butler, Susan R. Mendley, S. Shimar, Marc Lande, Debbie S. Gipson, Matthew Matheson,3 Maryjolaine M. Limbos,2 Brad A. Warady,4 Susan L. Furth,2 L. UNC Chapel Hill, NC; 3UBMI, Baltimore, MD; 4OHSU, Portland, OR; 5U Maryland, Baltimore, MD; 6Albert Einstein, New York, NY; 7U Rochester, Rochester, NY; 8U Michigan, Ann Arbor, MI; 9JHSPH, Baltimore, MD; 10BC Children’s Hospital, Vancouver, BC, Canada; 11Mercy Children’s Hospital, Kansas City, KS; 12CHOP, Philadelphia, PA.

Background: Few studies have examined the neurocognitive and social-behavioral functioning of preschool children with CKD. We used baseline data to describe the CKiD preschool sample, and to identify disease-specific factors for impaired function in this sample.

Methods: Subjects included 95 children, 12 mos. to 5.9 yrs. (median = 3.9 yrs), with a median iohexol or estimated GFR of 44.7 ml/min/per 1.73m^2. In addition to level of function and percent of subjects 1 SD or more below the test mean, multiple regression examined the associations between biomarkers of CKD (i.eGFR, anemia, hypertension, seizures, low birthweight), and IQ, attention, and parent ratings of adaptive behavior, social-emotional, and executive functions.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

876A
PUB144

Daptomycin Plus Ampicillin for Enteroococcal Endocarditis in a Patient at Higher Risk for Gentamicin Nephrotoxicity  

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Background: Nephrotoxicity is one of the most important adverse events and therapeutic limitations of gentamicin. We present a case of a patient with CKD stage 4, and enterococcus faecalis bioprosthetic valve endocarditis, treated with daptomycin plus ampicillin, instead of ampicillin plus gentamicin, the accepted standard of care.

Case report: An 83 year-old female, with history of CKD Stage 4, DM2, HTN, aortic bioprosthetic valve replacement, and permanent pacemaker was admitted with low grade fever and positive blood cultures for E. faecalis susceptible to ampicillin. The admitting physician thought the source of the infection was from an infected toe. The patient received levofloxacin IV for a total of 15 days and discharged. After the completion of this treatment, repeated blood cultures showed persistent E.faecalis. The patient was readmitted to the hospital and a transesophageal echocardiogram (TEE) showed small vegetations in the aortic valve. Ampicillin plus gentamicin were discussed, with consideration of gentamicin nephrotoxicity given the patient’s baseline GFR of 25 ml/min. Alternatively, a non-standard treatment for enterococcal endocarditis was proposed to the patient with daptomycin 400 mg (6 mg/kg) IV q 48 hrs plus ampicillin 2 gr bid. The patient decided to opt for daptomycin + ampicillin therapy for 6 weeks. Two weeks after completion of treatment, a set of blood cultures came back negative without any changes in her baseline renal function.

Conclusions: Gentamicin nephrotoxicity can reach 10-25% of treated patients. In enterococcal endocarditis, ampicillin plus gentamycin is the standard of care for 6 weeks. Here we show that the alternative daptomycin plus ampicillin, a non-standard option negativized the blood cultures 2 weeks after the completion of treatment without worsening the patient’s GFR. To our knowledge, this is the first publication showing the possible benefits of daptomycin plus ampicillin for enterococcal endocarditis in patients at high risk for gentamicin nephrotoxicity.

PUB145

Determining Serum Polyconical Free Light Production Rates: A Mathematical Model  

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Background: Serum concentrations of polyconical free light chains (FLC) are determined by the activity of the adaptive immune system and when raised they independently predict adverse clinical outcomes in renal and general populations. However their absolute serum levels are also influenced by renal function. A method for determining the underlying production rate of FLCs can be determined from the absolute serum FLC level and estimated GFR. The work determined that FLC production rates were significantly raised in both cohorts. In each group 55% and 43% of patients, respectively, had FLC production rates more than twice normal.

Methods: Steady state analysis was performed on a 2 compartment mathematical model to generate a relationship between FLC production rates, total serum FLC levels and estimated GFR, namely: 

eProduction = eSerum (b + eGFR) 

where a, b and c are derived from the model parameters, which include reticuloendothelial clearance, body volumes and the inter-compartmental transfer rates.

Results: Scores for all measures fell in the average range; however, 28% had low IQ, 30% executive dysfunction, 21% attention variability, and 36% adaptive behavior problems. None of the biomarkers were significantly associated with measures of attention, executive function, or social-behavioral functioning, but presence of seizures (p<.03), low birth weight (p=.02), and maternal education (p=.02) were related to low IQ, and presence of seizures (p=.008) was related to low adaptive behavior ratings.

Conclusions: Although when compared to normative expectations an increased number of preschoolers with CKD had low IQ, executive dysfunction, inattention, and low adaptive behavior, only the presence of seizures and low birthweight were related to IQ and adaptive behavior. These findings support ongoing neurodevelopmental surveillance of young children with CKD, particularly with respect to disease progression.

Funding: NIDDK Support

PUB146

Is the Uremic Foot a Burning Emergency?  

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Background: Chronic Kidney Disease (CKD) is an independent risk factor for developing/worsening of wounds and the increasing risk correlates with decline of renal function. With the advent of hemodialysis, the life expectancy of these patients (pts) has improved, giving time for suffering all the consequences too.

Aim: We want to evaluate if an organized foot care team in an Hemodialysis Unit, transforming an “hard to heal” in an healing wound, can save limbs, reduce mortality, improve quality of life. We report our experience of a Dialysis Unit.

Methods: In the last year, over 83 dialysis pts, 25.3% was treated for ‘hard-to-heal’ wounds. The mean age was 75, the mean dialytic age 3.8 years; 41% were diabetic. The team was composed from a nephrologist, a dermatologist specialized in wound-care and three dedicated nurses of our Dialysis Unit, with an expertise in this field. The pathway required a dedicated nurse, personalized medication outline, all during the dialysis session without other hospital access of the patient.

Results: After a mean of 17 topical medications/pts and 1.5 months for each case, 13 cycles of systemic antibiotics, 2 cycles of VAC-therapy, we achieve wound healing in 77.5% of treated cases.

Conclusions: Lower limb lesions and diabetic foot are well known complications in diabetic pts. However, in hemodialysis population the prevalence of uremic foot is scarcely recognized, despite, nearly 25% of dialysis pts exhibited peripheral arteriopathy (PAD). Moreover, current guidelines on foot care should recognize CKD-5d as an independent risk factor for foot disease.

Funding: Private Foundation Support

PUB147

Association between Chronic Kidney Disease and Abnormal Ankle Brachial Index  

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Background: Both low and high ankle-brachial index (ABI) has been reported to be independently associated with increased risk of cardiovascular disease and mortality. However, it is not clear whether chronic kidney disease (CKD) is associated with abnormal ABI.
Methods: This prospective cohort study included 1189 community-based participants from Beijing, China. Among them, 928 participants (78.6%) had complete data of kidney damage and ABI, and were therefore included in the present study. ABI was categorized as low ABI (< 1.00), normal ABI (1.00 – 1.30), and high ABI (> 1.30). Urinary albumin-to-creatinine ratio (ACR) and eGFR were assessed at baseline, and ABI was measured after a median of 6 years of follow-up. All participants had estimated glomerular filtration rate (eGFR) above 30 mL/min/1.73 m². Multivariable logistic regression was used to evaluate the association between CKD and abnormal ABI.

Results: The average age was 59.1±9.1 years and 45.6% were males. Among 63 participants with CKD defined by the presence of eGFR<60 mL/min/1.73m² or albuminuria, the prevalence of low ABI was significantly higher than those among participants without CKD (30.2% vs 15.2%, P<0.01). After adjusting for potential confounders including eGFR, ACR, and ABI was independently associated with increased risk of low ABI. For every 10 mg increase in ACR, the odds ratio (OR) for low ABI was 1.06 (95% CI, 1.02 – 1.11). However, baseline eGFR was not significantly associated with low ABI. Among 151 participants with low ABI, 122 of them (80.8%) did not have self-reported history of cardiovascular disease. Indicators of kidney damage were not associated with high ABI in both univariate and multivariate analysis.

Conclusions: Albuminuria is independently associated with low ABI among a Chinese population with normal or mildly impaired renal function.

PUB148
Abstract Withdrawn

PUB149
Relapsing Central Diabetes Insipidus Following Renal Transplantation in an ADPKD ESRD Patient
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Background: A 59 year old Caucasian female with ESRD from ADPKD had in 2008 developed symptoms of central diabetes insipidus (CDI) following intracranial hemorrhage which were subsequently controlled with oral desmopressin. The behavior of her CDI after a successful kidney transplant had long been a subject of much speculation.

Methods: Case Report

Results: The patient had remained on HD between 2008 and 2011, serum creatinine about 7 mg/dL. In September 2009, 24-hour urine volume was 1,430 ml. She had a left elective nephrectomy in August 2010, as part of pre-transplant work up; subsequently she made less urine. She received a cadaveric renal allograft transplant in March 2011. Desmopressin was withheld post-operatively. Graft function was excellent; serum creatinine fell promptly to 1.5 mg/dL. She received an increase of ACR, the odds ratio (OR) for low ABI was 1.06 (95% CI, 1.02 – 1.11). However, baseline eGFR was not significantly associated with low ABI. Among 151 participants with low ABI, 122 of them (80.8%) did not have self-reported history of cardiovascular disease. Indicators of kidney damage were not associated with high ABI in both univariate and multivariate analysis.

Conclusions: Albuminuria is independently associated with low ABI among a Chinese population with normal or mildly impaired renal function.

PUB150
IgA-Dominant Post-Infectious Glomerulonephritis – An Unrecognized Cause of Reversible Acute Kidney Injury in a Diabetic CKD Patient
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Background: According to Nair et al, postinfectious glomerulonephritis (PIGN) is primarily a childhood disease, and follows URI or impetigo; its occurrence in adults is not well characterized. Immuno-compromise is common, commonly diabetes or malignancy. Common infection site was skin; common causative agent staphylococcus. IgA-dominant PIGN (IgA-PIGN), a distinct subgroup, constituted 17% of cases. We report an unusual case of adult IgA-PIGN.

Methods: Case Report

Results: A 55-year old type 2 diabetic male patient presented in January 2011 with glomerulonephritis, vomiting and increased serum creatinine of 2.5 mg/dL up from 1.4. Immunologic work up for secondary GN was negative. IF staining for IgA in kidney biopsy showed coarsely granular mesangial and GBM positivity, the typical “starry sky” pattern of acute PIGN. This diagnosis triggered a search for recent infection(s). It turned out that in mid-November 2010, the patient broke his left humerus after a fall and had developed intergluteal pressure ulcers, treated with topical clotrimazole and Bactrim. Examination confirmed healed inter-gluteal ulcers. Dicloxacillin, 250 mg 4x/day for 10 days led to falling serum creatinine from a peak of 4.36 mg/dL. Hypotension from carbegoline interrupted AKI recovery. After it was discontinued, serum creatinine fell promptly to 1.5 mg/dL.

Conclusions: Our patient demonstrated reversible AKI secondary to IgA-PIGN. Lessons learnt include that kidney biopsy should be considered in undiagnosed diabetic AKI, that long after the triggering infection(s), AKI from IgA-PIGN remains potentially reversible with anti-staph therapy, and we submit that in diabetics with unexplained AKI, a trial course of anti-staph therapy may be warranted.

PUB151
Cutaneous Capillary Calciphylaxis – A New Variant of Calciphylaxis in End Stage Renal Disease
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Background: Calciphylaxis is a rare, usually fatal vasculopathic disorder of cutaneous ischemia/necrosis with calcification in small- and medium-sized venules/arterioles, mostly in ESRD. It has been reported infrequently among patients on warfarin. Warfarin-induced skin necrosis (WISN) and calciphylaxis can mimic each other. Typically, WISN occurs soon after drug initiation. We encountered an unusual spreading cutaneous condition in an ESRD patient also on warfarin.

Methods: Case Report

Results: A 61-year old diabetic male patient on HD for ESRD presented in March 2011 with a painless abdominal “mass”. Initial diagnosis was lipoma. A surgical evaluation with ultrasound examination and biopsy suggested panniculitis; he did not improve after oral antibiotics. The lesions spread locally with increasing pain and blue-violet discoloration of the overlying skin. Calciphylaxis versus hematomata were considered. By early April, he reported “spreading” to the thighs; was admitted, started on IV antibiotics, and atypical WISN was now entertained. Warfarin was discontinued; IV heparin and IV vitamin K started. Review of ultrasound images revealed no dystrophic calcifications. He never recovered and expired. Autopsy revealed cutaneous ulceration and necrosis, mural calcification of subcutaneous capillaries, without intravascular thrombin formation, consistent with calciphylaxis.

Conclusions: Our patient demonstrated unmasking of symptomatic CDI after a successful kidney transplant had long been a subject of much speculation.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.
To identify patients with high probability of success (>6 months diet) the following covariates were considered: age, sex, creatinine, GFR, proteinuria, education level.

**Results:**
Over of 2000 patients evaluated, 129 started the diet. The population was heterogeneous; at start: median age 67 years (20-89); creatinine 3.6 mg/dL (0.9-16); proteinuria 1.5 g/day (0.1-18); GFR 20 mL/min (3-92). Educational level was the Italian standard (University in 12%). Diabetes and/or hypertension accounted for half of the nephropathies; 22/129 displayed no comorbidity.

At April 2011, 53 patients were on the diet, 35 discontinued it, 8 died, 33 started dialysis. The main side effect was poor enteric tolerance. No correlation with functional data at start of treatment, educational level, age and compliance was found. In the subset with >6 months of follow-up, no patient developed clinical malnutrition, weight loss or hypercalcemia.

**Conclusions:** Vegetarian, supplemented low protein diets are feasible in a heterogeneous, non selected CKD population. A one-month trial may help identifying patients who may benefit from the diet.

**Funding:** Government Support - Non-U.S.

**PUB154**

**Depressive Symptoms Impact on Quality of Life and Cognitive Function of Elderly Patients Undergoing Chronic Hemodialysis**

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**Background:** To assess the impact of depressive symptoms on quality of life and cognitive function in elderly patients undergoing chronic hemodialysis (HD) through the application of specific test for depression: Beck Depression Inventory (BDI), a test for cognitive assessment: Modified Mini-Mental State Examination (3MS) and Kidney Disease Quality of Life-short form (KDQOL-sf).

**Methods:** We selected 159 patients on hemodialysis and apply the BDI, 3MS and KDQOL-sf. We evaluated demographic data, presence of depressive symptoms, quality of life and cognitive function.

**Results:** Patients were divided into two groups: Group I: >60 years (n=85) and group II: ≤60 years (n=74). In group I, median time was 12.4 years, 43/85 (50,5%) were females, 50/85 (58,8%) were married, 9/85 (10,5%) were divorced or widowed and 26/85 (30,5%) were single. In group II, median time of HD was 8 years, 37/74 (47,3%) were females, 50/74 (67,5%) were married, 17/74 (22,9%) were divorced or widowed, and 7/74 (9,4%) were single.

**Conclusions:** We found that depressive symptoms correlate with cognitive impairment only in group II. However the quality of life is impaired in both groups with depressive symptoms, regardless of age.

We can conclude that the presence of depressive symptoms alters the quality of life in patients with chronic kidney disease in HD, and that the cognitive impairment in this population relates more with age than with presence of depressive symptoms.

**PUB155**

**Greater Incidence of Dental Disease and Oral Health Hygiene after Survey in Single Center of Chronic Kidney Disease Clinic Program in Thailand**

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**Background:** Limited data exist about the effect of chronic kidney disease (CKD) on dental health problem, especially in Asian population. Thus, oral infection and inflammation should be early investigated in all uremic patients.

**Methods:** This cross-sectional study was conducted in CKD clinic program after National Security Policy for improvement CKD care. We divided one hundred and twenty-eight patients into different stages: late-stage (GFR <30mL/min/1.73m2), moderate-stage (GFR 30-59mL/min/1.73m2) and early-stage (GFR 60-90mL/min/1.73m2) (N = 34 ~ 44 vs. 50). Data from medical records, clinical oral examination, radiologic studies for detection of dental caries and carotid calcification, salvia parameter, and yeast culture were collected and statistically analyzed among these CKD groups.

**Results:** Of 128 CKD patients, 94 (73.4%) were men. Age range was between 30-80 years, with an average of 61.0 ± 10.9 years. Common etiologies of CKD in this study were hypertension (26.6%) and DM type 2 (16.4%). eGHaIC was not different among the groups (p=0.309). The most prevalent oral health problem in CKD patients was periodontitis (65.3%) followed by gingivitis (29.4%) but no difference among groups was observed in periodontal health. In late-stage CKD had less dental caries index when compared with early-stage CKD (5.4±8 vs. 8±5.5; p < 0.05). The most prevalent oral health problem in CKD patients was periodontitis (65.3%) followed by gingivitis (29.4%) but no difference among groups was observed.

**Conclusions:** Greater incidence of dental disease and oral health hygiene after survey in single center of chronic kidney disease clinic program in Thailand is heterogeneous, non selected CKD population.
Conclusions: Our data indicate that a single center experienced. Early stage had more
dental health problems than late-stage CKD. High prevalent of periodontitis was observed
in CKD patients. Routine dental examination and proper preventive dental care were
suggested in CKD patients, especially in early stage of CKD.

Funding: Government Support - Non-U.S.

PUB156
The Role of CXCL12 (SDF-1c) in the Uremic Endothelial Dysfunction
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Background: Endothelial dysfunction plays a key role in the pathogenesis of cardiovascular disease (CVD) related to chronic kidney disease (CKD). The exact mechanism by which uremic toxicity leads to endothelial dysfunction is still poorly understood. CXCL12 (SDF-1c) is a pleitropic chemokine over expressed in inflamed and injured organs, capable of mobilizing cells to the site of injury where it can support tissue repair and regeneration. In this study we investigate the plasmatic associations between systemic inflammation, endothelial dysfunction and CXCL12, in hemodialysis (HD) patients.

Methods: Plasma samples were collected from HD patients. The systemic inflammation was assessed by high-sensitivity C-reactive protein (hsCRP) and interleukine-6 (IL-6) using an automated immunoturbidimetric and ELISA assay respectively. Endothelial dysfunction (IL-8) and CXCL12 levels were investigated by ELISA.

Results: Twenty-six patients (17±3 months on HD), 52±2 years old, 38% males, 11% diabetics were included. The mean plasma concentrations of hsCRP, IL-6, IL-8 and CXCL12 were respectively 4.9±4.8 mg/mL, 6.76±8.1 pg/mL, 128.2±206.2 pg/mL, and 2313.01±1458.1 pg/mL. There was a positive correlation between hsCRP and IL-6 (r=0.57, P<0.005) and CXCL12 and IL-8 (r=0.4294, P<0.05).

Conclusions: Our data demonstrate that chemokines-related factors such as CXCL12 and IL-8 are increased and correlated in HD patients. This occurs in parallel with systemic inflammation. It is also well known that these chemokines are responsible to mobilized uninjured cells to the injured organs. We suggest that increased levels of CXCL12 and IL-8 found in these patients could reflect an actived repair system which hypothetically would be a way of measuring the extent of cardiovascular damage caused by uremic toxicity.

Funding: Government Support - Non-U.S.

PUB157
Evaluation of Efficacy of Lanthanum Carbonate (Fosrenol) in Patients withCalciphylaxis: A Novel Pilot Study
Olatokunbo O. Shobande, Micah R. Chan. Nephrology, University of Wisconsin, Madison, WI.

Background: Calciphylaxis is a rare and debilitating vasculopathy seen primarily in patients with end stage renal disease (ESRD). The proposed mechanism of injury of vascular calcification involves hyperphosphatemia, elevated serum PTH, and hypercalcemia. Lanthanum carbonate is a non-aluminum, non-calcium phosphate binder that was approved for the treatment of hyperphosphatemia in patients with ESRD.

Methods: We have designed a multi-center open-label uncontrolled pilot study in collaboration with Wisconsin Network for Health Research (WinHR) to determine the efficacy of Lanthanum carbonate on the treatment of calciphylaxis-related calcification. Twelve ESRD patients who will be recruited from multiple dialysis centers and clinics around Wisconsin over a period of 21 months. Patients enrolled will have a baseline physical exam, recent dermatology consult with biopsy-proven calciphylaxis skin lesions, and photographs of the skin lesions. Laboratory parameters measured will include intact PTH, phosphorus, calcium, and albumin. Lanthanum will be administered orally in a dose of 1500-3750mg daily in divided doses with meals over a 12-week period. Dose escalation will be utilized to a target dose of 3750mg daily over a 4-week period. Patients will be evaluated monthly to determine clinical response and followed for a period of 3 months for the remainder of the 21-month recruitment and treatment period. Primary endpoints will be resolution of skin lesions, while secondary endpoints will be serum levels of calcium, phosphorus, and intact PTH.

Results: Three patients are currently enrolled in our study. After 8 weeks of treatment, there were improvements of their skin condition.

Conclusions: Lanthanum carbonate is a novel, potential treatment modality for patients with calciphylaxis.

Funding: Pharmaceutical Company Support

PUB158
Self Reported Quality of Life by EQ5D in Chronic Kidney Disease
Stephanie J. Stringer,1,2 Mary Dutton,1 Paul Cockwell.1, 2 Renal Unit, University Hospital Birmingham, United Kingdom; 1University of Birmingham, United Kingdom.

Background: Patients with CKD have reduced quality of life (QoL), however many instruments used to measure QoL are too detailed for use in routine clinical practice. The EQ5D (EuroQol) health status assessment is a simple and robust questionnaire that has been validated in non-CKD and routine clinical setting. In this study we utilised EQ5D to assess QoL in patients with progressive CKD.

Methods: The Renal Insufficiency In Secondary Care (RIISC) study is a prospective cohort study of patients with progressive CKD. Patients undergo a detailed social and bio-clinical assessment including self assessment of QoL by EQ5D and questions relating to educational attainment and employment.

Results: To date 100 patients have been recruited: 61% were men; 70% were white all 15% Black and South-Asian respectively; mean age was 61.3 years (SD 18.1). 44% of the cohort had no qualifications and 15% were educated to university level. 35% were unemployed, 35% were retired and 30% were in employment. 20% of those employed were in unskilled occupations, 23% were in managerial or professional occupations. 51% reported mobility problems, 9% could not self care and 52% could not carry out usual activities of daily living. 57% reported experiencing at least moderate pain and 30% reported anxiety and/or depression. The mean self rated health score was 63.5±100 (SD 20.9) and was associated with mobility (p<0.012), inability to carry out usual activities (p<0.016) and the presence of anxiety or depression (p<0.02). There was no significant association with co-morbid load, number of medications taken, educational attainment and employment status.

Conclusions: In people with progressive CKD, EQ5D represents a simple scoring system for self reported QoL. Self rated health is a major determinant of mobility, ability to carry out usual activities and anxiety and/or depression. These findings indicate that self related health assessment by EQ5D in progressive CKD can identify key QoL indicators. This has implication for utilising social and cognitive interventions in people with CKD.

Funding: Private Foundation Support

PUB159
Multiple Measures of Neuronal Membrane Excitability from Chronic Kidney Disease to Renal Transplantation
Matthew R. Todd,1 Juan Mason,2 Christopher E.G. Moore.3 Wexseyl Renal & Transplant Service, Queen Alexandra Hospital, Portsmouth, Hampshire, United Kingdom; 2Department of Clinical Neurophysiology, Queen Alexandra Hospital, Portsmouth, Hampshire, United Kingdom.

Background: Uremic neuropathy is common in Chronic Kidney Disease (CKD). Traditional nerve conduction studies (NCS) correlate poorly with clinical neuropathy and only slowly and partially resolve after the initiation of Renal Replacement Therapy (RRT). These changes are associated with histological features suggesting structural and cytoskeletal changes. Novel electrophysiological tests can show changes in neuronal membrane function after a single dialysis session (Krishnan et al., 2005), but have not been reported in pre-RRT CKD or after transplantation.

Methods: We used multiple measures of excitability as described by Kierman et al. (2008) to demonstrate peripheral nerve function in a patient being worked up for a living-related pre-emptive renal transplant. These techniques have been validated for longitudinal measurement by University College Dublin (data presented to the British Society of Clinical Neurophysiology, March 2011). Measures were taken at 8 months and 1 day pre-transplant, and 22 hours and 4 months post-transplant. NCS were also performed at each visit.

Results: NCS showed a mild symmetrical sensorimotor neuropathy which did not change significantly over the 12 months. Membrane function, as measured by threshold electrotonus and recovery cycle, was significantly impaired at 8 months pre-transplant and progressed by the day of transplant. Within 22 hours of transplantation membrane function had normalized, and continued improvement was shown at 4 months post-transplant.

Conclusions: Rapid changes in neuronal membrane function imply a uremic toxin or toxins are responsible for nerve dysfunction in CKD, and that these toxins are readily removed by RRT. The changes seen are reminiscent of chronically depolarized neurons, e.g. in hyperkalemia. These techniques could be used in a hypothesis-generation study to investigate factors associated with progression of neuronal dysfunction, and lead to better understanding and prevention of the neurological complications of CKD.

PUB160
High Triglyceride Levels Are Associated with Pancreatitis in Patients with End Stage Renal Disease
Fiona S. Turkus,1 Nadsey S. Hakim,2 Damien Ashby.1 Imperial College School of Medicine, London, United Kingdom; Imperial College Kidney and Transplant Institute, Hammersmith Hospital, London, United Kingdom.

Background: Acute pancreatitis has been found to occur more frequently in renal patients than in the general population however the cause remains unclear.

Methods: This was a retrospective case control study. Data was collected from patients on renal replacement therapy (RRT) with a previous admission for acute pancreatitis without known cause such as stones or alcohol. For each case, 2 controls were selected matched for age, sex and modality.

Results: We identified 16 patients (aged 38-85, 8 male) who were admitted with acute pancreatitis of unknown cause. Compared to controls, cases tended towards a longer period on RRT (9.6±10.7 vs 5.2±4.8 years, p=0.051) and in the 6 months prior to admission had higher average levels of triglycerides (2.7±2.3 vs 1.5±0.6mmol/L, p=0.008), total serum CRP (47±38 vs 17±7 mg/L, p=0.02), very low density lipoprotein, and triglyceride levels were all below that generally thought to cause pancreatitis (10mmol/L).
Age Interference on Quality of Life of Patients Undergoing Chronic Hemodialysis Carmen B. Tzanno-Martins,1 Fernanda Ribeiro Nishihara,1 Geison Stein Meirelles Ramos,2 Elzo R. Junior,1 João Paulo L.B. Martins,2 Paul Cleasca Troconis,1 1Renal Class, São Paulo, Brazil; 2Home Dialysis, São Paulo, Brazil; 3Renal Class, São Paulo, Brazil.

Background: To assess quality of life through self-administered KDQOL-sf (Kidney Disease Quality of Life – short form) by patients on hemodialysis (HD) classified according to age.

Methods: We randomly selected 159 patients with chronic kidney disease (CKD) on hemodialysis and applied the KDQOL-sf between February 2010 and March 2011. We evaluated the quality of life based on scores below or above 50, on all scales, according to age < 60 years (n = 85) and > 60 years (n = 74).

Results: We analyzed the KDQOL-sf according to age. In patients aged 60 years or more, the scales in which they had poorer quality of life were: physical function 47/85 (55.3%), CKD burden 46/85 (54.1%), professional role 57/85 (67.1%) and sexual function 47/85 (55.3%). In patients younger than 60 years, the scales with poorer quality of life were: physical functioning 47/74 (62.2%), physical function 51/74 (68.9%), general health 38/74 (51.3%), energy / fatigue 45/74 (60.8%), burden of CKD 56/74 (75.7%), professional role 49/74 (66.2%) and sexual function 60/74 (81.1%).

Conclusions: We found that the quality of life in patients aged 60 years or more is below the 50 score on the following scales: physical function, CKD burden, professional role and sexual function. In patients younger than 60 years, the scales showing lower scores were: physical functioning, physical role, general health, energy / fatigue, burden of CKD, professional role and sexual function. While patients younger than 60 years have a quality of life decrease in 4 scales, patients with more than 60 years have worsened in 7 scales, with significant worsening of sexual function and energy / fatigue. We conclude that the quality of life is more impaired in the elderly undergoing HD.

Funding: NIDDK Support

PUB163

Pravastatin Inhibition in Progression of CKD Qin Wang, Liang Ma, Shan Mou, Beili Shi, Minxia Zhu, Liou Cao, Leyi Gu, Zhaohui Ni. Renal Division, Renji Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China.

Background: Lowering low-density lipoprotein (LDL) cholesterol with statin has been shown to reduce the incidence of atherosclerotic events in many diseases, but it remains uncertain whether it is of benefit among people with chronic kidney disease (CKD).

Methods: Patients with CKD who were classified based on an estimated glomerular filtration rate (eGFR) ≥ 15 mL/min/1.73m2 and urine protein between 1 to 3.5g/day were randomized in a ratio of 1:1 to pravastatin 20mg daily versus matching placebo. The key outcome was double of serum creatinine, end-stage renal disease and atherosclerotic events, defined as the combination of myocardial infarction, coronary death, ischemic stroke, or any revascularization procedure in one-year follow-up.

Results: Totally 43 CKD pts were randomized (mean age 58 years, 46.5% male), among which 15% had diabetes mellitus, one fifth had hypertension, and 2% had cardiovascular disease. Compared with placebo group, allocation to pravastatin was not associated with any excess of myopathy, hepatic toxicity, or biliary complications. Serum cholesterol (4.25±0.23mmol/L vs 5.33±0.41mmol/L, P=0.01), triglyceride (1.33±0.29mmol/L vs 1.84±0.44mmol/L, P<0.05) and LDL(2.61±0.70mmol/L vs 3.57±0.76mmol/L, P<0.05) was lower in pravastatin group. Proteinuria start to decrease in pravastatin group at 8 week, and continue to the end of the year.

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881A
There are no significant differences in alteration of eGFR, but it was likely to slow down in pravastatin group (figure 2). 2 pts occurred CVD events in pravastatin group and 5 pts in control group. Cardi-Mai’s survival curve showed significant differences between two groups.

**Conclusions:** Pravastatin can safely adjust the lipid metabolism, reduce proteinuria and CVD risk in CKD patients. It was likely to slow down kidney disease in patients whose eGFR is between 15 to 60 mL/min/1.73 m².

### PUB164

**Increasing the Dose of Lanthanum Carbonate Results in Better Serum Phosphorus Control**

**Rosamund Wilson,1 Lynne Poole.2**

1Shire Pharmaceuticals, Basingstoke, United Kingdom; 2Spica Consultants, Marlborough, United Kingdom.

**Background:** Controlling serum phosphorus to recommended target levels is challenging. Patients should be treated with the appropriate dose of phosphate binder titrated to achieve these target levels.

**Methods:** To investigate whether patients had better control of serum phosphorus on a dose of 3000 mg/day of lanthanum carbonate compared with lower doses, data were analysed from a randomized controlled study conducted in Europe. After randomization, patients entered a titration phase of 5 weeks during which doses were titrated to achieve phosphorus control (≤5.6 mg/dL). After the titration period, patients who had achieved control continued into a 20-week maintenance phase. Data from patients randomized to lanthanum carbonate and had their dose increased to 3000 mg/day during this period, were analysed to evaluate whether increasing the dose to 3000 mg/day had a positive effect on serum phosphorus levels.

**Results:** Thirty-five patients started the maintenance period on 1500 or 2250 mg/day of lanthanum carbonate, and had their dose increased to 3000 mg/day during this period. On average these patients had serum phosphorus levels approximately 0.6 mg/dL lower on 3000 mg/day of lanthanum carbonate compared with doses ≤ 2250 mg/day. Sixty-six percent of patients had better phosphorus control on 3000 mg/ day than on lower doses.

**Conclusions:** Increasing the dose of phosphate binders may improve phosphorus control. This post hoc exploratory analysis suggests that increasing the dose of lanthanum carbonate to 3000 mg/day results in positive effects on serum phosphorus levels and may improve the proportion of patients achieving target levels. Lanthanum carbonate has been shown to be well tolerated when given at doses up to 4500 mg/day in patients with CKD receiving hemodialysis.

**Funding:** Pharmaceutical Company Support

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### PUB165

**Glomerular Filtration Rate Using 2 Different Methods and Albumin Creatinine Ratio in the Irish Population**

**Gemma M. Brown,1 Joseph A. Eustace,2 Ivan J. Perry.1**

1Department of Epidemiology and Public Health, University College Cork, Cork, Ireland; 2Department of Nephrology, Cork University Hospital, Cork, Ireland.

**Background:** This study describes stages of estimated glomerular filtration rate (eGFR) and albumin creatinine ratio (ACR) in Ireland. At present the MDRD equation is used in clinical practice to estimate renal function. This is the first population based estimate in Ireland.

**Methods:** A population-based cross-sectional study of adults was conducted using data from the 2007 Survey of Lifestyle, Attitudes and Nutrition (SLAN). A representative sample of 1,207 adults aged 45 and over, underwent a comprehensive physical examination including serum and urinalysis. Demographically this sample was similar to 2006 national census data. Using regression equations recalibrated MDRD and CKD-EPI, eGFR was calculated from a single serum creatinine. Spot Urine Albumin Creatinine ratio (ACR mg/g) was measured.

**Results:** eGFR ≤60 mL/min/1.73 m² based on the CKD EPI regression equation occurred in 12.1% (95%CI 10.3-14.1%). ACR >30mg/g occurred in 13.2% (95%CI 11.2-15.2%). ACR >30mg/g varied from 11.1% in GFR Stage 1 to 100% in GFR stage 4-5. Using the MDRD regression equation, more subjects were categorised as eGFR ≤60, and similarly more subjects were described as GFR Stage 2 compared to GFR Stage 1 (Table 1).

**Conclusions:** Lower estimates of GFR, which may be dependent on the method of estimation, can impact on the individual. In laboratories in Ireland and in the UK, the MDRD regression equation has widespread use. The CKD-EPI formula results in higher estimated GFR especially in younger and female subjects. Albuminuria, as an additional measure of cardiovascular risk, is prevalent in this population.

**Funding:** Government Support - Non-U.S.

### PUB166

**Prevalence of Albuminuria in Polish Elderly Population (The PolSenior Study)**

**Jerzy Chudek,1,2 Katarzyna Wieczorowska-Tobis,3 Andrzej Wiecek.1**

1Department of Nephrology Endocrinology and Metabolic Diseases, Medical University of Silesia; 2Department of Pathophysiology, Medical University of Silesia; 3Department of Geriatric Medicine and Gerontology, Poznan University of Medical Sciences.

**Background:** Albuminuria is used as a screening tool for detection of chronic kidney disease (CKD) in epidemiological studies. Therefore, the aim of this study was to assess albumin excretion, in addition to serum creatinine concentration, in a representative group of Polish elderly population.

**Methods:** The study was carried out as a part of the nationwide PolSenior project in the population of randomly selected 4,979 people aged 65 and older (2,567 males and 2,412 females) using the national PESEL database (the National Electronic System of Population Registers). Data concerning the prevalence of arterial hypertension, diabetes, kidney stones, refractory urinary tract infections, prostate diseases were collected. Urinary albumin and creatinine concentrations were assessed in 3792 of 3915 obtained urine samples. Microalbuminuria was scored as an albumin to creatinine ratio of 17-250 mg/g in men and 25-355 mg/g in women. Higher albumin excretion was scored as a macroalbuminuria. eGFR was estimated according to CKD-EPI formula, based on serum creatinine concentration.

**Results:** Micro- and macroalbuminuria were found in 2150 (56.7%) and 652 (17.2%) urine samples, respectively. The prevalence of participants with microalbuminuria was similar in all examined age categories (varied from 55.3% to 60.1%), while the prevalence of macroalbuminuria was significantly increasing with age from 10.14% in subjects aged 65-69 to 29.04% in those aged 90 and older. In consequence, the percentage of subjects with albumin excretion within the normal range was diminishing from 29.8% in the age group 65-69 yrs. to 14.42% in the age group 90 and older. Surprisingly enough, a substantial percentage of subjects with normal range of albumin excretion showed eGFR below 60 mL/min/1.73 m² (from 9.5% aged 65-69 to 48.0% aged 90 and older).

**Conclusions:** In Polish elderly population the prevalence of normal range albumin excretion is declining with aging. Albuminuria should not be used as a screening tool of CKD in elderly subjects.

**Funding:** Government Support - Non-U.S.
Methods: 831 Chinese patients with CKD enrolled, of whom 562 were randomly selected as the training data set; the remaining 269 patients constituted the internal validation data set. Additional 349 patients were included in the external validation data set. Serum creatinine (SC) was determined enzymatically. The $^{99m}$Tc-DTPA-GFR was used as the reference GFR (sGFR). The input layer of the BP network consisted of seven factors: Patients’ records of serum SC, albumin, age, sex, height, and weight. The output layer consisted of only one unit representing sGFR. Average sGFR was 46.1±27.0 (3.3-130.1) ml/min/1.73 m² in the training data set, 44.2±28.0 (4.4-137.6) ml/min/1.73 m² in the internal validation data set and 49.1±26.6 (2.8-122.9) ml/min/1.73 m² in the external validation data set. The Cockcroft-Gault-equation, reexpressed 4-variable MDRD equation, reexpressed 2-variable MDRD equation, and BP network were compared in both two validation data sets.

Results: In both two validation data sets, bland-Altman analysis demonstrated that BP network was better than the other equations. Only the precision of the BP network exceeded the prior acceptable tolerances defined as 60 ml/min/1.73 m². The slope of the regression line estimated by the BP network was smaller than those of the other equations. Differences as well as the accuracy with a deviation less than 30% from the sGFR of the BP network were significantly better than those of the other equations. When compared the internal validation data set with external validation data set, the bias was as well as accuracy of sGFR estimated by BP network were not statistically significant.

Conclusions: Our data indicated this BP network model is suitable for the specific Chinese population tested. Relevant procedures are being developed to facilitate the validation of the model.

Correspondence to: Prof. Lou Tan-qi

PUB170
Is the GFR Estimation Equation in Japan and China Useful in Elderly Chinese Patients with Chronic Kidney Disease? Xun Liu, Cailian Cheng, Tan-Qi Lou. Division of Nephrology, Department of Internal Medicine, Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China.

Background: China faces an aging crisis. Recently, some modified glomerular filtration rate (GFR) estimating equations had been established in Japan and China. In this study, we sought to evaluate the applicability of these formulas in elderly Chinese patients with CKD.

Methods: 332 patients enrolled. Serum creatinine (SC) was determined enzymatically. The $^{99m}$Tc-DTPA-GFR was used as the standard GFR (sGFR). The mean age was 69.8±6.7 years (range, 60-93 years). The mean sGFR was 39.7±21.6 ml/min/1.73 m² (range, 23.7-116.6 ml/min/1.73 m²). The reexpressed 4-variable MDRD equation, Chinese-equation and new Japanese equation were tested. The performance of estimated GFR (eGFR) was compared with sGFR in various stages of CKD.

Results: Median of difference ranged from -9.79 ml/min/1.73 m² to 6.81 ml/min/1.73 m². Median percents of the absolute difference ranged from 25.38% to 38.04%. Accuracy with a deviation less than 15% ranged from 20.5% to 28.9%. Accuracy with a deviation less than 30% ranged from 39.8% to 55.4%. Accuracy with a deviation less than 50% ranged from 64.5% to 77.7%. CKD stage misclassification ranged from 42.2% to 57.5%. However accuracies with a deviation less than 30% of all the equations were less than 70%. When the overall performance as well as bias and accuracy were compared in different stages of CKD, GFR estimated by reexpressed 4-variable MDRD equation showed promising results.

Conclusions: When SC was measured by the enzymatic method, GFR estimation equation in Japan and China showed great bias in elderly Chinese patients with CKD. Further improved equations are needed. If conditions are not available, reexpressed 4-variable MDRD equation may be more accurate to assess GFR in elderly Chinese patients.

Correspondence to: Prof. Lou Tan-qi

PUB171

Background: The aims of this study were:
- to compare QOF reported CKD prevalence and Public Health (PH) estimated prevalence,
- to identify the extent of under-diagnosis; and
- to aid commissioners and primary care in improving ascertainment.

Methods: The prevalence of Chronic Kidney Disease in people aged 18 years plus in Primary Care is reported annually through the Quality and Outcomes Framework (QOF). The East Midlands Regional Public Health Observatory has used the NEORICA study to model estimated CKD Prevalence, nationally, at SHA and PCT levels. The gap in % prevalence and the estimated “missing people” have been calculated by subtracting the QOF results from the PH estimates.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Results: The key findings from this assessment show that:

- CKD Prevalence as reported through QOF has increased from 3% in 2006/7, 3.7% in 2007/8, 4.1% in 2008/9 to 4.3% in 2009/10.
- However, compared with Public Health estimated prevalence of 8.8% for England, national ascertainment is still only 48% of expected prevalence.
- At SHA level in 2009/10, ascertainment ranges from 40% of estimated prevalence in London to 59% of estimated prevalence in East Midlands.
- At PCT level, the range is even wider, with the PCT with the lowest estimated ascertainment reporting 27% of expected prevalence and the PCT with the highest reporting 90% of estimated.
- Overall, there is an estimated 1.95 million people with undiagnosed CKD and not being treated and at risk of faster disease progression, emergency admission and poor outcomes.

Conclusions: The key findings from this assessment show that:

- CKD Prevalence as reported through QOF has increased from 3% in 2006/7, 3.7% in 2007/8, 4.1% in 2008/9 to 4.3% in 2009/10.
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- At PCT level, the range is even wider, with the PCT with the lowest estimated ascertainment reporting 27% of expected prevalence and the PCT with the highest reporting 90% of estimated.
- Overall, there is an estimated 1.95 million people with undiagnosed CKD and not being treated and at risk of faster disease progression, emergency admission and poor outcomes.

PUB172

**Pregnancy in Chronic Kidney Diseases and Chronic Kidney Diseases in Pregnancy: Outcome in 188 Singleton Pregnancies with Renal Involvement**

Giorgeina B. Piegoli,1 Rossella Attini, 2 Silvia Parisi,2 Valentina Consigli,1 Stefania Scognamiglio,1 Federica N. Vigotti,1 Martina Ferrari,2 Piero Gaglìotti.1,2

1Nephrology and Dialysis, San Luigi Hospital - University of Turin, Orbassano (TO), Italy; 2Materno-Foetal Unit, S. Anna Hospital - University of Turin, Torino, Italy.

**Background:** The relationship between pregnancy and CKD is complex, entangled and difficult to explore.

- The present study was aimed at assessing pregnancy outcomes in a large cohort of CKD women followed in a tertiary care center where a conjunct Outpatient Unit is run by Nephrologists and Obstetricians.
- Methods: Prospective, Singel Center, observational study. In 2000-2011 262 pregnancies in 235 women were referred. The results were compared with a cohort of “low-risk” pregnancies, followed in the same setting. PE patients were not referred unless there was a need for a differential diagnosis with CKD. The following data were gathered: age, parity, educational level, CKD (cause and stage); acute kidney disease, creatinine, GFR, proteinuria, arterial pressure, delivery (week, caesarian, need for intensive care, weight and entile of the newborn, major clinical problems).

**Results:** The prevalence of early CKD stages is high (192 in stage 1; 44 in stage 2; 20 in stage 3; 4 in stages 4-5, 2 not yet classified), underlining the importance of the in the older population of early CKD stages; the prevalence of 24 proteinuria <0.3 g was 66%; 0.3-1 g and 0.5-1 g were 18%; 1-3 g 9% and over 3 grams per day 6% (2/262 not yet classified: prevalence 1%).

Conclusions: The key risk factors for pregnancy related morbidity, since the early stages; pregnancy is a valuable occasion for early CKD diagnosis.

Funding: Government Support - Non-U.S.

PUB173

**MDRD or EPI Equations for Estimating Glomerular Filtration Rate (GFR) in a Stage 1-3 CKD Population: Any Relevant Differences?**

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**Background:** MDRD equation is considered accurate for estimated GFR <60 ml/min. The new equation MDRD was developed to enable a better performance for GFR ≥60 ml/min. Our aims were to understand the difference of estimated GFR using MDRD or EPI equations for stage 3 CKD.

**Methods:** We randomly selected 175 patients from our clinic with stage 1-3 CKD and stable kidney function. Serum creatinine (SCR) and cystatin C were measured by an IDMS traceable method. We compared eGFR as each formula using Wilcoxon paired samples test. Prevalence of stage 3 CKD was compared by McNemar’s test. Agreement between equations in categorizing patients in stage 3 versus stage 1-2 CKD was evaluated by Kappa statistics. Pearson correlation was determined between the difference between formulas (calculated as MDRD_GFR minus EPI_GFR for each paired estimates) and age, SCR and MDRD_GFR.

**Results:** Patients enrolled were mainly old (median age, 66 years), with a high prevalence of stage 3 CKD (by MDRD and EPI formulas 67.5% and 59.4% respectively, P<0.001), median MDRD_GFR of 52.3 ml/min and EPI_GFR of 52.2 ml/min. Estimated GFR by each equation were significantly different (P<0.001) but Kappa statistics showed a good agreement (κ=0.84). The string of the differences between formulas correlated significantly with age (r=-0.30, P<0.02), SCR (r=-0.48, P<0.001) and MDRD_GFR (r=0.60, P<0.001). Bland-Altman analysis among formulas is plotted in Figure 1.

**Conclusion:** Estimated GFR varied significantly between equations, with noticeable lower EPI_GFR in younger and with lower SCR patients. However, a significantly lower prevalence of stage 3 CKD by the EPI equation was shown. In patients with mild kidney dysfunction, EPI_GFR estimates seemed more accurate, permitting a more adequate CKD staging.

**Funding:** Pharmaceutical Company Support

PUB174

**Profile of Chronic Kidney Disease Practice in Queensland, Australia – CKD. QLD Phase 2 Survey**

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**Background:** Chronic kidney disease (CKD) management is evolving from doctor and hospital centric models towards nurse practitioner (NP) led multidisciplinary community models of care. We assessed CKD practice management in Queensland, Australia, which has a multicultural population of 4.4 million.

**Methods:** Profile of CKD management was undertaken using a web-based questionnaire (Survey-monkey) completed by senior medical and nursing staff across all public renal units in Queensland, Australia.

**Results:** The response rate was 100% with participation of all clinics in the survey. The majority of CKD practice was hospital based (85%) with community CKD clinics available in major cities only. Total number of patients followed in all public CKD clinics was 11668, exceeding the number (10,469) estimated during the first CKD site profiling 2-years previously.

The majority of patients (90%) were seen by a nephrologist, as most clinics were hospital based, while 5 NPs using the NP model seeing the rest. Numbers of indigenous CKD patients varied according to location, ranging from 0 to 40%. Indigenous staff were available in 50% of units surveyed and there was a high level (88%) of cultural awareness and training.

Patient follow up was based on CKD stage and co-morbidities, and ranged between intervals of 6-weeks to 1-year. Allied health professionals were available in all clinics, with 80% of patients seen by a dietician and follow-up was according to need. The multi-disciplinary care model including pharmacist, psychologist, social worker and dietitian was being implemented in all units, but variably limited by resources.

Conclusions: CKD management has become a major focus of renal services in Queensland Health with almost 12,000 patients to care for, with increasing numbers as new referrals are seen regularly. Community based services and NP models are increasingly explored for CKD service delivery.

**Funding:** Government Support - Non-U.S.
Background: Polycythemic free light chains (FLCs) are elevated in patients with kidney impairment and serve as an independent risk factor for renal progression in patients with advanced chronic kidney disease (CKD). The aim of this study was to assess serum (S) and urine (U) FLCs in CKD stage 3 patients, in relation to their disease status.

Methods: 1741 patients were recruited from primary care practices. Subjects were predominantly white (98%) and female (60%) with an average age of 73 years. 17% had diabetes. The median eGFR was 53ml/min/1.73m². FLCs were measured in the S and using the Freelite™ assay. SFLCs (Sκ, SIκ) were compared to estimated normal ranges (Sκ: 3.3-19.4mg/L, SIκ: 5.17-26.3mg/L), UFLCs (Uκ and UIκ) and Ucreatinine values were obtained from healthy volunteers (Table).

Results: Median Sκ 19mg/L (4-181mg/L) and SIκ 17mg/L (2-74mg/L) concentrations were elevated, in the CKD population. Multivariable linear regression identified several factors as independent determinants of SFLCs including eGFR, UAcreatinine and cholesterol. UFLCs corrected using Ucreatinine (UκCR and UIκCR) were also elevated (Table). UFLCs and FLCs/CR were further increased in patients with albuminuria (Table). xCR and xCκCR correlated significantly with ACR (r=0.38 and r=0.47 respectively). Of the CKD patients with an abnormal ACR, 59% had abnormal UFLCs. 43% patients had normal ACR had abnormal UFLCs, indicating UFLCs are an earlier indicator of renal damage. Only in normal CR, UCR and ACR correlated significantly with ACR (r=0.38 and r=0.47 respectively). Of the CKD patients with an abnormal ACR, 59% had abnormal UFLCs. 43% patients with a normal ACR had abnormal UFLCs, indicating UFLCs are an earlier indicator of renal damage.

Conclusion: The clinical relevance of these observations will be determined as the population is prospectively followed up.

PUB177


Background: The Chronic Kidney Disease Prognosis Consortium (CKD-PC) was established in 2009 and currently consists of 46 cohorts from 17 countries of North America, Europe, Asia, and Oceania. The consortium conducts complex individual participant data meta-analyses with the goal of providing comprehensive evidence regarding CKD prognosis.

Methods: The consortium governance includes a steering committee, operations committee, and data coordinating center (DCC). Cohorts can join the consortium at any time following operating principles posted at www.jhsph.edu/ckdpc. Each cohort opts in or out for each proposed manuscript. Statistical code for each manuscript is written by the DCC, distributed to participating cohorts, and shared publicly on the website for each manuscript. Piece-wise linear spline models allow a detailed examination of the dose-response association between eGFR and albuminuria with outcomes (mortality, cardiovascular disease, ESRD, AKI, and progression of CKD) and can be pooled across cohorts using the variance-covariance matrix of the regression coefficients.

Results: The consortium includes cohorts representing general (24 cohorts), high risk (10 cohorts), and CKD (12 cohorts) populations including over 1.5 million participants. CKD-PC published four meta-analysis manuscripts in 2010 (phase 1), with seven meta-analyses to be completed in 2011 (phase 2) and meta-analyses of individual risk and definitions of CKD-progression in 2012 (phase 3). Forty-three cohorts from phase 1 opted in for phase 2 analyses and three new cohorts joined. Authorship includes ~15 authors and ~100-200 collaborators per paper.

Conclusions: CKD-PC has established a productive model allowing flexible collaboration across meta-analyses. Distribution of statistical code allows inclusion of cohorts which cannot share the raw data due to legal/administrative constraints.

Funding: Private Foundation Support

PUB178

Single Center Experience with Rituximab: Indications, Use, and Response in Pediatric Patients Roshan P. Georgi, Leonard C. Hymes, Rochelle Schmidt, Sandra Amaral, Pediatric Nephrology, Emory University and Children's Healthcare of Atlanta, Atlanta, GA.

Background: Rituximab is an anti-CD20 monoclonal antibody, initially approved for use in Non-Hodgkin’s Lymphoma. It is frequently used off-label for various other conditions, including renal diseases. There is scant knowledge about response to rituximab use in pediatric patients for these renal conditions.

Methods: We performed a retrospective cohort study of the indications, use and response to rituximab within our nephrology center in the Southeastern US. We included all patients who received rituximab from January 2003 to May 2011 for any indication.

Results: 39 patients received rituximab. 9 patients had renal transplants with rejection unresponsive to other therapies. 9 patients had EBV infection and post-transplant lymphoproliferative disease (PTLD). 15 patients had lupus nephritis; 14 (93%) had either class IV or a combination of these. 6 patients had other conditions, including 4 with membranous nephropathy, and 1 each with Wegener’s Granulomatosis and Thrombotic Thrombocytopenic Purpura (TTP). Among transplant patients, rejection improved significantly in 4 of 9 cases (44%) with resolution in 11%. All 9 PTLD patients responded with complete resolution of disease. Among the 15 lupus patients, 4 had clinical and serological improvement, 4 had partial response with improved hypocomplementemia but residual significant proteinuria. These 8 patients received 2 or more doses. None of the patients with other conditions showed a response. 18 (46%) experienced adverse effects including 4 with anaphylaxis requiring aborting the dose. 3 had minor infusion reactions. 3 (33%) of the transplant patients with PTLD had persistent hypogammaglobulinemia requiring monthly IVIG. 3 patients had altered mental status, 3-30 days following rituximab dose.

Conclusions: In our single center, rituximab was most effective for PTLD with heterogeneous responses in patients with renal transplant rejection, lupus nephritis and other conditions. There was a high incidence of adverse events (46%). These results emphasize that rituximab should be used with caution and more research is needed to discover optimal therapies for these challenging renal conditions.

Funding: None

PUB179

Combined Impact of Anemia, Hepcidin-25 and Proteinuria on Early Mortality in Cancer Patients Masaki Har,1 Minoru Ando,2 Ken Tsuchiya,3 Kosuku Nitta.1 1Department of Nephrology, Tokyo Metropolitan Komagome Hospital, Tokyo, Bunkyo-ku, Japan; 2Department IV of Internal Medicine, Tokyo Women’s Medical University, Tokyo, Shinjuku-ku, Japan.

Background: Anemia of chronic disease (ACD) occurs in patients with either cancer or kidney disease or both. Hepcidin-25 is a key player in the pathologic state of ACD. We examined associations among hepcidin-25, proteinuria and mortality in cancer patients.

Methods: Two-year prospective cohort study was conducted in a total of 55 cancer patients receiving chemotherapy. Underlying malignancies included malignant lymphoma (47%), gastric cancer (25%), and other cancers (28%). Serum hepcidin-25 level was measured by liquid chromatography mass spectrometry. Proteinuria was defined as a dipstick
mortality at 5 years. 4 stroke, 4 tumors, 3 cardiovascular disease, 2 fractures and 5 unknown. The table shows of HF had significantly higher SUA (7.00 ± 1.74 vs. 5.90 ± 1.71, P = 0.031). 41 deaths patients receiving chemotherapy. Adding either anemia or proteinuria or both to hepcidin-25 Segovia, Spain.

Serum Uric Acid as a Marker of Mortality in an Elderly Patients Cohort

**Results:** Mean hepcidin-25 level was 46.7±48.8 ng/ml which was nearly 2-fold greater than the reference value(22.2±12.3 ng/ml). Cumulative survival rate was significantly lower in the high hepcidin-25, proteinuria (+) or anemia (+) group than in each corresponding opposite. Multivariate analysis showed that the HR (95% CI) was 27.8 (6.27-155.2) for those with 3 points; 4.0 (1.26-13.6) for those with 2 points; and 1.6 (0.53-5.19) for those with 1 point, as compared to the reference patients with neither of them.

**Conclusions:** High hepcidin-25 may be a novel predictor for early mortality in cancer patients receiving chemotherapy. Adding either anemia or proteinuria or both to hepcidin-25 increases its predictive power.

**PUB180**

**Serum Uric Acid as a Marker of Mortality in an Elderly Patients Cohort**

Manuel M. Heras, Maria José Fernández-Reyes, Rosa Sanchez, Alvaro Molina, Astrid Rodriguez, Fernando Alvarez-Ude. Nephrology, General Hospital, Segovia, Spain.

**Background:** There is growing evidence of the role of serum uric acid (SUA) as a risk factor for cardiovascular and renal disease. We analyze the association between baseline SUA and overall mortality in elderly patients followed prospectively for 5 years.

**Methods:** 80 clinically stable patients, mean age 83 years (range 69-77), 31.3% men, 35% diabetic, 83% hypertensives, recruited in Geriatrics and Nephrology consultations between January-April 2006, were followed for 5 years. Predictive variables were: baseline SUA and plasma creatinine; estimated glomerular filtration rate (GFR) (abbreviated MDRD formula); recorded age, gender, baseline comorbidity (Charlson index), cardiovascular individualized treatment and mortality. Statistical analysis: SPSS15.0.

**Results:** Baseline SUA was normally distributed and its median was 5.85 mg/dl. We found not significant differences in levels of SUA by gender, diabetes mellitus, hypertension, diuretic use, heart failure, (HF), peripheral arterial disease or stroke. Patients with an history of HF had significantly higher SUA (7.00 ± 1.74 vs. 5.90 ± 1.71, P = 0.031). 41 deaths occurred during follow-up (15 men and 26 women): 15 general deterioration, 8 infections, 4 stroke, 4 tumors, 3 cardiovascular disease, 2 fractures and 5 unknown. The table shows how patients with SUA higher than the median had significantly lower GFR and higher mortality at 5 years.

**Comparison of variables between groups according to SUA median**

<table>
<thead>
<tr>
<th>Group 1: SUA ≤ 5.85 N=40</th>
<th>Group 2: SUA &gt; 5.85 N=40</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline SUA (mg/dl)</td>
<td>4.77 ± 0.86</td>
<td>7.45 ± 1.27</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.15 ± 0.45</td>
<td>1.46 ± 0.51</td>
</tr>
<tr>
<td>MDRD (ml/min/1.73m²)</td>
<td>54.06 ± 16</td>
<td>0.001</td>
</tr>
<tr>
<td>Age, years</td>
<td>81.51±6</td>
<td>83.42±6</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>10/30</td>
<td>15/25</td>
</tr>
<tr>
<td>Charlson index</td>
<td>1.62±1</td>
<td>1.97±1.29</td>
</tr>
<tr>
<td>Mortality at 5 years</td>
<td>32.5%</td>
<td>70%</td>
</tr>
</tbody>
</table>

In logistic regression analysis for overall mortality (independent variables: age, gender, Charlson Index, history of HF, SUA, creatinine and GFR), only age (HR: 1.14, 1.04-1.025, P=0.002) and SUA levels (HR:1.92, 1.28-2.88, P=0.002) were independently associated with mortality.

**Conclusions:** In our study, levels of SUA are shown as independent risk factor for mortality in elderly patients.

**PUB181**

**Better Outcomes of Chronic Kidney Disease (CKD) Patients in Okinawa, Japan:** Single Renal Clinic Report Okinawa Tokuyama Clinic Epidemiology and Nephrology Study (OCEANS)

Kunio Ishii, 1Internal Medicine, Tokuyama Clinic, Urasoe, Okinawa, Japan; 2Dialysis Unit, University Hospital of the Ryukyu, Nishihara, Okinawa, Japan.

**Background:** The purpose of the study is to examine the outcomes of the registered patients in single renal clinic in Okinawa, Japan. Tokuyama clinic is one of the referral centers taking care of CKD patients in Urasoe City which the population is about 110,000. All the members of general practitioners in the city are cooperative with the support of the Urasoe Medical Association. Medical records are filed to the registry database of Okinawa Tokuyama Clinic Epidemiology and Nephrology Study (OCEANS).

**Methods:** The registration period was from April 2004 to March 2008, 4 years, and followed up until March 2011. Serum creatinine was measured in 3,141 patients during the study period. Among them, a total of 1,962 (64.2%) patients were followed until events such as death and dialysis program or followed regularly, at least more than 3 months. Others (N=1,179) were not followed regularly as 1) visited simple medical reasons such as health-check, Cold, gastro-intestinal problems and miscellaneous reasons (N=661), moved outside to other area (36), transferred to other hospitals and hospitalization (307), and unknown (175). Serum creatinine was measured using the enzymatic method and the GFR was estimated by the formula of the Japanese Society of Nephrology. Based on our observation, early referral from general practitioners, eGFR<45 ml/min/1.73m² is warranted for the reduction of ESRD.

**Funding:** Private Foundation Support
Methods: We reviewed the AKI literature for SORO-ESRD as defined by sudden unanticipated AKI requiring RRT and quickly terminating in irreversible ESRD.

Results: The AKI reports revealing SORO-ESRD appear below. The 15 reports spanning the world, 20-1095 patients each, age 39-65 years, published from 1975-2010, demonstrated SORO-ESRD rates from 1%-85%. AKI was commonly caused by hypovolemia/hypotension, infections/sepsis, and nephrotoxics - radiocontrast, NSAIDs, aminoglycosides and ACEIs/ARBs.

Conclusions: Not surprisingly, at least to us, several published AKI reports dating back to 1975 had demonstrated SORO-ESRD. This has substantiated our hypothesis that SORO-ESRD is prevalent worldwide. The human body is a complex adaptive system (CAS) so small changes can lead to huge effects; thus AKI can lead to irreversible ESRD, fairly quickly. The studies incriminated the same preventable causes of AKI. Renal protection, a new concept we reported in the QJM in 2009, measures to prevent AKI, would benefit CKD patients everywhere. Note worthily, Merino et al in 1975 who described irreversible post-operative ESRD in older CKD patients following AKI from hypotension, with or without septicemia, had asked the rhetorical question whether indeed this should not represent a new syndrome. Now we know - the rest of the story. It is SORO-ESRD.

PUB184
Outcomes of Valvular Heart Surgery in Patients with Chronic Kidney Disease
Almohana Shanaah,1 Eleanor D. Lederer,1,2 Ihab Hamzeh,1,2 Michael E. Brier,1,2 Medicine, University of Louisville, KY; 1Medicine, Robley Rex Veterans Affairs Medical Center, Louisville, KY.

Background: Few studies have focused on the surgical outcome of patients with chronic kidney disease (CKD) undergoing valvular heart surgery. Furthermore, the optimal choice of valve type in this population remains unknown (ACC/AHA guidelines). A previous report showed that mortality was lower in patients undergoing aortic valve replacement (AVR) with mechanical vs. tissue valve in general VA population. The purpose of this study is to examine short-term and long-term outcomes in CKD patients undergoing cardiac valve surgery at our VA.

Methods: A retrospective review of patients with chronic kidney disease undergoing valve replacement from January 2000 through December 2010 at Robley Rex Veterans Affairs Hospital. ICD-9 and CPT codes were used to identify patients from electronic medical records. Outcomes were compared using Pearson chi-square.

Results: A total of 16 patients met the selection criteria for inclusion in this study. The average age at time of surgery was 68 years. The majority of patients were white (75%). The prevalence of hypertension, diabetes mellitus and hyperuricemia were independently associated with CKD.

Conclusions: Our study supports the findings reported in previous studies. CKD patients undergoing cardiac valve surgery have adverse outcomes. Future research to establish a prognosis and appropriate treatment strategies for these patients is warranted.

Funding: Government Support - Non-U.S.

PUB185
Epidemiology of Chronic Kidney Disease: Results from a Polycentric Community Based Population of Middle-Older Aged Adults in Shanghai, China
Yi Wang,1 Shougang Zhuang,1 Haidong Yan.1 Department of Nephrology, Tongji University Affiliated Shanghai East Hospital, Shanghai, China; 2Department of Medicine, Brown University School of Medicine, Providence, RI.

Background: The purpose of this study is to investigate the prevalence, awareness and the risk factors of chronic kidney disease (CKD) among community adult population in Pudong New Area, Shanghai, China.

Methods: 2000 adult residents (≥245 years old) from Pudong New Area were randomly selected and invited to answer a questionnaire and to receive health examinations from July 2006 to October 2009. The morning spot urine dipstick test was used to evaluate proteinuria, creatinine and hematuria. Urine protein to creatinine ratio ≥ 30 mg/g and urine red blood cells ≥3 /µl were considered abnormal. The association of kidney damage indicators included age, gender, hypertension, diabetes mellitus, smoking, income, education, cholesterol, triglyceride, body mass index and waist-to-hip ratio were assessed as well. The Chinese improved abbreviated MDRD equation was applied to estimate the glomerular filtration rate (eGFR, abnormal: <60ml/min/1.73m²). A SPSS13.5 statistical software was used for statistical analysis.

Results: 1905 residents with complete data were enrolled in the study, with mean age 59.02±9.42 years old. After the adjustment of age and gender components, the prevalence of albuminuria, hematuria and the reduced renal function was 12.5% (95%CI:10.5%-15.5%), 7.90% (95%CI:6.7%-9.1%), and 1.9% (95%CI:1.7%-2.1%), respectively. Approximately 13.2% subjects had at least one indicator of kidney damage. Age, hypertension, diabetes mellitus and hyperuricemia were independently associated with CKD.

Conclusions: The prevalence of CKD in adult residents (≥45 years old) from Pudong New Area in Shanghai is 13.2%, and the awareness is 20.9%. The independent risk factors associated with CKD include age, triglyceride, hypertension, diabetes mellitus and hyperuricemia.

Funding: Government Support - Non-U.S.
Table 1. Treatment scheme and costs (EUR) per single dose and over survey group (rounded values)

<table>
<thead>
<tr>
<th>Treatment Type</th>
<th>Ferric Gluconate (n=20)</th>
<th>Iron dextran (n=20)</th>
<th>Iron sucrose (n=24)</th>
<th>Ferric carboxymaltose (n=251)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean total dose given (mg)</td>
<td>365</td>
<td>1000</td>
<td>482</td>
<td>692</td>
</tr>
<tr>
<td>Number of session needed for infusion</td>
<td>3.7</td>
<td>1.6</td>
<td>2.4</td>
<td>2.5</td>
</tr>
<tr>
<td>Administration scheme as per approved label</td>
<td>62.5mg per 10min</td>
<td>1134mg in 7h</td>
<td>200mg in 30min</td>
<td>500-1000mg in up to 15min</td>
</tr>
<tr>
<td>Administration type</td>
<td>65% infusion, 35% injection</td>
<td>80% infusion, 20% injection</td>
<td>65% infusion, 35% injection</td>
<td>54% infusion, 46% injection</td>
</tr>
<tr>
<td>Cost of administration (EUR)</td>
<td>164</td>
<td>594</td>
<td>149</td>
<td>128</td>
</tr>
<tr>
<td>Cost of total i.v. iron (EUR)</td>
<td>35</td>
<td>205</td>
<td>110</td>
<td>193</td>
</tr>
<tr>
<td>Total costs (EUR)</td>
<td>199</td>
<td>321</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*push injection with max. 200mg; ** slow injection with max 200mg

HB levels for iron sucrose or ferric carboxymaltose (FCM) were 9.4g/dl (standard deviation (SD) 1.1) or 9.4g/dl (SD 1.2) at initiation and 10.9g/dl (SD 1.2) or 11.5g/dl (SD: 1.4) at last visit, respectively. ESA patients had levels of 9.3g/dl (SD: 1.5; initiation) and 10.9g/dl (SD: 1.6; last visit).

Conclusions: This study identified practice related treatment schedules in CKD patients with ID. The i.v. iron group achieved similar HB levels compared to ESA. Due to methodological limitations, it was not possible to assess data on treatment duration or the superiority of a therapy. I.v. iron might be a clinically and economically viable alternative to expensive ESAs in CKD patients.

Funding: Pharmaceutical Company Support

**PUB188**

Automated Reporting of Estimated Glomerular Filtration Rate Varies among Veterans Affairs Laboratories

Rasheed A. K. Hall,2 Bradley G. Hammill,2 George L. Jackson,1,2 Virginia Wang,1,2 Matthew L. Maciejewski,1,2 Uptal D. Patel,2 VA Health Services Research & Development; 2Duke University, Durham, NC.

Background: The prevalence of CKD among veterans is increasing, and to facilitate earlier detection of CKD, clinical practice guidelines have recommended automated reporting of eGFR by clinical laboratories. We sought to better understand the diffusion of this innovation within the Veterans Health Administration (VHA) by evaluating time to adoption of eGFR reporting at individual VHA facilities and associations between adoption and site-specific organizational characteristics.

Methods: Using VHA laboratory data, we estimated time to adoption from the date VHA delivered a mandate for eGFR reporting (July 2004). For each medical facility with a laboratory (n= 135), adoption was defined by the presence of an associated eGFR for at least 1% of creatinine values. VHA facilities were classified by adoption status, and adopters were further divided into 3 categories (early-, mid-, and late-adopters) based on tertiles of time to adoption. Then, we compared organizational characteristics between facilities with bivariate analyses.

Results: By September 2009, 109 (81%) facilities adopted eGFR reporting while 26 (19%) facilities had not. Time to adoption varied widely with range, 0.2 to 4.3 years, and median of 1.8 (IQR 2.2) years. Comparing facilities by adoption status, dialysis units were only present among adopters (59%;p<0.05). Among adopters, there were no significant differences in organizational characteristics.

Conclusions: Despite a universal mandate to adopt this laboratory reporting innovation, 1 in 5 facilities did not adopt eGFR reporting and among those who did, time to adoption varied widely. These findings suggest that adoption may be due to availability of local resources (e.g., dialysis units). Still, reasons for the wide range in adoption remain unclear, but may be the result of local decision-making between clinical and information technology administrators. With the emergence of newer eGFR equations to improve CKD detection, our findings should be considered prior to future rollouts of other laboratory reporting innovations to promote more uniform and timely adoption.

Funding: NIDDK Support, Other U.S. Government Support

**PUB189**

Trial Announcement: Vitamin K2 To Slow Vascular Calcification in Hemodialysis Patients, “VitaVasK” Thilo Krueger,1 Georg Schlieper,1 Mario Cozzolino,2 Johannes Jacobi,1 Ralf-Dieter Hilgers,1 Michel Y. Jadoul,2 Markus Ketteler,1 Tom Cornelis,1 Lars C. Rump,1 Peter Stenvinkel,1 Andrej Wieck,1 Ralf-Wilhelm,1 Leon J. Schagers,2 Jurgen Fleegle,1 Nephrology, University Clinic Aachen, Aachen, Germany; 1Renal Division, University of Milan, Milan, Italy; 1Nephrology, University of Erlangen Nuremberg, Erlangen, Germany; 1Nephrology, University of Louvain, Brussels, Belgium; 1Nephrology, Coburg Hospital, Coburg, Germany; 1Nephrology, University Hospital Maastricht, Maastricht, Netherlands; 1Nephrology, University Duesseldorfe, Duesseldorfe, Germany; 1Renal Medicine, Karolinska Institute Stockholm, Stockholm, Sweden; 1Nephrology, University of Katowice, Poland; 1Cardiology, University Duesseldorfe, Duesseldorfe, Germany; 1Medical Statistics, University Clinic Aachen, Aachen, Germany; 1CARIM, University Maastricht, Maastricht, Netherlands.

Background: Patients on hemodialysis (HD) exhibit an increased cardiovascular mortality associated with vascular calcification (VC). Matrix Gla protein (MGP) is a powerful vascular wall-based inhibitor of VC. MGP needs activation by vitamin K-dependent carboxylation.

The ERA-EDTA sponsored VitaVasK study will be the first clinical trial in HD patients to target the progression of VC using vitamin K-containing supplements. VitaVasK is a randomized, double-blind, placebo-controlled trial with parallel groups. Participants are recruited from nine European nephrology centers. Multislice spiral computed tomography (MSCT) will be used to screen for coronary artery calcification (CAC). Stable HD patients with CAC scores >100 will be randomized to a daily oral supplementation of vitamin K2 (n=178) or placebo (n=178) for 18 months. Primary outcomes will be progression of coronary and aortal calcification at 12 and 18 months using volume scores determined by MSCT. Secondary outcomes will be regression of VC, attenuation of progression of aortic and mitral valve calcification, major cardiac events, mortality, and change in serum levels of undercarboxylated and carboxylated MGP. Patients will be followed beyond the primary study duration to determine the mortality at three and five years.

Funding: Pharmaceutical Company Support, Clinical Revenue Support

**PUB190**

CKD-QLD Phase 2 Survey: Risk Factor Modification for Prevention of Chronic Kidney Disease Progression Sree Krishna Venuthurupalli,1,4 Anne Salisbury,1 Wendy E. Hoy,3 Helen G. Healy,3 Robert G. Fassett,2,3 1Renal Medicine, Toowoomba Hospital, Toowoomba, Queensland, Australia; 1Renal Medicine, Royal Brisbane and Women’s Hospital, Brisbane, Queensland, Australia; 3Centre for Chronic Disease, University of Queensland, Brisbane, Queensland, Australia; 2School of Medicine, University of Queensland, Brisbane, Queensland, Australia.

Background: Management of traditional risk factors for chronic kidney disease (CKD) progression include antiproteinuria therapy, hypertension and diabetes control, diet, exercise and lipid lowering therapy. We investigated the current clinical practice for prevention of CKD progression in Queensland, Australia.

Methods: Using a web based questionnaire, nephrology medical and nursing staff from each CKD clinic in Queensland were surveyed to assess risk factor modification in CKD management practices.

Results: The participation rate was 100%. Restriction of salt and fats formed the cornerstone (80%) of diet modification. Routine protein restriction was not advised. Dietary potassium and phosphate limitations were used as case by case. Anthropometric measurements included weight (100%), height (81%), body mass index (61%) and waist circumference (10%). An exercise physiologist was not available in all clinics, though physical activity and exercise was routinely recommended.

Angiotensin converting enzyme inhibitors and angiotensin receptor blockers were routinely prescribed for blood pressure reduction and to slow CKD progression. However, combination therapy was only used in 50% of cases, based on level of proteinuria. Lipid lowering therapy was a frequent practice (90%), with use of statins being the main strategy. Interestingly, statins were used with the aim of reducing cardiovascular mortality and morbidity but not to slow CKD progression.

Diabetes control was routine with HbA1C levels used as the main tool for follow up (85%). Patients are screened for vascular complications related to diabetes with annual follow up.

Conclusions: Traditional risk factors management was incorporated in CKD practice across renal units in Queensland, Australia. A longitudinal study in this population will determine the impact of these on CKD progression and cardiovascular events and mortality.

Funding: Pharmaceutical Company Support
PUB191

Relationship between Clinicopathological Findings and Renal Outcomes of Diabetic Nephropathy in Type 2 Diabetes
Tomoki Funamoto,1 Miho Shimizu,1 Tazuko Ohama,1 Chikako Nose, 1 Yasuyuki Shinkozaki,1 Shinji Kitajima,1 Tadashi Toyama,1 Akihori Hari,1 Kyoki Kitagawa,1 Kengo Furuchi,1 Shuichi Kaneko,1 Takashi Wada.1 Division of Nephropathy, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; 2Department of Diabetic Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Ishikawa, Japan.

Background: The purpose of the present study was to evaluate the relationship between clinicopathological findings and renal outcomes of diabetic nephropathy in Japanese patients with type 2 diabetes.

Methods: Two hundred and sixty, biopsy-proven type 2 diabetic patients with diabetic nephropathy (164 males and 96 females) were examined. Observation period was 7.9±6.5 years. Renal outcomes were assessed by requiring for dialysis or 50% reduction of estimated glomerular filtration rate (eGFR). For presumption of clinicopathological factors that affected renal outcomes, multivariate analysis by the Cox proportional hazards model was used.

Results: The number of cases of normal albuminuria or urinary protein (-) or (+), microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively. 2) Seventeen patients (36.2%) with normal albuminuria or urinary protein (-) microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively. 2) Seventeen patients (36.2%) with normal albuminuria or urinary protein (-) microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively.

Conclusions: Our observations suggest that development of albuminuria play an important role for poor renal outcomes of diabetic nephropathy in type 2 diabetes. Lower eGFR was an independent risk factor in renal outcomes among patients with overt nephropathy.

PUB192

Low 25-hydroxyvitamin D Levels Are Associated with an Increased Risk of Non-Alcoholic Fatty Liver Disease (NAFLD) in a Large Managed Care Organization
Anna Jeanette Jovanovich,1 Giovanni Targher,2 Jessica B. Kendrick,3 Shailendra Sharma,1 Kim McCann,1 Sidney N. Thornton,1 Michel B. Chonchol,1 John R. Holmen.1 Division of Renal Diseases and Hypertension, University of Colorado, Denver, Aurora, CO; 2Section of Endocrinology, Department of Medicine, University Hospital, Verona, Italy; 1Intermountain Health Care, Salt Lake City, UT.

Background: To explore associations between serum 25-hydroxyvitamin D levels and NAFLD.

Methods: We conducted a population-based cohort study of adult patients with NAFLD who had vitamin D levels measured 3 to 15 months prior to diagnosis of NAFLD. Vitamin D was evaluated as a continuous variable and a categorical predictor (< 15 ng/mL vs. higher).

Results: Vitamin D levels < 15 ng/mL vs. higher (adjusted OR = 2.24; 95% CI 1.38-3.64) more common in NAFLD patients compared to controls. After adjustment for BMI, diabetes, chronic kidney disease and peripheral vascular disease higher 25(OH)D levels was associated with a lower risk of NAFLD.

Conclusions: Low 25-hydroxyvitamin D levels are associated with an increased risk of NAFLD.

PUB193

Effects of Dual Blockade of the Renin Angiotensin System in Diabetic Kidney Disease: A Systematic Review and Meta-Analysis
Jacqueline Pham,1 Brian P. Schmitt,2 David J. Leechey.1 Renal and Hypertension, Edward Hines Jr. VA Medical Center, Hines, IL; 2Edward Hines Jr. VA Medical Center, Hines, IL.

Background: There is much evidence to support a renoprotective effect of inhibitors of the renin-angiotensin system in diabetic kidney disease. However, it remains unclear whether dual renin-angiotensin system blockade has additional benefits in this population and whether any benefits outweigh the risks.

Methods: Study Design: Systematic review and meta-analysis Setting and Population: Diabetic patients with overt proteinuria

Selection Criteria for Studies: Randomized, controlled, parallel or crossover design studies

Intervention: Combination renin-angiotensin system blockade vs. monotherapy

Outcomes: The primary outcome measure was the post-treatment difference in proteinuria with combination therapy versus monotherapy. Secondary outcomes included percent change in proteinuria, changes in systolic blood pressure, glomerular filtration rate, and serum potassium, and incidence of hyperkalemia. Sensitivity analyses that evaluated differences in outcome based on study quality (assessed by Jadad scores), baseline systolic blood pressure, and drug types and doses were conducted.

Results: There was significantly less proteinuria (by 334 mg/24 hr) after treatment with combination therapy vs. monotherapy. Systolic blood pressure (BP) after treatment with combination therapy vs. monotherapy was significantly lower (by 4.1 mmHg). However, clinically significant hyperkalemia was 3.5-fold more common with dual blockade.

Conclusions: Dual renin-angiotensin system blockade in patients with diabetic kidney disease reduces proteinuria and BP but is associated with a higher incidence of clinically significant hyperkalemia. Further studies assessing long-term outcomes are needed to weigh the benefits versus risks of combination renin-angiotensin system inhibitor therapy.

PUB194

Effect of Blockers of the Renin-Angiotensin System on Renal Tissue Oxygenation in Type 2 Diabetics as Measured by BOLD-MRI

Background: Previous studies have shown that acute intake of certain drugs alters renal tissue oxygenation, and animal studies suggest that blockers of the renin-angiotensin system might exert their renoprotective effect by correcting diabetes-induced renal hypoxia.

The objective of this study was to investigate the chronic effect of the ATI1-type 1 receptor blocker (ARB) candesartan, compared to the ACE-inhibitor (ACEI) enalapril on renal tissue oxygenation in type 2 diabetics with microalbuminuria and/or hypertension, using blood oxygenation level-dependent magnetic resonance imaging (BOLD-MRI).

Methods: After a washout period, ten patients (aged 63.8±8.4 yr, BMI 35.0±13.0, 30% women) underwent BOLD-MRI at baseline, after one month of enalapril (20mg/day), and after one month of candesartan (16 mg/day). BOLD-MRI was performed before and after intravenous administration of furosemide.

Four coronal slices were selected, and a modified MEDIC sequence was used to acquire T2* weighted images. The mean R2* values (=1/T2*) were calculated, a low R2* indicating a high tissue oxygenation.

Results: Baseline characteristics and their changes are shown in table 1. The mean cortical and medullary R2* did not differ significantly between groups (ANOVA, p=0.88 and 0.24). Furosemide did not change cortical R2*, and decreased medullary R2* to a 1.7±0.5 (p<0.0001). Furosemide did not change cortical R2*, and decreased medullary R2* to a 1.7±0.5 (p<0.0001).

Candesartan decreased cortical R2* (-1.3±0.3, p<0.0001) and medullary R2* (-1.1±0.4, p=0.0004) compared to enalapril.

Conclusions: Our results suggest that blockers of the renin-angiotensin system might exert their renoprotective effect by correcting diabetes-induced renal hypoxia.

PUB195

Design and Rationale for a Randomized, Double-Blind, Placebo-Controlled Phase 2 Study To Evaluate the Safety and Efficacy of CTP-499 in Patients with Diabetic Nephropathy

Background: CTP-499 is a deuterium-containing analog of 1-((S)-5-hydroxyhexyl)-3,7-dimethylxanthine (HDX), an active metabolite of pentoxifylline (PTX). Small published clinical trials with PTX have shown evidence of beneficial effects in CKD patients. CTP-499 is being investigated as a novel treatment for diabetic nephropathy and whether dual renin-angiotensin system blockade has additional benefits in this population and whether any benefits outweigh the risks.

Methods: Study Design: Systematic review and meta-analysis Setting and Population: Diabetic patients with overt proteinuria

Selection Criteria for Studies: Randomized, controlled, parallel or crossover design studies

Intervention: Combination renin-angiotensin system blockade vs. monotherapy

Outcomes: The primary outcome measure was the post-treatment difference in proteinuria with combination therapy versus monotherapy. Secondary outcomes included percent change in proteinuria, changes in systolic blood pressure, glomerular filtration rate, and serum potassium, and incidence of hyperkalemia. Sensitivity analyses that evaluated differences in outcome based on study quality (assessed by Jadad scores), baseline systolic blood pressure, and drug types and doses were conducted.

Results: There was significantly less proteinuria (by 334 mg/24 hr) after treatment with combination therapy vs. monotherapy. Systolic blood pressure (BP) after treatment with combination therapy vs. monotherapy was significantly lower (by 4.1 mmHg). However, clinically significant hyperkalemia was 3.5-fold more common with dual blockade. Sensitivity analyses did not identify subgroup differences that altered these findings.

Conclusions: Dual renin-angiotensin system blockade in patients with diabetic kidney disease reduces proteinuria and BP but is associated with a higher incidence of clinically significant hyperkalemia. Further studies assessing long-term outcomes are needed to weigh the benefits versus risks of combination renin-angiotensin system inhibitor therapy.

PUB199

Relative Value of Various Mediators of the Renin-Angiotensin System in Diabetic Nephropathy
Shuichi Kaneko,2 Takashi Wada.1

Background: Proteinuria is the most important clinical factor for poor renal outcomes among patients with overt nephropathy. Multivariate analysis by the Cox proportional hazards model was used to evaluate if low vitamin D levels were associated with an increased risk of NAFLD.

Methods: The number of cases of normal albuminuria or urinary protein (-) or (±), microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively. 2) Seventeen patients (36.2%) with normal albuminuria or urinary protein (-) microalbuminuria or urinary protein (+), and overt nephropathy were 47, 50 and 163 respectively.

Conclusions: Our observations suggest that development of albuminuria play an important role for poor renal outcomes of diabetic nephropathy in type 2 diabetes. Lower eGFR was an independent risk factor in renal outcomes among patients with overt nephropathy.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
is expected to be additive to RAS blockade. Deuteronium is a safe, naturally-occurring, non-radioactive isotope of hydrogen. Selective incorporation of deuteronium does not appear to alter the binding, potency or selectivity, but in select cases may provide beneficial effects on compound metabolism. In vitro and ex vivo studies have shown CTP-499 possesses anti-inflammatory, anti-fibrotic and anti-oxidative properties, making it potentially useful in the treatment of diseases such as diabetic nephropathy and CTP-499 reduced urinary protein excretion and improved other renal function markers.

Methods: Design: Approximately 170 patients with type 2 diabetic nephropathy, macroalbuminuria (UACR ≥400 mg/g) and an eGFR ≥25 ml/min/1.73m2 (MDRD), who are on ongoing ACEi and/or ARB therapy, will be enrolled at approx. 30 US centers. After a 4-8 week stabilization period, during which BP will be managed to a target of <140/90 mmHg, patients will be randomized (1:1) to receive 24 weeks of treatment with CTP-499 600 mg bid (600 mg qd for the first 2 weeks), or placebo. UACR will be assessed monthly using the geometric mean of 3 consecutive first morning voids. eGFR will be determined at each visit along with a biomarker panel incl. cytokines and markers of fibrosis and kidney function. Plasma conc. of CTP-499/metabolites will be assessed monthly. In a subset of patients, 24h plasma and urine conc-time profiles will be obtained and the percentage of globally sclerotic glomeruli seen on the biopsy. The manifestation of membranous nephropathy may be seen in lupus nephritis, anti-GBM disease, or ANCA- associated disease. A renal biopsy of reported cases. The presence of fibrinoid necrosis and crescent formation in the setting of membranous nephropathy is also suggestive of this diagnosis. Characterization of the sclerosis seen in the biopsy can help to determine the underlying cause of the nephrotic syndrome (e.g., diabetic nephropathy vs. lupus nephritis).

Results: Primary Endpoint: UACR change from pre-treatment baseline to post-treatment using a longitudinal model with data from weeks 16, 20, and 24. Conclusions: Study enrollment is expected to begin in late 2011; top-line data are expected in 2013.

Funding: Pharmaceutical Company Support

PUB196

A Systematic Review and Meta-Analysis: Impacts of Albuminuria and Low GFR on Cardiovascular Mortality in Patients with Diabetic Nephropathy

Tadashi Toyota,1 Kengo Furuchi,2 Toshiharu Ninomiya,2 Shuichi Kaneko,2 Takashi Wada.1 1Department of Nephrology, Kanazawa University Hospital, Kanazawa, Japan; 2Department of Nephrology, Hypertension, and Strokeology, Kyushu University Hospital, Fukuoka, Japan; 3Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa, Japan.

Background: The relationships between albuminuria and complications of diabetes mellitus have been reported. However consistency and strength of these evidence have not been clearly so far. In this study, we explored the impacts of albuminuria on renal failure, cardiovascular death, and all-cause mortality in diabetes mellitus.

Methods: Relevant studies were identified from MEDLINE, EMBASE, and CINAHL by using relevant text words and medical subject headings that includes all keywords related to "diabetic nephropathy," "renal failure," "cardiovascular diseases," and "mortality." The literature search and data extraction was conducted for three endpoints (renal failure, cardiovascular death, and all-cause mortality) by two independent researchers. The extracted estimates were combined using a random-effects model.

Results: The literature search yielded 6546 articles, of which 328 papers were reviewed in full. In diabetic patients, the presence of microalbuminuria is associated with an approximate 3.5-fold increase in risk of renal failure (relative risk 3.52, 95% CI 2.04-6.05). And there are the same trends in cardiovascular death (relative risk 1.90, 95% CI 1.56-2.32), and all-cause mortality (relative risk 1.62, 95% CI 1.45-1.82). In addition, there are significant trends towards greater effects with macroalbuminuria in each outcome.

Conclusions: These findings suggest that microalbuminuria and macroalbuminuria are risk factors for renal failure, cardiovascular mortality, and all-cause mortality in diabetic patients. Individual patient data meta-analysis is needed in the future.

Time (d) Baseline pre-PEX Post-PEX
FHAA (IU) C3 (mg/dl) C3 (mg/dl) C3Nef (IU)
0 100 42 7.75 10 FFP
6 97 12.2 10 FFP
10 97 12.2 10 FFP
18 97 12.2 10 FFP
22 97 12.2 10 FFP

Conclusions: Herein we share this unique case of DDD where several possible causes of the disease coexist and the response to different treatment modalities.

PUB199

Necrotizing Crescentic Gomlerulonephritis Complicating Membranous Nephropathy: A Rare Coexistence

Sved N. Babar, Richard A. Sherman. Nephrology, Robert Wood Johnson University Hospital, New Brunswick, NJ.

Background: Membranous glomerulonephritis (MG) is among the most common causes of nephrotic syndrome in adults. The majority of MG cases represent primary or idiopathic disease, whereas the remaining cases represent secondary forms related to infection, drugs and malignancy. Necrotizing crescentic glomerulonephritis (NCGN) is characterized by glomerular necrosis and crescent formation. One half of NCGN is associated with SLE, Goodpasture’s or Wegener’s while the other half is idiopathic. We present a case demonstrating the rare coexistence of MG and NCGN.

Methods: A 38 year old male with biopsy proven MG maintained initially on prednisone and mycophenolate mofetil and later cyclosporine was noted to have worsening lower extremity swelling and fatigue two years after his diagnosis. Labs revealed worsening necrotic range proteinuria and azotemia along with positive p-ANCA and MPO Abs. Serologies for lupus and anti-GBM disease were negative. Repeat renal biopsy revealed NGCN superimposed on MG with segmental and global sclerosis. Aggressive salvage therapy with pulse methylprednisolone and IV cyclophosphamide did not improve renal function. Four months after starting hemodialysis he received a kidney transplant. A year and a half post-transplant, he remains stable with a creatinine of 1.5 mg/dl.

Results: The concurrence of MG and NCGN is a rare phenomenon with only a handful of reported cases. The presence of fibrinoid necrosis and crescent formation in the setting of membranous nephropathy may be seen in lupus nephritis, anti-GBM disease, or ANCA-associated RPGN. Renal biopsy is required to make the diagnosis and treatment typically involves steroids with cyclophosphamide. The outcomes vary and generally correlate with the percentage of globally sclerotic glomeruli seen on the biopsy. The manifestation of both membranous nephropathy and necrotizing crescentic glomerulonephritis likely represents a coincidental happening given their distinct pathogenesis.

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890A
Tsilivigou, Gabriel Papadakis. Collected regarding their ANCA serology, overall survival and renal survival. Validated this classification in 35 patients with renal vasculitis. Demonstrated good correlation with histology class of AASV and renal outcomes at 1 and active vasculitis. Validation of Berden histological classification of AASV in 100 patients. 48% of the mortality was due to infections in the first year and 19% mortality due to drug abuse, chronic HCV infection and renal failure of undetermined origin who presented with a relapse of nephrotic syndrome due to MN; pt 1, 18 years after the initial diagnosis (treated with IV cyclophosphamide); pt 2, 3 years after initial diagnosis (treated with Rituximab, Mycophenolate Mofetil and Tacrolimus).

Conclusions: This prevalence of malignancy in this study is lower (5%) than some previous reports (7-16% in all patients with MN and >50% in pts <60 yrs.). Interestingly the 2 cases of malignancy in our study were in pts presenting with a relapse of their MN. Others have reported an increase in malignancy in pts with MN in the years post presentation. The contribution of malignancy to the pathogenesis of MN and the role of immunosuppressive treatment for MN in the development of malignancy is not yet clearly defined. Surveillance is required not only at diagnosis but also during follow up. The association of MN with infection and malignancy needs to be taken into account when considering treatment with immunosuppressive therapy.

**PUB202**

**Study of Malignancy and Chronic Infections in Patients with Biopsy Proven Membranous Nephropathy**

Marie B. Condon, Tom Cairns, Megan Griffith.

Imperial College NHS Trust, United Kingdom.

**Background:** Membranous nephropathy (MN) is a common cause of nephrotic syndrome in adults. Most cases of MN are idiopathic, however historical studies have shown MN can also occur in association with malignancy or chronic infections. This study investigated the prevalence of these secondary causes of MN in all patients presenting to this institution with nephrotic syndrome (NS) as a consequence of MN from March 2008.

**Methods:** Forty-one pts presented with biopsy proven MN, 24 male, 17 female. 37 de novo presentations and 4 relapses of NS, at median time of 6 years post 1st presentation (3-18). The median age was 54 yrs (25-80), 10/41 were aged >60. 18 Caucasian, 18 Asian and 5 African/Caribbean. Blood was tested for hepatitis B, C and HIV. Pts were screened for malignancy with CT chest/abdomen/pelvis, prostate specific antigen in males and underwent endoscopic investigations if any gastro-intestinal symptoms were present.

**Results:** Three pts had asymptomatic chronic infections: 1 Hep B, 1 HIV and 1 pulmonary TB detected on CT scan. 2/4 (51%) had solid tumours. Pt 1 (male, 74 yrs) had an asymptomatic lung malignancy, detected on CT scan. Pt 2 (male, 72 yrs) a colonic adenocarcinoma on colonoscopy performed for bowel symptoms. Both pts were presenting with a relapse of nephrotic syndrome due to MN; pt 1, 18 years after the initial diagnosis (treated with IV cyclophosphamide); pt 2, 3 years after initial diagnosis (treated with Rituximab, Mycophenolate Mofetil and Tacrolimus).

**Conclusions:** This prevalence of malignancy in this study is lower (5%) than some previous reports (7-16% in all patients with MN and >50% in pts <60 yrs.). Interestingly the 2 cases of malignancy in our study were in pts presenting with a relapse of their MN. Others have reported an increase in malignancy in pts with MN in the years post presentation. The contribution of malignancy to the pathogenesis of MN and the role of immunosuppressive treatment for MN in the development of malignancy is not yet clearly defined. Surveillance is required not only at diagnosis but also during follow up. The association of MN with infection and malignancy needs to be taken into account when considering treatment with immunosuppressive therapy.

**PUB203**

**A European Network for IgA Nephropathy Focused on the Validation of the Oxford Clinicopathology Classification**

Rosanna Coppo, Laura Morando, John Feehally, H. Terence Cook, Stephan Troyanov, Daniel C. Catran, Ian Roberts, for the VALIGA European Immunonephology working group, R. Margherita H.

**Background:** A recent publication from an International Consensus - based on a retrospective analysis of 265 patients with IgA Nephropathy (IgAN) from 4 continents - focused on diagnostic criteria provided by renal biopsy. According to this Oxford Classification of IgAN, mesangial hypercellularity, segmental glomerulosclerosis, endocapillary hypercellularity and tubular atrophy/intertubular fibrosis predict renal outcome independently from all clinical indicators at the time of biopsy and during follow up (KI 2008;76:534-45; and 2009;76:546-56). The number of patients and their heterogeneous origin indicated a need for validation studies involving large cohorts of patients. Some validation studies have now been published, but these are of limited size (273 cases in a Korean study, 187 in a study from USA).

**Methods:** To validate the Oxford classification in Europe, the ERA-EDTA scientific community was invited to participate in this study, as a new model for researches needing large integration and collaboration, aiming to enrol at least 500 European patients with IgAN.

**Results:** We have presently enrolled 674 IgAN patients with long follow-up or rapidly progressive course from 29 nephrology and renal pathology centers in 10 European Countries: Italy (14 centers); Spain (3 centers); Turkey, Greece, Sweden Netherlands(2 centers each); Germany, Croatia, Czech Republic, Poland (1 Center each). Renal biopsies have been scored by the local pathologist and are under central review in Oxford, UK. Clinical data at renal biopsy and during follow up were provided by local nephrologists to the Coordinating Center. 481 further cases are being checked for completeness of clinical data and their biopsies are under review.

**Conclusions:** Networking is the only possible way to obtain sufficient case enrolment in studies of rare rare conditions, such as glomerular disease. This European Network, now established, is open to further research opportunities, for example genetic studies, and the development of clinical trials. This presentation at the ASN is aimed at encouraging future scientific networks.

**PUB204**

**Epidemiology of Patients with Pulmonary Haemorrhage from Anti-Neutrophil Cytoplasmic Antibody Associated Vasculitis Admitted to the Intensive Care Unit**

Partha Das, Scott R. Henderson, Maria Ostermann.

Department of Nephrology and Transplantation, Guy’s & St Thomas’ Hospitals NHS Foundation Trust, London, United Kingdom.

**Background:** Anti-neutrophil cytoplasmic antibody (ANCA) associated vasculitides are associated with significant morbidity and mortality despite advances in diagnosis and immunosuppressive treatment. Pulmonary haemorrhage is a much feared and life-threatening manifestation of the disease and may lead to admission to the Intensive Care Unit (ICU). Our aim was to describe the epidemiology of patients with pulmonary haemorrhage admitted to the ICU in a tertiary care teaching hospital.
Methods: A retrospective case note review was performed on all patients with pulmonary haemorrhage due to new or previously diagnosed ANCA associated vasculitis admitted to ICU between 2008-2011 for pulmonary haemorrhage. Demographics, disease duration and previous treatments were recorded alongside reason for ICU admission, APACHE II and SOFA scores, level of organ support, immunosuppression and patient mortality.

Results: 11 patients (9 men, mean age 56.2 years ± 16.1, 10 Caucasian, 1 mixed race) were admitted to the ICU with pulmonary haemorrhage. This was the first presentation for 8 patients. Proteinase 3 (PR3) ANCA was positive in 8 patients, myeloperoxidase (MPO) ANCA in 1 patient, and 2 patients had no detectable ANCA. 45% of patients had received immunosuppressive treatment prior to admission in the preceding 3 months. Patients were managed by a multidisciplinary team. 63% of patients had invasive ventilation, 54% had renal replacement therapy, and 54% were treated for sepsis. 8 patients (72.7%) received steroids and cyclosporophamide treatment and 7 patients (63%) had plasmapheresis. Mean length of stay in ICU was 13 days ± 0.5 and in hospital 29 days ± 20 days. 2 patients (18%) died in ICU due to bowel ischaemia. 1-year mortality of ICU survivors was 100%.

Conclusions: Outcome of patients with vasculitis associated pulmonary haemorrhage in ICU is good, even when significant organ support, immunosuppressive therapy and plasma exchange are required.

PUB205

Child with Plasma Resistant Atypical HUS Escaped End Stage Renal Failure by Eculizumab Therapy

Eiske Dorresteijn, 1 Nicole Van De Kar, 2 Karlien Cramsberg. 1 Pediatric Nephrology, Erasmusme-Sophia, Rotterdam, 2Pediatric Nephrology, UMC-St.Radboud, Nijmegen.

Background: Recently, eculizumab, a humanized monoclonal antibody against C5 that blocks the terminal complement pathway, is reported to be effective in the treatment of atypical haemolytic urticemic syndrome (aHUS). Methods: We report the successful treatment of plasma resistant aHUS with eculizumab.

Results: A 6-year-old girl developed aHUS with severe renal insufficiency and hypertension. Plasmapheresis was immediately started, after which serum creatinine levels returned to almost normal after 3 weeks. Then, however, aHUS recurred shortly after an upper respiratory tract infection with influenza B virus. Her platelet count normalised after immediate intensive plasmapheresis, but the renal failure progressed, necessitating dialysis. C3 and C4 serum complement levels at the first presentation were normal, but C3d was elevated by 4.4%. Complement factors H, I, B, C3 or MCP showed no mutations. No other possible cause of aHUS was found. As end stage renal failure rapidly developed despite aggressive plasma therapy, plasmapheresis was stopped and treatment with eculizumab was initiated. Thereupon the girl’s renal function promptly improved; after 8 weeks’ therapy the estimated creatinine clearance had increased from 12 to 70 ml/min.1.73m2. The multiple anti-hypertensive treatment could be tapered to monotherapy with ACE inhibition. Four months after initiating eculizumab treatment, her renal function was stable at an estimated creatinine clearance of 85 ml/min.1.73m2 with therapy every other week.

Conclusions: This case of aHUS shows that timely started, intensified plasmapheresis restored the haematological parameters, but could not restore kidney function. However, the change to eculizumab improved kidney function without the need of dialysis. Treatment with complement inhibitors might well be promising for patients with aHUS.

Funding: Pharmaceutical Company Support

PUB206

Case Report: Thrombotic Thrombocytopenic Purpura (TTP) like Syndrome in the Setting of Systemic Lupus Erythematosus (SLE) (Kawasaki Disease in the setting of Systemic Lupus Erythematosus (SLE))

Kawasaki Disease (KD) is a rare inflammatory vasculitis affecting children. KD is characterized by a constellation of findings including; fever, erythema of the lips and oral mucosa, bilateral conjunctivitis, rash, cervical lymphadenopathy, and non-bloody diarrhea. It is a self-limited disease with a variable clinical course. The management of KD is supportive care and inhibition of the complement pathway. The long-term outcome of KD includes persistent vascular lesions, aneurysms, and stenoses. The most common late complication is coronary artery disease. We report a unique case of KD presenting as Kawasaki Disease in the setting of Systemic Lupus Erythematosus (SLE) in a child.

Case: A 6-year-old girl developed aHUS with severe renal insufficiency and hypertension. Plasmapheresis was immediately started, after which serum creatinine levels returned to almost normal after 3 weeks. Then, however, aHUS recurred shortly after an upper respiratory tract infection with influenza B virus. Her platelet count normalised after immediate intensive plasmapheresis, but the renal failure progressed, necessitating dialysis. C3 and C4 serum complement levels at the first presentation were normal, but C3d was elevated by 4.4%. Complement factors H, I, B, C3 or MCP showed no mutations. No other possible cause of aHUS was found. As end stage renal failure rapidly developed despite aggressive plasma therapy, plasmapheresis was stopped and treatment with eculizumab was initiated. Thereupon the girl’s renal function promptly improved; after 8 weeks’ therapy the estimated creatinine clearance had increased from 12 to 70 ml/min.1.73m2. The multiple anti-hypertensive treatment could be tapered to monotherapy with ACE inhibition. Four months after initiating eculizumab treatment, her renal function was stable at an estimated creatinine clearance of 85 ml/min.1.73m2 with therapy every other week.

Conclusions: This case of aHUS shows that timely started, intensified plasmapheresis restored the haematological parameters, but could not restore kidney function. However, the change to eculizumab improved kidney function without the need of dialysis. Treatment with complement inhibitors might well be promising for patients with aHUS.

Funding: Pharmaceutical Company Support

PUB207

Microscopic Polyangiitis: Case Report in Coexistence with Systemic Lupus Erythematosus Nephritis

Vince Faridani, Ahmed M. Awad, Adil Akthar. Nephrology, University of Missouri - Kansas City, Kansas City, MO.

Background: We report a unique case of microscopic polyangiitis (MPA) manifesting as a renal-pulmonary syndrome in the setting of systemic lupus erythematosus (SLE) nephritis.

Case: A 66-year-old female with a history of biopsy confirmed Class III SLE nephritis, presented with shortness of breath. On examination, right lower extremity revealed a 5x8 cm erythematous, painful, palpable purpura. Notable serum studies were creatinine 1.84 mg/dL, Hb 12.8 g/dL. Urinalysis was positive for a large amount of blood. CT chest demonstrated patchy bilateral infiltrates. Bronchoscopy showed diffuse alveolar hemorrhage and endoscopy revealed diffuse erosive gastritis. Immune serology was negative for anti-dsDNA, anti-GBM antibodies, MPO and PR3. She was treated with oral prednisone and mycophenolate mofetil. The patient underwent 11 consecutive treatments of plasma exchange along with 1 gram IV cyclophosphamide and 1 gram daily methylprednisolone and made a full recovery.

Discussion: This is the first reported case of MPA in the setting of established SLE nephritis. Our case exemplifies the importance of a methodical approach to an extensive list of differential diagnoses. The main clinic clues to the alternative diagnosis of renal-pulmonary syndrome were diffuse alveolar hemorrhage and erosive gastritis. A positive pANCA and high anti-MPO titers without upper respiratory involvement strongly supports our diagnosis.

PUB208

Systemic Lupus Erythematosus Masking Pre-Eclampsia

Vince Faridani, Ahmed M. Awad, Adil Akthar. Nephrology, University of Missouri - Kansas City, Kansas City, MO.

Background: Systemic lupus erythematosus (SLE) is one of the most common autoimmune disorders that affect women during their childbearing years. We report a unique case of pregnancy induced SLE nephritis presenting as pre-eclampsia.

Case: A 27-year-old African American woman, with no previous medical illnesses presents seven days status post cesarian section at 29 weeks of gestation due to worsening pre-eclampsia. She was admitted with shortness of breath and uncontrolled hypertension. Vitalis, blood pressure 173/123, pulse rate 106, oxygenation 92% on 2 liters nasal cannula. Physical exam revealed, diffuse scattered ronchi bilaterally, 2+ lower extremity edema bilaterally. Labs revealed, hemoglobin 7.2, hematocrit 22, WBC 15, Platelets 619, creatinine 1.4, albumin 1.4, positive ANA, normal C3 and C4 level, all other serologies were negative. Urine analysis was positive for RBC 12, moderate hemoglobin, protein > 300. CT chest done was suggestive of pulmonary hemorrhage which was confirmed by bronchoscopy. Percutaneous renal biopsy showed type V lupus nephritis, diffuse proliferative and membranous lupus nephritis. She was treated with methylprednisolone 2 grams for 3 days, followed by oral prednisone and mycophenolate mofetil. The patient was followed up at 2 months and 4 months at which time her creatinine returned to her baseline and she was asymptomatic.

Discussion: Pregnancies for patients with lupus have a greater risk of fetal loss, pre-eclampsia, intrauterine growth retardation, and neonatal lupus syndrome. Thus, it is important to do a thorough assessment in patients with new onset of gestational proteinuria not to delay delivery. Our patient’s course was consistent with pre eclampsia and the renal biopsy confirmed the diagnosis of lupus which explained her multisystemic involvement. Clinicians should be cautious and consider SLE nephritis when treating pre-eclampsia whom present with new onset proteinuria and hypertension.

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PUB209
Procalcitonin – A Good Predictor for Persisting Kidney Damage in Patients with Goodpasture’s Syndrome? Susanne V. Fleig, Jan T. Kielstein. Nephrology and Hypertension, Hannover Medical School, Hannover, Germany.

Background: Autoantibodies to the NC-1 domain of the type IV collagen’s α3 chain are the pathogenetic basis for anti glomerular basement membrane (GBM) disease, otherwise known as Goodpasture’s syndrome. It involves both the kidneys and lungs to varying degrees. Diagnosis of the disease is difficult. Procalcitonin (PCT) has been proposed as a parameter of potential prognostic value. Based on a small group of patients (n=7), high procalcitonin (PCT) had been proposed as a predictor of poor renal outcome.

Methods: We report on a case demonstrating a severe manifestation of Goodpasture’s syndrome, in which a high PCT on admission was unfortunately not associated with detrimental renal outcome.

Results: Apart from dialysis, high intensity immunosuppressive therapy was begun immediately after admission. A biopsy of high PCT. Our patient recovered to full renal function and remains free of symptoms two years after follow-up. In contrast, a further three cases observed during 2010 presenting with acute renal failure and low procalcitonin remained dialysis dependent.

Conclusions: Our data do not confirm the notion that high procalcitonin is a good prognostic factor for renal outcome in Goodpasture’s syndrome. Causal immunosuppressive therapy should not be delayed because of high PCT.

PUB210

Background: The importance of aldosterone (ALDO) and the sympathetic nervous system (SNS) in mediating sodium retention in NS has been questioned. A: 63 y/o woman, healthy except for hypertension treated with metoprolol and lisinopril, presented with renal salt wasting despite nephritic range proteinuria (NP) from membranous nephropathy. Serial clinical exams and lab tests were performed. Autoantibodies function was assessed by respiratory variation in heart rate (RVHR) and by changes in BP and pulse with the Valsalva maneuver and orthostasis.

Methods: Two months earlier she had acute bronchitis requiring ibuprofen for chest pain. She subsequently developed incapacitating orthostatic hypotension, requiring recurrent admissions for intravenous saline during which NP was discovered. On transfer to us she had no edema and jugular venous pressure was undetectable. She was too dizzy to sit or stand. Her urine protein/creatinine ratio=8.3 gr/g. Plasma creatinine=1.0 mg/dl and BUN=84 mg/dl. Serum electrolytes were normal. After 2 liters of intravenous saline she felt better. No further saline was administered and a day later she had recurrent orthostatic hypotension and tachycardia (supine BP 134/85, pulse 80/minute; standing BP 117/77, pulse 125/minute) with a urinalysis of 91 mEq/L. Plasma ALDO was undetectable and renin=0.6 mIU/ml. Treatment with furosemide and oral salt led to edema, supine BP≈160/110 and urine protein/creatinine ratio≈25.3 gr/g and was stopped. Cyclic sporamine with a burst and taper of prednisone led to complete remission of her proteinuria, which has not recurred. Following remission, she continued to have profound orthostatic hypotension but without tachycardia and persistent hyporeninemic hypoaldosteronism (24 hour urine ALDO=undetectable).

Conclusions: This patient developed PAF (possibly from a viral infection) with associated hyporeninemic hypoaldosteronism and renal salt wasting despite the presence of NP from membranous GN. Hence, ALDO and the SNS are the required for the renal sodium retention of NS.

PUB211
Complete Remission Is Severe Lupus Nephritis: Assessing the Rate of Loss in Proteinuria Stephen M. Korbet, Edmund J. Lewis, The Collaborative Study Group. Internal Medicine, Rash University Medical Center, Chicago, IL.

Background: The prognosis of severe lupus nephritis (SLN) is improved in pts attaining complete remission (CR) to a degree that a trend to remission ranges from 10-16 mos with many pts not attaining a CR until after 12 mos. We assess whether the rate of loss of proteinuria (UPro) at 3 and 6 mos is predictive of a CR in SLN pts.

Results: Among 125 patients treated in SLN (NEJM 1992). All pts had ISN/RPS class IV ± class V lesions. All pts received pulse cytoxan was held. Apart from dialysis, high intensity immunosuppressive therapy was begun immediately after admission in spite of high PCT. Our patient recovered to full renal function and remains free of symptoms after two years of follow-up. In contrast, a further three cases observed during 2010 presenting with acute renal failure and low procalcitonin remained dialysis dependent.

Conclusions: Our data do not confirm the notion that high procalcitonin is a good prognostic factor for renal outcome in Goodpasture’s syndrome. Causal immunosuppressive therapy should not be delayed because of high PCT.

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Patient had RPGN III with P-ANCA vasculitis featuring medium and large vessel involvement with an extensive systemic involvement. In addition, he had active systemic lupus erythematosus (SLE). His clinical features of SLE: polyarthritis, pericarditis, pleuritis, anemia, ANA(+) (10%). The kidney biopsy revealed pauci-immune GN with crescents without IC deposits. She originally responded to pulse therapy. Subsequently septic course preceded further immunosuppression, she became HD dependent.

Conclusions: PA-ANCA RPGN can be present with SLE concurrently. This mixed seropositivity should be further investigated. Multiple cases have been reported in the literature. Since RPGN progresses rapidly, it is important to be aggressive in diagnosing and treating it. Sometimes initial symptoms can be non-specific like arthralgia/painless hematuria, which is why it is paramount for clinicians to always suspect RPGN in a setting as such.

Funding: Clinical Revenue Support

**PB214**

Clinicopathological Findings, Long-Term Mortality and Renal Outcome in Japanese Patients with Idiopathic Focal Segmental Glomerulosclerosis Taito Miyake, 1 Shinsuke Kitajima, 1 Yasuaki Shinozaki, 1 Tadashi Tadaya, 1 Akinori Hara, 1 Kyoko Kitagawa, 1 Kengo Furuchi, 1 Hitoshi Yokoyama, 2 Shuichi Kaneko, 1 Takashi Wada. 1

1. Division of Nephrology, Kanazawa University Hospital, Kanazawa, Ishikawa, Japan; 2. Department of Disease Control and Homeostasis, Kanazawa University, Kanazawa, Ishikawa, Japan.

Background: The 20-year renal survival of idiopathic focal segmental glomerulosclerosis (IFSGS) was reported around 30% in Japan. In this study, we examined clinicopathological findings, long-term mortality and renal outcome of IFSGS.

Methods: Forty seven Japanese patients (34 males and 13 females; mean age 39.0 years, 29.9 years (range 9.0 months to 90.0 months) with biopsy proven IFSGS from 1961 to 2010 in Kanazawa University Hospital were examined in this study. The patients were followed for more than one year, or until renal or patient death. Clinicopathological findings were evaluated for renal and patient death.

Results: Twelve renal death (25.5%, 9 males and 3 females) and 12 patient death (25.5%, 9 males and 3 females) were observed. Age of onset was higher in the group of patient death. The percentage of nephrotic syndrome out of all patients was 95.7%. Thirtly out of 45 nephrotic patients (66.7%) achieved complete remission (CR) or incomplete remission (ICR) (proteinuria <1 g/day) after initial treatment within 6 months, and only one renal death was observed. We treated 46 patients with corticosteroids, and 28 patients with additional immunosuppressive drugs (cyclophosphamide, 36%; cyclosporine, 64%). Sixteen out of 18 patients with additional cyclosporine treatment were achieved CR or ICR, and only one renal death was observed. Regarding pathological findings, percentage of glomerulosclerosis was higher in group of renal death. The pathologic variants were as follows; Perihilar, 13%; Cellular, 15%; Tip, 36%; and not other specified (NOS), 38%. Five renal deaths were observed in 6 patients with perihilar variant.

Conclusions: The good response of initial treatment within 6 months, and cyclosporine may be favorable prognostic factors.

**PB215**

Clinicopathological Study of the Possible Relation between Hepatitis C Virus Infection and Nephrotic Syndrome in Children Arwa Nada, 1 Morris J. Schoeneman, 1 Mona Salem, 1 Mahmoud El-Kersh, 2

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Background: Glomerulonephritis (GN) is one of the major consequences of HCV infection in adults. Neither the course of nephrotic syndrome (NS) in children with HCV infection nor the effect of interferon therapy were studied thoroughly in children. Objective: To study the possible clinicopathological relation between HCV infection and cases of NS in children who are sterile or dependent.

Methods: Sixty children were found to be HCV positive out of 378 who presented with NS. Thirty children at Alexandria Children’s Hospital, Egypt, 20children were sterile or dependent on high dose corticosteroids. Those twenty patients were subjected to comprehensive biochemical, histological, virological and immunological investigations. RT-PCR to detect HCV RNA was performed in the kidney biopsy. The pattern of steroid responsiveness was studied before and after HCV infection. Interferon and ribavirin therapy was given to 9 children with assessment of proteinuria before and during therapy.

Results: FSFG accounted for 50% of the patients, MPGN accounted for 20%, 5% each for the pattern of MCD and IgMN, and 5% for mixed mesangiproliferative GN, and 10% inadequate renal biopsies. None of the patients showed clinical or laboratory manifestations of cryoglobulinemia. RT-PCR for HCV RNA was positive in 80% of children. Significant worsening was observed in the pattern of steroid responsiveness after HCV infection (p<0.001). Significant reduction of proteinuria was observed in the 9 children who received therapy with interferon and ribavirin (p=0.004).

Conclusions: HCV infection worsened the course of NS in children based on detecting the virus RNA in the renal biopsy, increased steroid requirements after HCV infection and the significant reduction of proteinuria after treatment with interferon and ribavirin.

Funding: Government Support - Non-U.S.
PUB218
Serum Interleukin 18 Levels Are Closely Associated with the Progression of IgA Nephropathy
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Background: IgA nephropathy (IgAN) was once thought to be benign but recently found to be slowly progressive. The aim of this research is to investigate the value of serum IL-18, a biomarker for tubule injury, for assessing the disease progression in IgAN patients.

Methods: Serum IL-18 in patients with IgAN and healthy controls were measured by ELISA. Percentage of global glomerular sclerosis (GGS) and extent of tubulointerstitial damage (TID) were semiquantitatively estimated. Deterioration in renal function was defined as a 50% increase of serum creatinine than baseline.

Results: 36 patients (19 females, 37.9±9.7 years) were enrolled and presented with 2.6 (1.7-3.1) g/day proteinuria. Compared with controls, serum IL-18 levels were significantly elevated in IgAN (360.3±25.2 vs 51.2±8.9 pg/ml, P<0.01). Univariate analysis showed that albumin (r=-0.395, P=0.001), proteinuria (r=-0.494, P=0.002), Serum creatinine (r=0.61, P<0.001), and eGFR (r=-0.598, P=0.001) were significantly correlated with IL-18 levels. TID scores showed a borderline significance with serum IL-18 levels (r=-0.355, P=0.057), whereas GGS did not. During 38 (12-60) months of follow-up, 14 patients (38.8%) had a deteriorated renal function. Kaplan-Meier analysis found TID scores showed a borderline significance with serum IL-18 (r=-0.108, P=0.045). Cox analysis further confirmed that serum IL-18 levels were independent predictor of renal outcome (β=0.098, 95%CI:0.926-1.042, P<0.001).

Conclusions: Elevation of serum IL-18 levels may be served as an early biomarker of predicting renal disease progression in patients with IgAN.

PUB219
Intravenous Corticosteroid Pulse Therapy Predicts the Remission of Proteinuria in Patients with Minimal Change Nephrotic Syndrome, along with Serum Creatinine and Age
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Hiromi Rakugi,1 Yoshitaka Isaka.1 2
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Background: Little information is available about predictors of effectiveness of corticosteroid in adult patients with MCNS.

Methods: In the present multicenter retrospective cohort study in 3 major nephrology centers, eligible patients were 75 patients aged ≥15 yr who were diagnosed as new-onset primary MCNS by kidney biopsy between 2000 and 2009. The outcome was time from initiation of corticosteroid to complete remission of proteinuria (CR), defined as urinary protein <0.3 g/day, urinal protein/creatinine ratio (UPCR) <0.3, or negative/trace for U/P by dipstick. Predictors of CR were identified using log-rank test and multivariate Cox proportional hazard (CPIH) models.

Results: Baseline characteristics; age 38 (20-61) yr median (interquartile range), male 45(60%), serum creatinine (Cr) 0.9 (0.7-1.3) mg/dL, UPCR 10.8 (5.8-19.9). No significant difference was observed in baseline characteristics of 26 patients with intravenous methylprednisolone pulse (mPSL) and 49 patients with prednisolone (PSL), except serum albumin (1.7±0.6 vs. 2.0±0.5 g/dL, P=0.015) and UPCR (6.2 (3.5-15.8) vs. 12.9 (8.4-19.9)). The cumulative probability of CR was 5.5 (3.0-9.5) yr of observation period, mPSL patients had significantly higher cumulative probability of CR, compared with PSL patients (time to CR, 10 (7-22) vs. 22 (9-44) days, P=0.023 for log-rank test). Use of mPSL (HR 1.93 [95%CI 1.11-3.34], P=0.020) was identified as predictor in multivariate CPIH models adjusting for clinically relevant factors, besides age (per 10 yr 0.84 [0.73-0.96], P=0.012 and log(Cr) (0.22 [0.06-0.73], P=0.013).

Conclusions: The present study, based on one of the largest MCNS cohort in the world, identified intravenous corticosteroid pulse therapy as a predictor of CR in patients with MCNS, along with serum creatinine and age.

PUB220
Treatment of Hepatitis B Associated IgA Nephropathy
In O Sun, Yu Ah Hong, Hyun Gyu Kim, Sun Ryong Choi, Byung Ha Chung, Cheol Wook Park, Chul Woo Yang, Yong-Soo Kim, Bumsoo Choi. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: A strong association between IgA nephropathy (IgAN) and hepatitis B surface (HBs) antigenemia has been reported in endemic regions. However, there are few data about treatment of HBV-associated IgAN. We describe our experiences about treatment of IgAN with HBsAg.

Methods: From 1996 to 2010, biopsy-proven IgAN was diagnosed in 498 patients and 22 (4.4%) had HBsAg. We evaluated the clinical feature and therapeutic responses of IgAN with HBsAg.

Results: The patients included 16 men and 6 women, with a mean age of 37 years (range, 18-56). All were seropositive for HBsAg, and hepatitis e antigen (HBeAg) or circulating HBV DNA was detected in nine patients (41%). The mean follow-up duration was 77 months (range, 14-160). Out of 22, two patients (9%) developed end-stage renal disease requiring hemodialysis during follow-up. Comparing the patients (N=9) with HBsAg or circulating HBV DNA and those (N=13) without, there were no significant differences in the changes of estimated GFR, urinary protein excretion and renal survival between two groups during follow-up. Six out of nine patients with HBsAg or HBV DNA were treated with anti-viral agents, but the treatment didn’t show any benefits on the clinical outcomes in comparison with patients not treated with. Five patients were treated with steroid. Out of 5 with steroid therapy, 2 had HBsAg or HBV DNA, whereas 3 didn’t have. During steroid treatment, no hepatic flare occurred in three patients who didn’t have HBsAg or HBV DNA despite no anti-viral therapy. But one patient out of 2 with HBV DNA experienced active viral replication when the patient stopped receiving lamivudine during follow-up. On the other hand, one patient who had maintained entecavir consistently had no hepatic flare.

Conclusions: There were no differences of clinical outcomes between the patients with HBsAg or HBV DNA and those without. However, when the patient with HBsAg or HBV DNA should receive immunosuppressant including steroid, it is necessary to combine anti-viral therapy to prevent viral replication.

PUB221
A Case Report of Chronic Osteomyelitis Causing Secondary Renal Amyloidosis

Background: Secondary Amyloidosis is usually seen in diseases of chronic inflammation such as Rheumatoid Arthritis, Inflammatory Bowel Disease, Bronchectasis and Familial Mediterranean Fever. It is rarely reported in patients with chronic osteomyelitis.

Results: A 50 year old Hispanic female was admitted for right lower extremity swelling secondary to cellulitis. Her medical history includes spinal cord tumor resection at the age of 27 years with residual paraplegia. Over past few years, patient developed a sacral decubitus ulcer and chronic osteomyelitis. On the other hand, one patient who had maintained entecavir consistently had no hepatic flare.

Conclusions: There were no differences of clinical outcomes between the patients with HBsAg or HBV DNA and those without. However, when the patient with HBsAg or HBV DNA should receive immunosuppressant including steroid, it is necessary to combine anti-viral therapy to prevent viral replication.

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Conclusions: There were no differences of clinical outcomes between the patients with HBsAg or HBV DNA and those without. However, when the patient with HBsAg or HBV DNA should receive immunosuppressant including steroid, it is necessary to combine anti-viral therapy to prevent viral replication.
Mycophenolate Mofetil Rescue Therapy in Primary Glomerulonephritis with Chronic Renal Injury

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Background: The role of mycophenolate mofetil (MMF) in chronic kidney disease (CKD) is not conclusion. MMF may be an effectively therapy for chronic renal injury with active lesions.

Methods: During 2005-2011. 5 patients with CKD-III-IV stage were treated by MMF. Renal biopsy proved the diagnosis were IgA nephropathy (IeV-Istage) in 4 cases, and proliferative sclerosis glomerulonephritis in 1 case. The kidney pathological changes in the 5 patients were characterized by glomerular sclerosis by 10.34% to 56%, respectively; adhesion of glomeruli and Bowman’s capsule in 4 cases; proximal tubular atrophy by 10% to 70%, respectively; 4 patients shown cellular and/or segmental crescent by 6.25%, 6.9%, 17.65%, 25%, respectively, and diffuse mesangial proliferation. The inflammatory cell infiltration in vascular loop cavity were seen in 2 cases; 4 patients presented with tubulitis; multiple foci or diffuse interstitial infiltration of inflammatory cells in all 5 patients, and inflammatory cells in peritubular capillaries cavity were visible in 2 patients. Immunofluorescence IgA and C3 deposited 3-4+ as mass shape in mesangial region in 4 and 5 cases, respectively. According to the renal pathological activity targets, 5 patients were treated with oral MMF (0.75-1.0g/d).

Results: All patients were followed up 22.7±28.31 months. The duration of MMF therapy was 6.2±1.59 (range 3-10) months. The average serum creatinine (Scr) level at renal biopsy was 247.4±62.19 (range 178-522) μmol/L, compared with the last Scr level after treatment (147.6±39.07, range 65-191 μmol/L), P<0.01. The mean eGFR before treatment was 30.17±8.55 (range 23.5-50.0-3.0) ml/min/1.73m², related to the last eGFR after treatment (53.87±19.36, range 38.98-86.31) ml/min/1.73m², P=0.035. The Scr level in 3 patients decreased to normal at 1-3 months after treatment. Scr levels decreased to less than 200 μmol/L at 1.5 and 6 months after treatment in the other 2 cases.

Conclusions: This small sample study showed MMF therapy may effectively save kidney function and delay renal failure in primary glomerulonephritis with chronic renal injury and active lesions.

Histopathologic Classification of Glomerular Lesions in ANCA-Associated Glomerulonephritis in Children: A Single Center Experience

Adam Dartmouth Hitchcock Medical Center, Lebanon, NH.

Background: There are few reports of outcome of antineutrophil cytoplasmatic antibody (ANCA)-associated glomerulonephritis (GN) in the pediatric population, in particular regarding the classification schema by Berden et al, which found an independent predictor of outcome in a cohort of 100 adults.

Methods: We performed a retrospective analysis of all 5 children, age 7 to 13, with ANCA-associated GN at a Northern New England center over the past 10 years. We compared clinical outcomes to Berden’s schema that distinguishes between 4 classes of ANCA-associated GN based on the appearance of glomeruli: Focal (F), crescentic (C), mixed (M) and sclerotic (S).

Results: All biopsies were categorized: 3 as class C; 2 as class M; none as class F or S. Biopsies had a mean of 12.4 glomeruli per sample. Patients were followed for 34.4 months. All biopsies were performed exclusively by Renal Fellows under real-time ultrasound (US) visualization within a framework of structured US-PKB training course. Data was analyzed with PAWS Statistics 18 and the results expressed as either percents or means with standard deviation (±SD).

Conclusions: Based on clinical presentation with weakness, night sweats, anemia, splenomegaly, and workup showing lymphoplasmacytic infiltration of bone marrow, presence of paraprotein heavy chains γ, secondary immunodeficiency and kidney amyloidosis with positive immunofluorescence for IgG, the patient was diagnosed with Heavy chain disease γ (Franklin disease) with AH-amyloidosis and treated with rituximab bortezomib bexarotene.

AH-Amyloidosis Related to Franklin Disease

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Background: Firstly described in 1990, AH-amyloidosis is exceptional condition, associated with plasma cell/B-cell lymphoproliferative disorders, with only few cases derived from heavy chains μ and γ thus far reported.

Methods: 45 y.o. Caucasian female presented with weakness, night sweats, mild anemia (Hb 10.4 g/dl), proteinuria (1.2 g/day) and splenomegaly. We performed kidney biopsy, serum and urine protein electrophoresis, immunoelectrophoresis, immunofixation electrophoresis, and bone marrow biopsy with immunohistochemical examination.
Methods: We analysed the excretion of FLC in urine in patients with MLC associated disease. We performed a prospective study in 30 patients because of unclear diagnosis. Patients were grouped according their histological findings: 1) CN 2) light chain deposit disease (LCDD), 3) AL amyloidosis (AL), 4) other renal disease (ORD). Urine FLC was determined by immune electrophoresis and immunoassay.

Results: Kidney biopsy of 94 patients with MM (n=52), B-cell Non Hodgkin lymphoma (B-NHL in n=5), AL-amyloidosis (AL-A in n=15) and monoclonal gammapathy of undetermined significance (MGUS in n=22) were analyzed. The findings in kidney biopsy were CN n=27, LCDD n=6, AL n=19 and ORD n=42. The critical value for FLC was 15 mg/l. The critical value for detecting a CN was defined with >50mg/dl, the sensitivity was 85%, the specificity 79%, With FLC concentration >75mg/dl the sensitivity was 78% the specificity was 85%, respectively. Adjusted to the renal function (FLC x 1/ GFR) the sensitivity and specificity was 93% and 87% for a quotient > 2 and 81% and 94% for a quotient > 5, respectively.

Conclusions: This data demonstrate that the examination of FLC in urine is helpful to find patients with CN. Patients with LCDD or AL had significant lower FLC concentrations in urine and better renal function at the time of diagnosis. This might be important in patients who are not eligible for kidney biopsy. Because CN decreases very often rapidly with renal function the correct diagnosis of renal involvement is important for treatment decisions.

PUB229
Renal Function in Myeloma Patients Treated with Ibandronate

Background: Treatment of myeloma bone disease (MBD) with bisphosphonates (BP) is standard of care. However, renal toxicity due to BP treatment tends to be a major problem in myeloma patients (MP), especially. At highest risk are patients with reduced renal function (RF) and patients who had switched the BP. Toxicity appears typically after few months of BP treatment.

We evaluated the renal safety of ibandronate (IBD) in MP, who were treated with IBD for MBD. The patients were stratified according to pretreatment and kidney function.

Methods: In a prospective noninterventional study (NIS) about safety and efficacy of IBD in breast cancer patients it turned out that MP were also included unintentionally. Out of 3540 documented patients 105 MP were identified. The data from these MP were evaluated separately. The patients were subdivided in 4 groups according to their RF: GFR >90 (1), 60–90 (2), 30–59 (3) and <30 (4) ml/min. Since pretreatment with other BP could be of influence, patients were analysed according to former pretreatment: no BP, IBD, and other BP. The RF was calculated every month over a period of six month by the MDRD-formula.

Results: 105 patients were available for evaluation. In 99 patients RF was documented over a minimum of 5 months. The initial RF was (1) n=14, (2) n=36, (3) n=38 and (4) n=17, respectively. At baseline there were no differences in RF according to their pretreatment. The IBD dosage was 6 mg in 90% of all infusions, 4 mg in 3,6%, 3 mg in 2% and 2 mg in 4.4%, respectively. The GFR was stable over time in the groups 1-3 and improved significantly in group 4: GFR +15.6 ml/min [95%CI 1.0–32.9], respectively.

In 9 patients the treatment was terminated prematurely, due to disease progression (n=3), death due to myeloma during study period (n=3). Two patients were lost by follow up and one patient declined further treatment.

Conclusions: The data of this NIS demonstrate, that there is no evidence for renal toxicity of IBD in MP in all stages of RF. Quite in contrary to previous observations with other BP patients with worse kidney function (stage 4) had a significant improvement of GFR over the study period.

Funding: Pharmaceutical Company Support

PUB230
The Role of a CT Urogram in a Stone Clinic
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Background: Medullary sponge kidney (MSK) is traditionally diagnosed by intravenous urography. When Computed Tomography Urography (CTU) is combined with three-dimensional post-processing using MultiDetector-Raw Computed Tomography (MDC), the diagnosis of MSK can be made with high accuracy.

Methods: Patients with multiple bilateral stones or with a finding of medullary calcinosis were referred for a CTU in addition to standard laboratory testing, and a 24 hour urine collection for evaluation of metabolic risk factors for recurrent stones. A retrospective analysis of the first 16 of these patients is presented. Patients were seen between January 2008 and March 2011 for initial evaluation of stones. Characteristic findings of MSK on CTU include the appearance of the collecting tubes manifested as a papillary “blush”, “patterned” or “bouquet of flowers” pattern. In addition, medullary calcinosis and medullary cysts were also evaluated. This information was assessed in light of demographic and laboratory data of the patients.

In four patients were diagnosed with MSK based on the characteristic radiologic features seen on the CTU. All 4 of these patients had features of medullary calcinosis, while only three had medullary cysts. Three of the four patients also had features suggestive of distal RTA. Six patients had medullary calcinosis without MSK. 3 of these 6 patients (without MSK) also had features of distal RTA. 2 of 8 patients with features of distal RTA did not have medullary calcinosis or MSK.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only
Underline represents presenting author.
Conclusions: UTUrography effectively demonstrates characteristics radiologic findings of the renal medullary cysts. The medullary cysts may not be distinguishable in the right kidney, but were not seen in all cases of MSK. Most but not all cases of distal RTA were associated with medullary cysts.

PUB231
Kidney Cysts in a Patient with Systemic Toxicity from D-Penicillamine
Farrukh M. Koraishy,1 Gary M. Israel, 2 Neera K. Dahl. 1

Background: D-Penicillamine (PCA) has been used for treatment of cystinuria due to its cysteine binding properties. PCA has also been known to cause impaired collagen deposition and dysfunction in the elastic fibers resulting numerous systemic toxicities. Necrotizing angiitis is a comparatively uncommon glomerulonephritis. We report a case of a patient who developed large bilateral kidney cysts during long-term treatment for PCA with cystinuria.

The patient was treated effectively for cystinuria for over 20 years with a PCA dose of 2.0 grams per day. His renal function remained normal and he had no proteinuria. In 1990, an Intravenous pyelogram (IVP) with tomography showed normal kidney sizes and no cysts were noted. In 2003, the patient was admitted progressive dyspnea. A CT of the chest and abdomen revealed new multiple, bilateral cysts in the kidneys. The largest cyst on the right was 11.9 x 8.8 cm, the largest on the left was 8.3 x 5.8 cm. Both kidneys were between 9.10 cm in size. Patient also had a rise in his serum creatinine. PCA was discontinued in 2005 when the patient developed systemic toxicities of PCA including loosening of the skin, keratocous and popliteal aneurysms. A skin biopsy diagnosed cutis laxa. Other systemic toxicities included cardiomyopathy, severe lung disease and thickening of the walls of the esophageous and duodenum. During these 2 years, the size of his kidneys had decreased and his renal function also worsened. He also developed proteinuria that ranged between 0.5 to 1.0 grams per day.

Since the discontinuation of PCA, the size of his kidneys and his renal function have been stable. Other systemic toxicities have also remained stable.

Conclusions: To our knowledge, this is the first report of kidney cysts acquired during PCA therapy. The etiology of cyst formation due to PCA is unknown. The possible mechanisms include the alteration in the connective and elastic fibers of the kidney and the malfunction of the extracellular matrix and epithelial cell interactions.

Cystic renal disease should be evaluated in patients treated with PCA.

PUB232
Estimating the Range of Kidney Length in Adults with Normal GFR by Automatically Extracting Data from Dated Ultrasound Reports
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Background: Abnormal kidney size can reflect renal disease. However, normal kidney size is not well-characterized, particularly in the mixed ethnicity population in Hawaii. Maximum ultrasound kidney length is the most practical way to estimate kidney size. Ultrasound dictations are unstructured freetext having data that must be extracted manually, and this limits the number of data points available. To analyze the largest possible sample, we create a tool to automatically extract kidney lengths from the ultrasound reports.

Methods: Freetext ultrasound reports were extracted by using natural language processing principles. Programs to download and parse the text and extract data were written in SAS and Excel/VBA to extract the longest kidney length from 45,020 renal and abdominal ultrasound reports from 2002-2010. The top 1% of outliers were manually reviewed. To validate the data extracted from the program, we compared computer-extracted maximum kidney lengths from 500 reports to two independent physicians extracting the same data manually.

To determine which ultrasounds were eligible to represent “normal” kidneys, we excluded patients 18 years old, diabetes, HIV, polycystic kidneys, eGFR ≥60 mL/min/1.73 m², kidney or other organ transplant, and abnormal echogenic kidneys.

Results: The computer-extracted data was validated, with no significant difference in human vs computer error rate. Of the 45,020 dictated reports, 4,493 qualified for this study. The maximum kidney length was 11.62 cm (SD 1.09) and 11.18 cm (SD 1.11) in male and female adults, respectively. The kidney length peaked in the 30-39 age group and decreased with increased age. Kidney length was positively correlated with both height and weight.

Conclusions: We created a novel technique to collect and analyze a massive amount of data and characterize the normal range of kidney length within Hawaii population. The maximum kidney length depends on age, gender, height, and weight. Ultrasound kidney length can be used as a diagnostic tool for early detection of renal disease. We plan to examine the use of kidney length as a predictor of risk of progressive loss of kidney function in a future study.

PUB233
Urine Neutrophil Gelatinase-Associated Lipocalin in Early Diagnosis of Urinary Tract Infection in Children
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Background: Neutrophil gelatinase-associated lipocalin (NGAL) is a protein mainly found in activated neutrophils and renal tubular cells. The expression of NGAL can rise 10,000-fold in response to renal tubular injury, so that it has been proposed as an early biomarker of acute kidney injury. But, there are a few studies for NGAL level in urinary tract infection (UTI) so far. And result of urine culture that is the most accurate method for diagnosing UTI suggests that the classical technique is swift and as least as accurate would be valuable. The present study assessed whether urine and serum NGAL levels could be an early biomarker of UTI.

Methods: Fifty febrile UTI patients and a control group of 50 febrile non-UTI patients were enrolled. Serum and urine NGALs were quantified using the BioPorto® NGAL enzyme-linked immunoabsorbent assay within 48 hours after fever onset in both patient groups.

Results: Mean urine NGAL level was significantly higher in UTI patients than in control (49.0±12.9 ng/mL vs 12.9±9.0 ng/mL, p<0.001). But there was no significant difference of mean serum NGAL level in the UTI and control groups (116.2±9.1 ng/mL vs 101.9±9.1 ng/mL, p=0.651). Using 10.4 ng/mL of urine NGAL as the cutoff value for diagnosis of UTI, sensitivity was 95.8% and specificity was 66.7%.

Conclusions: The results indicate that urine NGAL may be useful for additive marker to urinalysis in the early diagnosis in UTI in children.

PUB234
Baclofen Toxicity in Renal Failure: An Underappreciated Occurrence
Julie Ann T. Linagoe, Dominic A. Sci, Daniel E. Carl. Nephrology, Virginia Commonwealth University, Richmond, VA.

Background: Baclofen (BA) is used for the treatment of musculoskeletal spasticity and occasionally for hiccup. It is mainly eliminated by renal mechanisms and its half life of 4 to 7 hours in normal subjects, extends to 3 to 4 times higher in patients with advanced chronic kidney disease (CKD). This pattern of drug accumulation puts patients with impaired renal function particularly prone to BA toxicity.

Methods: We report 6 patients with impaired renal function and BA-related toxicity seen at our institution from Oct 2010 to Feb 2011.

Results: These 6 cases all had altered mental status (AMS) as the major presenting symptom following BA administration. In all cases, either conventional hemodialysis or continuous renal replacement therapy (RRT) were used to accelerate BA clearance and where obtained blood levels dropped proportionately. Central nervous system (CNS) symptoms resolved in all patients following initiation of RRT. The time to resolution of BA-related symptoms varied substantially reflective of the known CNS compartmentalization.

Conclusions: Characteristics of 6 patients with Baclofen Toxicity

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (y)/Sex</th>
<th>GFR (ml/min/1.73 m²)</th>
<th>Reason for use</th>
<th>Cumulative dose (mg)</th>
<th>Time (days)</th>
<th>Duration (days)</th>
<th>Symptoms</th>
<th>Intervention</th>
<th>Recovery</th>
<th>Abbreviations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>53/F</td>
<td>30</td>
<td>low back pain</td>
<td>25</td>
<td>7</td>
<td>3</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
<tr>
<td>Case 2</td>
<td>52/F</td>
<td>30</td>
<td>low back pain</td>
<td>30</td>
<td>10</td>
<td>3</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
<tr>
<td>Case 3</td>
<td>51/M</td>
<td>25</td>
<td>low back pain</td>
<td>20</td>
<td>5</td>
<td>1.5</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
<tr>
<td>Case 4</td>
<td>50/M</td>
<td>25</td>
<td>low back pain</td>
<td>20</td>
<td>5</td>
<td>1.5</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
<tr>
<td>Case 5</td>
<td>50/M</td>
<td>25</td>
<td>low back pain</td>
<td>20</td>
<td>5</td>
<td>1.5</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
<tr>
<td>Case 6</td>
<td>50/M</td>
<td>25</td>
<td>low back pain</td>
<td>20</td>
<td>5</td>
<td>1.5</td>
<td>AMS, spasticity</td>
<td>4 hr HD</td>
<td>2.5 days</td>
<td>30</td>
</tr>
</tbody>
</table>

NGAL levels could be an early biomarker of UTI.

NGAL enzyme-linked immunosorbent assay within 48 hours after fever onset in both patient groups.

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NGAL levels could be an early biomarker of UTI.
Results: Of 1238 participants, 505 general population excluding medical professionals were included. The mean age was 60 years and 85% were male. The awareness of CKD was 30.9%, and complication of hypertension, diabetes mellitus or dyslipidemia (33%) did not affect CKD awareness. In regard to CKD diagnosis, proteinuria (61%) was recognized sufficiently, but eGFR (14%) was poorly recognized. In regard to the symptoms of CKD, hypertension (73%) was recognized sufficiently, but renal anemia (49%) and mineral bone disorder (38%) were poorly recognized. Mild (34.3%) and heavy proteinuria (1.7%) was observed. The degree of actual life-style modification significantly correlated with proteinuria (r=0.11, P=0.016) and self-reported renal function (r=0.25, P=0.01).

Conclusions: The results suggest that awareness of CKD remains low in Japan, and efforts to improve knowledge of CKD and to promote life-style modification may play important roles in delaying the progression of CKD.

Funding: Government Support - Non-U.S.

PUB236
Urine Protein-to-Creatinine Ratio Does Not Correlate with 24-H Urine Total Protein Excretion in Nephrotic Proteinuria

Nuria Montero,1 María Jose Soler,1 María Jose Pascual,1 Clara Barrios,1 Eva Marquez,1 Eva Rodriguez,1 Maria Antonia Orfila,1 Luis Coca,2 Julio Pascual.1 Nephrology, Hospital del Mar, Barcelona, Spain; 2Laboratori de Referencia de Catalunya.

Background: Measurement of protein content of a timed 24 h urine collection is the definitive method for establishing presence of abormal proteinuria, however, urine collection is cumbersome. Spot urine protein to creatinine ratio seems to be a reliable diagnostic tool for urine protein measurement. Our aim is to evaluate spot urine protein-to-creatinine ratio compared to 24 h urine total protein excretion in different proteinuria ranges.

Methods: Observational, cross-sectional study of 159 consecutive pair less paired determinations of 24 h urine total protein excretion and spot urine protein-to-creatinine ratio in renal patients. The strength of the correlation was determined by calculating intraclass correlation coefficient (ICC) and Spearman correlation coefficient (SCC).

Results: Among all groups, ICC was 0.756 (CI 95%, 0.680-0.816) and SCC was r=0.91 (p<0.05). There is an excellent significant correlation between the spot urine protein to creatinine ratio and 24 h urine total protein excretion in different proteinuria ranges.

Conclusions: The spot urine protein to creatinine ratio seems to be a reliable diagnostic tool for urine protein measurement. Our aim is to evaluate spot urine protein-to-creatinine ratio compared to 24 h urine total protein excretion in different proteinuria ranges.

TABLE: Intraclass correlation coefficient between spot urine protein to creatinine ratio and 24-h urine total protein excretion

<table>
<thead>
<tr>
<th>Proteinuria 24h (mg)</th>
<th>&lt; 300</th>
<th>300-3499</th>
<th>≥ 3500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>60</td>
<td>97</td>
<td>22</td>
</tr>
<tr>
<td>Intraclass correlation coefficient (Confidence interval 95%)</td>
<td>0.456 (0.230-0.653)</td>
<td>0.656 (0.508-0.766)</td>
<td>0.340 (-0.041-0.650)</td>
</tr>
<tr>
<td>Spearman correlation coefficient (r)</td>
<td>0.498</td>
<td>0.828</td>
<td>0.181</td>
</tr>
<tr>
<td>p</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.420</td>
</tr>
</tbody>
</table>

When patients were stratified according to eGFR, the correlations between spot urine protein-to-creatinine ratio and 24 h urine total protein excretion were similar between groups.

Conclusions: In summary, a strong correlation is observed between spot urine protein to creatinine ratio and 24 h urine total protein excretion when the level of proteinuria is <3500mg/day. In our experience, there is no relevant correlation between spot urine protein to creatinine ratio and 24 h urine total protein excretion in nephrotic-range proteinuria. Further studies with larger sample sizes are needed to confirm these results.

PUB237
Impact of Reduced Glomerular Filtration Rate on Japanese Acute Stroke

Naoki Nakagawa. Asahikawa Medical University, Japan.

Background: Patients with chronic kidney disease (CKD) are at high risk of stroke and other cardiovascular diseases, and recent guidelines for the management of stroke stress the importance of managing such patients. However, the characteristics of the subtypes of stroke that occur in patients with CKD remains to be determined. The present study investigates stroke subtypes in patients with or without CKD using the stroke database at our hospital.

Methods: We analyzed data from 470 (male, 251; mean age, 70.6 y) patients admitted to our hospital with stroke between 2006 and 2010. Relationships between the type of stroke and estimated glomerular filtration rate (eGFR) for Japanese, age, sex, hemoglobin and the presence or absence of various risk factors for arteriosclerosis were investigated.

Results: The most common stroke was transient ischemic attack in 17 patients (4%), lacunar infarction in 40 (9%), atherothrombosis in 36 (8%), cerebral embolism in 129 (27%), unclassified cerebral infarction in 90 (19%), subarachnoid hemorrhage in 41 (9%), and cerebral hemorrhage in 104 (22%). Among the 470 patients, 140 (30%) had CKD with eGFR < 60 mL/min/1.73 m². Compared with a group without CKD (mean eGFR 85 mL/min/1.73 m²), the group with CKD had a significantly lower incidence of cerebral infarction (42% vs. 30%, P < 0.01), lacunar infarction (20% vs. 30%, P < 0.01), atherothrombosis (30% vs. 60%, P < 0.01), and hypertension (73% vs. 49%, P < 0.05) and a significantly lower incidence of subarachnoid hemorrhage (3.6% vs. 11%, P < 0.01) in the group with, than without CKD.

Conclusions: About 30% of patients with stroke admitted to our hospital had CKD. Those with CKD were older, had a higher prevalence of atrial fibrillation and a significantly higher rate of cardiogenic cerebral embolism. Thus, strict control of blood pressure and prevent the onset of atrial fibrillation should be important to prevent stroke among patients with CKD.

PUB238
Evaluation of Rectus Sheath Hematoma Risk Factors

Heeja S. Sheeth, Hoda Kaldas. Medicine, UPMC, Pittsburgh, PA.

Background: The following risk factors for rectus sheath hematoma (RSH) are known: systemic or prophylactic anticoagulation, trauma, abdominal surgery or injections, cough, old age, female gender and pregnancy. Platelet dysfunction associated with kidney disease, administration of antiplatelet agents, steroids, or immunosuppressants may increase the risk of bleeding complications such as RSH. RSH may increase morbidity/mortality, length of stay and readmissions.

Purpose: To evaluate the risk factors for RSH such as Chronic Kidney Disease, antiplated therapy, steroids and immunosuppressants.

Methods: The patients who developed RSH during 2006-6/2009 were identified from the CT reports from UPMC MARS data system. Patient charts were reviewed for demographics and risk factors. Descriptive statistics are reported for the risk factors.

Results: The MARS query identified 114 RSH CT reports. 12 patients were ineligible: NO RSH (10) and inadequate documentation (2). The 102 patients evaluated, demographics were: 60.8% females, 10.8% AA, mean age 62.6 years (SD 16.9, range18-97.6). 6 patients (5.8%) were overweight and 35 (34.3%) obese. 23 (22.5%) were admitted for RSH management. 24 (23.5%) patients had died in the first 30 days of the anticoagulant treatment in the hospital, 18 (17.7%) had been treated prothrombolytic therapy. The presenting symptoms were pain, drop in hematocrit, hypotension, or incidentally diagnosed on CT. Among the 42 patients administered prophylactic dose of or no anticoagulants, 11 (26%) had CKD stage 4/5. Stroke subtypes revealed a significantly higher incidence of cardiogenic cerebral embolism (34% vs. 25%, P < 0.01), followed by hemorrhagic stroke (31% vs. 15%, P < 0.01) and a history of cardiovascular disease (38% vs. 19%, P < 0.01) in the group with CKD, but the difference did not reach significance in the group without CKD.

Conclusions: The risk of bleeding complications such as RSH and death in patients with CKD remains low in Japan, and efforts to improve knowledge of CKD and to promote life-style modification may play important roles in delaying the progression of CKD. The results suggest that awareness of CKD remains low in Japan, and efforts to improve knowledge of CKD and to promote life-style modification may play important roles in delaying the progression of CKD.
Infected alkaline urine is an important clue to the presence of persistent progressive struvite stone formation with complications of acute kidney injury and urosepsis.

**Results:** The patient is a 59-year-old man with a history of *P. mirabilis* UTI 5 years ago. Urine pH was 8. He was successfully treated with antibiotics but lost to follow up. One day prior to admission, he presented with fevers, chills, and severe sepsis. Abdominal exam was unremarkable. He had no costovertebral angle tenderness. Digital rectal exam showed 5 fingerbreadths nontender prostate gland. His scrotum was erythematous, mildly tender, but with no swelling or penile discharge. He developed acute kidney injury. His serum creatinine was 7.6 mg/dl rising from baseline serum creatinine of 1 mg/dl. Urinalysis showed an alkali urine with pH of 8. Blood and urine cultures grew *Proteus mirabilis*. Abdominal CT scan revealed a 1.5 * 5 cm left distal ureteric stone extending across the UVJ. He underwent left percutaneous nephrostomy tube placement, and then laser lithotripsy of the left ureteral stone. Stone analysis was consistent with struvite stone. His sepsis ultimately resolved and serum creatinine returned to his baseline.

**Conclusions:** *P. mirabilis* is a common cause of struvite stone which is difficult to treat medically and needs surgical treatment. Incomplete treatment of *P. mirabilis* can result in progressive struvite stone formation with complications of acute kidney injury and urosepsis. Infected alkaline urine is an important clue to the presence of persistent *P. mirabilis* UTI.
This analysis revealed that compared to the average, kidney development genes show significantly higher DNA methylation level in promoter regions but lower methylation levels at TSS. This unique pattern at these genes was not found in the adenocarcinoma cell lines, which show higher methylation level at TSS and lower level at the promoter region. This analysis suggests that regulation of CpG islands methylation flanking TSS is different in kidney development genes than an average gene.

**Conclusions:** This suggests that renal development may involve transcriptional mechanisms that are unique.

### PUB245

**The Impact of Gene Polymorphisms of Interleukin-18 (IL-18), Transforming Growth Factor-β (TGF-β) and Vascular Endothelial Growth Factor (VEGF) on Development of IgA Nephropathy or Thin Glomerular Basement Membrane Disease**

**Background:** Interleukin-18 (IL-18), Transforming Growth Factor-β (TGF-β), and Vascular Endothelial Growth Factor (VEGF) play a role in renal development and the pathogenesis of autoimmune diseases such as IgA nephropathy (IgAN) and thin glomerular basement membrane disease (TGBMD). Specifically, polymorphisms in the IL-18, TGF-β, and VEGF genes have been reported to be associated with these diseases.

**Methods:** We genotyped 146 normal subjects (control group) and biopsy-proven cases of IgAN (N=70) or TGBMD (N=60) for polymorphisms in the IL-18, TGF-β, and VEGF genes. We analyzed the distribution of genotypes and alleles in each group and compared them using statistical methods.

**Results:** Polymorphisms in the IL-18, TGF-β, and VEGF genes were significantly associated with the development of IgAN and TGBMD. Specifically, the presence of certain alleles was significantly more common in patients with IgAN and TGBMD compared to the control group.

**Conclusions:** Our findings suggest that polymorphisms in the IL-18, TGF-β, and VEGF genes play a role in the development of IgAN and TGBMD. Further studies are needed to confirm these findings and to explore the mechanisms underlying the association between these polymorphisms and the development of these diseases.

### PUB246

**Changing Demographics of Autosomal Dominant Polycystic Kidney Disease over Four Decades**

**Background:** Understanding the demographics of ADPKD has important implications for the management and prevention of the disease. However, the demographics of ADPKD patients have changed over time due to improvements in diagnosis and management of patients with ADPKD.

**Methods:** We analyzed the demographics of ADPKD patients who were seen at our center from 1961-1992 and 1993-2007. We compared the demographics of these two time periods for several factors, including age, gender, and family history of ADPKD.

**Results:** Significant differences were observed between the two time periods. For example, the average age at diagnosis was higher in the 1993-2007 cohort compared to the 1961-1992 cohort. Additionally, the proportion of patients with a family history of ADPKD was higher in the 1993-2007 cohort.

**Conclusions:** The demographics of ADPKD patients have changed over time, likely due to improvements in diagnosis and management of the disease. These changes have important implications for the care of patients with ADPKD.
Congenital Heart Disease in Autosomal Dominant Polycystic Kidney Disease
Maria V Irazabal,1 Amber Harmon,2 Heidi M. Connolly,3 Marie C. Hogan,4 Jamie L. Sundsbak,1 Sandro Rossetti,1 Peter C. Harris,1 Vicente E. Torres.1 Division of Nephrology and Hypertension, Mayo Clinic, Rochester, MN; 2Division of Cardiovascular Diseases, Mayo Clinic, Rochester, MN.

Background: PKd2 and Pkd11, but not Pkd1, are expressed in the embryonic node and required for left-right patterning. Disturbances in embryonic left-right patterning are an important cause of disrupted cardiac development and congenital heart disease (CHD). PKd2 and Pkd11 null mice exhibit cardiaco atrophy and outflow tract defects. Although cardiac valvular abnormalities occur with increased frequency in Autosomal Dominant Polycystic Kidney Disease (ADPKD), it is uncertain whether an association between ADPKD and CHD exists.

Methods: To examine this question we reviewed the echochardiogram reports obtained between 1999 and 2010 in ADPKD patients (554 patients).

Results: A diagnosis of CHD (including previous diagnoses, excluding patient formam oval and atrial septal aneurysm) was present in 25 patients (4.5%). Atrial or ventricular septal defect (n=4–0.7%), patent ductus arteriosus (n=1), isolated bicuspid aortic valve (BAV=9–1.6%), aortic coartation (n=3–0.7%, 3 with associated BAV), hypoplasia of upper descending thoracic aorta (n=1), left coronary artery to pulmonary artery fistula (n=1), congenital pulmonic stenosis (n=1), Ebstein anomaly (n=1), tetralogy of Fallot (TOF n=1), tricuspid atresia (n=1), and double inlet left ventricle with transposed great vessels and pulmonic stenosis (n=1). Four cases had been referred to our institution for evaluation of CHD, in the remaining diagnosis of CHD was incidental to evaluation for ADPKD or other conditions. Three additional ADPKD patients with TOF, VSD and isolated left superior vena cava were seen during the same period of time but had no echochardiogram.

Conclusions: CHD is rarely associated with ADPKD, but the observed prevalences seem higher than those in the general population. 2.9 vs 0.8% (excluding isolated BAV).

PUB248
Mutations in KIF7 Link Joubert Syndrome with Sonic Hedgehog Signaling and Microtubule Dynamics
Max C. Liebau,1,2,3 Gudrun Nürnberg,1,2 Max C. Liebau, 1,2 and Microtubule Dynamics

Background: Joubert syndrome (JBTS) is characterized by a specific brain malformation with various additional pathologies including a nephronophthisis-like cystic renal phenotype in around 25% of the JBTS patients. JBTS can result from mutations in at least 10 different genes and has been linked to dysfunction of primary cilia. We have recently found that modified microtubule stability and growth direction caused by loss of KIF7 function may be an underlying disease mechanism contributing to JBTS.

Methods: We report a case of DDS in a baby boy who was born with ambiguous genitalia and transferred to our NICU.

Results: He was found to have persistent HTN and proteinuria in the NICU. His karyotype was XY and WT1 gene showed mutation P. D 396N. c.1186G>C. A consistent with DDS. We followed him in Nephology clinic for 8 months and his proteinuria reached nephritic range and he became albumin transudation dependent.

Conclusions: DDS is the result of mutations in the WT1 gene on chromosome band 11p13. The WT1 protein is a transcription factor predominantly expressed in the embryonic kidneys and gonads. Patients with Denys-Drash syndrome develop early-onset nephrotic syndrome, have a high prevalence of severe hypertension, and experience rapid progression to end-stage renal disease (ESRD). The vast majority of patients with DDS is destined to develop Wilms tumor in the native kidneys and are at significant risk for development of gonadaloblastoma in the dysgenetic gonads. DDS therapy includes management of fluid and electrolyte balance, treatment of hypertension, renal replacement therapy for ESRD or after bilateral nephrectomy and renal transplant. DDS and WT1 related syndromes are under recognized. Late diagnosis of DDS may lead to late diagnosis of Wilms’ tumor, which is often associated with this syndrome. Diagnosis of WT1 related syndromes and molecular testing for WT1 mutations should be considered in the presence of ambiguous genitalia and hypertension or proteinuria.

PUB251
Genetic Factors in the Development of Primary Chronic Glomerulonephritis
Magdalena Mostowski,1 Stanislaw Czekalski,2 Pawel J. Jagodzinski,3 Andrezj F. Oko.1 Department of Nephrology, Transplantology and Internal Medicine, Poznan University of Medical Sciences, Poznan, Poland; 2Department of Biochemistry and Molecular Biology, Poznan University of Medical Sciences, Poznan, Poland.

Background: Genetic factors can play a significant role in the onset of primary chronic glomerulonephritis (GN). Genes encoding proteins of the renin - angiotensin - aldosterone system (RAAS) are candidate genes for primary chronic GN because of influence on renal function in physiological and pathological conditions. The aim of the study was to evaluate the association of 10 polymorphisms of RAAS genes (ACE rs4646994; AGT rs699; AGTR1 rs5186; ATP6AP2 rs2968917; rs2971597; CYP11P2 rs1799998; REN rs11571080 and rs2368564) with the risk of primary chronic GN in the Polish population.

Methods: The analysis of selected single nucleotide polymorphisms (SNPs) was conducted in the group of 148 patients from the Polish population with biopsy proven primary chronic GN. Control group consisted of 193 healthy persons matched by sex, age, and place of birth. SNP genotyping was performed using PCR-RFLP (Restriction Fragment Length Polymorphism) or HRM (High - Resolution Melting) analysis.

Results: The significant result was found for the rs1799998 polymorphism of the CYP11P2 gene. Individuals carrying two copies of the rs1799998 C allele has nearly twofold increased risk of primary chronic GN (ORc=CC/CT+TT=1.94; 95%CI: 1.171 - 3.218; p = 0.0095). The genetic variations in ACE (rs4646994), AGT (rs699), AGTR1 (rs5186), ATP6AP2 (rs2968917, rs2971597), CYP11P2 (rs1799998) were not significantly associated with the risk of primary chronic GN in the Polish population.
Acute Caffeine Overdose Requiring Hemodialysis
Syed N. Babar, John A. Walker. Nephrology, Robert Wood Johnson University Hospital, New Brunswick, NJ.

Background: Caffeine is consumed on a large scale in the form of beverages like coffee, tea, and soft drinks. In the brain, it acts as an antagonist of adenosine receptors, and with adenosine having vasodilatory properties, it is a common composition of various headache pills. We present a rare case of caffeine intoxication requiring hemodialysis.

Methods: A 40 year old woman was found unresponsive after she had consumed approximately 100 pills of Fioricet (Composition: Caffeine 40 mg, Acetaminophen 325 mg, Butalbital 50 mg). Labs revealed hypokalemia, lactic acidosis, and an elevated serum acetaminophen level. Gastric detoxification was initiated with activated charcoal along with IV N-acetyl cysteine. The patient became agitated and developed seizures. Three hours of emergent hemodialysis was administered empirically. Immediately post dialysis, she responded to her name and the next day was fully awake and oriented. Pre and post hemodialysis serum caffeine levels were 51 and 4 mcg/ml respectively (therapeutic level 5-15 mcg/ml).

Results: In quantities found in most foods and beverages, caffeine is unlikely to cause any acute medical problems. However, its presence in higher concentrations in OTC products like energy drinks, diet aids, and prescription medications may be a potential cause for acute toxicity. Average doses may result in feelings of alertness and decreased fatigue, whereas high doses (>250-500 mg) results in restlessness, nervousness, insomnia, and tremors. Even higher doses can cause a hyperadrenergic syndrome resulting in seizures, cardiovascular instability and altered mental status. Hypokalemia, lactic acidosis, and hyperglycemia are classic features of caffeine overdose.

Treatment of severe acute intoxication is generally supportive and includes providing treatment of the immediate symptoms. However, extracorporeal therapy is indicated for caffeine concentrations of >100 mcg/ml or in patients who develop seizures or arrhythmias regardless of the caffeine concentration.

Conclusions: With the widespread availability and consumption of caffeine, its toxicity, (intentional or unintentional) should be recognized by physicians and appropriate treatment provided in a timely fashion.

Atenolol Overdose Successfully Treated with Hemodialysis: A Case Report
Shih-Han S. Huang, Rita Suri. Medicine, Nephrology, London Health Sciences Centre, London, ON, Canada.

Background: Atenolol overdose is common. Because of its hydrophilic characteristic, a few case reports have demonstrated the benefits of hemodialysis treatments. However, the amount of atenolol clearance throughout hemodialysis sessions has not been studied.

Methods: In this case report, a patient with impaired renal function was successful treated with two 5-hour intermittent high-flux high-efficiency hemodialysis therapies after atenolol overdose. Serial atenolol levels were measured during his hemodialysis treatments. Atenolol in plasma was determined using liquid chromatography, tandem mass spectrometry.

Results: We demonstrated an over 50% atenolol reduction after each 5 hours hemodialysis therapy. The parameters for mean arterial blood pressure (mmHg), heart rate (BPM), the numbers of inotropes and vasopressors and the plasma atenolol levels required during the first 48 hours of hospital admission, are presented in Figure 1. The zero hour was set at time when the patient first present to emergency room.

Conclusions: Hemodialysis therapy is an effective treatment for atenolol overdose, especially in patients with impaired renal function.
Continuous Renal Replacement Therapy with Regional Citrate Anticoagulation Induces a Negative Calcium Balance

**Background:** Continuous Renal Replacement Therapy (CRRT) associated with regional citrate anticoagulation (RCA) is commonly employed in intensive care units, especially in patients with acute kidney injury (AKI), hemodynamic instability and high risk of bleeding. However, this therapy carries the potential risk of negative calcium (Ca) balance associated with a worsening of hemodynamic instability. The aim of the study was to better understand the safety aspects of CRRT with RCA.

**Methods:** In order to assess the effects of CRRT with RCA in Ca kinetics, 10 continuous venovenous haemodialysis (CVVH) sessions, performed with Ca-free dialysate, were evaluated. The delivered dialysis dose and the blood flow were fixed, and intravenous Ca reposition was titrated based on ionized Ca values.

**Results:** During therapy, our patients (7 males, 66±11yrs, 6 with AKI) presented a diuresis rate of 0.3L (range, 0.1-2.4L). No cardiac arrhythmia neither any circuit clotting was observed during therapy. Biochemical parameters are shown below. Comparison of biochemical parameters at the baseline and the end are presented as mean±SD. *p<0.05

**Conclusions:** In conclusion, this study showed a negative calcium balance in patients submitted to CRRT therapy, mainly in those with a high UF rate.

**PUB256**

Outcomes of Patients with End Stage Renal Disease (ESRD) under Chronic Hemodialysis and Patients without ESRD in Acute Renal Failure Requiring Continuous Renal Replacement Therapy: A Single Center Study

**Background:** The purposes of this study were to compare the survival of conventional hemodialysis (HD) patients with the survival of non-end stage renal disease (ESRD) patients. Moreover, we aimed to compare the survival of non-ESRD and ESRD patients requiring CRRT.

**Methods:** We evaluated adults (> 18 years) requiring CRRT who were treated in the ICU of Kosin University Gospel Hospital, Busan, Korea from January 1, 2008 to November 30, 2010. A total of 100 (24 ESRD, 76 non-ESRD) patients received CRRT during the study period. Predictors of all-cause death were examined using Kaplan-Meier analysis and Cox proportional hazards analyses in both treatment groups.

**Results:** For non-ESRD patients, the 90 day survival rate was 41.6 %. For ESRD patients, the 90-day survival rate was 55.3 %. Multivariate Cox proportional hazards analyses demonstrated that conventional HD was not a significant predictor of mortality. However, this therapy carries the potential risk of negative calcium (Ca) balance associated with a worsening of hemodynamic instability. The aim of the study was to better understand the safety aspects of CRRT with RCA.

**Conclusions:** The survival rates of non-ESRD and ESRD patients requiring CRRT did not differ, and conventional HD was not a significant predictor of mortality.

**Table 1. Comparison between ESRD Group and Non-ESRD Group**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>ESRD (n=24)</th>
<th>Non-ESRD (n=76)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>35.6±6.4</td>
<td>41.6±9.2</td>
<td>0.324</td>
<td></td>
</tr>
<tr>
<td>Admission to CRRT, day</td>
<td>6.4±5.9</td>
<td>8.4±2.8</td>
<td>0.819</td>
</tr>
<tr>
<td>APACHE score</td>
<td>89.2±34.9</td>
<td>89.0±32.5</td>
<td>0.982</td>
</tr>
<tr>
<td>FOSCHI score</td>
<td>20.8 (33)</td>
<td>54 (71.1)</td>
<td>0.295</td>
</tr>
<tr>
<td>No. of organ failure (range)</td>
<td>1.4±0.8</td>
<td>1.8±0.9</td>
<td>0.131</td>
</tr>
<tr>
<td>Serum BUN (mg/dl)</td>
<td>59.2±33.9</td>
<td>53.2±28.5</td>
<td>0.391</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>6.5±4.0</td>
<td>3.5±2.3</td>
<td>0.002</td>
</tr>
<tr>
<td>Leukocyte (× 10³/L)</td>
<td>12.6±5.1</td>
<td>14.6±10.2</td>
<td>0.351</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.3±7.7</td>
<td>10.4±2.1</td>
<td>0.701</td>
</tr>
<tr>
<td>Platelet (× 10³/L)</td>
<td>126.2±53.1</td>
<td>105.6±82.2</td>
<td>0.554</td>
</tr>
</tbody>
</table>

**Results:** For non-ESRD patients, the 90 day survival rate was 41.6 %. For ESRD patients, the 90-day survival rate was 55.3 %. Multivariate Cox proportional hazards analyses demonstrated that conventional HD was not a significant predictor of mortality. However, this therapy carries the potential risk of negative calcium (Ca) balance associated with a worsening of hemodynamic instability. The aim of the study was to better understand the safety aspects of CRRT with RCA.

**Conclusions:** The survival rates of non-ESRD and ESRD patients requiring CRRT did not differ, and conventional HD was not a significant predictor of mortality.

**Fig. 2. Patient survival in conventional hemodialysis group and non-ESRD group**

**Conclusions:** The survival rates of non-ESRD and ESRD patients requiring CRRT did not differ, and conventional HD was not a significant predictor of mortality.

**PUB257**

The Efficacy of Ferumoxytol in Peritoneal Dialysis Patients

**Background:** Iron deficiency is one of the major elements contributing to anemia in CKD patients. Oral iron is often not tolerated and ineffectively absorbed. Intravenous infusion is time consuming and inconvenient in peritoneal dialysis (PD) patients self-treating at home. A new iron preparation, ferumoxytol, which can be administered as a bolus intravenous injection, would allow PD patients to more easily comply with current IV iron dosing regimens. Therefore, we evaluated the effect of ferumoxytol on hemoglobin, hematocrit, ferritin, and iron saturation.

**Methods:** We reviewed the medical records of PD patients aged ≥ 18 years, who received at least one dose of ferumoxytol between January 2010 and August 2010 at our institution.

**Results:** 9 males and 8 females, aged 53.5 ± 16.6 years with average weight of 83.94 ± 22.8 kg were included. 15 patients received 2 doses and 2 patients received only 1 dose of ferumoxytol.

**Conclusions:** The average time interval between the two doses of ferumoxytol was 6.9 ± 3.6 days. The average intervals between the 2nd dose and subsequent labms were 24.6±11.7 days (Wk 4), 56.1±18.4 days (Wk 8), 87.3±13.6 days (Wk 12), and 116.7±13.8 days (Wk 16). Data presented as mean±SD. Epoetin dose presented as mean.

**Table 1.**

<table>
<thead>
<tr>
<th>Lab</th>
<th>Baseline</th>
<th>4 Wk Post Feraheme</th>
<th>8 Wk Post Feraheme</th>
<th>12 Wk Post Feraheme</th>
<th>16 Wk Post Feraheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>10.4±1.0</td>
<td>11.0±0.9</td>
<td>11.3±0.9</td>
<td>11.3±1.0</td>
<td>10.9±1.2</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>32.3±3.9</td>
<td>33.8±3.5</td>
<td>34.6±3.2</td>
<td>34.6±3.4</td>
<td>35.6±3.3</td>
</tr>
<tr>
<td>Ferritin (ng/mL)</td>
<td>329±152</td>
<td>625±248</td>
<td>743±259</td>
<td>648±367</td>
<td>329±129</td>
</tr>
</tbody>
</table>

*p<0.05 versus baseline

**Conclusions:** Ferumoxytol has the expected efficacy of an intravenous iron compound, with improvements in anemia and iron being evident as early as 4 weeks post ferumoxytol. Additionally, significant decrease in monthly epoetin dose was noted at 12 weeks post ferumoxytol dosing. Ferumoxytol is a desirable therapeutic option in peritoneal dialysis patients, who typically visit the clinic at monthly intervals. Alternative iron therapies would require lengthy infusions, or frequent visits to achieve comparable iron delivery.


**PUB259**

**Statins, Vitamin D, Cholesterol in End-Stage Renal Disease**  
Ishir Bhan, Sagar U. Nigwekar, Ravi I. Thadhani.  
Department of Medicine, Division of Nephrology, Massachusetts General Hospital, Boston, MA.

**Background:** Randomized trials of statins in end-stage renal disease (ESRD) have failed to convincingly demonstrate an effect on cardiovascular mortality. However, recent studies in the general population have suggested a possible effect of statins on the metabolism of 25-hydroxyvitamin D (25-OH D). We sought to examine the relationship between statin use, 25-OH D levels, and total cholesterol levels in ESRD.

**Methods:** We obtained statin usage status, and 25-OH D levels, total cholesterol levels in a subset of patients in the Accelerated Mortality in Renal Replacement (ArMORR, n=10044) cohort of incident hemodialysis patients. We then determined associations between these factors using univariate and multivariate modeling, controlling for potential confounding factors.

**Results:** 26% of individuals were on statins at baseline. 25-OH D levels were available in 12.3% of subjects. In univariate analysis, there was no association between statin use and 25-OH D levels (p=0.4). However, both statin use and higher 25-OH D levels were associated with lower total cholesterol (Figure 1). In a multivariate analysis controlling for age, race, sex, albumin, and season, both statin use (p=0.001) and 25-OH D (p=0.017) continued to be independently associated with lower cholesterol levels. There was no evidence for interaction between the effects of 25-OH D and statins on cholesterol.

**Conclusions:** Mortality rates are specific for each gender in hemodialysis patients. In men, old age, hyperalbuminemia, low parathormone, heart failure, excessive daytime sleepiness and comorbidity severity were associated with mortality; in women, old age, diabetes, depressive symptoms and comorbidity severity. In the final model and in all cases, old age and comorbidity severity were associated with increased mortality; in women, comorbidity severity was the determinant of mortality; in men, old age, hyperalbuminemia, low parathormone and excessive daytime sleepiness were determinant of mortality.

**Funding:** Government Support - Non-U.S.

**Figure 1:** 25(OH) D levels were categorized by tertile. Increasing 25(OH) D levels were associated with lower cholesterol levels among statin users (p=0.003) and non-users (p=0.004). Statin use was also associated with lower total cholesterol (p=0.001).

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only  
Underline represents presenting author.

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**PUB260**

**Frequent Epoetin Alfa Dose Titrations Observed in End-Stage Renal Disease (ESRD) Patients**  
T. Christopher Bond, Steven Wang, Jaime Rubin.  
DaVita Clinical Research, Minneapolis, MN.

**Background:** There is increasing pressure to maintain hemoglobin (Hb) levels in the range of 10-12 g/dL in U.S. dialysis patients (pts). Physicians use dose titration to attempt to keep patients in Hb range. We sought to characterize EPO dose titrations patterns in patients at a large dialysis organization.

**Methods:** We examined all EPO doses in patients treated at DaVita dialysis clinics between 1/2009-12/2010. Pts were age ≥18 years, prevalent (>120 days on dialysis), receiving in-center hemodialysis 3 times per week. We segmented EPO utilization into three categories. "Dose holds" were defined as ≥3 consecutive sessions with zero EPO dose. "Stable periods" were defined as consecutive doses within 10% of 1st dose in the period, ignoring zero EPO doses lasting ≥3 consecutive sessions. Non-dose hold and non-stable periods were defined as "transition periods". We defined titrations as absolute differences of ≥10% between mean EPO dose in consecutive periods. Dose changes on either end of a dose hold were considered 1 titration if the dose returned to within ±10% of the dose in the period immediately prior to the dose hold; they were considered 2 titrations otherwise.

**Results:** Titrations were calculated at the clinic level as the total number of tetrations divided by the total number of patient-months. Of the observed tetrations, there were an equal number of up and down tetrations not from/ to a dose hold (41%). The remaining tetrations were to or from a dose hold.

**Conclusions:** Results suggest that physicians titrate EPO in ESRD patients very frequently, with the majority of patients receiving at least 1 dose titration per month. Further research is needed to determine if these frequent dose titrations are associated with time in target range.

**Funding:** Pharmaceutical Company Support

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**PUB261**

**The Effect of Long Acting ESA on Maintaining Stable Hb Levels in Chronic HD Patients; 3 Year Observational Study**  
Christopher Brown, Ruipinder Rai, Jennifer Williams, Sadananda V. Aithal, Ashraf I. Mikhail.  
ABM UHBB.

**Background:** Increasingly, the focus of anaemia management of CKD has shifted from absolute Hb levels to achieving stable Hb with minimal variability. The risk of failing to maintain or overshoot Hb levels in chronic dialysis patients has been a concern with long acting ESA.

We assessed the efficacy of long acting ESA in maintaining Hb levels in chronic HD patients.

**Methods:** This study used data from 83 patients switched from short acting sc epoetin β to CERA. Six months treatment period with epoetin β were compared with upto 3 years treatment with CERA, Hb levels was analysed using Hb levels, number of Hb excursions and residual standard deviation of Hb regression. Two methods of analysis was used to assess Hb stability:

Method 1: Quantifying excursions of ≥1 g/dL outside individual mean Hb levels.

Method 2: 1. Regressional residual SD, a surrogate marker for Hb stability. RSD was then validated to be one of the best measure of Hb stability.

**Treatment approach to iron was not changed throughout the study.**

**Results:** Throughout the observation, ESA dose was modified to maintain the Hb within the target range. During the observation period and due to the publication of large multi-centre studies and regulatory advice, target Hb range was lowered from (10.5 - 12.5) to (10 - 12) g/dL. 6 monthly Hb parameters before and after switch

**Mean Hb**  
Pre-switch (months 6 to 1) Mean Hb 11.8  
Month 12 to 17 Mean Hb 11.2  
Months 18-23 Mean Hb 11.2  
Months 24-29 Mean Hb 11.5  
Months 30-35 Mean Hb 11.5

**Epoetin (IU/wk)**  
Pre-switch (months 6 to 1) Epoetin 8475  
Month 12 to 17 Epoetin 575  
Month 18-23 Epoetin 230  
Month 24-29 Epoetin 272  
Month 30-35 Epoetin 260

**CERA (mcg/Mth)**  
Pre-switch (months 6 to 1) CERA 212  
Month 12 to 17 CERA 230  
Month 18-23 CERA 272  
Month 24-29 CERA 260  
Month 30-35 CERA 260

**Hb excursions/pt**  
Pre-switch (months 6 to 1) Hb excursion 0.07  
Month 12 to 17 Hb excursion 0.94  
Month 18-23 Hb excursion 0.95  
Month 24-29 Hb excursion 0.84  
Month 30-35 Hb excursion 0.88

**No. excursions/pt**  
Pre-switch (months 6 to 1) No. excursion 0.53  
Month 12 to 17 No. excursion 0.49  
Month 18-23 No. excursion 0.44  
Month 24-29 No. excursion 0.34  
Month 30-35 No. excursion 0.42

**No. high excursion/pt**  
Pre-switch (months 6 to 1) No. high excursion 0.54  
Month 12 to 17 No. high excursion 0.45  
Month 18-23 No. high excursion 0.51  
Month 24-29 No. high excursion 0.50  
Month 30-35 No. high excursion 0.46

**Residual SD**  
Pre-switch (months 6 to 1) Residual SD 0.7  
Month 12 to 17 Residual SD 0.63  
Month 18-23 Residual SD 0.61  
Month 24-29 Residual SD 0.60  
Month 30-35 Residual SD 0.62

**Figure 1:** Total cholesterol by 25-OH Vitamin D level (ng/ml)

**Conclusions:** In patients initiating hemodialysis, both statin use and higher 25-OH D are independently associated with lower total cholesterol levels. Future studies of statins in ESRD should examine the additional value of optimizing vitamin D status.

**Funding:** NIDDK Support

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**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only  
Underline represents presenting author.
Influence of Different Erythropoietin Stimulating Agents on Stability of Hemoglobin Levels in Patients on Maintenance Hemodialysis Maciej Drozd, Dominik Cieniawski, Karolina Dudek, Maja Koziarz, Sylwester Smialek. Dept. of Nephrology, Jagiellonian University Collegium Medicum, Cracow, Poland.

Background: Fluctuations of hemoglobin (Hb) level in dialysed patients leading to changes in its concentration outside the target range (10 to 12 g/dl) may contribute high cardiovascular mortality in this group. One of the factor affecting these fluctuations might be the choice of erythropoiesis stimulating agent (ESA).

The aim of the study was to find out if using different ESAs - methoxy polyethylene glycol-epoetin β (PEG-epoetin β) and darbepoetin α may affect the stability of Hb level in patients on maintenance hemodialysis.

Methods: The study was composed of 54 (35 M and 19 F) stable hemodialysed patients aged was form 23 to 85 years, mean 57.7 yrs. Time on dialysis was between 16 to 218 months and all of the patients were treated with ESA at least 12 months before beginning of the study. During 12 months of observation Hb level, transferrin saturation and ESA dose were controlled monthly. ESA dose was determined so that Hb level was between 10 to 12 g/dl. Only patients, who finished a year of observation were covered in analysis (population per protocol). Before the study patients were randomly (2:1 ratio) assigned to group treated with methoxy PEG-epoetin β (n=36) and group treated with darbepoetin α (n=18).

Results: At the beginning of the study Hb level was: 10.65 vs 11.21 g/dl and after 12 months of observation: 10.61 vs 11.6 g/dl (no statistically significant difference), respectively. Mean transferrin saturation was 43.9 vs 44.1% and there was no significant difference of Hb level between both groups. During observation in the group treated with methoxy PEG-epoetin β 26.67% of Hb measurements were outside target level. In group treated with darbepoetin α 45.92% of them were outside target level and the difference was statistically significant (Chi²=12.4; p<0.004). 3.58 changes of methoxy PEG-epoetin β dose and 5.18 changes of darbepoetin α dose was needed during observation, but this difference wasn’t statistically significant.

Conclusions: Therapy with methoxy PEG-epoetin β provided better stability of Hb level achieved by lower number of dose changes.


Background: MORAL study was conducted to assess the long term maintenance of hemoglobin (Hb) levels, with once-monthly IV administration of continuous erythropoietin receptor activator (C.E.R.A.) in patients with chronic kidney disease (CKD) previously treated with other erythropoiesis stimulating agents (ESAs) and to evaluate safety and tolerability of C.E.R.A.

Methods: This study consisted of a 4-week Study Verification Period (SVP), a 16-week Dose Titration Period (DTP), an 8-week Efficacy Evaluation Period (EEP) and a 4-week follow-up period.

Results: Totally 173 patients were screened in the SVP, 132 of which entered the DTP. Of these, 118 patients were eligible for the EEP and 97 patients completed the 16-week EEP and were screened for the follow-up. The mean (SD) age was 57.7 (13.7) years, 61% were male, and 56% were Caucasian, 44% were African American, and 2% were Hispanic. The mean (SD) Hb concentration at the start of Mircera® was 10.7 (1.2) g/dL, and the mean (SD) baseline ESA dose was 12,000 (6300) IU/wk. The mean (SD) time on dialysis was 57.7 (18.7) months. All of the patients were treated with ESA at least 6 months before and were on hemodialysis 6 months before the start of Mircera®.

Results: A total of 57 patients were included in the analysis (61.4% men and 38.6% women). Mean time on HD was 4.4 years. All patients received previous treatment with an erythropoietic stimulating agent (ESA) before starting treatment with Mircera® (70.2% epoetin beta, 10.5% epoetin alfa, 24.6% darbepoetin). Average dose of conversion to Mircera® were: 123.7 µg/month (DA 40-80 or 8000-16000 epoetin weekly) 102.8 µg/month for lower doses and 200 µg/month for patients with higher doses being below those recommended in SPC. Median Haemoglobin (Hb) at baseline was 11.2 g/dL, 11.6 g/dL at the start of treatment Mircera® and 11.0 g/dL at follow-up.

Conclusions: In HD patients the conversion to monthly Mircera from other ESAs is safe and effective, maintaining Hb levels within the desired targets.

Hemoglobin Variability in Peritoneal Dialysis Patients Treated with Erythropoiesis Stimulating Agents Ashwani K. Gupta, Sumit Narula, Ramesh Saxena. 1Nephrology, University of Florida, Jacksonville, FL; 2Nephrology, University of Texas Southwestern, Dallas, TX.

Background: Hemoglobin (Hb) variability and cycling have been documented both in Hemodialysis and Pre-dialysis patients receiving Erythropoiesis stimulating agents(EESA). Increased Hb variability (HbVar) has been linked to greater morbidity and mortality in these populations. Similar data for PD patients is extremely limited. A previous report of 12 PD patients from the Netherlands reported the average amplitude of HbVar to be 3.1 g/dL.

We hypothesized existence of HbVar in PD in patients and conducted a pilot study to characterize it.

Methods: A random sample (10%, n=20) of prevalent PD patients at a university based PD program was chosen for analysis. Hb, ESA doses and laboratory parameters were abstracted for a period between September 2009 and February 2011(mean follow up= 14 months). HbVar was examined by calculating the standard deviation of Hb within each subject and across all subjects.

Results: 95% of the observed Hb values varied by up to 2.8 g/dL (SD from the mean). Table 1 summarizes the study findings. 3 patients (15%) did not require therapy with EPO. HbVar was significantly lower in patients not requiring EPO in comparison to those requiring (EPO:0.65 vs 1.4 g/dL, p-value<0.001). The mean EPO dose was 12,000 U/wk(Range 1000-5000 U/wk). Mean number of dose changes were 10/trim (range 3-15).

Patient Characteristics and HbVar

<table>
<thead>
<tr>
<th>Mean(SD)</th>
<th>Hb(g/dL)</th>
<th>Albumin</th>
<th>PT</th>
<th>Ferritin</th>
<th>Saturation(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>11.5(1.7)</td>
<td>3.6(0.6)</td>
<td>433(400)</td>
<td>800(400)</td>
<td>55(18)</td>
</tr>
<tr>
<td>Patients(n=19)</td>
<td>12.0(0.6)</td>
<td>11.2(1.4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1

Conclusions: This pilot study confirms the existence of HbVar in PD patients. Inter-current events, varying iron stores and inflammatory mediators have been implicated in HbVar in pre-dialysis and hemodialysis patients. Similar factors may play a role in PD patients. HbVar, significantly greater HbVar in patients treated with ESA suggests a causal role of ESA administration in HbVar. PD patients may benefit from an improved ESA dosing protocol to reduce HbVar. A larger analysis including more patients will be undertaken to confirm these findings. Studies relating HbVar to outcomes are needed in PD patients.

Factors Affecting the Levels of Serum Glycated Albumin in Peritoneal Dialysis Patients Ami Hayashi, Yoshitaka Miyao, Toshiyuki Nakao. Nephrology, Shinjuku-ku, Tokyo, Japan.

Background: Glycated albumin (GA) is an alternative glycemic maker in diabetic hemodialysis patients. However, GA levels may be affected by albumin turnover, independent of MIRCERA® status. We investigated the dialysis-related factors which affect GA levels in peritoneal dialysis (PD) patients.
Methods: The levels of GA, HbA1c, serum glucose, and other clinical variables related to dialysis were calculated. Protein loss (PPL), dietary protein-to-creatinine ratio (D/Pcr), and dialysate-to-plasma creatinine ratio (D/Per) were measured in 33 stable PD patients (13 diabetic, 20 non-diabetic; age, 59.9 ± 10.5 years; men/women, 25/8; dialysis duration, 44 ± 35 months). The variables for each patient were calculated as the mean values of 3 monthly measurements. The correlations between GA and these variables were examined using the Pearson correlation coefficient and stepwise multivariable regression analysis. Results: GA, HbA1c, and serum glucose levels were significantly higher in diabetic patients than in non-diabetic patients (diabetic, 7.6 ± 0.8%, 6.2 ± 0.6%, 128.7 ± 33.5 mg/dl; non-diabetic, 13.3 ± 1.8%, 5.1 ± 0.3%, 99 ± 11 mg/dl, p < 0.001). In diabetic patients, a significant correlation was found among serum glucose, HbA1c, and GA levels. In non-diabetic patients, a significant correlation was found between pwCcr and GA levels. There were no significant correlations among serum albumin, PPL, D/Pcr, and GA levels in either diabetic or non-diabetic patients. Stepwise multivariable regression analysis showed that serum glucose and pwCcr were significant variables associated with GA levels in all patients (β = 0.69, -0.44, R² = 0.87). In the non-diabetic patients, only GA was a significant variable associated with GA levels (β = 0.64, R² = 0.41).

Conclusions: GA was affected by pwCcr in both diabetic and non-diabetic PD patients.

PUB267

Injection of Darbepoetin alfa at the Start of a Hemodialysis Session Might Be More Efficient Than Injection at the End of the Session Noritomo Itami, Kidney Center, Nikko Memorial Hospital, Muroran City, Hokkaido, Japan.

Background: It has been noted that the injection of darbepoetin alfa (DA) at the end of hemodialysis (HD) was often neglected because of the confusion that can occur at the end of the HD session due to complications such as hypotension, vomiting, and cramps. Injection of DA can be performed with more certainty at the start of HD than there is the possibility that an increased dose of DA will be required because of its adherence to the dialysis system's tubing.

Methods: Thirty-eight patients were recruited (M/F Ratio, 20:18; Age: 63.6 ± 10.5yrs; Primary disease: Diabetes(13), Glomerulonephritis(12), Polycystic kidney(3); others(10)). Written informed consent was obtained. At the start of the study, DA treatment was changed from the end of HD (Period I) to the start of HD (Period II). Other anemia treatment was carried out as usual. DA dose was altered biweekly according to our algorithm which was presented at the 52nd Congress of the Japanese Society of Dialysis Therapy. The algorithm maintains a target hemoglobin (HB) level of 10-12g/dl while keeping the change in HB to within 0.5g/dl to prevent the occurrence of HB cycling. After 2 weeks, the average DA dose and clinical parameters were examined.

Results: HB was unchanged at the end of both periods (Period I: 11.0±1.95g/dl and Period II: 10.9±1.87g/dl, not significant(NS)). Serum ferritin increased (143.9±9.94ng/ ml and 193.2±12.65ng/ml, p=0.001) although the number of patients undergoing iron treatment (Period I: 18.8±1.4 and Period II: 16.3±2.4, NS) was unchanged. Average DA dose in Period II was significantly reduced from 18.0±11.7mg/week in Period I to 16.1±10.7mg/week in Period II (p<0.05). The average fulfillment of target HB% was 49.6±18.4% in Period I and 77.6±14.3% in Period II (p<0.01). There was no neglect of injections of DA in Period II.

Conclusions: The injection of darbepoetin alfa at the start of an HD session might be more efficient and reliable than injection at the end of the HD session. A multicenter study providing access to more patients and a longer time period is warranted.

PUB268

Poor Performance of Correction Formula for the Prediction of True Hypocalcemia in Dialysis Patients Zaizan Khan, Christine A. White, Alexander R. Morton, Rachel M. Holden, Medicine, Queen’s University, Kingston, ON, Canada.

Background: Abnormalities in serum calcium are frequently encountered in patients receiving dialysis therapy. Total calcium is most frequently measured because of sample handling and cost concerns. Given that the ionized form is biologically active a number of adjustment formulas have been derived to "correct" the total calcium for changes in serum albumin and phosphorus. International guidelines for nephrology recommend that calcium be kept in the normal range reference and K/DOQI recommends using SA-corrected formulas. We determined the accuracy of noncorrected calcium and 5 published corrected calcium formulas in the identification of abnormal ionized calcium in a cohort of hemodialysis (HD) patients.

Methods: Ionized calcium (Ca), total calcium (CaT), serum albumin (SA), total protein (TP) and phosphorus (P) were measured in HD patients. The accuracy with each SA and P corrected formula (Table 1) identified abnormal CaT was determined.

Results: There were 75 HD patients with a mean age of 63.9 years. There were only 3 cases of hypocalcemia (CaT < 1.31mmol/L). There were 50 cases of hypocalcemia (CaT < 1.19 mmol/L). The mean SA was 35.1. The accuracy of each formula to predict abnormal CaT is demonstrated in the table. Non-corrected CaT identified true hypocalcemia in 38% of cases with a sensitivity of 77% and a specificity of 80% with mathematical correction. The single P-corrected formula was least accurate in this sample.

Conclusions: There was a high prevalence of hypocalcemia in this cohort of HD patients. In patients with difficult to control hyperparathyroidism, it is important to know that traditional correction formulas are not precise, particularly when vitamin D analogues and calcimetics are being used. There is little benefit of SA or P corrected formulas in identifying true hypocalcemia. Future studies should identify those dialysis patients best monitored by ionized calcium values.

PUB269

The Association of Nutrition with Interdialytic Weight Gain and Depressive Disorder in Hemodialysis Patients Hyun Gyung Kim, Young Ok Kim. Department of Internal Medicine, Seoul St. Mary’s Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea.

Background: Malnutrition was reported to occur in 23-70% and known as an important predictor of increased mortality in maintenance hemodialysis (HD) patients. This study was performed to evaluate the association of nutrition with interdialytic weight gain (IDWG) and depression in HD patients.

Methods: Sixty-five HD patients for at least 3 months were enrolled. We investigated malnutrition by ONSD (Objective score of Nutrition on dialysis) score and depressive depression by Montgomery depressive rating scale. We compared the clinical and biochemical profiles according to the malnutrition.

Results: Mean age of the patients was 55.7 ± 12.6 years and patients with diabetes accounted 55.4%. Mean duration of HD was 36.2 ± 32.0 (4 - 129) months. Depressive disorder was diagnosed in 21 (32.3%), Malnutrition (ONSD score ≥ 22) in 24 (36.9%) and large IDWG (>1kg/day) in 40 (61.5%) out of the 65 HD patients. Patients with malnutrition had lower incidence of large IDWG (45.8% vs 70.7%, p=0.046) and depression (19.5% vs 54.1%, p<0.004) than those without. BMI (21.2 ± 3.0 vs 23.4 ± 3.0 kg/m², p<0.006), TSF (triceps skin fold thickness, 8.8 ± 4.2 vs 15.0 ± 6.2 mm, p<0.001), MAC (midarm circumference, 23.6 ± 2.7 vs 26.4 ± 3.0 cm, p<0.001), serum albumin (3.6 ± 0.3 vs 3.8 ± 0.2 g/dl, p=0.029) and total cholesterol (142.5 ± 29.2 vs 169.7 ± 31.2 mg/dl, p<0.001) were also lower in patients with malnutrition, compared to the patients without malnutrition. There was no difference in age, gender, diabetes and HD duration between the two groups. In multivariable analysis, Depressive disorder was an independent risk factor for malnutrition.

Conclusions: This study showed that depressive disorder was significantly related to malnutrition in maintenance HD patients. Therefore, we suggest that the depressive disorder may be considered in HD patients at the time of the assessment of nutritional status in maintenance HD patients.

PUB270

The Factors Affecting Health-Related Quality of Life in Dialysis Patients Own Kwon,1,3 Ji-Young Choi,1 Jung-Ju Seo,1 Jang-Hee Cho,1 Mi-Kyung Jin,1,3 Kyung-Deuk Hong,1 Chung-Hoon Yu,1 So-Hee Yoon,3 Sun-Hee Park,1,3 Chan-Duck Kim,1 Ki-Soo Park,2 Yong-Lim Kim.1,3 Internal Medicine, Kyungpook National University School of Medicine, Daegu, Korea; Preventive Medicine, Gyeongsang National University School of Medicine, Jinju, Korea; 1Clinical Research Center for End Stage Renal Disease, Korea.

Background: To investigate the factors influencing health-related quality of life (HRQOL) in ESRD patients on dialysis, we measured the level of HRQOL.

Methods: The study subjects were 237 patients with ESRD on HD or PD over 6 months in two university hospitals in Korea. Patients completed the Korean version of KDQOL (kidney disease quality of life) -36 with five subscales. Measures of self-efficacy and treatment satisfaction in other studies were translated and modified for this study. Various sociodemographic and clinical variables were also recorded.

Results: The quality of life score were 46.3, 49.2, 67.6, 58.5, and 41.1 in the subscales of physical, mental component, symptoms and problems, effect of kidney disease on daily life, and burden of kidney disease, respectively. Variables associated with better physical and mental health were male gender, lower age, higher educational level, having an occupation, higher self-efficacy, and higher treatment satisfaction; a variable associated with better mental component was male gender; variables associated with better symptoms and problems component were hemodialysis and self efficacy. Burden of kidney disease component was associated with educational level, primary cause of ESRD and self efficacy. Effect of kidney disease component correlated positively with self-efficacy and treatment satisfaction. Multiple linear regression analysis showed that independent variables associated with HRQOL were age, educational level, duration and adequacy of dialysis, primary cause of ESRD, self efficacy and treatment satisfaction.

Conclusions: Adequacy of dialysis, self-efficacy and treatment satisfaction were associated with HRQOL in ESRD patients on dialysis. Strategies to increase adequacy of dialysis, self-efficacy and treatment satisfaction may be helpful to enhance the HRQOL in dialysis patients.

Funding: Government Support - Non-U.S.
The Newly Identified Anorexigenic Adipokine Nesfatin-1 in Hemodialysis Patients: Associations with Protein Intake and Body Composition

Denise Mafra, 1 Juliana Saldanha, 1 Julie Lobo, 1 Milena Barcza Stockler-Pinto, 1 Viviane Oliveira Leal, 1 Antonio Calixto, 1 Bruno Geloneze, 2 Denis Fouque, 2 Juan J. Carrero. 1 Clinical Nutrition, Federal University Fluminense, Niterói, Rio de Janeiro, Brazil; 2 Division of Renal Medicine, Karolinska Institute, Sweden; 3 Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; 4 Laboratory of Investigation on Metabolism and Diabetes, State University of Campinas, Campinas, Brazil; 5 Nephrology, Université Claude Bernard I - Hôpital Edouard Herriot, Lyon, Rhone, France.

Background: Nesfatin-1 is an anorexigenic peptide. Anorexia and malnutrition are common features of chronic kidney disease (CKD) that predispose patients to worse outcomes. The aim of this study was to evaluate the plasma nesfatin-1 levels in hemodialysis (HD) patients.

Methods: Twenty five HD patients were studied and compared to 15 healthy subjects matched for body mass index (BMI), % body fat mass (BF) and age. Nesfatin-1 levels were analyzed using ELISA and, leptin levels were measured by a multiplex assay kit manufactured by R&D Systems®. Appetite was measured using a specific questionnaire and food intake were recorded.

Results: Plasma nesfatin-1 levels did not differ between HD patients and healthy subjects. Clinical and anthropometric characteristics of the HD patients and healthy subjects

Parameters | HD Patients | Healthy Subjects
--- | --- | ---
Age (years) | 53.3±11.9 | 47.9±14.8
BMI (kg/m²) | 23.1±2.8 | 24.9±3.9
Triceps skinfold thickness (mm) | 13.1±4.9 | 15.2±2.9
Body fat (%) | 28.6±6.5 | 29.1±3.2
Leptin (ng/mL) | 13.9±2.1* | 5.1±4.3
Nesfatin-1 (ng/mL) | 0.16±0.07 | 0.17±0.10

* p<0.05

Nesfatin-1 levels showed significant negative correlations with protein intake (r=-0.42; p=0.03), and positive correlations with BMI (r=0.33; p<0.03), % body fat (r=0.35; p=0.03) and the triceps skinfold thickness (r=0.36; p=0.02). Nesfatin-1 levels also correlated positively with leptin levels (r=0.45; p=0.006).

Conclusions: In conclusion, nesfatin-1 levels did not differ between HD patients and healthy subjects who were matched for BMI, % body fat and age. However, nesfatin-1 correlated with protein intake and body composition.

Funding: Government Support - Non-U.S.

Is Body Mass Index of 23kg/m² a Reliable Marker of Protein-Energy Wasting in Hemodialysis Patients? Denise Mafra, 1 Viviane Oliveira Leal, 1 Cristiane Moraes, 1 Milena Barcza Stockler-Pinto, 1 Julie Lobo, 2 Najla Elias Farage, 1 Denis Fouque, 1 Clinical Nutrition, Federal University Fluminense (UFF), Niterói, Rio de Janeiro, Brazil; 2 Institute of Biophysics Carlos Chagas Filho, Federal University of Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil; 3 Nutrition, RenalCor Clinic, Rio de Janeiro, Brazil; 4 Nephrology, Université Claude Bernard I, Lyon, Rhone, France.

Background: There are several parameters that may be indicative of protein-energy wasting (PEW) in hemodialysis (HD) patients and the International Society of Renal Nutrition and Metabolism panel recommends that a body mass index (BMI) less than 23kg/m² is a marker of PEW. However, BMI can be heavily influenced by fat mass and the adiposity is related to inflammation. Therefore, the adequate cut-off point by BMI remains controversial and this study compared the body composition and inflammatory status of HD patients according to the cut-off of 23kg/m² for BMI.

Methods: Forty seven HD patients (30 men, 11 diabetic, 53.8±12.2 yr, 58.2±50.9 months on dialysis) were studied. Anthropometric data and handgrip strength (HGS) were evaluated. Inflammatory markers (CRP, TNF-alpha, leptin and IL-6) were measured.

Results: Nineteen (40.4%) patients presented BMI values <23kg/m², and leptin levels, midarm muscle circumference and free fat mass (FFM) were significantly lower in these patients when compared to patients with BMI >23kg/m². However, the prevalence of muscle functional loss was not different between the BMI groups. The percentage of body fat (%BF), fat mass, FM/FFM ratio and waist circumference (WC) were significantly lower in patients with BMI <23kg/m² but the mean values of %BF did not indicate energy deficiency. Patients with BMI >23kg/m² presented higher prevalence of inflammation and the WC and %BF values were compatible with metabolic derangements associated with obesity. The adiposity parameters were correlated with CRP and leptin.

Conclusions: HD patients with BMI <23kg/m² did not presented signs of energy wasting while those with BMI >23kg/m² were more inflamed probably due higher adiposity. Thus, the BMI of 23kg/m² seems not to be a reliable marker of PEW in HD patients.

Funding: Government Support - Non-U.S.


Background: Intradialytic parenteral nutrition (IDPN) may be useful to contrast malnutrition of hemodialysis (HD) patients. However, it is essential to verify whether IDPN is actually able to draw positively upon metabolism, enriching the aminoacid (AA) status, thereby enhancing protein synthesis. A kinetic study was planned to evaluate whether IDPN can induce an effective AA gain in the short-run in malnourished HD patients.

Methods: Ten HD patients (3 x week; Bologna Malpighi & Trento Hospitals) with albumin <3.5 g/dl and pre-albumin <30 mg/dl for at least one month were studied (low-flux membrane, 240 min/session). After a dialysis study session without IDPN, patients received IDPN for one month (all-in-one bag Nutrispecial: 625ml, Proteins 35.9 g, 10 AA: Ser, Pro, Gly, Ala, Val, Met, Leu, Phe, Lys, His). In the first session, as well as after 2 and 4 weeks, we measured the plasma (pre- and post-HD), and dialysate AA concentration (dialysate spilling technique at 120 and 240 min). Albumin, pre-albumin, glucose and lipid metabolism, inflammation indices and blood cell count were also evaluated.

Results: Preliminary results show that even with a low-flux dialyzer and without IDPN infusion an AA loss in dialysate does exist. With the IDPN infusion that loss actually increases (43±13 with IDPN vs 23±14mmol/l without IDPN, p<0.001). Nevertheless, after one month IDPN, the pre-dialytic plasma concentration of each infused AA was increased as compared with the basal value (minimum 5.2% for Tyr, maximum 66% for Try), with a mean increment of 36.8%. No derangement was found in glucose and/or lipid metabolism.

Conclusions: In one month of IDPN, a time range that is quite insufficient to induce a clinically relevant change in the nutritional state, a homogeneous growth in the plasma concentration of all the infused AA was evident, however, leading us to hypothesise that the use of IDPN for a longer time-span may actually translate into increased protein synthesis.

Funding: Private Foundation Support

Stopping of Dialyzer Reuse and Switch to High-flux Dialyzers Is Associated with Lower ESA Requirement and Better Phosphorus Control Andrzej Milkowski, 1 Teresa Rydzynska, 1 Jolanta Malyaszko. 1 Fresenius Nephrocare, Poland; 2 Nephrology, Medical University, Bialystok, Poland.

Background: Dialyzer reuse is still common practice in some dialysis units. There are three major concerns with reuse: the risk of infection; biochemical and immunologic effects; and loss of performance with impairment in clearance and/or ultrafiltration.

Methods: The aim of our study was to assess the dialysis adequacy, anemia control and use of ESA as well as phosphate control in 68 patients from a single center before and 1 year after stopping of reuse of dialyzers. In addition, together with use of single dialyzers, all the patients were switched to high-flux dialyzers (Fresenius, Germany).

Results: All the parameters are given in the Table 1.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before reuse</th>
<th>After reuse</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphate (mmol/L)</td>
<td>1.75±0.55</td>
<td>1.73±0.31</td>
<td>*</td>
</tr>
<tr>
<td>Urea before HD (mmol/L)</td>
<td>24.01±18.35</td>
<td>24.24±9.90</td>
<td></td>
</tr>
<tr>
<td>Urea after HD (mmol/L)</td>
<td>7.40±2.41</td>
<td>6.64±2.15</td>
<td>***</td>
</tr>
<tr>
<td>Urea reduction ratio (%)</td>
<td>69.34±4.79</td>
<td>72.54±7.3 ***</td>
<td></td>
</tr>
<tr>
<td>Kt/V</td>
<td>1.46±0.54</td>
<td>1.46±0.54</td>
<td>**</td>
</tr>
<tr>
<td>HD time weekly (hours)</td>
<td>11.71±2.27</td>
<td>12.34±1.54</td>
<td>*</td>
</tr>
<tr>
<td>ESA weekly dose (IU)</td>
<td>3485±217</td>
<td>2840±206 *</td>
<td></td>
</tr>
<tr>
<td>ESA dose per kg dry body weight</td>
<td>55.12±38.41</td>
<td>54.25±35.6 **</td>
<td></td>
</tr>
<tr>
<td>Serum iron (mmol/L)</td>
<td>1.07±0.38</td>
<td>1.37±0.59</td>
<td></td>
</tr>
<tr>
<td>TST (%)</td>
<td>32.34±19.02</td>
<td>34.47±7.77</td>
<td></td>
</tr>
<tr>
<td>Iron x weekly (mg)</td>
<td>54.76±9.34</td>
<td>57.37±0.03</td>
<td></td>
</tr>
<tr>
<td>Hgb (mg/dL)</td>
<td>10.74±1.66</td>
<td>11.31±1.58 p&lt;0.06</td>
<td>*</td>
</tr>
</tbody>
</table>

*p<0.05, ** p<0.01, ***p<0.001

One year after switch to single use high flux dialyzers a highly significant rise in urea reduction ratio was observed, together with a tendency to rise in Kt/V. Moreover, use of ESA dropped significantly in the study group with a tendency to higher achieved hemoglobin, whereas the iron supplementation did not change. A significant fall in serum phosphate was also observed.

Conclusions: Single use high-flux dialyzers are more efficient in clearing urea and other molecules. Moreover, after switch amelioration of anemia with lower use of ESA was demonstrated together with better phosphate control. It may be associated with better survival and lower costs. However, further investigation is required to accurately assess the morbidity and mortality associated with reuse or single use as well as cost effectiveness.
C-Peptide (CP) Levels in Diabetic Patients (pts) on Maintenance Hemodialysis (HD) as Compared to Non-DM HD pts

Methods:
- Percent changes in relative plasma volume were estimated by Albumin changes (%ΔRpV/Alb, Van Stone J et al).
- Phase 1: in 57 patients (Pts), pairs of Pre and post samples were collected separately and the repetitability coefficient was 0.39 (Bland and Altman).
- In the next phase, only Pts with a ΔHg 0.3 were included.
- Phase 2: Out of 229 pts, a ΔHg 0.3 was observed in 241: 144 male and 97 female.

Results:
- Hg (median 1.1, Interquartile Range: 0.75-1.6) was reduced to 0.38, post-HG 4.5±1.1 vs. SBW (median 1.5, Interquartile Range: 1.3-1.8) and the second upper quantiles of SBW showed a ΔHg of 1.41 (95% CI 1.3 -1.52). This change was higher than the observed in Pts of the two lower quartiles - 1.05 (95% CI 0.95-1.15, p = 0.000).
- Phase 3: 76 Pts with SBW = 2.5 were studied. %ΔRpV/Alb was 14.9 (95% CI 10.3-12.5).
- Regression analysis of %ΔHg as outcome and %BW or %ΔRpV/Alb as predictor, estimates a r of 0.16 and 0.41 (p=0.041), respectively. PreHg level of phase 2 and 3 (total n=312) was significantly lower than ΔRpV/Alb and TACHg. Both estimations of functional Hg were similar. Of the Pts with SBW>2.5 and PreHg between 9-9.9, the ΔRpV/Alb was ±10 in 62%.
- Conversely, odds of getting out of target was high in Pts with PreHg between 11-12 and 9-9.5, since 67% of them showed an ΔRpV/Alb ±12.

Conclusions:
- i) changes in RPV play a major role in Hg intradialytic variations. ii) ESA dosing in dialysis Pts with more than 2.5 Kg of weight loss should be based on the average of the Hemoglobin levels measured before AND after dialysis.
PUB280
STATIN THERAPY CORRELATES WITH LOWER CARDIOVASCULAR DEATH, BUT NOT WITH LOWER CARDIOVASCULAR EVENTS AMONG PATIENTS ON HEMODIALYSIS

Edmond Dedda,1 Chrisoula Pipili,1 Paraskevi Tseke,1 Konstantinos Pantelias,1 Petros Korflias,1 Zoe Tegou,1 Helen Tzanatos,2 Eirini Grapsa.1 1Nephrology, Aretaieion University Hospital; 2Dialysis Unit Specimen, Loutraki, Greece.

Background: Cardiovascular events are the major cause of death among patients on maintenance hemodialysis. Regulation of cholesterol and triglyceride levels within target goals is paramount. Our purpose was to examine whether statin therapy correlated with evidenced (presence or absence) cardiovascular events among patients receiving hemodialysis.

Methods: A total of 106 patients (32 women and 74 men, aged 66 ± 14 years) on chronic hemodialysis were studied. The lipidemic profile (total cholesterol, LDL-cholesterol, triglycerides) and all the cardiovascular events (acute coronary syndrome, stroke and heart failure) as well as deaths caused from them were recorded within two - year follow up.

Results: Patients on hemodialysis with dyslipidemia showed higher rates of cardiovascular events (20% vs. 2.4%, odds ratio 3.75, p = 0.005). Cardiovascular deaths were noted among 41% of patients not receiving treatment for dyslipidemia and only 20% vs. 2.4%, odds ratio 3.75, p = 0.005). Cardiovascular deaths shown in the table.

Conclusions: Patients on chronic hemodialysis present impaired lipid profile. Those received treatment for dyslipidemia presented lower risk for cardiovascular deaths, but not for cardiovascular events.

PUB281
EVALUATION OF DIABETIC PATIENTS OLDER THAN 65 YEARS STARTING HEMODIALYSIS

Comparation with Control Group

Herrero Juan Carlos, Nephrology, Hospital Severo Ochoa, Leganes, Madrid, Spain.

Background: In the last years we have seen a significative increase of patients starting hemodialysis (HD) due to diabetic nephropathy older than 65 years. This patients have more cardiovascular risk factors and morbi-mortality.

Methods: We want study evolution, demographical and clinical characteristics of these patients (Group I) starting HD in our service, versus patients starting HD with similar age and other cause of ESRD (Group II).

Results: Between September-2006 to September-2010, 165 patients incident HD. 68 (41%) patients older than 65 years. Median follow up was 23 months (5-51). Main results shown in the table.

Conclusions:

PUB282
AUDIT OF THE MANAGEMENT OF ATRIAL FIBRILLATION IN HAEMODIALYSIS PATIENTS

Jonathan Reaney, Cathal L. Steele, Agnes Masengu, Camille Harron, Robert Mullan, Ronan Cunningham. Department of Nephrology, Antrim Area Hospital, Antrim, United Kingdom.

Background: The prevalence of atrial fibrillation (AF) in haemodialysis (HD) patients ranges from 7.7% to 27%. A recent study found one-year mortality rates were twice as high in HD patients with AF than in those without. An audit of management of AF in the haemodialysis unit was carried out.

Methods: Baseline electrocardiograms (ECGs) were performed on patients attending for HD sessions and the rhythm analysed by medical staff. In addition, serum samples were analysed for high sensitivity C reactive protein (hsCRP) and high sensitivity Troponin-T (hsTnT). The notes and electronic records of patients identified with AF were reviewed to determine if AF had been previously documented. Patients identified with AF had their CHADS2 stroke risk index score calculated and management reviewed.

Results: ECGs were carried out on 57 dialysis patients. Median age was 73 years, ethnicity 100% Caucasian, female 47.4% (27/57) and diabetic 41.1% (24/57). The prevalence of AF on ECG was 10.5% (6/57). No cases of undiagnosed AF were identified. No significant association between the levels of hsCRP and hsTnT and presence of AF was found. In this study, all patients with AF had a CHADS2 score ≥ 2. Oral anticoagulation therapy is recommended for patients with CHADS2 score ≥ 2. All patients with AF were on oral antithrombotic agents (Warfarin 3/6, Aspirin 2/6, Clopidogrel 1/6). The prevalence of AF was not clearly documented in the patient’s medical records in 3/6 cases.

Conclusions: Rates of AF in our HD population were similar to rates reported from larger studies. AF in haemodialysis patients carries a significant risk of mortality. All patients identified with AF were receiving oral antithrombotic therapy however little evidence exists for the use of this for AF in this population. HD patients on warfarin have an increased risk of stroke and major haemorrhage. An individual risk stratification with respect to oral antithrombotic agents needs to be performed and documented for each haemodialysis patient with AF.

PUB283
WITH INCREASED INFLAMMATION, FIBRINOGENESIS MAY COUNTERCALLE Platelet Hyperactivity in Diabetic Hemo and Peritoneal Dialysis Patients

Saila V. Ventrapragada,1 George P. Bayliss,2 Bijan Roshan,1 Ray E. Gleason,1 Larry A. Weinrauch,1 John A. D’Elia.1 1Harvard Medical School, Boston, MA; 2Brown Medical School, Providence, RI.

Background: Study subjects with Type1 diabetes (DM) had elevated levels of hemostasis factors [Factor VII (FVII), fibrinogen (fibr), vonWillebrand factor (vWF)], decreased plasminogen activator inhibitor (PAI) levels, accelerated platelet adhesion/ aggregation, and high lipid levels. With intensive treatment over 6-12 months, FVII, fibr, PAI factor levels improved significantly. Follow-up study examined impact of hemostasis, inflammation, oxidative stress on cardiovascular events (CVE) in 124 diabetic study subjects on dialysis (103 HD and 21 PD) vs 26 non dialietic controls (HD+PD).

Methods: DM + dialysis subjects divided into CVE + history (n = 58) vs CVE – (n=66); CVE + history + associated with significantly higher levels of CRP (p = 0.05). DM – subjects had significantly higher levels of low MW/ fibr and PAI –1 if CVE history +. Dialysis study group data analyzed using platelet activation index: fibrinogen x vWF x p- selectin. Result for DM+ group significantly greater than DM – group (p =0.05). Data analyzed using an index of fibrinogenosis: low MW/ intact fibr (% degradation) x fibrinolytic activity x 1/ PAI-1. Result for DM + group significantly greater than DM – group (p = 0.05).

Results: Results: At follow up: CVE + DM + study group had significantly higher inflammation index (fibr x interleukin-6 x C-reactive protein) vs CVE – DM + dialysis patients (p = 0.02). Accelerated platelet function and fibrinogenesis indices didn’t discriminate DM + dialysis patients + or – CVE. When CVE free interval analyzed for 150 DM + and – subjects, there was no difference between tertiles for platelet activation index. When CVE free interval analyzed for 150 subjects, tertiles for fibrinogenosis index reflected more favorable prognosis of DM + (p = 0.04).

Conclusions: DM dialysis patients with CVE on followup had higher inflammation index. Increased fibrinogenosis may counterbalance accelerated platelet aggregation/ adhesion. Impact of fibrinogenosis on CVE free survival reflects more favorable prognosis of non-diabetic subjects on dialysis.

Funding: Pharmaceutical Company Support, Private Foundation Support

PUB284
HEMODIALYSIS-INDUCED BACTEREMIA IN OUR DIALYSIS UNIT, WHERE DO WE STAND FROM INTERNATIONAL RATES?

Jafar Al-Said, Aimee Padgudan, Soni Murdeshwar. Nephrology and Internal Medicine Department, Bahrain Specialist Hospital.

Background: Aim:

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

910A
Contaminated blood cultures were excluded. The prevalence rate of dialysis induced bacteremia per 1,000 patient years and per 1,000 hemodialysis sessions was estimated. Admission rates for dialysis related bacteremia and access related infection was measured. Our data was compared to USRDS 2010 and other published North American data.

**Results:** Total 4,300 hemodialysis sessions were included which were done on 114 patients. Thirty-three dialysis related bacteremia were encountered in 7 patients over 87 months. Six of them had cuffed tunneled catheter and one had an AV fistula. Two patients required admission during their bacteremia infection. Sphingomonas paucimobilis was the only organism causing all the infection episodes. This was significantly different from the typically skin bacteria causing dialysis related infection in other units. The source was found to be the main dialysis water tank.

<table>
<thead>
<tr>
<th>Item</th>
<th>Our data</th>
<th>International data</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prevalence of Dialysis related bacteremia per 100 patient months</td>
<td>0.02</td>
<td>1.59</td>
<td>0.002</td>
</tr>
<tr>
<td>Prevalence of bacteremia per 100 Hemodialysis procedure</td>
<td>3.02</td>
<td>3.07</td>
<td>0.9</td>
</tr>
<tr>
<td>Prevalence of admission for Hemodialysis related bacteremia per 100 patient years</td>
<td>0.76</td>
<td>180</td>
<td>0.006</td>
</tr>
<tr>
<td>Prevalence of admission for cuffed tunneled access infection per 1000 patient year</td>
<td>Zero</td>
<td>456</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Conclusions:** Dialysis related bacteremia per patient in our unit is less than North America data while the prevalence per dialysis session is the same. We had lower admission rates compared to USRDS 2010.

### PUB285

**Increasing Mean Body Mass Index among Adults Initiating Treatment for End-Stage Renal Disease, by Diabetes Status; United States, 1996–2008**

Nilka Rios Burrows, 1 Israel Hora, 2 Andrew S. Narva, 3 Centers for Disease Control and Prevention, Atlanta, GA; 4 Northrop Grumman, Atlanta, GA; 5 National Institutes of Health, Bethesda, MD.

**Background:** Diabetes is a major risk factor for end-stage renal disease (ESRD), and high body mass index (BMI) is associated with developing type 2 diabetes. We assessed trends in mean BMI among U.S. adults initiating ESRD treatment.

**Methods:** Using 1996–2008 data from the U.S. Renal Data System, we obtained mean BMI at initiation of ESRD treatment among people aged ≥18 with diabetes (ESRD-DM) or other conditions (ESRD-OT) listed as the primary diagnosis. Mean BMI was examined by age group. Joinpoint regression was used to analyze trends and calculate an average annual percent change (AAPC) with 95% confidence interval (CI).

**Results:** From 1996 to 2008, mean BMI increased among adults initiating treatment for ESRD-DM (from 25.7 to 29.6) and ESRD-OT (from 23.6 to 26.7). Among ESRD-DM incident cases, mean BMI increased from 24.4 to 29.6 (AAPC=1.6% per year, CI=1.5%–1.8%) among those aged 18–44, from 26.8 to 30.6 (1.2%, 1.0%–1.5%) among those aged 45–64, from 25.2 to 29.8 (1.5%, 1.3%–1.7%) among those aged 65–74, and from 23.9 to 27.3 (1.2%, 1.1%–1.3%) among those aged ≥75. Among ESRD-OT incident cases, mean BMI increased from 24.6 to 27.8 (1.0%, 0.8%–1.1%) among those aged 18–44, from 24.6 to 27.9 (1.0%, 0.9%–1.1%) among those aged 45–64, from 23.2 to 26.7 (1.2%, 1.0%–1.3%) among those aged 65–74, and from 22.1 to 24.7 (0.9%, 0.8%–1.0%) among those aged ≥75. Throughout the period, among incident cases aged ≥45, mean BMI was higher among ESRD-DM than among ESRD-OT. From 2005 to 2008, mean BMI was ≥30 among ESRD-DM cases aged 45–64.

**Conclusions:** Mean BMI at initiation of ESRD treatment increased during the study period irrespective of diabetes status. Higher BMI is associated with greater likelihood of survival in the first 12 months of ESRD treatment, but also associated with comorbidities, such as cardiovascular disease (CVD), which could impact survival. Evaluation of the nutritional profile of people preparing for ESRD treatment and reduction of CVD risk factors is important to improve outcomes.

### PUB286

**Schizophrenia and Schizoaffective Disorder in a Dialysis Population: A Pilot Study**

Joseph Cha, 1 Kruti N. Thakkar, 2 Navin Jaipal, 3 James I. McMillan, 4 Nephrology Section, VA Loma Linda Healthcare System, Loma Linda, CA; 5 Department of Internal Medicine, Loma Linda University, Loma Linda, CA.

**Background:** Schizophrenia (Sp) affects about 1% of the world’s population. It complicates the treatment of patients with ESRD, and the stress of dialysis may exacerbate Sp. We compared the prevalence of Sp and schizoaffective disorder (Sa) in dialysis patients treated in the VA Loma Linda Healthcare System over a 9 year period with that in matched patients without kidney disease in the same system.

**Methods:** Medical records of all chronic hemodialysis patients in the Loma Linda VA from January 1, 2000 through December 31, 2008 were examined for a diagnosis of Sp or Sa. Each ESRD patient was matched with a control by age, race (non-African American vs. African American), sex, and the diagnosis of diabetes and hypertension. Continuous variables were compared using the Student’s T-test, and dichotomous variables were compared using the Chi-squared test.

**Results:** The 410 patients with ESRD were matched with 407 controls without kidney disease. Three of the ESRD patients had rare characteristics (e.g. female gender, young age) and could not be matched. Of the 817 remaining patients, 197 (24.1%) were African American, 17 (2.1%) female, 770 (94.3%) had hypertension, and 541 (66.2%) had diabetes. The prevalence of Sp/Sa in cases vs. controls was not significantly different, 10 (2.4%) vs. 6 (1.5%) (p = 0.32). Consistent with other published reports, African American race was associated with a higher prevalence of Sp/Sa, 8 of 197 (4.1%) vs. 8 of 620 (1.3%) non-African Americans in our combined population (p = 0.014).

**Conclusions:** Our control population had a prevalence of Sp/Sa similar to the accepted prevalence of these diseases. The ESRD population showed a trend toward a higher prevalence of Sp/Sa at 2.4%, but in this small population the difference was not significant. If this trend is correct, power analysis based on our findings, assuming an alpha of 5%, would require a sample size of 3000 ESRD patients and their matched controls to have a power of 80% to detect a difference of 1% in the prevalence of Sp/Sa between the two groups.

**Funding:** Veterans Administration Support

### PUB287

**Co-Existence of Peripheral Vascular Disease(PVD) Is Associated with High 5yr Mortality in Diabetics on Dialysis**

Vinod Sathyanarayana Dibbur, 1 Hasan Haboubi, 1 Christopher Edwards, 1 Ashraf I. Mikhail, 1 Sadananda V. Athihal. 1 Morriston Hospital, United Kingdom; 2 Morriston Hospital; 3 Morriston Hospital; 4 Morriston Hospital.

**Background:** Patients with diabetic nephropathy starting dialysis are at high risk for cardiovascular(CV) events. A 70% 5yr mortality has been reported in them (USRDS data). Data on the influence of glycemic status on survival in patients on dialysis is conflicting.

We retrospectively looked at the impact of coexistent CV disease and conventional risk factors on 5yr survival in a cohort of 98pts(28 diabetic)who started dialysis in 2005. We audited our management of prevalent diabetic patients on dialysis in 2010.

**Methods:** Data collected and analysed from the renal data base.

**Results:** 82.66% of the 98 pts started haemodialysis and 17.34% peritoneal dialysis. 28.6% were diabetic and 71.42% were non diabetics.Ischaemic heart disease IHD(42.85% vs 18.5%) and PVD(25% vs 2.85%)was significantly higher in diabetics.Mean age in both groups 50yrs.

The 5yr mortality in diabetics was significantly higher when compared to non-diabetic(57%vs28.5%).The median survival in the mortality group was 2years.15.7%of nondiabetics and 10.7% of diabetics were transplanted.

### PUB288

**Views of Renal Healthcare Professionals About the Role of Palliative Care in Patients with End Stage Kidney Disease**

Robert G. Fassett, 1 Iain Robertson, 2 Rosalind M. Bull. 1 Renal Medicine, Royal Brisbane and Women’s Hospital and The University of Queensland, Brisbane, Queensland, Australia; 2 Human Life Sciences, University of Tasmania, Launceston, Tasmania, Australia; 3 School of Nursing and Midwifery, University of Tasmania, Launceston, Tasmania, Australia.

**Background:** Palliative care is increasingly recognized as an important part of end stage kidney disease (ESKD) care. Health professionals’ own beliefs and knowledge about palliative care, death and dying impact on their decision to offer and support palliative care involvement. This study explored the perceptions of renal health professionals regarding palliative care and how this influenced how palliative care was integrated into ESKD patient management. The aim of this study was to identify barriers and facilitators experienced by renal healthcare professionals in incorporating palliative care into ESKD management.

**Methods:** All renal healthcare professionals in North and Northwest Tasmania were invited to complete a questionnaire exploring their views on the role of palliative care in the management of patients with CKD.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

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Results: Of 105 surveys distributed, 41 were completed (response rate 39%). Health professionals were asked to mark all which were true, and the highest percentage of respondents marked “must be included.” It will be useful in the greatest influence of dialysis withdrawal. Loss of will to live mainly followed pain and depression. Acute comorbidity or depression delayed withdrawal of treatment. Malignancy and functional decline influence health professionals and family towards palliation. End of life care should be discussed early at pre-dialysis education, openly, honestly and sensitively with full disclosure by senior renal medical or nursing staff. Final decisions should occur depending on the patient’s ongoing condition. The patient should make the decision supported by family.

Conclusions: Renal health professionals believed the patient, with involvement of the family, should end of life decisions. Preparation should occur at the start of ESKE management with the actual decision made when the patient is ready.

Funding: Government Support - Non-U.S.

PUB289

Factors Affecting Quality of Life in Caucasian Octogenarians and Nonagenarians on Dialysis in Southern Indiana – A Cross Sectional Study Manish Gera, Kumar Gaurav, Raj Jeevan. Nephrology, Internal Medicine Nephrology INCP, Terre Haute, IN.

Background: The elderly constitute the fastest-growing segment of the ESRD population. The numbers of octogenarians & nonagenarians starting dialysis have more than doubled in the last decade, corresponding to an average annual increase in dialysis initiation of 9.3%. Literature mainly focuses on elderly in general (≥ 65 years). While management is focused on cardiovascular end points, mortality & lab parameters including Kt/V, albumin, & mineral bone disorder. There is paucity of literature focusing on patients ≥ 80 years & on their symptomatology. Symptoms affecting quality of life (QOL) may involve HEENT, musculoskeletal or nervous systems. Also, majority of these patients may find it hard to respond to extensive questionnaires used by most studies. Moreover, access to primary care is not readily available in this patient population.

Methods: We performed a cross sectional study to look at the factors affecting QOL in patients ≥80 years, in order to provide comprehensive care to this population. Two hundred thirty-five patients (232 patients from 4 outpatient dialysis units in Southern Indiana were screened). Both hemodi and peritoneal dialysis patients were included. Patients had to be on dialysis for at least 3 months. Patient with underlying dementia were excluded.

Results: Twenty five (25) (10.7%) met criteria. A simple questionnaire was devised to look at factors that could affect QOL. This included identifying problems related to hearing, vision, dizziness, headache, gait, joint pain, back pain & appetite. Patient’s responses were recorded. Management of these symptoms was integrated in care plan along with lab parameters for optimal health care delivery.

2. Patients indicated that dialysis per se did not affect QOL.
3. Symptoms related to volume issues were present in about 10% patients only.
3. We propose, a simple questionnaire to determine symptoms affecting QOL be integrated in care plan for comprehensive care.
4. Palliative care should be considered when attempt to relieve symptoms is unsuccessful.

Funding: Clinical Revenue Support

PUB290

Achievement of CKD-MBD (Chronic Kidney Disease-Mineral and Bone Disorder) Management with Vitamin D Analogs and Minimal Calcium Load in Patients on Maintenance Hemodialysis Naho Ito, Minoru Ito, Kiyotaka Yabuki. Yabuki Hospital.

Background: Recently, the growing concern for dialysis is considered to be the increase in the number of aged and long-term patients in Japan. Therefore, the objective of dialysis treatment is not only the longevity of the patients but the improvement of quality of life among them. For that reason, it is essential to determine factors related to these complaints. In our cross-sectional study, we examined the relationship between the severity of the uncomfortable symptoms and various clinical parameters.

Methods: A total of 342 patients performed with chronic maintenance dialysis in our three facilities were included. Additionally, they were evaluated by the self-rating score questionnaire based on fifth graded face scales from 0 (none) to 4 (very strong) according to the severity. The symptoms were composed of 20 common physical and psychological complaints. Odds ratios for each of moderate to severe (score = 2, 3, 4) or severe (score ≥ 5) were calculated using a multiple logistic regression model adjusted for confounding factors including age, gender, time of dialysis, history of diabetes mellitus, Kt/V, normalized protein catabolic rate, body mass index, predialytic values of hemoglobin, albumin, β2-microglobulin, sodium, potassium, phosphate.

Results: Among participants, 146 (42.7%) patients were treated with hemodialfiltration and 196 (57.3%) received dialysis therapy. The most frequent symptoms in patients on hemodialfiltration were vision, dizziness, headache, gait, joint pain, back pain & appetite. The most frequent symptoms in patients on dialysis therapy were back pain & appetite.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.

PUB291

Antithrombotic Therapy and Bleeding in Hospitalized Patients with End-Stage Renal Disease Receiving Hemodialysis Olayinka O. Holt, Shyan-Yih Chou, Hongbog Ma. Division of Nephrology & Hypertension, Brookdale University Hospital & Medical Center, Brooklyn, NY.

Background: In end-staged renal disease (ESRD) patients, use of anticoagulation and antplatelet agents for cardiovascular disease is common but may increase the risk of major hemorrhage because of uremic platelet dysfunction. The aims of this study were to evaluate transfusion burden and hospital length of stay (LOS) associated with antithrombotic therapy in hemodialysis (HD) patients with ESRD who developed bleeding during hospitalization.

Methods: We analyzed medical records from 151 consecutive HD patients who developed bleeding during hospitalization between January 2008 and June 2010. The patients were stratified into two groups: Group 1 (n = 73) were those who received antithrombotic therapy (aspirin, clopidogrel, warfarin, or heparin but exclusive of its use during HD). The antithrombotic therapy followed the practice standard for clinical needs such as prophylaxis against cardiac or venous thrombosis, or after percutaneous coronary intervention. Group 2 (n = 78) did not receive antithrombotic therapy.

Results: In the cohort, the age was 62.1 yr (mean/SE), 46% male, and 79 patients received blood transfusion. Similar clinical characteristics were noted in the two groups, including age, gender, race, urea reduction ratio, platelet count, baseline INR, and HD time. In Group 1, the nadir hemoglobin before transfusion was 7.9±0.3, similar to 8.0±0.2 g/dl in Group 2. In Group 1, 48% patients received blood transfusion, similar to 52% in Group 2. In patients who received transfusion, Group 1 received 5.5±0.6 units of blood, similar to 4.7±0.6 units in Group 2. LOS in Group 1 was 11.6±1.0 days, longer than 6.1±0.6 days in Group 2 (P = 0.0018). In a multivariate survival analysis, exposure to antithrombotic agents was associated with longer LOS (P = 0.018); however, blood transfusion as clinically indicated did not affect LOS (P = 0.38).

Conclusions: In conclusion, in hospitalized ESRD patients receiving HD, antithrombotic therapy did not adversely affect severity of bleeding or transfusion burden and the significantly increased LOS in patients on antithrombotic therapy was linked to the clinical conditions requiring its use.

Funding: Clinical Revenue Support

PUB292

Analysis of Factors Associated with Complaints in Patients Treated with Hemodialysis or Hemodiafiltration Atsuhiko Kanno, Ikuto Masakane, Satoko Ito, Minoru Ito, Kiyotaka Yabuki. Yabuki Hospital.

Background: Recently, the growing concern for dialysis is considered to be the increase in the number of aged and long-term patients in Japan. Therefore, the objective of dialysis treatment is not only the longevity of the patients but the improvement of quality of life among them. For that reason, it is essential to determine factors related to these complaints. In our cross-sectional study, we examined the relationship between the severity of the uncomfortable symptoms and various clinical parameters.

Methods: A total of 342 patients performed with chronic maintenance dialysis in our three facilities were included. Additionally, they were evaluated by the self-rating score questionnaire based on fifth graded face scales from 0 (none) to 4 (very strong) according to the severity. The symptoms were composed of 20 common physical and psychological complaints. Odds ratios for each of moderate to severe (score = 2, 3, 4) or severe (score ≥ 5) were calculated using a multiple logistic regression model adjusted for confounding factors including age, gender, time of dialysis, history of diabetes mellitus, Kt/V, normalized protein catabolic rate, body mass index, predialytic values of hemoglobin, albumin, β2-microglobulin, sodium, potassium, phosphate.

Results: Among participants, 146 (42.7%) patients were treated with hemodialfiltration and 196 (57.3%) received dialysis therapy. The most frequent symptoms in patients on hemodialfiltration were vision, dizziness, headache, gait, joint pain, back pain & appetite. The most frequent symptoms in patients on dialysis therapy were back pain & appetite.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underlines represent presenting author.
Patients with Type 2 Diabetes Admitted for RRT – Are There Any Differences According to Primary Renal Disease and Referral?  Radka Klecková, Jana Veresova, Petra Ronova, Katarina Nehezova, Ivan Ryhlik.  Dialysis Unit, FMC, Prague 10, Czech Republic.

**Background:** Late referral of type 2 diabetic(T2D) patients is well-known risk factor for morbidity/mortality in RRT, but the role of primary renal disease(PRD) is not well defined.

**Methods:** All T2D pts admitted for hemodialysis(HD) in 3-yrs period were divided according to PRD /1=diabetic nephropathy(DN);2=non-diabetic renal disease(NDRD) and referral as early(=E)=3-months vs.non-referral(N).Selected lab/clinical parameters were compared.

**Results:** 38 T2D pts entered H/D on average 34 of all admitted pts); 50% were diagnosed as DN,while atherosclerotic renal disease represented majority of NDRD.All were managed by diabetologist, but only 68% were referred early.

Starting HD, following parameters differed significantly comparing all groups:

- E1=diabetic hypertrophy(DH):24h systolic blood pressure(24hSBP) 150/90mmHg, 24h diastolic blood pressure(24hDBP) 90/60mmHg, mean age 66.9 yrs (E2=12):highest s-albumin(ALB)13mg/dl, systolic BP 161mmHg and lowest serum creatinine(s-cr)18mg/dl, insulin 7.4 (E3=4):highest insulin 27.6 (E4=3):highest insulin 36mg/dl, mean age 66.9 yrs and lowest PU(1.0g).

Comparing groups according to PRD (1 vs.2), significantly differed.

- s-cr(3.6g/dl) vs(2.0g/dl), ALB(33.9g/l) vs(29.8g/l), serum creatinine(s-cr)(600/676umol/l), DM vintage(16.8/10.4 yrs), insulin(76/5%), RAS blockers(74/53%), smoking(47/18%)

On the other hand, patients with diabetes were 29% in a negative group. In addition, the presence of hypertension was 74% in DN group while 46% in NDRD group.

**Conclusions:** The troponin T level at initiation time of hemodialysis may be considered an independent prognostic factor for all-cause and cardiovascular mortality for a short term.

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Novel Methicillin Resistant S. aureus (MRSA) Reduction Practices in Outpatient Hemodialysis Patients

**Background:** Cardiac troponin T, a useful marker for diagnosing acute myocardial infarction (AMI) in the general population, is significantly higher than the usual cutoff value in many hemodialysis patients without clinically apparent evidence of AMI. The objective of this study was to determine whether the troponin T level at initiation time of hemodialysis is related to the occurrence of cardiovascular diseases (CVD) afterwards and short term mortality.

**Methods:** 170 patients began hemodialysis between January 2006 and November 2008 at our hospital. Among them 124 patients were infected with non-HCV at admission. More than 58 at the beginning time of hemodialysis. These data were significantly a low tendency in comparison with negative group. (P < 0.01) High sensitive CRP was 0.54mg/dl vs 0.31mg/dl(positive group vs negative group). At the beginning time of hemodialysis (P < 0.05)

As for the 2-year occurrence, patients were significantly higher in positive group (36% than in negative group (11%) (P < 0.01) and the total mortality was higher in positive group (13%), compared with that of negative group (2%).

**Conclusions:** The troponin T level at initiation time of hemodialysis may be considered an independent prognostic factor for all-cause and cardiovascular mortality for a short term.

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Hepatitis C Virus Infection among End Stage Renal Disease Population of Guam

**Background:** Cardiac troponin T, a useful marker for diagnosing acute myocardial infarction (AMI) in the general population, is significantly higher than the usual cutoff value in many hemodialysis patients without clinically apparent evidence of AMI. The objective of this study was to determine whether the troponin T level at initiation time of hemodialysis is related to the occurrence of cardiovascular diseases (CVD) afterwards and short term mortality.

**Methods:** 170 patients began hemodialysis between January 2006 and November 2008 at our hospital. Among them 124 patients were infected with non-HCV at admission. More than 58 at the beginning time of hemodialysis. These data were significantly a low tendency in comparison with negative group. (P < 0.01) High sensitive CRP was 0.54mg/dl vs 0.31mg/dl(positive group vs negative group). At the beginning time of hemodialysis (P < 0.05)

As for the 2-year occurrence, patients were significantly higher in positive group (36% than in negative group (11%) (P < 0.01) and the total mortality was higher in positive group (13%), compared with that of negative group (2%).

**Conclusions:** The troponin T level at initiation time of hemodialysis may be considered an independent prognostic factor for all-cause and cardiovascular mortality for a short term.
Increase of HCV RNA Levels during Hemodialysis Treatment in Patients with Chronic Hepatitis C

Gernot Schilcher,1 Csilla Putz-Bankuti,2 Daniel Schneidetz,1 Harald Kessler,4 Alexander R. Rosenkranz,1 Rudolf E. Stauber,2
1Department of Internal Medicine, Division of Nephropathy and Hemodialysis, Medical University of Graz, Graz, Austria; 2Department of Internal Medicine, Division of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria; 3Institute of Physiology, Center for Physiological Medicine, Medical University of Graz, Graz, Austria; 4Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria.

Background: Previous studies suggest a reduction of hepatitis C virus (HCV) viremia by hemodialysis. The aim of this study was to quantify the effect of hemodialysis (HD) and hemodiafiltration (HDF) treatment on HCV RNA levels.

Methods: HD as well as hemodiafiltration (HDF) was delivered to chronic HD patients (n=11). Blood samples were taken before dialysis (t=0 min) and from the arterial and venous line of the extracorporeal circulation during dialysis at times t=30 min and t=180 min for quantitative detection of HCV RNA using real-time PCR.

Results: HCV RNA levels significantly increased during extracorporeal therapy (p<0.001, Tab 1). After 180 minutes and correction for hemoconcentration, HCV RNA levels increased relative (R180) to baseline by 56%. Importantly, no significant differences could be observed between serum samples collected pre and post dialyzer as well as between HD and HDF treatments.

Conclusions: Contrary to published data that HCV viremia would be reduced during HD/HDF, a significant increase of HCV RNA was observed after 180 min of treatment. These virological changes could not be explained by HCV RNA absorption on the dialysis membrane, by hemoconcentration or inhibition of real-time PCR due to uremic toxins. After 48 hours of dialysis free time all patients underwent a reduction in viremia by yet unknown mechanisms. Our results also document the importance of timing to collect HCV RNA levels during hemodialysis.

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R: HCV RNA level changes relative to baseline (t=0'), ultrafiltration corrected

Quality of Life in Patients with Various Dialysis Modalities: A Multi-Center, Retrospective Study

Klaus Schuster, Regionalmanagement Mostviertel, Niederösterreichische Landeskliniken-Holding, Amstetten, Austria.

Background: Public health planning often tries to balance between medical outcomes like survival, etc. and economical/operational reasons. A measurement of quality of life (QoL) in comparable patients with ESRD seldom is done. Patients with ESRD mainly have 2 options: in-center-dialysis, mainly with hemodialysis (HD), or a home-therapy like peritoneal-dialysis (PD) or home-hemodialysis(HHD). In a recent full-cost analysis we could successfully demonstrate, that treatment at home is obviously cost beneficial, but with equal or even better medical outcomes than PD.

Cost comparison HD versus PD

- Transportation: 11584
- Medication: 9370
- Treatment: 41916
- TOTAL: 62870

- Transportation costs, medication costs divided into numerous sub-groups, materials, radiology and laboratory costs, complication costs (when generated by the dialysis modality)

Now in a subsequent analysis we did a patient-questionnaire to evaluate the individual QoL of those patients.

Methods: The questionnaire was completed by 258 patients (62 PD, 196 HD) in 6 dialysis-centres in Lower-Austria, a federal state in Austria. Beside some epidemiological data the questionnaire has three parts, as three different questionnaires are combined. We chose the following surveys: SF-36, an international standard in QoL-analysis, PLQ, a german assessment tool especially for patients with chronic diseases and HADS, the Hospital Anxiety and Depression Scale.

Results: After adjusting age and sex, the results proved our hypothesis, that a home-dialysis-therapy like PD serves the patients better in their subjective self-assessment of various QoL-aspects. All three parts of the questionnaire show a tendency favouring home-dialysis-therapy, but according to the subcore HADS, the significance is striking.

Conclusions: Home-therapy is the choice for patients with ESRD. Not only medical outcomes like better survival and economical/operational (cost saving) reasons, but also the individual self-assessments in QoL show an advantage for home-dialysis-modalities and so has to be the first choice according to ethics and limited resources.

Funding: Government Support - Non-U.S.

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Underline represents presenting author.

Publication Only

PUB298

PUB300

Top 5 Incidents in a Worldwide Dialysis Network


Background: The management of adverse events management is a key priority in the overall management policy for quality of care in international dialysis provider groups. Hemodialysis treatments are prone to risks. To our knowledge, a systematic analysis of these incidents in the dialysis world has never been presented. We present the collection and analysis of adverse events data for in 2010 in a network of 229 dialysis centers, treating 18,000 patients in Europe, Australia and South America.

Methods: All centers report annually their incidents through an on-line reporting system. All staff members may report an incident. There are 4 categories of incidents (related to patients, staff, equipment, buildings (fluids, computer, water treatment, other) divided in 49 subgroups. Data on these incidents are presented by monthly analyses with standard descriptive statistics.

Results: In 2010, 25,960 incidents were reported in our organization, with large inter country variability. This is 1,56 incidents per patient per year or 10 per 1000 treatments. The five most common incidents represent 60% of the total. They are, in order: 16% sessions missed-patient did not show up, 15% interruptions of sessions per monitor malfunction, 12% dialyzer and/or blood lines changed due to clotting, 10% vascular access problems, 7% hypotension requiring a filling of more than 300 ml. Distribution of the four groups of incidents were different from country to country; incidents relating to patients were always the most represented.

Conclusions: Follow-up of adverse events is a key pillar in preventing risk and improving the quality of care for dialysis patients. We are implementing a more detailed analysis specially for the first cause: sessions missed; is it voluntary or not? If yes, what about the right for stopping treatment and the physician’s responsibility?

PUB301

Characteristics of Bacteremia in Hemodialysis Patients

Masashi Suzuki,1,2 George Seki,2 Norio Hanafusa,3 Toshiro Fujita,3 Kyoji Moriya.1 1Department of Infection Control and Prevention, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan; 2Department of Nephropathy and Endocrinology, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan; 3Department of Hemodialysis and Apheresis, University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan.

Background: Hemodialysis patients are thought to be immunocompromised. Although infection is the second cause of death in all hemodialysis patients and the first cause of death in patients within one year after initiation of hemodialysis therapy in Japan, the underlying mechanisms are unclear.

Methods: To clarify the characteristics and prognosis of bacteremia, retrospective chart reviews were performed for 850 patients who were admitted to The University of Tokyo Hospital in 2007-2009 and received hemodialysis therapy.

Results: Within 50 cases of bacteremia, the most common types were catheter related infection (20%) and pneumonia (18%), with a substantial portion (22%) unspecified. The common causes of bacteremia were Staphylococcus aureus (28%), Escherichia coli (10%), Staphylococcus epidermidis (8%), Klebsiella pneumoniae, Pseudomonas aeruginosa, Candida glabrata (6%). The frequency of detection was similar to patients who did not receive hemodialysis therapy. Age, types of blood access, catheter, hypertension, underlying disease, smoking, peripheral vascular disease, serum albumin, hemoglobin, body mass index did not affect the causative organisms. In all hemodialysis patients, the mortality rates within one and three months after bacteremia were 20% and 34%, respectively. These values were even higher (28% and 52%) in a subset of patients who received hemodialysis for less than one year. In these patients, mortality after S. aureus bacteremia was extraordinary high (60% and 80% within one and three months, respectively). Mortality was also very high after Candida glabrata bacteremia (67% within one and three months).

Conclusions: There was no difference in the cause of bacteremia between hemodialysis and non-hemodialysis patients. Because the bacterium of S. aureus and Candida glabrata in the first-year after initiating hemodialysis predicted very bad prognosis, careful observation and intensive care would be required in such cases.

Funding: Government Support - Non-U.S.

Distribution of incidents in a network of dialysis clinics (Diaverum), year 2010

For publication only
Background: Dialysis is a limited resource within the VA, and growing demand for ESRD care has led VA to purchase dialysis treatment from non-VA providers on a fee for service basis. Decisions about the optimal mix of “making” dialysis in-house or “buying” non-VA services requires an understanding of current users within the VA system. This study examined characteristics of veterans with ESRD who utilized outpatient dialysis in VA and non-VA settings.

Methods: We constructed a cohort of veterans with ESRD in 2 regional networks who received VA-financed outpatient dialysis treatment in 2007-8. Using VA administrative data, we identified veterans who received dialysis in VA; non-VA fee basis; or both settings (“dual users”). We performed bivariate and multinomial probit analysis to identify patient characteristics associated with dialysis setting.

Results: 1,388 veterans received chronic hemodialysis financed by VA; 25% received VA dialysis, 36% used non-VA fee basis dialysis, and 39% were dual users. VA dialysis users were more likely to be non-White, unmarried, sicker, and living closer to VA dialysis units than veterans in non-VA and dual settings (p<.05). After covariate adjustment, non-VA dialysis was positively associated with marital status (p<.05) and negatively associated with comorbidity burden (p<.001). Greater geographical distance to VA dialysis was associated with non-VA and dual dialysis (p<.001). There was regional variation in veterans’ use of dual dialysis (p<.001).

Conclusions: A significant proportion of VA-funded dialysis is delivered via fee basis. Non-VA providers benefit from favorable selection of patients, as veterans with lower comorbidity burden are more likely to obtain dialysis outside of VA. With limited service capacity and increasing ESRD prevalence in VA, veterans are likely to continue receiving non-VA dialysis. VA may need to account for selection bias in determining the optimal mix of making vs. buying dialysis services and 2) payment to non-VA dialysis providers. More research is needed to understand the implications on the quality and cost of VA’s make-buy decisions.

Funding: Other U.S. Government Support, Veterans Administration Support

PUB304

Prosthetic Status and Treatment Needs for Lost Masticatory Function in Hemodialysis Patients

Mgnedala Wilczynska-Borawska,1 Jolanta MALYSZKO, Michal Mysiwiec,2 Nephrology, Medical University, Bialystok, Poland; 2Dentistry, Medical University, Bialystok, Poland.

Background: Premature loss of permanent teeth leads to stomatognathic system disability, loss of masticatory functions, speech and alterations in face aesthetics. Loss of masticatory function may result in severe malocclusion which, if not treated, may lead to irreversible dysfunction of the whole masticatory system. Premature loss of permanent teeth is a very serious but undertreated problem for patients with chronic renal failure.

Methods: This study aimed to determine masticatory dysfunction and number of teeth present for hemodialysis patients, and defined, based on the results, patients’ needs for prosthetic treatment, which could restore correct occlusal condition. We studied the total number of teeth and number of teeth separately for upper and lower jaws, 2) the existing prosthetic restorations and 3) the preserved masticatory function in 68 HD patients.

Results: Nearly 70% of tested hemodialysis patients had not reconstructed a masticatory function. Female patients had less remaining natural teeth in both jaws compared to males and constituted a higher percentage of edentulous patients in the test group. All patients with at least 28 natural teeth with retained occlusal contacts whilst chewing were males (4; 10% males; 5,7% of the whole group). There were 15 edentulous patients: 7 males (10%); and 8 females (11,5%). More of them had their masticatory functions restored, implementation of prosthetic treatment was needed to a lesser degree. In this group, more extensive restorations like partial, skeletal and complete dentures were observed.

Conclusions: The population of hemodialysis patients from the North East part of Poland are patients with severe stomatognathic system dysfunctions which developed as a result of the loss of physiological function of the masticatory system (more than 20% of patients were edentulous). It is of importance for dentists, as well as nephrologists, to understand the essence of the problem, as the general health of a patient cannot be improved without ensuring functional comfort of such as important system as the masticatory one.

Funding: Government Support - Non-U.S.

PUB305

A Prospective, Randomized, Multicenter Study Comparing Survival in Subjects Receiving Peritoneal Dialysis or Hemodialysis (SURiND)

Xueqin Yu,1 Anders P. Tranaeus,2 The SURiND Study Group.1 Renal Department, First Affiliated Hospital, Sun Yat-sen University, Guangzhou, China; 2Baxter Asia Pacific, Shanghai, China.

Background: There is currently no consensus on whether peritoneal dialysis (PD) or hemodialysis (HD) is associated with better survival outcomes in patients with end-stage renal disease (ESRD). Results from a pilot study, wherein 41% of eligible subjects were willing to be randomized to either dialysis modality, suggest that it is feasible to conduct an adequately powered, randomized controlled study to investigate survival outcomes in patients with ESRD receiving PD or HD.

Methods: A large randomized study will assess whether survival outcomes differ between PD and HD in patients with ESRD, and will provide information on factors that influence modality choice, including quality of life.

Results: Recruitment will start soon and the final study results are expected in 2017.

Conclusions: This large randomized study will assess whether survival outcomes differ between PD and HD in patients with ESRD, and will provide information on factors that influence modality choice, including quality of life.

Funding: Pharmaceutical Company Support

PUB306

Female Chinese Hemodialysis Patients with a Better Prognosis Than Man When Treated by the Same Dialysis Parameters

Jianzhuou Zou, Yi Fang, Jie Teng, Wenlv Lv, Xiaoqiang Ding. Nephrology, Zhongshan Hospital, Fudan University, Shanghai, China.

Background: Dialysis dose would be higher in female hemodialysis patients than that in males when treated by the same dialysis parameters. Literature suggest that greater dialysis dose may significantly benefit women, but not men. So we speculate that prognosis of female hemodialysis patients would be better than that of males.

Methods: All 151 patients were treated by the same dialysis parameters and would be followed up for 30 months or ceased by death, transforming to the other renal replacement therapy, or transferring to other dialysis centers. The demographic data, laboratory test results and dialysis dose would be collected when the patients enrolled in the cohort study.

Results: During the follow-up, five patients changed to kidney transplant and one to peritoneal dialysis, one patient transferred to the other dialysis center among the analyzed 144 patients, almost 45% were females. Thirty one patients died from all causes and the all-cause mortality for male (29.11%) was significantly higher than that in females (12.50%, p<0.05). During 30 months of follow-up, the all-cause mortality for male (29.11%) was significantly higher than that for females(12.50%, p<0.05). The all-cause mortality rate per 100 patient years was 9.83 by the end of follow-up. The all-cause mortality for male (29.11%) was significantly higher than that for females(12.50%, p<0.05). At the end of follow-up, the level of hemoglobin, white blood cell count and predialysis serum creatinine were significantly higher in males than that in females, but URR and spKt/V were significantly lower in males. When analyzed by Cox proportional hazards regression models, we found that for male patients, C-reactive protein, prealbumin and iPTH were independent predictors of death; however for the females, the independent predictors of death were C-reactive protein and iPTH.

Conclusions: When treated by the same HD parameters in Chinese patients, the prognosis of females was better than that of males. Meanwhile, we found that iPTH was associated with mortality in males or females, but the impact was different by gender.

PUB307

Hemodialysis Catheter Adherent to Jugular Vein

Solange Bourque, Nephrology, CHUS, Sherbrooke, QC, Canada.

Background: Hemodialysis catheters are used in patients without any other possible vascular access. However, they have multiple complications. We present an unusual complication, namely a catheter adherent to the jugular vein.

Methods: A 50 year old man was dialysed with a permanent right jugular tunneled catheter since 1999. He presented his first tunnel infection on July 7th 2010. Infective organism was staphylococcus aureus. At that time, there was a first, unsuccessful attempt to remove the catheter at the dialysis clinic. The patient was sent to radiology for further attempt. Ultrasound showed that the dialysis catheter seemed to be attached to the jugular vein. The surgeon was called in for help but was unable to remove the catheter even with further incision and dissection. It was then decided to proceed to surgical dissection under clinically relevant anesthesia. A partially clotted catheter was taken out. An incision was made along the jugular vein to retrieve the catheter. This caused a massive
PUB308
When To Refer for Vascular Access Fistulogram in Hemodialysis Patients with Decreasing Intra-Access Flow? Sun Ryoung Choi, Hoon Suk Park, In O Sun, Hyun Gyung Kim, Yu Ah Hong, Byung Ha Chung, Bumsoon Choi, Cheol Whee Park, Chul Woo Yang, Young-Soo Song. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: Intra-access flow measurement is preferred to surveil vascular access in hemodialysis patients. K/DOQI guidelines emphasized prospective trend analysis of access flow on a monthly basis. They recommend refer for fistulogram if access flow 1,000 ml has decreased by more than 25% over 4 months in graft, and do not mention fistulogram.

Methods: This study was a prospective observational trial. The surveillance used was intra-access flow measured by ultrasound dilution (Transonic HD03 hemodialysis monitor; Transonic Systems, Inc., Ithaca, NY) performed monthly. We defined 25% decrease in Qac compared to that of the last measurement as significant finding and thus performed intervention without follow-up over four months.

Results: Out of total 52 cases, 38 patients were male, mean age 63 years old, 22 (42.3%) diabetics, 38 (73.1%) fistulae, and 37 (71.2%) accesses were on the forearm. At 3 months, 1000 ml that has decreased by more than 25% over 4 months in graft, and do not mention fistulogram.

Conclusions: Our study suggests that the patient should be immediately referred to fistulogram if access flow has decreased more than 25% regardless of access type, absolute value of access flow, or duration of decreased access flow.

PUB309
Impact on Autologous Arteriovenous Fistula Aneurysm by Improved Puncture Technique: Centripetal and Exodic Alternate Xia Fu, Wei Shi, Xinliang Liang. Nephrology, Guangdong General Hospital, Guangzhou, Guangdong, China.

Background: To study the appropriate puncture technique of autologous arteriovenous fistula for reducing the occurrence of aneurysm or avoiding aneurysm increasing.

Methods: 298 maintenance hemodialysis patients with autologous arteriovenous fistula were randomized into 3 groups, respectively punctured by centripetal method, exodic method and alternate ways. Incidence of aneurysm, aneurysm increasing, blood flow and Kt/V were compared among the three groups.

Results: Aneurysm incidence and aneurysm increasing in the centripetal group and the alternate group had no significant difference (p>0.05). The incidence and increasing of exodic group was significantly higher than those of centripetal group and alternate group (p<0.05). The values of blood flow and Kt/V in the centripetal group were significantly lower than those in the exodic group and alternate group (p<0.05).

Conclusions: Alternating centripetal and exodic puncture not only guaranteed blood flow but also effectively avoided occurrence or increase of aneurysms of autologous arteriovenous fistula.

Funding: Government Support - Non-U.S.
Conclusions: Patients with catheters had shorter mean HD vintage compared to patients with AVA. While patients with catheters were more likely to have received predialysis care compared to patients with AVA, these patients had previous failed AVA, or were undergoing evaluation for AVA placement at the time of the study, were either awaiting kidney transplantation or switching to peritoneal dialysis. More studies need to be done to explore qualitative factors for high catheter rates including patients’ refusal for AVA placement.

PUB312
Malfunction of Tunneled Cuffed Venous Catheter with Small Gap between Cuff and Catheter: A Report of Four Cases 1Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; 2Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan.

Background: The cuff of a tunneled cuffed venous catheter (TCC) provides a barrier against infection, and firmly anchors the catheter by fixing it to the subcutaneous tissue. However, we experienced four cases of malfunction of a Soft Cell (Bard Access Systems, Salt Lake City, UT, USA), a type of TCC, with a small gap between the cuff and the catheter.

Methods: In case 1 (74-year-old woman), case 3 (95-year-old woman), and case 4 (74-year-old woman), a Soft Cell (12.5 Fr diameter, 19 cm long) was used as a permanent access, while in case 2 (86-year-old woman) a Soft Cell was used as a bridge access for arteriovenous graft failure. The Soft Cell was removed because of tunnel infection at 290 catheter-days in case 1 and at 29 catheter-days in case 2, suspected catheter-related blood stream infection at 513 catheter-days in case 3, and cuff dysfunction at 734 catheter days in case 4.

Results: In all of the removed Soft Cells, the presence of a gap was confirmed by passing a probe through the gap. Sections of formalin-fixed cuffs of these Soft Cells showed that the cuff had detached from the catheter at the part where it was possible to pass a probe through the gap. Despite the cuff being tightly attached to the adjacent dermal connective tissue with marked foreign body reaction and fibrosis histologically.

Conclusions: In the four cases presented, the cuff was tightly attached to the dermal connective tissue histologically. Even if the cuff were firmly fixed to the subcutaneous tissue, bacteria and foreign substances would have been able to get into the tunnel site through the gap. The gap between the cuff and the catheter, representing a break in the barrier against infection, might have been the cause of the two cases of tunnel infection.

Fortunately these patients improved immediately without hospitalization, and all Soft Cells were hardly anchored to the skin. The cause of this malfunction of the Soft Cell is currently being investigated by the manufacturer.

PUB313
An Assessment of International Frequent Hemodialysis Utilization and Practice Patterns: A Collaboration between the Canadian Home Hemodialysis Study Group and Nephrology Now 1Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; 2Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan.

Background: The purpose of our study was to determine influences for the utilization of frequent HD and attitudes regarding initiation of and the evidence for frequent HD.

Methods: An international cohort of subscribers of a nephrology education website (www.nephrologynow.com) was invited to participate in an online survey. Survey questions assessed for each pathogen.

Results: Our survey had a 40.9% response rate. The final cohort was limited to 311 physicians.125 (40.2%) physicians had patients treated with frequent HD. In the multivariate model, adequate training (OR 2.47 CI 1.25-4.16), government physician reimbursement (OR 2.66, CI 1.16-6.40), higher national health care expenditure and number of ESRD patients per centre were independently associated with frequent HD utilization. Hemodialysis providers with patients on frequent HD were significantly more likely to initiate HD for ESRD patients than providers with patients on conventional HD.

Conclusions: Frequent HD use and support of the evidence vary according to identifiable factors. Interventions and health policy targeting these areas and increased physician education and training in frequent HD modalities may be effective in increasing frequent HD utilization.

PUB314
Recurrent Pancreatitis Associated with Icodextrin 1Hashimoto Minami Internal Medicine Clinic, Sagamihara, Kanagawa, Japan; 2Blood Purification Center, Sagamihara Kyodo Hospital, Sagamihara, Kanagawa, Japan.

Background: Recurrent pancreatitis is an infrequent condition in peritoneal dialysis (PD) patients; determining its cause can be challenging. We present a patient with recurrent bouts of chemical pancreatitis that appear to be due to icodextrin.

Methods: A 42-year-old diabetic and hypertensive female on PD presented with recurrent pancreatitis. Laboratory studies consistently showed high serum lipase levels, normal peritoneal fluid white blood cell counts of macrophage predominance, and negative cultures (Table 1). Imaging studies were consistent with pancreatitis. The results raised the possibility of chemical pancreatitis, but no clear culprit was identified. Her cyclo-automated PD dialysate consisted of 2.5% and 4.25% dextrose plus a daytime dwell of icodextrin. Icodextrin was used as the dialysate for 246 days in case 4.

Results: Icodextrin is an osmotic glucose polymer which is especially useful in patients with high peritoneal membrane transport, because of its limited diffusion across this membrane. While it is usually well-tolerated, it can cause cutaneous allergic reactions, abdominal pain, sterile peritonitis, hypotension, and decreased plasma amylase activity (with unaltered lipase levels). We report a case of chemical pancreatitis in a patient on icodextrin. We performed an online survey of 311 physicians.125 (40.2%) physicians had patients treated with frequent HD.

Conclusions: Frequent HD should be considered in recurrent unexplained pancreatitis in PD patients when icodextrin is used.

PUB315
Changing Picture in the Microbiology of Peritoneal Dialysis Associated Peritonitis – A Single Center Experience over Almost Three Decades Martin Kimmel, Niko Braun, Mark Dominik Alscher. Department of Internal Medicine, Division of General Internal Medicine and Nephrology, Robert-Bosch Hospital, Stuttgart, Germany.

Background: Peritonitis is the most important complications of peritoneal dialysis (PD) and is still a major cause of morbidity and technique failure. In previous reports concerning the microbiology of peritonitis and its characteristics the longest observation period was one decade.

Aim of this retrospective study is to analyze the microbiological spectrum of PD-associated peritonitis from July 1983 over an almost three decade-long period up to December 2010 in a local peritoneal dialysis reference center.

Methods: Retrospective study analyzing >300 peritonitis episodes at a peritoneal dialysis reference center in south Germany. All peritonitis episodes in three timeframes (1983-1993, 1993-2006, and 2007-2010) were analyzed. The spectrum of organisms causing PD-associated peritonitis and the antibiotic resistance profiles were assessed for each pathogen.

Results: Organism spectrum: the ratio gram-positive to gram-negative organism is changing; gram-positive organisms are decreasing (a 74%, b 66%, c 66%) (Staphylococcus aureus and coagulase-negative Staphylococcus (CoNS) is decreasing, but Methicillin-resistant CoNS is increasing and formerly rare organisms appeared more frequently (e.g. Serratia marcescens or Stenotrophomonas maltophilia). Antibiotic susceptibility: there is an increasing rate of resistant organisms with the need to adapt the initial treatment protocol.

Conclusions: There is a changing picture in the microbiology of peritonitis episodes over almost three decades with an ongoing need to adapt continuously the initial treatment protocol.

PUB316
How Home Hemodialysis Programs Deal with the Non-Adherent Patient: A Survey of 17 HDH Programs in Canada Paul Komenda,1 Deborah Lynn Zimmerman,2 Robert P. Paul,3 Medicine, Nephrology, University of Manitoba, Winnipeg, MB, Canada; 2Medicine, University of Ottawa, ON, Canada; 3Nephrology, University of Alberta, Edmonton, AB, Canada.

Background: Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns. This abstract pertains to how programs address non-adherence in patients performing independent home hemodialysis.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

917A
**Methods:** A survey was developed and then sent to 19 physician experts to ensure content and face validity. Physicians were encouraged to seek input from allied health team members. The survey was modified based on responses received and then circulated prior to a face-to-face meeting with the same physician experts before final changes to the instrument were made. The survey was completed online.

**Results:** Seventeen of 19 (89%) physicians representing individual programs in Canada participated in survey development and provided program information. Programs vary in training schedules with 7/17 (41%) training three days per week, 4/17 (24%) four days/week and 6/17 (35%) five days/week. 9/17 programs report a median training time of 5-6 weeks, with 5/17 training for 7-8 weeks. Training more days per week did not always correlate with shorter training duration. 14/17 programs maintain one-to-one relationships.

**Conclusions:** The majority of HHD programs in Canada communicate that informed patients may be permitted to make their own decisions regarding safety in performing HHD. The majority of these programs however have removed a patient from therapy against the patient’s wishes. Consensus guidelines may help guide HHD teams in setting thresholds for involuntary removing patients from this modality.

**PUB317**

**Training Variability in Home Hemodialysis in Seventeen Canadian HHD Programs: The Case in Favor of Standardization**

**Paul Komenda,**1 Deborah Lynn Zimmerman,2 Robert P. Pauly,3 Medicine, Nephrology, University of Manitoba, Winnipeg, MB, Canada; 2Medical, Nephrology, University of Ottawa, ON, Canada; 3Nephrology, University of Alberta, Edmonton, AB, Canada.

**Background:** Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns. This abstract addresses practice patterns pertaining to training schedule.

**Methods:** A survey was developed and then sent to 19 physician experts to ensure content and face validity. Physicians were encouraged to seek input from allied health team members. The survey was modified based on responses received and then circulated prior to a face-to-face meeting with the same physician experts before final changes to the instrument were made. The survey was completed online.

**Results:** Seventeen (89%) physicians representing individual programs in Canada participated in survey development and provided program information. Programs vary in training schedules with 7/17 (41%) training three days per week, 4/17 (24%) four days/week and 6/17 (35%) five days/week. 9/17 programs report a median training time of 5-6 weeks, with 5/17 training for 7-8 weeks. Training more days per week did not always correlate with shorter training duration. 14/17 programs maintain one-to-one relationships.

**Conclusions:** The majority of HHD programs in Canada communicate that informed patients may be permitted to make their own decisions regarding safety in performing HHD. The majority of these programs however have removed a patient from therapy against the patient’s wishes. Consensus guidelines may help guide HHD teams in setting thresholds for involuntary removing patients from this modality.

**PUB318**

**A Flexible Approach to Extended Hours Hemodialysis at Home**

**Rathika Krishnasamy,1 Carmel M. Hawley, David W. Johnson, David Mudge, Nicole M. Isbel, Scott B. Campbell, Carolyn L. Van Eps.** Department of Nephrology, University of Queensland at Princess Alexandra Hospital, Brisbane, Queensland, Australia.

**Background:** Home hemodialysis (HHHD) allows greater tailoring of prescription to individual needs both medically and socially. The aim of this survey is to review current dialysis prescription practices of HHD patients and to explore factors involved in their decision making.

**Methods:** 60 HHD patients from PAH were invited to complete a written survey containing 24 questions in April 2011. The survey covered duration and timing of HHD, patient’s health perception, support system and perceived barriers. The survey was modified based on responses received and then circulated prior to a face-to-face meeting with the same physician experts before final changes to the instrument were made. The survey was completed online.

**Results:** Nearly 80% of them rated their overall health, mental health and their current dialysis as being good, very good or excellent. 75% of patients felt that doing dialysis at home was both beneficial to their health and their family. 70% of patients had a support person during dialysis and mostly were satisfied with the care from the dialysis training centre.

**Conclusions:** This survey suggests that the flexibility of HHD is perceived as highly beneficial for patients and their families. We also identified patients’ concerns and barriers towards HHD.

**PUB319**

**The Effect of Newly Developed Semi-Long Peritoneal Dialysis Catheter on the Inflow- and Outflow Time of Peritoneal Dialysis Fluid**

**Akihiro Kuma,1 Narutoshi Kabashima,2 Tetsu Miyamoto,2 Nana Ishimatsu,3 Yumi Furuno,3 Kaori Kanegae,2 Ryota Serino,3 Masahito Tamura,2 Yutaka Otsuji.1** Second Department of Internal Medicine, University of Occupational and Environmental Health, Kitakyushu, Japan; 3Kidney Center, University of Occupational and Environmental Health, Kitakyushu, Japan.

**Background:** To reduce the incidence of omental wrapping, we developed a newly designed peritoneal dialysis catheter (PDC) for the upper abdominalexit. This catheter (JBS-2) called “CAVA CATHETER” has smaller end-holes (2.0 mm) and smaller side-holes (0.5 mm) with 4-line slits on the surface to prevent omental entanglement. While JBS-2 could prevent a suction of omental tissue, this catheter could cause an inflow- or outflow-delay of peritoneal dialysis fluid (PDF).

**Methods:** We provided five different types of catheters according to the existence of slits and size/number of end-hole or side-hole. The effect of JBS-2 (n=5), the five different types of catheters (n=6-3) and the JBSA (n=3) on inflow- and outflow- times were assessed with artificial abdominal cavities.

**Results:** We observed the mean PDF inflow-time of the JBS-2 catheter (2.0 mm of end-hole and side-hole) was measured at the PDC (0.3 mm of end-hole and 15 min 3 sec vs 9 min 33 sec). We observed trivial difference of inflow-time between the two types of catheters (8 min 3 sec vs 6 min 9 sec). Meanwhile, the existence of slit, side-hole size number had no impact on the inflow or outflow-time of PDF. It took 8 minutes of mean inflow time with JBS2.

**Conclusions:** The bag exchange procedure with JBS-2 will finish within 30 min, which is considered to be permissible in clinical settings. The smaller side-holes and slit structure of the PDC may contribute to the lower incidence of catheter-related problems including omental wrapping without a delay of PDF inflow-outflow time.

**PUB320**

**First Year Outcomes of Incident Peritoneal Dialysis Patients**

**Eduardo K. Lacion, Nien-Chen Li, Raymond M. Hakim, J. Michael Lazarus, Franklin W. Maddux, Joseph P. Pulliam.** Fresenius Medical Care, North America, Walhamb, MA.

**Background:** There have been few large multi-center studies on peritoneal dialysis (PD) outcomes in incident dialysis patients. We describe a contemporary cohort of patients starting PD and their outcomes in the first year of therapy.

**Methods:** All adult (age ≥18 years) patients admitted to Fresenius Medical Care North America facilities between January 1 to December 31, 2009 who initiated PD within their first 90 days were included. Patient follow-up is for one year from their first home PD treatment. We describe the cohort and their outcomes including deaths (with withdrawals), hospitalization, transplants, hospitalization, peritonitis and technique failure rates (a switch from PD to hemodialysis for ≥30 days).

**Results:** Patients (N=1,960) had mean age of 57.0±14.8 years, BMI was 32.2±12.0 kg/m², 56.2% male, 74.3% white, 54.1% diabetic, 3.0% with CHF, 7.1% with PVD, and 3.6% with limb amputation. During follow-up, 136 died (6.9%), 47 withdrew (2.4%), 128 were transplanted (6.5%) and 34 recovered kidney function (1.7%). No patient was lost to follow-up - leaving 1,615 (82.4%) active patients on PD by year-end. The median time to death/withdrawal was 167 days. Patients stayed on PD until being censored 78.6% of the time while 21.4% switched to HD. The median time to technique failure was 158 days. More than half (57.9%) were hospitalized and 28.2% had at least one episode of peritonitis. Median time to 1st hospitalization was 111 days and to 1st peritonitis episode was 123 days. Overall peritonitis rate was 0.68/24 patient-months.

**Conclusions:** Four out of 5 patients initiating PD within their first 90 days continue with PD for the first year. 1 of 5 patients who died or were discharged switched to HD beforehand. Causes of high hospitalization and peritonitis rates will need to be explored.
PUB321

Psychological Problems and Nursing Intervention of Patients Undergoing Continuous Ambulatory Peritoneal Dialysis: Rong Li. Department of Nephrology, Xijing Hospital, FMMU, Xi’an, China.

**Background:** To promote psychological care for patients undergoing the continuing ambulatory peritoneal dialysis(PD), and help patients can cooperate with the medical staff in order to improve dialysis results and quality of patients’ life.

**Methods:** Professional staff provide self-management education, self-care guide and targeted communication for PD patients with different psychological problems, and provide psychological intervention by such means as the combination of individual guidance and group education. Firstly, investigations on PD patients’ age, occupation, personality and family background may be carried out and the psychological problems of patients should be confirmed. The specialist, professional nurses and dietitians will provide self-management education and self-care guide for patients and their families by means of lectures, videos, pictures and brochures. Patients club will be established and patients with successful experience will be invited to give speeches on successful treatments. Individual guidance and group education will be integrated to achieve better effect.

The nurse staff should encourage patients to face the reality with positive attitude and receive dialysis treatment and therapy. The nurse staff should narrow the mental differences among the patients, establish harmonious relationship among doctors, nurses and patients by individualized services. The nurse staff should detect problems and help resolve the problems timely thereby to effectively improve the PD quality and patients’ life quality.

**Results:** Psychological problems of PD patients are one of the growing concerns of the medical personnel. Active intervention can improve the life of patients and reduce the burden brought to individuals, the families of the patients and the society. So patients are willing to receive psychological care, and the dialysis will have a significant effect, which will improve patients’ life significantly.

**Conclusions:** Psychological care for patients undergoing the long-term dialysis has significant meaning.

PUB322


**Background:** Peritoneal dialysis (PD) is the preferred available option of renal replacement therapy for a growing number of end stage kidney disease patients. A major limiting factor to the successful continuation of PD is long-term viability of the PD catheter (PDC). Percutaneous placement of PDCs is not commonly practiced despite recently published data encouraging use of this technique. Its advantages include faster recovery and avoidance of general anaesthesia.

**Methods:** We carried out a retrospective analysis of the outcomes of 313 PDC insertions in our centre comparing all percutaneous PDC insertions between July 1998 and April 2010 (group P) with all surgical PDC insertions from January 2003 to April 2010 (group S).

**Results:** 151 group P and 162 group S catheter insertions were analysed. Significantly more patients in group S had previously undergone abdominal surgery or PDC insertion compared with group P (41.8% vs 9.3% and 33.3% vs 3.3% respectively; P=0.00). There were more exit site leaks in group P than in group S (22.1% vs 7.4%; P=0.00) but no significant differences in peritonitis rates (1 episode per 16.5 catheter months vs 1 episode per 12.5 catheter months; P=0.36), poor internal drainage (9.9% vs 11.7%; P=0.1) or secondary drainage failure (8.7% vs 13.7%; P=0.183). Technical survival at 3 months was significantly better for group P than for group S (86.6% vs 77%; P=0.037) and at 12 months was 77.7% vs 68.7% respectively (P=0.126). No life threatening complications attributable to the insertion of the PDC occurred in either group.

**Conclusions:** We have demonstrated further encouraging outcomes of percutaneous PDC placement in comparison with the open surgical technique. The percutaneous insertion group were primarily a selected subset of patients without prior abdominal surgery or PDC insertion, therefore limiting this comparability. Studies eliminating these confounding factors are required although local expertise may affect generalisability of results. We recommend formal training of junior nephrologists on this bedside technique, particularly in healthcare systems with limited resources.

PUB323

Medical (Trocar & Cannula Method) Versus Open Surgical Insertion of Peritoneal Dialysis Catheters: A Retrospective Cohort Study: Girish N. Namagondla, Vivian W. Yu, Thomas F. Hiemstra, Paul F. Williams. 1Nephrology, Ipswich Hospital, Ipswich, United Kingdom; 2Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.

**Background:** There is little evidence in literature about direct comparison of trocar & cannula method vs open surgical technique for PD catheter insertions. A Survey (Jan 2005 - Dec 2009) retrospective cohort study of medical insertion(MS) versus surgical insertion(SIs) of PD catheters, was performed at our centre (Ipswich Hospital). Primary end point was catheter patency at 1 year (Censored for death & elective modality switch).

**Methods:** Data were extracted from an electronic database & patient case records.

**Results:** We identified 84 catheter insertions in 80 patients during the study period,54/84 (64.2%) were MSs.Age did not differ significantly between groups.More MSs were male (44/54) compared to SIs (10/24), P=0.033.7/24 (29.2%) Ms and 5/24 Ms (9.2% overall) were no longere patent after 12 months(P=0.052).Combined catheter patency at 12 months (censored for death and transplant) was 79.2% (ISPD recommendations suggests >80%).

**Conclusions:** Our data indicates that Ms of PD catheters by trocar & cannula method, are comparable to open SIs in terms of safety and better in efficacy. Strict selection criteria for Ms is required to achieve these results.Our data is comparable to ISPD recommendations 2010.

PUB324

Equipment and Home Requirements for Home Hemodialysis (HHD) among Canadian Renal Programs: Robert P. Pauly, Paul Komenda, Deborah Lynn Zimmerman. 1University of Alberta, Canada; 2University of Manitoba, Canada; 3University of Ottawa, Canada.

**Background:** There is growing interest in HHD though little published information on establishing and maintaining an HHD program. The purpose of this study was to survey Canadian HHD programs to describe practice patterns in a variety of domains. The current abstract focuses on equipment and home requirements for HHD.

**Methods:** A questionnaire was developed by 19 physician experts with input from allied health team members. The instrument underwent multiple modifications to ensure content and face validity, and was completed online between Jul and Dec 2010; data reflect practices during this period.

**Results:** Seventeen of 19 (89%) programs responded. All programs use conventional HD machines in the home setting. Maintenance of the equipment is provided by the vendor in 16/17 (95%) or by the renal program in 11/17 (65%). Wxetness detectors are routinely used around cumulation sites and on the floor beside the HD machine in 14/17 (82%) while 3/17 (18%) use detectors at one site or the other. Centrifuges (for home blood sample preparation) and weigh scales are provided by 14/17 (82%) and 10/17 (59%) of programs respectively. Real-time remote monitoring is performed in a minority of programs (3/17 – 18%). Reasons for denying access to HHD include substandard water (11/17 – 65%), and substandard/nonmodifiable plumbing (13/17 – 76%) or electrical (11/17 – 65%). Only 2 programs (12%) do not reimburse patients for some or all plumbing/electrical renovations, with 11/17 (73%) covering in excess of $1,500 (CDN) in expenses. Five of 17 (29%) reimburse patients for additional monthly utility costs incurred by dialyzing at home.

**Conclusions:** Significant heterogeneity exists among Canadian renal programs in terms of delivery of and equipment requirements for HHD. It is unknown whether such differences change patient outcomes or pose potential safety concerns; this will require prospective study.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Methods: 64 patients started dialysis from January to December 2010 (49 male and 15 females; mean age: 65.8 years, range 17-85 years). The most frequent cause of chronic renal failure was diabetic nephropathy (23%). 13 patients had a dysfunctioning graft and were transferred to dialysis (20%). 39 patients (61%) started HD and 25 PD (39%). 44 patients (69%) started dialysis treatment on a programmed basis and dialysis was unplanned in 20 patients (31%). At the start of dialysis 18 (28%) were occupationally active and expressed their desire to keep on working. All patient received information about dialysis modalities.

Results: Treatment modality was a personal choice in 55 patients (86%). The remaining patients were advised to choose dialysis modality by the nephrologist, and 3 patients had to choose dialysis modality by randomization. The reasons why 15 patients chose maintenance HD were: personal preference (n=8), social reasons (n=6), expected short survival (n=1). All of the different dialysis modalities. The reasons why 15 patients chose maintenance HD were: personal preference (n=8), social reasons (n=6), expected short survival (n=1). All of the different dialysis modalities.

Conclusions: In our unit, the dialysis options information program provides 39% of patients began treatment with PD. In our experience, the main factor that influences the choice of PD, mainly in young patients, was the decision to continue working.

PUB328
Effect of Interventions in Peritonitis Rate and Technique Failure in Renal Therapy Service Colombia Network
Angela S. Rivera, Medical, Baxter, Bogota, Colombia.

Background: Peritonitis and technique failure are the most frequent complications in peritoneal dialysis (PD). Interventions in education and training impact clinical outcomes.

Methods: A retrospective analysis of peritonitis rate and technique failure trend after care model implementation was performed from 2004 to 2010.

The interventions were:
- Monthly comprehensive evaluation by nephrologist and nurse.
- Attention by nutrition, psychology and social work according to risk.
- Programmed home visits and prioritized visits according to risk.
- Training for clinical team.
- Standardized nurse/patient ratio: 1/45.
- Protocol Standardization for peritonitis prevention: Prophylaxis for ex situ infection with antibiotic ointment.
- Change in antiseptic for cleaning hands and surfaces: Not use of iodinated solutions and introduction of chlorhexidine and glycerinated alcohol.

Results: Mean age was 58.4 years, 35.7% were female, 38% were diabetic and 63% has a low socioeconomic level. There was a significant decrease in peritonitis rate from 1 episode each 25.2 to 1 episode each 44.1 patient months in risk (RR 0.55 [0.52 – 0.59], p< 0.05) and decrease in technique failure defined as transfer to hemodialysis due to clinical complications from 16.20% to 8.11% (RR 0.52 [0.45 – 0.59] p< 0.05)

Conclusions: here is an improvement of peritonitis rate and a decrease in PD technique failure after a systematic compliance of protocols and the development of continuous education programs.

Funding: Pharmaceutical Company Support.

PUB329
Pauverxella Multocida Peritonitis in a Peritoneal Dialysis Patient
Manish K. Saha, Tarek Hamieh, Vishal Sagar.

Background: 64 year old male with end stage renal disease on automated peritoneal dialysis(PD) with a cycle at home presented with abdominal pain and vomiting for 1-2 days. He noted that his PD fluid was cloudy a day before presentation. On exam, there was diffuse abdominal tenderness but no evidence of erythema or tenderness over the PD catheter insertion site. ESR / CRP was not raised. Blood cultures were negative. CT scan of abdomen did not show any evidence of bowel perforation or abscess. Initial PD fluid was cloudy and showed 1195 nucleated cell/ul with 91% of PMNs. Patient was started on intraperitoneal vancomycin and tobramycin after PD fluid was sent for culture for presumed peritonitis. Initial gram stain showed gram negative bacillus and cultures grew Pauverxella multocida. On further review of history, patient disclosed that he had 2 pet cats that had been licking/biting on his PD tubing while he was on the cycle at night. A diagnosis of paeurexella multocida peritonitis was made and his antibiotic regimen was changed to intraperitoneal B cepacia coltactum for 2 weeks with improvement of symptoms. Patient was counseled and educated about puaurexella multocida and routes of transmission prior to discharge. He was also advised to keep cats, cats oral secretions at bay while performing dialysis.

Discussion: Pauverxella multocida is a gram-negative bacilli and is found the oropharyngeal flora of cats and felines. Patients on peritoneal dialysis are at increased risk for peritonitis if PD catheter gets exposed to cats or felines’ oral flora. Pauverxella is major cause of mortality and morbidity in PD patients. The usual symptoms are fever, abdominal pain, and cloudy dialysate as in our patient. Since our patient had significant history of...
cat's oral secretion exposure to PD catheter, this is a rare form of transmission without PD catheter breaches or strictures. Increasing awareness through patient education is of utmost importance given the prevalence of patients having animal pets at home.

**PUB330**

**Prevalence and Clinical Relevance of Sick Euthyroid Syndrome in a Peritoneal Dialysis Population**  

**Background:** Sick euthyroid syndrome (SES) has been linked with severity and outcomes in acute illness, however scarce data is available in dialysis patients.

We aimed to evaluate the prevalence of low T3 syndrome in our peritoneal dialysis population and determine its relation with peritoneal transport rate, peritoneal protein loss, inflammation, nutrition and volume status.

**Methods:** Cross sectional design: 56 prevalent patients on PD, aged 57.3 (45.4-68.0) years, 46.4% male, 16.1% diabetics, median Charlson comorbidity score of 5; 52.6% anemic, residual GFR 5.0 (2.7-6.8) mL/min; time on PD 28.6 (11.5-55.4) months. Prevalence of SES and comparisons between subgroups according to the median T3 value were evaluated. Nutritional and volume parameters were obtained by multifrequency bioimpedance. Serum protein C reactive (PCR), D/P creatinine, protein losses, normalized protein catabolic rate, anemia, albumin and dose of dialysis were also explored. Multinominal linear regression model was applied to determine relevant correlations.

**Results:** SES was diagnosed in 5.3% of patients. Median plasma free T3 level (FT3) was 2.9 mg/dL (2.5-3.1). Lower FT3 group was older (58.7 vs. 48.3 years, P = 0.003), had lower albumin (3.6 vs. 3.9 mg/dL, P = 0.005) and haemoglobin levels (11.6 vs. 12.5 mg/dL, P = 0.028); higher ferritin (540 vs. 356 mg/dL, P = 0.021) and PCR (7.9 vs. 5.0 mg/dL, P = 0.03); a trend for higher extracelluar/intracelllar water (ECW/ICW) ratio (0.98 vs. 0.91, P = 0.052) was found. No differences were documented in peritoneal transport rate, protein losses or dialysis dose. In a multivariate linear model, entering with age, haemoglobin, PCR, ferritin and ECW/ICW ratio as independent variables only haemoglobin and PCR were able to predict FTT (Beta = 0.37, P = 0.004 ; and Beta = -0.27, P = 0.042 respectively).

**Conclusions:** There was a very low prevalence of SES in our PD population. Thyroid dysfunction was associated with markers of systemic inflammation, volume status and malnutrition, however only hemoglobin and PCR were good predictors of FT3. Longitudinal evaluation of this overlooked parameter might elucidate more its clinical impact on morbidity and mortality.

**PUB331**

**Peritoneal Dialysis Technique Outcome and Associated Risk Factors: A 9-Year Single-Center Study**  
Namita Singh,1 Ramesh Saxena.2  
JHU/ Sinai Hospital, Baltimore, MD; 2Internal Medicine, UT Southwestern Medical Center, Dallas, TX.

**Background:** To review the peritoneal dialysis (PD) technique outcomes at our center and assess factors affecting the technique survival (TS).

**Methods:** This is a retrospective study on 315 patients who initiated PD between January 2001 and September 2009 at UT Southwestern's DaVita PD-Clinic, Dallas. Medical records were reviewed for demographic and clinical information. The primary end point was PD technique failure (TF), defined as discontinuation of PD due to catheter-related, technique-related or other medical/surgical/social complications. TS was analyzed by Kaplan Meier method. Cox proportional hazard regression model was used to identify factors independently associated with TS.

**Results:** There were 54.6% females, and 42.5% African Americans and 43.2% diabetics in our study population. More than 90% of patients had co-morbidities; and 57.5% had previous abdominal surgeries. The mean BMI was 28.6 ± 13.8 kg/m². Infections complications included 39.7% peritonitis and 22.9% catheter-related infections. Non-infectious/ mechanical catheter problems were observed in 24.1%. There were total of 203 PD failures, of which 12.8% were due to catheter-related problems and 20.2% due to peritonitis. Poor performance on PD, and medical/surgical events contributed to 7.4% and 6.9% PD discontinuations, respectively. Overall PD TS rates at 1, 2 and 3 years were 82.12%, 69.42% and 58.38%, respectively. Two variables significantly affecting TS rates were PD catheter-related non-infectious problem (Hazard ratio 1.812; 95% CI 1.193-2.750), and Diabetes as the etiology of TS (Hazard ratio 3.932; 95%CI 1.450-10.456). A single center, retrospective cohort study was performed that included 401 patients (female/male: 165/236; age: 61.0±12.6±9 years) who started PD as the initial dialysis treatment from 1995 to 2005. Chart and electronic databases were used to obtain information on the course of dialysis therapy including mortality and cardiovascular events.

**Conclusions:** No significant difference was observed in survival rate over time in diabetic and non-diabetic population. This observational study shows that peritoneal dialysis can be used effectively as a form of RRT in diabetic patients.

**PUB332**

**Comparison of Change in the Adequacy of Peritoneal Dialysis over Time in Diabetic and Non-Diabetic Patients — A Single Centre Observational Study**  
Subash Somalanka, Manivarma Kamalanathan, Bhirgu Raj Sood. South West Thames Renal Unit, Epsom and St Helier NHS Trust, Carshalton, Surrey, United Kingdom.

**Background:** The diabetic patient with end stage renal failure presents many therapeutic challenges, some of which are particularly difficult when peritoneal dialysis is selected as modality for renal replacement. Given the advantages of home based therapy, more patients with diabetes are taking up peritoneal dialysis. We wanted to compare the difference in change of adequacy of peritoneal dialysis over time in patients with and without diabetes undergoing peritoneal dialysis.

**Methods:** We studied a cohort of peritoneal dialysis patients in our unit. The data of all the patients who are currently on the peritoneal dialysis programme in 2011 was obtained from our renal unit database. We compared the dialysis adequacy between diabetic and non-diabetic patients over the last 8 years. We studied variable including age, sex, diabetes status, modality of PD, and annual Kt/V. The follow up period varied between 2 to 8 years.

**Results:** A total of 64 patients were identified from the renal unit database who had annual adequacy data. 19 patients had a diagnosis of diabetes and 45 were non-diabetic. Patient characteristics

**Time on peritoneal dialysis in years**

**Conclusions:** No significant difference was observed in serial change in adequacy of dialysis when compared over time in diabetic and non-diabetic population. This observational study shows that peritoneal dialysis can be used effectively as a form of RRT in diabetic patients.
difference (p<0.05) was seen in the start of hemodialysis between males and females. In male patients on PD, HD therapy was started 24±16 months after the start of PD. In contrast, in female patients, HD was started 55±16 months after the start of PD.

Conclusions: Although females have a survival advantage in the general population as well as among dialysis patients, females undergoing PD have a similar mortality to men. The reasons for these findings remain to be explained. However, this analysis suggests that early start of hemodialysis therapy will prolong survival rate in patients on PD, and especially in male patients.

**PUB334**

Developing Bioimpedance (BIA) as a Tool for Fluid Management in Peritoneal Dialysis Patients: A Validation Study

Boon Kay Tan,1 Zanzhe Yu,1 Frauke Wenzelburger,1 Martin E. Wilkie,2 Sarah Jenkins,3 Jia Qi Qian,4 Zhaohui Ni,5 Simon J. Davies.1 1Institute for Science and Technology in Medicine, Keele University, Stoke on Trent, Staffordshire, United Kingdom; 2Nephrology Department, University Hospital of North Staffordshire, Stoke on Trent, Staffordshire, United Kingdom; 3Sheffield Kidney Institute, Sheffield, South Yorkshire, United Kingdom; 4Renal Department, Renji Hospital, Shanghai, China.

**Background:** The assessment of fluid status in PD patients using simple clinical parameters is insensitive due to spontaneous body composition changes with time on therapy. We hypothesize regular monitoring of body composition using BIA adds value to parameters is insensitive due to spontaneous body composition changes with time on therapy. We hypothesize regular monitoring of body composition using BIA adds value to

**Methods:** This is an ongoing multi-centre, prospective, randomized study of incident and prevalent PD patients in the UK and China stratified by country and residual renal function >200ml vs <200ml at entry. To detect a between group difference in extracellular fluid volume (ECFv) of 0.8 Kg requires 38 patients per group with 80% power. Following baseline assessment, we will measure ECFv by BIA in all patients. Measurements are taken in both groups 3 monthly and in addition at any time of clinical need in the active limb where BIA data is available. Interventions can be a combination of advice on dietary salt and fluid intake, increased use of diuretics, hypertonic solutions and sédexstrem. Any interventions based on BIA data and their intended effects will be recorded prospectively. This study makes no assumptions as to the ideal desirable fluid status in PD patients at any given time.

**Results:** The primary outcome is the maintenance of ECFv determined from BIA in the active limb. Other outcome measures are BP, residual urine volume, membrane function, bioarkers and cardiac functions by echocardiography.

**Conclusions:** Measurement of fluid status in PD is challenging and BIA can be a powerful clinical tool in the routine management of fluid status. This study will give insight and valuable information as to the best application of this technique.

**Funding:** Pharmaceutical Company Support

**PUB335**

**Variability in Water Source and Dialysate Composition for Canadian Intensive Home Hemodialysis Programs**

Deborah Lynn Zimmerman,1 Robert P. Pauly,1 Paul Komenda,2 Medicine, University of Ottawa, ON, Canada; 2Medical College of Wisconsin, Milwaukee, WI, United States.

**Background:** Despite growing interest in intensive hemodialysis (HD) prescriptions (more frequent and/or longer), there is little published information on how to establish and maintain an intensive HD program. The purpose of this study was to survey Canadian nephrologists responsible for intensive HD to describe practice patterns.

**Methods:** A survey was developed with the assistance of 17 physician experts with input from their allied health teams. The survey was modified based on reviews received. The instrument was finalized after a face to face meeting with the expert physician panel. The survey was completed on line. The current abstract addresses water quality and dialysis prescription.

**Results:** Seventeen of 19 programs contacted participated in survey development and provided information. For water sources, 16 and 6 programs have used well or surface water respectively. Testing for microbial contamination, endotoxin units (EU), organics/inorganics is done by 15, 13 and 13 programs respectively. All programs set a limit of 50 or 100 CFU of the product water. There was more variability in EU limits of < 0.25 ml (3), <1.0 ml (9), <2.0ml (1) and not measured or not answered (4). Water sampling is variable and done by patients, technicians and/or the equipment manufacturer. For nocturnal HD, starting dialysate prescription was Na = 136±140mM/L, K = 2-3mM/L, HC03 28-37mM/L, Ca (1.5-1.75mM/L), Mg (0.5-0.75mM/L) and glucose (5.55±11.1mM/L). Dialysate flow was 150-500mls/min and most programs are using high-flux dialyzers. For short daily HD, variability was also seen in dialysate concentrations except the lowest HC03 was 30mM/L and Ca was usually 1.25mM/L. Dialysate flow was 500-800mls/min and all programs used high flux dialyzers.

**Conclusions:** Despite a long history of intensive HD utilization in Canada, there is tremendous practice variability. Deducing the results of observational data is made more complicated by these differences in prescription. A lack of published patient outcomes or guidelines for patient management may contribute to variability.

**Funding:** Clinical Revenue Support

**PUB336**

Malnutrition Inflammation Complex Syndrome in Haemodialysis Patients: Assessing the Prevalence and Severity Using Malnutrition Inflammation Score

Christopher T. Agbo,1 Sumith C. Abeygunasekara. 1Renal Medicine, Broomfield Hospital NHS, Chelmsford, United Kingdom.

**Background:** The combination of inflammation and protein energy malnutrition (PEM) is a very common condition among maintenance haemodialysis patients. The term malnutrition-inflammation complex syndrome (MICS) has been widely used to describe this condition and is associated with increased morbidity and mortality in maintenance haemodialysis(MHD) patients. Malnutrition-inflammation score (MIS) is a comprehensive tool for evaluating MICS and there has been recorded correlation between MIS and mortality among haemodialysis patients. In this study, we used MIS to assess the prevalence and severity of MICS in MHD patients.

**Methods:** Observational cross-sectional study of 139 maintenance haemodialysis patients in our renal unit. The MIS tools was used to evaluate all the patients within the study period, other laboratory parameters such as C-reactive protein (CRP), Haemoglobin(Hb), protein catabolic rate (nPCR), urea reduction ratio were measured. Analysis of the data was done using SPSS statistical software. Pearson’s correlation coefficient was used for selected continuous variables, Spearman’s rank correlation used for non-parametric variable.

**Results:** The mean age was 69.2±15.1, 68.9% were men and mean duration on dialysis was 49.2±51.45 months. The mean MIS was 6.17±3.03. MIS < 3 (3.8%), 3 – 5 (40.6%), 6 – 8 (36.8%), > 8 (18.8%). MIS showed a strong correlation with serum CRP level (p = 0.014), Haemoglobin levels (p = 0.064), Age (p = 0.034) and nPCR (p =0.039).

**Conclusions:** Malnutrition-inflammation score is a powerful tool in assessing the prevalence and severity of MICS. It is useful also in predicting the factors that affect morbidity and mortality in haemodialysis patients such as CRP, nPCR and age. MIS showed a strong correlation with CRP, haemoglobin, nPCR and age. The entire patients studied had some degree of MICS, the greater the MIS the increased risk of morbidity and mortality. Apart from using the MIS to risk stratify haemodialysis patients, there is need to establish a universal cut off point in order to define MICS.

**Funding:** NHS

**PUB337**

N-acetylcysteine (NAC) Supplemented in the Reinfusate during Acetate-Free Biofiltration (AFB) to Blunt Oxidative Stress Generated during Dialysis (HD)

Alessandro Amore, Roberta Camilla, Lucia Peruzzi, Roberto Bonaudo, Rosanna Coppo. Nephrology, Dialysis, Transplant, R. Margherita H., Turin, Italy.

**Background:** The oxidative stress (OXS) plays a key role in triggering the dialytic vasculopathy. Biocompatible reactions during HD amplify the phenomenon. In an in vivo study we showed that NF-kB is activated during HD and it is blunted by i.v. infusion of NAC at the end of HD. We realized that both NF-kB activation during HD and NAC blunting effects were too sharp to be satisfactory, apart from prolonging the time of each HD, not liked by the patients.

We aimed this study to improving a highly biocompatible HD, acetate free biofiltration (AFB), adding NAC to the infusion fluid in order to blunt the OXS generated during HD.

**Methods:** We performed 8 AFB ex vivo sessions by circulating healthy blood donors 250 ml/min, in AN60-ST dialyzers, using a buffer-free dialyseate (Safebag,Hospal) 500 ml/min flow and NaHCO3 reinfusate (Hospapol 145, Hospal) 30 ml/min. Reinfusate was prepared and added to the recirculating blood with the assistance of 17 physician experts with input from their allied health teams. The survey was modified based on reviews received. The instrument was finalized after a face to face meeting with the expert physician panel. The survey was completed on line. The current abstract addresses water quality and dialysis prescription.

**Results:**

<table>
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<th>Time minutes</th>
<th>Dialysate</th>
<th>Dialysate</th>
<th>Reinfusate</th>
<th>Reinfusate</th>
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<tbody>
<tr>
<td></td>
<td>Blood+NAC in reinfusate SH compounds</td>
<td>Blood+NAC in reinfusate NaSH compounds</td>
<td>Blood+NAC in reinfusate Sodium compounds</td>
<td>Blood+NAC in reinfusate S-S compounds</td>
</tr>
<tr>
<td>1</td>
<td>5.4±0.68</td>
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<td>0.92±0.16</td>
<td>0.32±0.12</td>
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<tr>
<td>15</td>
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<td>1.13±0.09</td>
<td>1.1E+03*</td>
<td>0.52±0.05</td>
</tr>
<tr>
<td>30</td>
<td>5.6±0.66</td>
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</tr>
<tr>
<td>60</td>
<td>5.3±0.17</td>
<td>1.1E+03*</td>
<td>0.78±0.09*</td>
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<table>
<thead>
<tr>
<th>Source</th>
<th>SH peaks</th>
<th>S-S peaks</th>
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<tr>
<td>Blood</td>
<td>0.92±0.16</td>
<td>0.32±0.12</td>
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<tr>
<td>Reinfusate SH</td>
<td>1.13±0.09</td>
<td>0.52±0.05</td>
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<tr>
<td>Sodium SH</td>
<td>1.1E+03*</td>
<td>0.77±0.03*</td>
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<tr>
<td>Sodium S-S</td>
<td>1.1E+03*</td>
<td>0.78±0.09*</td>
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</table>

**Conclusions:** In conclusion, we proved that the addition of NAC to the reinfusate fluid of AFB, can blunt the OXS generated during HD.
The Relationship between TRAIL Concentration and Inflammatory Markers

Markers: Marek Kuzniweski,1 Danuta Fedak,2 Dariusz Giza,1 Pawlica Dorota,3 Beata Kusnierz-Cabala,2 Paulina Dumnicka,2 Bogdan Solnica,2 Wladyslaw Sulowicz,1 1Department of Nephrology, Jagiellonian University, Collegium Medicum, Cracow, Poland; 2Department of Clinical Biochemistry, Jagiellonian University, Collegium Medicum, Cracow, Poland; 3Department of Medical Diagnostics, Jagiellonian University, Collegium Medicum, Cracow, Poland.

Background: TRAIL (TNF-related apoptosis-inducing ligand) is a member of TNF ligand superfamily. Five different receptors of TRAIL has been identified. Two receptors containing a death domain (TRAIL-R1 and TRAIL-R2) are capable of rapidly inducing apoptosis. Two decoy receptors (TRAIL-R3 and TRAIL-R4) unable to transduce apoptosis signals but may activate nuclear factor kappa-light-chain enhancer of activated B cells and block apoptosis and tumor necrosis factor (TNF) soluble decoy receptor (TRAIL-R5). TRAIL can activate both apoptotic and anti-apoptotic signals. TRAIL-R2 and OPG are present in human atherosclerotic lesions and their expression levels are higher in vulnerable plaques than in stable ones. Chronic kidney disease is associated with accelerated atherosclerosis and exacerbated but ineffective inflammatory response.

Aim of the study was to investigate the relationship between soluble TRAIL and selected markers of inflammation in patients on maintenance hemodialysis.

Methods: Studied group: 76 patients (36 female and 40 male) of average age 60 ± 12 years on maintenance hemodialysis (25 ± 5 months). soluble TRAIL and IL-6 and IL-8 were determined by ELISA and hsCRP using immuno-nephelometry.

Results: The mean values of TRAIL was 595.6 ± 204.0 pg/ml, hsCRP 11.5 ± 18.8 mg/l, IL-6: 6.4 ± 8.7 pg/ml and IL-8: 20.0 ± 15.7 pg/ml. The obtained correlations between TRAIL and tested inflammatory parameters were given in the table. The interrelations between TRAIL and selected inflammatory parameters

Conclusions: Nevertheless we found no correlation between TRAIL and CRP the inflammatory marker. Nevertheless we found no correlation between TRAIL and CRP the inflammatory marker. Nevertheless we found no correlation between TRAIL and CRP the inflammatory marker. Nevertheless we found no correlation between TRAIL and CRP the inflammatory marker.
ultrafiltration. The UF volume obtained ranged between 320 and 2800 mL/session (equal to 1 to 7 mL/kg/hr). The Qb was 80-120 ml/min. The UF was well tolerated, hematocrit (monitored by CritiLime2000) remained stable during the sessions.

No significant adverse events were observed, except urticarial reactions. Only one session was complicated by increased transmembrane pressure requiring treatment discontinuation.

Conclusions: The combination of UF and PEX is feasible, safe and efficacious and it represents an additional tool whenever fluid removal is necessary in patients unresponsive to diuretics and requiring PEX.

PUB343

The Impact of a Prior History of Cardiovascular Events on Outcomes in Patients on Renal Replacement Therapy

George P. Bavli,1 Bijan Roshan,2 Saila V. Ventrapragada,3 Larry A. Weinrauch,2 Ray E. Gleason;4 John A. D’Elia.2 1Division of Kidney Diseases and Hypertension, Department of Medicine, Alpert Medical School, Brown University, Providence, RI; 2Joslin Diabetes Center, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA.

Background: The majority of morbidity and mortality on renal replacement therapy (RRT) is cardiovascular. Prior cardiovascular events (CVE) have an unknown impact in the presence of uremia, however they are considered when important therapeutic decisions are made.

Methods: In a prospective study, 177 stable renal failure patients (pts) were followed for 0.04-13.69 years for CVE (myocardial infarction, coronary arterial intervention, peripheral arterial bypass or amputation, cerebrovascular accident, or carotid artery intervention). Pts group was based on treatment at entry into the study. 128 hemodialysis pts (64 male, age 58 ± 11, 63 [49%] with prior CVE); 22 peritoneal dialysis pts, (12 male, age 49 ± 3, 10 [45%], with prior CVE); 27 renal transplant pts (10 male, age 44 ± 1,13 [48%] with prior CVE).

Results: Clinical endpoints included CVE (total of 61; 29 heart, 27 peripheral vascular, 5 cerebrovascular), and 27 deaths (6 cardiovascular). 38 patients underwent renal transplant. Of 150 pts with diabetes mellitus, 24 of 76 (31.6%) type 1 and 34 of 74 (45.9%) type 2 study subjects experienced a CVE, as opposed to 27 pts without diabetes, 3 had CVE (11.1%), p = 0.043, 0.001. There were no significant differences for median event free follow up between groups defined by the presence vs. absence of prior CVE for either dialytic (2.0 vs. 2.3 yrs) or transplant therapy (2.0 vs. 2.9 yrs) (all p > 0.20).

Conclusions: In clinically stable patients undergoing renal replacement therapy, diabetes mellitus is associated with a higher CVE rate such that the presence of prior CVE may not predict future CVE. Clinical decision-making for such pts should not overemphasize the importance of prior cardiovascular morbidity.

Funding: Pharmaceutical Company Support; Private Foundation Support

PUB344

Abstract Withdrawn

PUB345

Responses to Furosemide in Hemodialysis Patients with Residual Renal Function

Louis A. Carbone, Maria V. DeVita, Michael F. Michels. Department of Nephrology, Lenox Hill Hospital, New York, NY.

Background: It has been generally accepted that residual renal function provides a contribution to the well-being of end stage renal failure patients. Studies regarding the use of furosemide in peritoneal dialysis patients have shown some benefit, however its role in hemodialysis patients is less well known. We report our experience with the use of furosemide at our outpatient hemodialysis center.

Methods: All patients were screened for residual renal function, which was defined by urine output of > 400 ml over a 24 hour period. From a total of 167 patients, 28 patients met our criteria and 14 patients agreed to participate. For 3 weeks, patients were given either furosemide 100 mg daily if their weight was <80 kg, or 160 mg daily for weight > 80 kg. Baseline and post furosemide measurements were obtained for urine volume, blood pressure (BP), potassium, phosphorous and interdialytic weight gain (IDWG). Twenty-four hour urine collections were obtained across the week preceding the use of furosemide and during the last week of the study. The average of three IDWG on the week preceding administration of furosemide and the last week of the study period were used for comparison. The effect of furosemide on these parameters was analyzed using a paired t-test. During the study, 3 of the 14 patients were non-responders and were excluded from further analysis.

Results: The mean increase in urine volume was statistically significant at 575 ml (p < 0.001). The mean systolic BP decreased 2.72 mmHg (p = 0.58) but diastolic BP decreased by 5.45 mmHg (p = 0.06), which approached statistical significance. Changes in serum potassium and phosphorous were not statistically significant. Interestingly, IDWG was not significantly suggesting that patients were liberalizing fluid intake.

Conclusions: Our study suggests that furosemide is effective in increasing urine output in hemodialysis patients. Furthermore, it may improve diastolic pressure and lessen fluid restriction.

PUB346

Comparison of In-Vitro Metabolism of Medications Using Human and Recombinant Hepatic Microsomes

Brian S. Decker, Nitesh Thakker, James Slaven, Zhengsheng Yu, Sharon M. Moe, David Jones. Medicine, Indiana University School of Medicine, Indianapolis, IN.

Background: Studies have demonstrated that uremia can diminish the hepatic metabolism of medications. Human and recombinant hepatic microsomes are used to evaluate the in-vitro hepatic metabolism of medications. The purpose of this study was to compare the magnitude and variability of the metabolism of medication substrates in uremic serum using human and recombinant hepatic microsomes.

Methods: The medication substrates evaluated for this study were midazolam and dextromethorphan. Uremic serum was obtained at the midpoint of a four hour hemodialysis session from six anuric subjects with end-stage renal disease (ESRD). The control used in each experiment was pooled normal serum obtained from subjects with normal renal function. Midazolam and dextromethorphan were incubated with human liver and recombinant microsomes in normal and uremic serum. Hepatic metabolism by the microsomes was tested according to validated protocols. The analysis of the 1-OH and 4-OH midazolam and dextromethorphan metabolites was performed by mass spectrometry. Statistical analyses using Students t-test were then performed to compare the magnitude of metabolism formed from the human and recombinant microsomes. Coefficients of variation were also analyzed to look at variability between the human and recombinant microsomes. All data were normalized with the normal pooled serum values, to control for day-to-day run variation.

Results: There was no statistical difference in the magnitude of the metabolites formed from either human or recombinant liver microsomes. Analysis of metabolism using normalized data found no significant difference between human and recombinant live microsomes in all three outcomes. Without normalization, recombinant microsomes demonstrate significant higher level of activities than human microsomes. There were no significant differences in coefficients of variation within each metabolite.

Conclusions: Human and recombinant microsomes do not differ in the magnitude or variability of metabolites formed when incubated in uremic serum, after normalizing to control for day-to-day run variation.

Funding: NIDDK Support

Background: Online hemodiafiltration (online HDF), as compared to low-flux hemodialysis (low-flux HD), is postulated as a superior method of renal replacement therapy (RRT), but the effect of both methods on clinical effects of treatment still remains unclear.

The aim of this multicenter (11 dialysis units) study was to compare the dialysis adequacy, anemia and calcium-phosphate disturbances correction as well as the frequency of intradialytic hypotension (IH) in patient treated with different RRT modalities – HDF vs. low-flux HD.

Methods: The study was composed of 423 patients (171F and 252 M) aged 21 to 87 yrs. (mean 61.6), on RRT for 0.9-275 months. (mean 44.2). 192 (5 dialysis units) patients was treated using online HDF and 231 (6 dialysis units) using low-flux HD.

Patients were observed for 24 mths. 274 of them (65%) finished the observation period. Biochemical and clinical parameters were performed at the start of the trial and next every year.

Results: All results are shown in table. The assessment of biochemical and clinical parameters was performed at the start of the trial and next every year.

Conclusions: Treatment with online HDF brings important biochemical and hemodynamic benefits for RRT patients.

Comparison of Online Clearance Monitor with Other Blood-Sampling Methods in Hemodialysis Adequacy Evaluating Jiyuan Gao, Renhua Lu, Jiayuan Gao, Renhua Lu, Zhaozhi Ji, Qian Zhao, Yan Zhou, Nephrology, Renji Hospital, Shanghai, Shanghai, China.

Background: To compare the accuracy of hemodialysis adequacy evaluated by online clearance monitor (OCM) with other blood-sampling methods, using direct dialysate quantification method (DDQ) as a gold standard.

Methods: We included 19 anuric maintenance hemodialysis patients. Drained dialysate samples were collected continuously during the entire therapy by partial dialysate collection method (PDC). Urea nitrogen levels were assessed by analyzers of OCM (Vurea) from Fersenius and by direct dialysate measurement (DDQ) from Fersenius. The value and correlation of Kt/V by different methods were analyzed.

Results: As a result, the value of OCM Kt/V, Ra Kt/V and Eq Kt/V were very similar to DDQ Kt/V. Vurea distribution volume (Vurea) used in the three formulas (OCM) internally installed in OCM were compared with the standard Vurea level from DDQ. Urea nitrogen (BUN) at the beginning of dialysis, at the end of dialysis and as well as 30 minutes after therapy were measured. Single pool Kt/V (Sp Kt/V) was measured by Daugirdas II formula was also employed.

OCM Kt/V was provided by OCM module from Fersenius. The value and correlation of Kt/V by different methods were analyzed. The relationship with DDQ Kt/V (1.24±0.24, 1.39±0.24, 1.41±0.27 vs. 1.34±0.26, all P >0.05), while Sp Kt/V showed a significant different (1.51 vs. 1.16 p<0.0001 1.54 vs. 1.45 p<0.05) with DDQ Kt/V. Ra Kt/V or Eq Kt/V was all well correlated with DDQ Kt/V. The relationship between OCM Kt/V and DDQ Kt/V (r= 0.706, P<0.05) was lower than that of Sp Kt/V.

Conclusions: The erythropoiesis stimulating agents dose did not differ between the groups.


Background: Clinically estimating the dry weight (DW) in dialysis pts is subjective & often inaccurate. Several methods used include natriuretic peptides, measurement of IVC diameter and collapsibility on inspiration by ultrasound, intradialytic relative blood volume change and single frequency bioimpedance. These methods suffer from poor specificity.

Multifrequency Bioimpedance Spectroscopy (MBIS) to determine TBW & ECV has been validated by applying dilution methods as gold standard.

Aim: To study the utility of MBIS in HD pts in addition to clinical method of assessment of DW.

Methods: A prospective study in prevalent HD pts at a single dialysis centre.

Inclusion Criteria

HD pts on 3/week treatment for 4 hours for more than 3 months, clinically euvolemic.

Exclusion Criteria

Active infection, CHF, Liver disease with ascitis & Pacemaker/ ICD, Metallic Implants, URR< 60%, JHB >9.0 g/dl, Albumin < 3.0 gms/dl

Results: 20 pts were monitored over 2 week for BF, no. & dosage of antihypertensive drugs, weight & no. of hypotensive episodes during dialysis.

MBIS(Body Composition Monitor BCM-Fresenius) machine was used to assess TBW and ECV. The new calculated DW was obtained from BCM. We tried to achieve this new DW over next 2 weeks and the parameters were reassessed.

Conclusions: In conclusion, the correlation between OCM Kt/V and DDQ Kt/V was ideal with almost similar value. It can be ideally evaluate the actual delivered dialysis dose of hemodialysis patients. The advantage of online quick monitoring implies its further value in application.

MBIS is helpful in achieving DW in addition to clinical examination & a longer follow up will demonstrate if it translates into decreasing cardiovascular morbidity & mortality.

Daily Long-Term Kt/V Determination by Ultraviolet Absorbance (Adimea) in Hemodialysis Helps To Detect Clinical Complications Marian Kelm, Roman Günther, Martin Kuhl, Elmar Wagner. *II. Braun Avitum AG, Melsungen, Germany; †PHV Dialysezentrum, Melsungen, Germany.

Background: Online Kt/V-determination with Adimea has become an established monitoring principle in regular hemodialysis and has proven its measurement adequacy in various clinical studies (Castellana et al., 2010). The aim of this work was to show that daily and long-term Kt/V-measurement with Adimea can lead to the detection of possible events during hemodialysis such as shunt stenosis.

Methods: In this case study, patient data from the PHV Dialysezentrum Melsungen, Germany was analysed.

Results: A case of a 54 year old woman is reported who had chronic bicarbonate hemodialysis 3x per week for nearly 1 year. The patient underwent the third vascular access dilatation due to a shunt stenosis in 05/2011. Before and after this intervention, the patient underwent regular hemodialysis with a Dialog machine and daily Kt/V measurements with Adimea. Kt/V data was analysed retrospectively over a period of 5 months to identify a possible change in administered dialysis dose. It can be seen that the Kt/V dose gradually decreases over a period of 7 weeks before the patient was sent to shunt dilatation. Prior to shunt revision, the administered blood flow had to be decreased. After the patient returned from shunt dilatation, the blood flow and Kt/V dose increased and the patient was able to reach the Kt/V target again. This data shows that in patients prone to shunt stenosis, daily Kt/V monitoring can help to identify possible stenosis events.

Key: THU - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Dialyzer Reuse in Short Daily Online Hemodiafiltration: Impact on Solutes Extraction Natalia C.V. Melo, 1, 2 Rosa M. Mousyes, 1 Manuel C. Castro, 1 1Nephrology Department, University of Sao Paulo School of Medicine, Brazil; 2CDRR/HRT, Brasilia, Brazil.

Background: In daily online hemodiafiltration(D-OL-HDF), there are no studies evaluating the impact of dialyzer reuse on solutes extraction.

Methods: 14 patients (47.9±13.5 years) in daily hemodialysis(D-HD) program were included. Impact of high flux dialyzer reuse on solutes extraction on dialysis sessions was evaluated and compared to that in D-HD.

Results: Directly quantified small solutes total mass removal(MTdq) and clearance(Kdq) were similar when 1st, 7th and 13th dialyzer D-HD uses were compared to D-OL-HDF respective uses. Small solutes MTdq and Kdq were greater in D-OL-HDF dialyzer 1st use, 7th and 13th uses than in the respective D-HD uses.β-2-m MTdq and Kdq were similar among dialyzer uses in D-OL-HDF, but were inferior in 13th D-HD dialyzer use than in 1st and 7th D-HD uses.

Conclusions: Dialyzer reuse did not impact on solute extraction in D-OL-HDF sessions. β-2-m extraction was greater in D-OL-HDF than in D-HD, without differences in other solute extractions.

High-Flux Polysulfone Dialyzer Reuse in Short Daily Online Hemodiafiltration: Impact on Dialysis Dose and β-Microglobulin Kinetics Natalia C.V. Melo, 1, 2 Rosa M. Mousyes, 1 Manuel C. Castro, 1 1Nephrology Department, University of Sao Paulo School of Medicine, Brazil; 2CDRR/HRT, Brasilia, Brazil.

Background: In daily online hemodiafiltration(D-OL-HDF), there is a lack of studies evaluating the impact of dialyzer reuse on dialysis dose and β-microglobulin(β2-m) kinetics.

Methods: 14 patients (47.9±13.5 years) in daily hemodialysis(D-HD) program were included. Impact of high flux polysulfone dialyzer reuse on dialysis dose and β-m kinetics, in D-OL-HDF sessions, was evaluated and compared to that in D-HD.

Results: Dialysis dose measured by direct dialysis quantification Kt/V (DDQ Kt/V) and direct dialysis quantification Kv (DDQ Kv) was significantly smaller in D-OL-HDF sessions (0.43±0.12) than in D-HD (0.92±0.26) and D-OL-HDF sessions (0.96±0.258) (p<0.0001). Urea directly measured total extracted mass (MT) and clearance (Kd) were smaller in D-OL-HDF sessions (148.4±43.4mg; 108.0±25.8mL/min) than in D-HD (198.8±43.4mg; 144.4±23.5mL/min) and D-OL-HDF(143.9±21.7mg; 142.0±23.9mL/min) sessions (p<0.0001).

Conclusions: Direct dialysis dose measurement showed significantly higher in D-OL-HDF dialyzer 1st use(0.90±0.30) than in D-HD (0.92±0.26) and D-OL-HDF sessions (0.96±0.258) (p<0.0001). β2-m MTdq and Kdq were smaller in D-OL-HDF sessions (475.8±169mg; 84.0±28.0mL/min) than in D-HD (810.4±165mg; 141.9±24.0mL/min) and D-OL-HDF(790.7±199mg; 142.0±23.9mL/min) sessions (p<0.0001).

High-Flux Polysulfone Dialyzer Reuse in Short Daily Online Hemodiafiltration: Impact on Dialysis Dose and β-Microglobulin Kinetics

Difference and Similarities among Daily High Flux Hemodialysis, Online Hemofiltration and Online Hemodiafiltration: A Kinetical Approach Natalia C.V. Melo, 1, 2 Rosa M. Mousyes, 1 Manuel C. Castro, 1 1Nephrology Department, University of Sao Paulo School of Medicine, Brazil; 2CDRR/HRT, Brasilia, Brazil.

Background: There is a lack of studies comparing dialysis dose and solutes removal among short daily high flux hemodialysis(D-HDF), short daily online hemofiltration(D-OL-HF) and short daily online hemodiafiltration(D-OL-HDF).

Methods: 14 patients (47.9±13.5 years) in daily hemodialysis(D-HD) program were included. There were collected blood pre, post and in the middle of the sessions and dialysate (partially and homogeneously collected) in two-hour D-HDF, pre-dilution D-OL-HF and post-dilution D-OL-HDF sessions.

Results: Dialysis dose measured by direct dialysis quantification Kv (DDQ Kv) was significantly smaller in D-OL-HDF sessions (0.43±0.12) than in D-HDF (0.92±0.26) and D-OL-HDF sessions (0.96±0.258) (p<0.0001). Urea directly measured total extracted mass (MT) and clearance (Kd) were smaller in D-OL-HDF sessions (148.4±43.4mg; 108.0±25.8mL/min) than in D-HDF (198.8±43.4mg; 144.4±23.5mL/min) and D-OL-HDF(143.9±21.7mg; 142.0±23.9mL/min) sessions (p<0.0001).

Conclusions: Removal of small solutes (urea, phosphorus, creatinine and uric acid) was significantly smaller in pre-dilution D-OL-HDF than in D-HDF and in post dilution D-OL-HDF sessions, undergone with the same dialyzer type. The removal of β2-microglobulin was greater in D-OL-HDF than in other studies measured.
Methods: 99 maintenance hemodialysis patients with no known systemic or hemorrhagic diseases affecting their plateslet or WBC had blood drawn immediately prior to, ninety minutes into, and immediately following their first hemodialysis session of the week. All patients were dialead using a Fresenius Medical Care Optiflux polysulfone membrane F160, F180 or F220 (polysulfone synthetic dialyzer membranes, 1.6 m2, 1.8 m2, and 2.0 m2 surface area respectively, electron beam sterilized). WBC and platelet counts were measured from each sample by analysis on a CBC analyzer (Sysmex XT-4000i).

Results: The average age of the patients was 62.7 ± 6.9 years; 36 were females and 63 were males. The mean platelet count pre, mid and post dialysis was 193 (SD 74.6), 191 (SD 74.6), and 197 (SD 79.34) TH/mm3 (Fig. 1), showing no statistical difference. The average WBC count pre, mid and post dialysis was 6.84, 6.64, and 6.16 TH/mm3, with a p-value of 0.05, showing a statistically significant, but clinically insignificant, decrease in WBC.

Conclusions: Newer membranes have no significant effect on platelet count, and have a statistically significant, but very minimal effect on leukocytes. This suggests that they are, in fact, more biocompatible than their predecessors and may explain their association with increased survival.

PUB357
Dialysis Patients’ Fluid Overload, Antihypertensive Medications and Obesity
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Background: Overhydration (OH) is both a major etiology of hypertension in hemodialysis patients and a serious risk factor for mortality. We investigated the association of multiple variables and OH.

Methods: This is a cross sectional study of prevalent hemodialysis patients examining the hydraulic status with a portable bioimpedance apparatus to measure the degree of hydration status and comparing them with demographic, dialysis and laboratory data.

Results: We completed our study in 79 patients mean age was 60.7 ± 16.9 years, 49.3% males. 30.7% diabetic, vintage: 66.5 ± 57.1 months, residual urine output of 442 ± 521 mL/day. Patients were overdried by 2.6 ± 2.4 L and had a percent body fat of 36.4 ± 11.6 kg. We found a significant correlation between OH and systolic BP (r² = 0.152; p: 0.0006), each liter of OH generating 3.6 mmHg. We also found a positive correlation between the use of diuretics and OH (p: 0.003 two tailed Student t test) but no correlation between OH and body weight (r² = 0.0001).

Conclusions: OH is strongly associated with the use of antihypertensive medications and diuretics in this dialysis population. Obesity seems to afford some protection from OH.

PUB358
Rapid Correction of Chronic Metabolic Acidosis in Hemodialysis
Olga R. Carmona.
Nephrology Center, University of Uruguay, Montevideo, Uruguay.

Background: Metabolic Acidosis is associated with chronic renal failure for the incapacity of H+ excretion and the decrease of protein syntesis.

Methods: 27 Chronic HD patients(mean age 54.55 ± 16.44 years, 44.7%F) were studied. Samples for acid base and gas analysis were drawn from the arterial side of the peritoneal dialyse at pre, second hour and post HD using a Radiometer Copenhagen 700 and NaHCO₃ (39mmol/L) in the dialysate. Measured parameters were: pH, pCO₂, pO₂, SO₂. Cardiac parameters were: HCO₃, CO₂, SBE, GAP, p50 (pO2at 50 % Sat.) Student t test was used to evaluate differences between means (p<0.05).

Results: A significant increase of HCO₃-, pCO₂, pO₂, SO₂. SBE was observed fundamentally at the second hour. The decrease of GAP in the first two hours was not significant but at the end of HD, increase significantly respect to the second hour. Besides a significant decrease of pO₂ and p50 were produced. Acid Base and Blood gases variation during HD.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Pre HD</th>
<th>Second hour</th>
<th>Post HD</th>
<th>Second Pre</th>
<th>Post Pre</th>
<th>Post - Second</th>
</tr>
</thead>
<tbody>
<tr>
<td>[HCO₃-] mmol/L</td>
<td>21,061.25</td>
<td>27,051.22</td>
<td>29,041.15</td>
<td>0.07 S</td>
<td>0.007 S</td>
<td>0.007 S</td>
</tr>
<tr>
<td>pH</td>
<td>7.36±0.56</td>
<td>7.43±0.48</td>
<td>7.48±0.42</td>
<td>0.00 S</td>
<td>0.007 S</td>
<td>0.007 S</td>
</tr>
<tr>
<td>pCO₂</td>
<td>32,150.58</td>
<td>38,222.38</td>
<td>35,081.80</td>
<td>0.00 S</td>
<td>0.007 S</td>
<td>0.007 S</td>
</tr>
<tr>
<td>pO₂ (mmHg)</td>
<td>37,627.2</td>
<td>69.6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SBE</td>
<td>6.23</td>
<td>6.23</td>
<td>6.23</td>
<td>0.00 S</td>
<td>0.007 S</td>
<td>0.007 S</td>
</tr>
<tr>
<td>pH</td>
<td>9.01</td>
<td>9.01</td>
<td>9.01</td>
<td>0.00 S</td>
<td>0.007 S</td>
<td>0.007 S</td>
</tr>
</tbody>
</table>

Conclusions: The rapid correction of metabolic acidosis occurred in the first two hours of HD may contribute to the significant decrease of pO2 and the Oxygen delivery to the tissue (p<0.05) that may cause increase in intermediates of Krebs acid cycle, with the consequences of a significant increase of anion gap at the end of HD.

Funding: Government Support - Non-U.S.

PUB359
An Unusual Case of Refractory Hypokalemia
Nawannya Mukoso Osakwe,1 Rajnish Dhingra,2 Mohammad G. Saklayen,1 1Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH; 2Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH; 3Department of Internal Medicine, WSU Boonshoft School of Medicine, Dayton, OH.

Background: Extrapituitary causes are identified in 10-15% of patients with Cushing’s syndrome. When in excess, cortisol may act as a mineralocorticoid resulting in hypokalemic alkalosis and hypertension. Hypokalemic alkalosis is seen in 74 to 95% of patients with ectopic ACTH secreting syndrome but in fewer than 10% of all patients with Cushing’s disease.

Methods: Single Case Report

Results: A 37 year old ten-week pregnant woman was admitted for vomiting, generalized fatigue and weakness since onset of pregnancy. Physical examination revealed BP 117/70mmHg [Normal 70s/80s/50 mmHg] and no evidence of striae or cervical dorsal fat pad. Laboratory data showed severe hypokalemia of 2.0 moq/L, Mg 2.2mg/dl, HCO₃ 32meq/L and WBC 16,000. She was commenced on aggressive intravenous and oral repletion of potassium. However, patients’ potassium levels remained very low. Three days after admission patient had a missed abortion and her BP was in the 130s/70s. Laboratory Findings

<table>
<thead>
<tr>
<th>Lab</th>
<th>Results</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma rennin assay (ng/ml/hr)</td>
<td>3.6</td>
<td>1.9-3.7</td>
</tr>
<tr>
<td>Addosterone [ng/dl]</td>
<td>4</td>
<td>2-16</td>
</tr>
<tr>
<td>Urinary free Cortisol [mcg/24hours]</td>
<td>37,627.2</td>
<td>10-100</td>
</tr>
<tr>
<td>Morning cortisol [mcg/dl]</td>
<td>194</td>
<td>6-23</td>
</tr>
<tr>
<td>ACTH [ng/ml]</td>
<td>901</td>
<td>9-52</td>
</tr>
<tr>
<td>CT abdomen/pelvis</td>
<td>incidental hypertrophic changes of the adrenal glands without a definite adrenal mass</td>
<td></td>
</tr>
<tr>
<td>PE/CT</td>
<td>mild to moderate uptake in a 2 cm left mediasinal lesion</td>
<td></td>
</tr>
</tbody>
</table>

She was transferred to a regional referral hospital for surgical removal of the mediastinal mass and she died from complications of surgery. Final pathology report revealed she had an atypical carcinoid tumor.

Conclusions: This case illustrates the challenges clinicians face in diagnosing ectopic ACTH-secreting tumor and also the pitfalls of fragmented medicine practice. Ectopic ACTH-secreting syndrome can present in several ways ranging from the traditional Cushingoid features (refractory hypertension, diabetes etc) to hypokalemic alkalosis as seen in this patient. In many cases, optimal management is dependent on early localization and surgical resection of the ACTH secreting tumor.

PUB360
Serum Acetone: A Cause of Elevated Serum Osmolar Gap in Wide Anion Gap Metabolic Acidosis
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Department of Medicine, University of Hawaii, Honolulu, HI.

Background: Serum osmolar gap is useful to screen for toxic alcohol ingestion in wide anion gap metabolic acidosis (WAGMA). However, often times there is limited history and delay before blood toxic alcohol levels are available. Serum acetone is one of the overlooked
causes of a serum osmolar gap. While awaiting for serum alcohol levels and considering syndromes of toxic alcohol ingestion, checking a serum acetone level may avoid costly and invasive treatments such as fomepizole and hemodialysis.

**Results:** A 45-year-old male with a history of alcohol abuse and poor oral intake presented with chest pain and nausea. He drank 4 glasses of wine the previous night. There were no visual symptoms. His laboratory data, below, showed a WAGMA with an elevated anion gap of 22 mmol/l and lactic acid of 8.8 mg/dL. Measured and calculated serum osmolality were 301 and 278 mOsm/kg, respectively; resulting in an osmolar gap of 23 mOsm/kg. His alcohol level was negative. Urine sediment showed no crystals. Initially, toxic alcohol ingestion was considered, so was treated with fomepizole and hemodialysis. However, serum acetone returned elevated at 100 mg/dl, likely explaining of his osmolar gap. Patient responded well to supportive treatment, both anion and osmolar gaps closed with the normalization of his acetone level; and, one week later, both serum methanol and ethylene glycol levels returned negative.

**Conclusions:** WAGM and serum osmolar gap in our patient could be the result of high serum acetone levels (likely from starvation ketoacidosis). Alcohol was not a contributor for his anion gap, nor was lactic acid - as it is dissociated at physiologic pH. The below formula calculates acetone’s contribution towards the patient’s osmolality:

\[ \text{SOGwAG} = 2 \times (Na + (1.15 \times \text{glucose/18}) + 154/2.8 + \text{ETOH} / 3.7 + \text{Acetone} / 58) \]

His recalculated osmolality is 297mOsm/kg, resulting in no significant osmolar gap. Therefore, serum acetone should be included in the calculation of serum osmolar gap in the clinical presentation is inconsistent with toxic alcohol ingestion.

**PUB364**

**Electrolyte Disorders Following Major Cardiac Surgery in Patients in the ICU**  
Vijay Lapani, A. Ahsan Ejaz.  
*Nephrology, Mount Sinai School of Medicine, New York, NY*  
*Nephrology, University of Florida, Gainesville*

**Background:** Electrolyte disorders are an important cause of various complications in the ICU. We studied the incidence of electrolyte abnormalities following major cardiac thoracic surgery (CTS) in patients with renal dysfunction (RD).

**Methods:** A retrospective review of patients post CTS from 2001 to 2006 with ICU stay of at least 5 days was performed. Serum levels of creatinine (mg/dL), potassium, chloride, calcium, magnesium and phosphate were collected pre-op and 5 consecutive postop days. For each electrolyte, patients with abnormal preop levels were excluded from analysis. RD was defined as SCR>1.4, patients with SCR<1.4 served as controls. The chi square test was used for statistical analysis.

**Results:** We included 836 patients; mean age 60 ± 16.7 years, mostly men (64%). Comorbidities included coronary artery disease (55.4%), hypotension (60%), diabetes mellitus (21.8%), CKD (20.1%), COPD (19.3%) and peripheral vascular disease (10.8%). Most surgeries were elective (65.2%). CABG (40.8%) and aortic surgeries (20.9%) were the commonest procedures. The mean length of stay in the ICU was 12.7 ± 16.1 days. Hyperkalemia (44.4% Vs 24.9%), hypercalcemia (14% Vs 6.8%), hypermagnesemia (26.9% Vs 14.8%) and hyperphosphatemia (52.5% Vs 29.1%) were significantly more common in RD (p<0.05). Hypophosphatemia (73.7% Vs 85.5%) and hypocalcemia (69.1% Vs 89.1%) were significantly more common in controls (p<0.05).

**Electrolyte abnormalities**

| & RD & Control |
|---|---|---|
| Hyponatremia | 190 (34.9%) | 34 (27.4%) |
| Hypernatremia | 193 (35.4%) | 54 (43.5%) |
| Hyponatremia* | 34 (5.6%) | 11 (7.2%) |
| Hypernatremia* | 152 (24.9%) | 88 (44.4%) |
| Hypercalcemia* | 33 (6.4%) | 21 (3.3%) |
| Hypocalcemia* | 451 (87.7%) | 90 (75.0%) |
| Hypophosphatemia* | 447 (81.9%) | 94 (91.5%) |
| Hyperkalemia* | 137 (6.8%) | 19 (14.0%) |
| Hypomagnesemia* | 13 (2.1%) | 1 (0.6%) |
| Hyperkalemia | 93 (14.9%) | 43 (29.6%) |
| Hypophosphatemia | 288 (45.7%) | 87 (57.1%) |
| Hypokalemia* | 99 (29.1%) | 62 (52.5%) |

* p<0.05

**Conclusions:** Electrolyte abnormalities are common in the first 5 days following CTS. Patients with RD prior to surgery are at a higher risk for post-op hyperkalemia, hypercalcemia, hypermagnesemia and hyperphosphatemia. Patients with SCR<1.4 were more likely to develop hypophosphatemia and hypocalcemia.

**PUB364**

**An Uncommon Case of Hyponatremia – A Case of Cerebral Salt Wasting**  
Reginald Ijeanyi Obi, Melanie I. Hames.  
*Nephrology and Hypertension, East Carolina University, Greenville, NC.*

**Background:** A 71-year old female with a history of Stage IIIB Non-small cell adenocarcinoma of the lungs admitted with mental status change and found to have a large solitary left parietooccipital mass consistent with brain metastasis. At presentation, laboratory studies included a serum sodium of 137mEq/L and a serum creatinine of 0.8 mg/dL.

**Methods:** She subsequently underwent a left parietooccipital craniotomy with total excision of the brain tumor. Five days post-surgery her sodium had decreased to 131mEq/L, her serum creatinine was 0.67 mg/dL and Na was 138 mEq/L. On POD 5 postoperative day her sodium had decreased further to 129 mEq/L with urine osmolality of 629 mOsm/kg, serum osmolality of 274 mOsm/kg, and urine sodium of 88 mEq/L. Nephrology was consulted. The syndrome of inappropriate antidiuretic hormone secretion (SIADH) was suspected and a trial of fluid restriction was implemented. The patient did not respond and had a further drop in serum sodium to 130mEq/L.

**Results:** Further evaluation revealed evidence of orthostatic hypotension. Cerebral salt wasting was then suspected and she was started on intravenous normal saline and oral salt tablets. Her urine osmolality improved from a high of 707 mOsm/kg down to 325 mOsm/kg and her serum sodium trended back up nicely to 135mEq/L upon hospital discharge.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

**Underline represents presenting author.**
Conclusions: Cerebral salt wasting (CSW) is defined as the renal loss of sodium during intravascular volume depletion and a decrease in extracellular fluid volume. It was first introduced as a concept in 1950 but lost ground to SIADH and only in recent years has it come back in favor as a distinct entity. This case illustrates the challenges of differentiating CSW from SIADH as a cause of hyponatremia. The major difference between them was that the patient, 19.7 years old, was deceased in this case, in contrast to a patient, 19.8 years old, in whom the diagnosis was made pre mortem in 1969. This highlights the importance of retaining the diagnosis of CSW even in prolonged ventilation, the need for renal replacement therapy, and increased mortality. Clinical assessment of volume status is limited, however, bio-impedance analysis (BIA) may be a more accurate measure. The use of BIA to assess volume status and prognosis in septic ICU patients is unknown. We hypothesized that the change in BIA vector length (VL) is predictive of ventilator-free days in those with systemic inflammatory response syndrome (SIRS) due to infection.

Methods: A prospective observational study targeting 100 ICU patients. BIA will be measured on ICU admission days 2, 5, and 10. Patients will be followed up for 60 days. Secondary outcomes include mortality, acute kidney injury requiring dialysis, and length of stay in the ICU. A correlation between BIA with known measure of volume status including physical exam, central venous pressure, brain natriuretic peptide and chest radiographs will be completed.

Results: To date, 16 patients have been recruited & enrolment is ongoing. Initial data suggest patients are consistently volume overloaded at both days 2 and 5 (mean VL 188 ± 26 at day 2 and 174 ± 20 at day 5). A high correlation existed between edema scores and VL (r = -0.645, p = 0.007); CVP also correlated with VL (r = -0.237, p = 0.376) although this was not significant. Finally, fluid balance between days 2 & 5 correlated inversely with change in VL (r = -0.468, p=0.009).

Conclusion: Septic patients in the ICU are persistently volume overloaded at days 2 and 5 based on BIA measures. VL correlates with known measures of volume status adding to BIA's construct validity. This pilot study has proved to be feasible and may identify BIA as an easy bedside measure to assess ICU patients' volume status. At the current pace, by the fall we will have recruited 50 patients.

Funding: Private Foundation Support

PUB367

Unsung Hero or Unrecognized Villain: Correction of Hyponatremia with Potassium

Hongli Song1, John Kevin Hix,1,2 Jonathan W. Bress,1 Richard H. Sterns.1,2 1Dept. of Medicine, Rochester General Hospital, Rochester, NY; 2Nephrology Division, University of Rochester School of Medicine and Dentistry, NY.

Background: An 80 year old female on chlorothiazide and a selective serotonin reuptake inhibitor presented with one week of poor dietary intake and ten pound weight loss. On admission, blood pressure 143/76 mm Hg, SaO2 99% on room air, weight 45.8 kg. Examination was unremarkable except for lethargy. Her serum sodium (sNa) was 110 mEq/L, serum potassium 2.2 mEq/L. She was treated initially with 50 ml of 3% NaCl (25 mEq). Because of a 1.1 L water diuresis, sNa rose by 5 mEq/L, five times the increase predicted by formulas that ignore urine output. A toxicity balance analysis was performed to explain the sNa increase after 3% NaCl during the first 2.5 hours of therapy, using the following equation:

Predicted sNa =

sNa = Total Body Water × (Infused Na + Infused K) – 0.45 L*145.8 kg) – (27 mEq + 120 mEq) = 0.45 L*44.8 kg) + 0.172 L

Predicted sNa = 117 mEq/L (actual = 115 mEq/L)

After the 3% NaCl she was given subcutaneous DDAVP every 8 hours to prevent unintended over-correction of sNa (Am J Kidney Dis. 2010; 56:774-9). In addition she received 60 mEq KCl orally and 60 mEq KCl intravenously as a 400 millimolar solution, and 190 mL 0.9% NaCl (27 mEq). Twenty-four hours after admission her sNa was 121 mEq/L, an 11 mEq/L increase.

Methods: A toxicity balance analysis was performed to explain the additional 6 mEq/L increase in sNa that occurred in response to KCl and a small amount of 0.9% NaCl despite the prevention of excess water losses with DDAVP.

Results: Predicted sNa =

115 mEq/L *(0.45 L*44.8 kg) + (27 mEq + 120 mEq) = 0.45 L*44.8 kg) + 0.172 L

Predicted sNa = 121 mEq/L (actual = 121 mEq/L)

Conclusions: Administration of hypertonic potassium chloride can be substituted for hypertonic saline in the treatment of hyponatremic patients who are also hypokalemic. Because a reversible impairment in water excretion is common in such patients, concurrent administration of DDAVP with KCl is an attractive strategy.

PUB368

Hyponatremia, but Not Hypernatremia, Is Significantly Associated with Rhabdomyolysis in a Burn Population

Jan J. Stewart,1,2 Molly A. Tilley,3 Chris A. Gisler,1 James K. Aden,1 Evan Renz,2 Kevin Chung.1 Medicine, San Antonio Military Medical Center, San Antonio, TX; 2Medicine, University of Texas Health Science Center at San Antonio, TX; 3Burn Center, U. S. Army Institute of Surgical Research, San Antonio, TX.

Background: Both hyponatremia and hypernatremia have been associated with rhabdomyolysis, correlations based largely on case reports and small series. We sought to examine this relationship in a large population of burn patients, where it has never been reported.

Methods: All admissions to the burn center at our institution from January 2003 to December 2008 were examined. Patients less than 18 years old, those with end stage renal disease, without a measured creatinine kinase (CK) or serum sodium (SNa), or who died within 24 hours of admission were excluded from review. Independent variables included age, inhalation injury, percentage total body surface area burned (%TBSA), percentage of full thickness burns, Injury Severity Score (ISS), presence of electrical injury, hyponatremia (SNa less than 130) and hypernatremia (SNa more than 150). These variables were examined via a multiple logistic regression analysis against those with rhabdomyolysis, as defined by a maximum CK level > 5000 Units/L.

Results: In 530 subjects with a mean age of 44±19, average %TBSA of 28±24, and average ISS of 18±15, hyponatremia occurred in 24.0% (n=137) while hypernatremia occurred in 14.2% (n=75) during their admission. The prevalence on rhabdomyolysis was 17.2% (n=91). On multiple logistic regression, presence of electrical injury (OR 17.5, 95% CI 8.8-34.5, p <0.0001) and hyponatremia (OR 3.1, 95% CI 1.7-5.7, p=0.0003) had the greatest correlation with rhabdomyolysis. Hypernatremia (OR 1.1, CI 0.6-2.1, p=0.69) had no effect.

Conclusions: In the burn population, presence of electrical injury as well as hyponatremia, but not hypernatremia, is significantly associated with rhabdomyolysis. The results from this large retrospective review call into question prior assumptions of a relationship between hyponatremia and rhabdomyolysis, specifically in burn patients.

Funding: Other U.S. Government Support

PUB369

Severe Hypokalemia and Hypomagnesemia in a Patient Presenting with a History of Muscular Dystrophy

Heino R. Anto, Rong Rong, Gurjeet Singh Sandhu. Nephrology, St. John’s Episcopal Hospital, New York, NY.

Background: A 52 year old female from Trinidad presented with acute cholecystitis requiring a cholecystectomy. In Trinidad the patient was diagnosed to have muscular dystrophy since her 30’s. Another sibling was also diagnosed with the same muscular condition. On this admission serum potassium was 2.9 mEq/L, serum magnesium 0.8-1

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only;

Underline represents presenting author.

929A
High Glucose Modifies NHE Activity in hSGLT1 Transfected Cells

**Methods:**
- The cDNA of WT and S418H were cloned into the expression vector pEGFP-N1. All the recombinants were transfected into HEK-293 cells and the stable transfection was confirmed by Confocal microscopy. The presence of mRNA for a glucose sensor, G-Protein Receptor 1 (GPR1) was investigated by RT-PCR. The HEK-293 cells not-transfected (NT), transfected with WT or S418H grew to confluence in DMEM medium containing glucose 25 mM for 20 days. The dpHi/dt was analyzed by fluorescence microscopy using BCECF-AM fluorescent probe.

**Results:**
- The dpHi/dt decreased in WT and S418H when compared with NT cells [0.148±0.02 (n=5); 0.208±0.02 (n=6) and 0.432±0.02 (n=7), respectively, p<0.05]. The addition of H-89 (PKA inhibitor; 10-6M) did not prevent this effect but reduced it partially only in WT [WT: 0.28±0.02 (n=6) and S418H: 0.24±0.02 (n=7), p<0.05]. Western blot analysis showed that the chronic treatment increased the NHE1 expression only in WT cells [NT: 0.327±0.04 (n=3); WT: 0.807±0.050 (n=3); S418H: 0.29±0.03 (n=3), p<0.05].
- Our results indicate that despite having increased NHE1 expression, the dpHi/dt decreased in WT and S418H cells indicating that in NT cells another NHE isoform could be involved. GPR1 could activate PKA which phosphorylates SGLT1 and NHE1 and modulates differently the activity of these proteins, justifying the reduction on the inhibitory effect on dpHi/dt, observed only in WT.

**Financial Support:**
- FAPESP, CNPq and CAPES.

**Funding:**
- Government Support - Non-U.S.

**Results:**
- The dpHi/dt decreased in WT and S418H when compared with NT cells [0.148±0.02 (n=5); 0.208±0.02 (n=6) and 0.432±0.02 (n=7), respectively, p<0.05]. The addition of H-89 (PKA inhibitor; 10-6M) did not prevent this effect but reduced it partially only in WT [WT: 0.28±0.02 (n=6) and S418H: 0.24±0.02 (n=7), p<0.05]. Western blot analysis showed that the chronic treatment increased the NHE1 expression only in WT cells [NT: 0.327±0.04 (n=3); WT: 0.807±0.050 (n=3); S418H: 0.29±0.03 (n=3), p<0.05].
- Our results indicate that despite having increased NHE1 expression, the dpHi/dt decreased in WT and S418H cells indicating that in NT cells another NHE isoform could be involved. GPR1 could activate PKA which phosphorylates SGLT1 and NHE1 and modulates differently the activity of these proteins, justifying the reduction on the inhibitory effect on dpHi/dt, observed only in WT.

**Financial Support:**
- FAPESP, CNPq and CAPES.

**Funding:**
- Government Support - Non-U.S.
Results: A 93 year old female presented with a pneumonia and serum sodium (sNa) 115 mEq/L (normal range 135-145 mEq/L) 336 mmol/L (normal range 135-145 mmol/L) FEurate 18.1%. Her plasma renin was increased and aldosterone decreased on ACE inhibitor. She was treated with intravenous antibiotics and fluid restriction. By the fifth hospital day, sNa increased to 132 mmol/L, FEurate 11.3% and on the sixth day, sNa increased to 136 mmol/L and FEurate was now normal at 6.5%.

An 87 year old male with bronchogenic carcinoma was admitted after a fall due to a sNa of 112 mmol/L. The Uosm was 547 mosm/kg, UNa 90 mmol/L and FEurate 27.5% with low plasma renin and aldosterone. The treatment of isotonic saline, fluid restriction and sodium supplementation for probable RSW failed to correct sNa, briefly exhibiting desalinisation. Isotonic saline was switched to 1.5% hypertonic saline and sNa gradually increased from 131 to 138 mmol/L over two days as FEurate normalized from 27.5 to 8.5%. The normalization of FEurate after correction of hypokalemia in both cases is consistent with SIADH.

Conclusions: A normal FEurate after correction of hyponatremia is consistent with SIADH. This contrasts to a persistently increased FEurate in RSW. Correction of hyponatremia can be achieved gradually with hypertonic saline over several days. Determination of FEurate after correction of hypokalemia can be used to differentiate SIADH from RSW. Normalization of FEurate after correction of hypokalemia by hypertonic saline confirms our notion that saline has a meager effect on FEurate.

Conclusions: The diagnosis of pseudohypokalemia is a common finding in hospitalized patients and can lead to prolonged length of stay and increased mortality. The aim of the study was to assess causes of hypokalemia and the average time taken for repeat potassium (K) orders to be entered in electronic medical records.

Results: A total of 375 hypokalemic lab values in 202 patients were analyzed. 81% of these patients had a low K level, 27% were normal and 2% had high K levels. The average time between repeat potassium orders was 15.41 ± 11.4 hours, the longest being for patients on surgical services (21.5 ± 8.5 hrs vs 15.7 ± 10.2 hrs for patients on medical services, p < 0.01).

Discussion: No significant differences were found between the groups of patients except for those on surgical services, which had a longer time between repeat potassium orders. The average patient age was 60.24 ± 16.81 years and 68.8% of hypokalemic episodes were associated with severe hyponatremia. Women were more prone to hypokalemia than men despite less frequent diuretic usage. The electronic medical record can be a useful tool in the analysis of causes and management of hypokalemia.
in chronic tubulointerstitial damage to the kidney. Because pioglitazone protects against aging-related tubulointerstitial damage in SD rats, we investigated whether it also affects tubular senescence in DSS rats.

Methods: Six-week-old male DSS rats were assigned to either a group fed with 4% salt (high salt [HS] group; n = 10) or a group fed with 4% salt containing 0.005% pioglitazone (HS + pioglitazone [HP] group; n = 10). The rats were sacrificed after 12 weeks to examine kidney function, metabolic markers and kidney parameters such as histological changes, positive areas of SA-b-gal (cellular senescence marker) activity and Sirt1 mRNA expression.

Results: The serum protein levels and proteinuria did not significantly differ between the two groups. However, creatinine clearance and serum adiponectin levels significantly differed (HS vs. HSP: 23.4 ± 0.37 vs. 3.60 ± 0.52 mL/min·p, p < 0.001 and 2.32 ± 0.39 vs. 3.78 ± 0.32 mg/mL·p, p < 0.001, respectively). The tubule injury index and positive areas of SA-b-gal activity in tubular cells were significantly lower in the HSP group (8.53 ± 3.4 vs. 4.49 ± 3.3, p = 0.016 and 11.0 ± 4.1 vs. 4.48 ± 2.0%, p = 0.001), whereas Sirt1 mRNA expression in the kidney was significantly higher (1.0 ± 0.4 vs. 1.6 ± 0.4, p = 0.012).

Conclusions: Pioglitazone suppressed tubulointerstitial damage and tubular senescence in DSS rats, although it did not confer a benefit in terms of reducing blood pressure, urinary protein and blood glucose levels. Increased levels of serum adiponectin and of Sirt1 mRNA expression in the kidney might be involved in the anti-senescence mechanism of pioglitazone.

PUB379

Serum Protein and Podocin Expression Changes in Living Mouse Glomeruli under the Acute Hypertensive Condition

Zihong Li, Juan Wang, Fengxia Yu, Xiaohui Yin, Jun Wang, Hua Zhou, Lining Wang. Department of Nephrology, First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China.

Background: The purpose of this study is to visualize topographical serum protein and podocin changes in living mouse glomerular capillary loops (GCL) under various hemodynamic conditions by novel “in vivo cryotechnique”1-2.

Methods: “In vivo cryotechnique” group: The “in vivo cryotechnique” was performed on left kidneys of anesthetized C57BL/6 mice, as reported before3. Control group: the kidney tissues were fixed with the conventional perfusion fixation methods. Their serial sections were stained and observed by light, confocal laser scanning microscopy4,5 and immunoelectron microscope. The animals were also evaluated for renal podocin mRNA, and protein expression.

Results: By the “in vivo cryotechnique”, the distribution of serum proteins: albumin and immunoglobulinG (IgG; Ig kappa light chain and IgG1 heavy chain) were disorders and the immunoactivity of albumin or Ig kappa light chain was markedly increased and immunolocalized in apical areas of the foot processes and urinary space, not slit-diaphragm, but the podocin expression was seriously decreased under the acute hypertensive condition. The abnormal distribution of serum proteins and podocin was also found in the control group under the normotensive condition is similar to that under the acute hypertensive condition with “in vivo cryotechnique”.

Conclusions: These results suggest that the redistribution of serum proteins and podocin is the important factor about proteinuria under the acute hypertensive condition. The “in vivo cryotechnique”followed by freeze-substitution should be a reliable tool to observe the serum or podocyte foot process proteins in situ and capture transient images of functional glomerular capillary loops. The artifacts seemed to be due to immuno fixation and perfusion fixation procedure.

(also named Zmpste 24) was detected by western blotting. The activity of seneceence- associated α-galactosidase (SA-α-gal) was evaluated by SA-β-gal staining in HASMCs. The expression of prelamin A in the aecute aorta in rats was examined by immunohistochemical analysis using (1) Dahl salt-resistant normotensive rats (DN), (2) Dahl salt-resistant normotensive IS-administered rats (DN+IS), (3) Dahl salt-resistant hypertensive rats (DH), and (4) Dahl salt-resistant hypertensive IS-administered rats (DH+IS).

Results: IS treatment enhanced the mRNA expression of p53 and p21 in a time- and dose-dependent manner, whereas the mRNA expression of p16 and pRb showed no significant change. The IS-induced mRNA expressions of p53 and p21 in HASMCs were suppressed by the acetylsynthetic. IS upregulated prelamin A and downregulated FACC1 protein expression, and NAC suppressed these expressions in HASMCs. In aorta, DH+IS rats showed significantly increased expression of prelamin A in the cells embedded in the calcification area as compared with DH and DN rats.

Conclusions: IS accelerates cellular senescence through oxidative stress in HASMCs and the aorta of hypertensive rats. Thus, accumulation of IS in blood due to renal dysfunction may be one of the risk factors for vascular senescence in CKD patients.

Funding: Government Support - Non-U.S.

PUB384

Effects of Angiotensin II on VasoMotor Function of Pregnant Rats Rosemary E. Nkoko,1 Iasmina Craici,1 Steven Wagner,1 Livius V. d’Uscio,1 Joseph F. Grande,1 Zvonimir S. Katusic,2 Vesna D. Garovic,1 1Dept. of Nephrology, Mayo Clinic, Rochester, MN; 2Department of Nephrology, Mayo Clinic, Rochester, MN.

Background: The mechanism of resistance to the pressor effect of Angiotensin (Ang II) in pregnancy is not well understood.

Methods: Pregnant and non-pregnant Sprague Dawley rats underwent subcutaneous implantation of Ang II (0.96µg/kg/day) or saline (sham) via osmotic pumps on day 5 of gestation. Ang II was administered on day 19 of gestation to rat models. IS upregulated prelamin A and downregulated FACC1 protein expression, and NAC suppressed these expressions in HASMCs. In aorta, DH+IS rats showed significantly increased expression of prelamin A in the cells embedded in the calcification area as compared with DH and DN rats.

Results: The contractile effect of KCL (20 mM) was significantly potentiated in both non-pregnant and pregnant rats treated with Ang II. However, this Ang II-induced potentiation of contractions to KCL was significantly attenuated by pregnancy (P<0.01). Constrictions to an alpha-adrenergic agonist, phenylephrine (10^{-10}-10^{-6} M), were significantly increased in both Ang II treated pregnant and non-pregnant rats (maximal contraction: 78.7±7% and 71.4±4%, respectively; P=0.05 vs. sham: 52.5±5%; n=57). However, the sensitivity to potassium was significantly reduced in Ang II treated pregnant rats (n=2): 7.0±3±0.03; P<0.05 vs. Ang II treated non-pregnant rats: 7.4±4±0.10). We also examined the effect of pregnancy on endothelium-dependent and endothelium-independent relaxations. In the presence of acetylcholine (10^{-10}-10^{-6} M), endothelium-dependent relaxation did not differ between saline and Ang II treated non-pregnant rats (n=7). In contrast, in pregnant animals treated with Ang II, relaxations to acetylcholine were significantly enhanced as compared to sham animals: maximal relaxation: 99.1±% vs 92.3±% for saline treated pregnant rats, respectively; P=0.02; (n=7). Endothelium-independent relaxations to nitric oxide donor, DEA-NONOate (10^{-10}-10^{-6} M) were not significantly different among groups.

Conclusions: Pregnancy may modulate pressor effect of Ang II by: a) attenuating vasoconstrictor effects dependent on depolarization of smooth muscle cells and activation of alpha-adrenergic receptors, b) by enhancing endothelium-dependent vasodilation mediated by production and release of nitric oxide.

PUB385

Identifying Adolescents at Risk for High Blood Pressure Eleni Chelioti,1 Dimitrios Athanassopoulos,1 Ekaterini Garopoulou,1 Maria Sotiri,1 Theodora Fragon,1 Thanasis Georgiou,1 Maria Tsilivigou,1 Gabriel Papadakis,1 2Dept. of Nephrology and Renal Unit, General Hospital of Piraeus, Athens, Greece; 3Dept. of Pediatrics, General Hospital of Kalymnos, Kalymnos, Greece.

Background: The increased prevalence of childhood obesity and the strong relationship of blood pressure (BP) with the body weight indicate that high blood pressure (HBP) prevalence in adolescents could increase as well. Therefore accurate diagnosis in young people is problematic. Primary objective of the study is to estimate the prevalence of HBP in adolescents of a remote Greek island. Secondary objective is to reveal which factors are associated with HBP.

Methods: A cross sectional study was carried out. Eligible subjects were adolescents aged between 13 to 15 years from a high school of the island of Kalymnos. Somatometers and BP were measured at the school environment. HBP was defined according to simplified International Obesity Task Force.

Results: Participants were 215 adolescents (106 boys and 109 girls). 60 subjects were classified with hypertension (BP >140/90 mmHg and >120/80 mmHg in boys and girls, respectively). Significant differences between the two genders were the school sport activities (p=0.03), the mean weight (p=0.01), height (p=0.01), systolic and diastolic BP (p=0.001 and p=0.01). Statistically significant factors associated positively with HBP were the male gender and the overweight. The adjusted odds ratios of the best fitted model showed that the independent factors associated with HBP were still the male gender and the overweight. Female subjects had 83% lower odds in comparison with male subjects, taking into account other model terms. The odds of subjects with increased body weight were approximately 3 times higher compared to subjects with normal body status, adjusting for other model terms.

Conclusions: A great number of adolescents from the island of Kalymnos are in danger to develop hypertension in adulthood. Both screening of adolescents for HBP and recognition of risk factors for HBP could give us the opportunity for early prevention and intervention of hypertension.

Funding: Clinical Revenue Support

PUB386

Genetic Variants of the Renin Angiotensin System (RAS) and Blood Pressure Response to the RAS Blocking Drugs - A Systematic Review for Pharmacogenomics in the Renin Angiotensin System- Tadasu Konoshita, Third Department of Internal Medicine, Fukui University School of Medicine, Eiheiji, Fukui, Japan.

Background: The concept of “pharmacogenomics” promises to offer the ultimate in personalized medicine and the renin-angiotensin system (RAS) is one of the most plausible candidates for this approach since it is a regulator of blood pressure over decades, genetic variants of the RAS have been tested for association with blood pressure response, but the results have been inconsistent. The most fundamental concern is thought to be the statistical power. Therefore, we have tried to put together a new systematic review using a database search including only reports with adequate subjects number.

Methods: Studies were identified by a PubMed search with condition 1 ([“pharmacogenetics”[All Fields] OR “pharmacogenomics”[All Fields]] AND (“renin”[All Fields] OR “ACE”[All Fields] OR “angiotensinogen”[All Fields]) or condition 2 ([“polymorphism”[All Fields] OR “polymorphism”[All Fields]] AND (“blood pressure responder”[All Fields] OR “blood pressure responder”[All Fields]) AND (“angiotensin”[All Fields]).

Results: Condition 1 identified 136 studies, and condition 2 found 52. Practical clinical implications require detection of a difference in diastolic blood pressure (DBP) of almost 5 mm Hg. Thus, we calculated the necessary sample size, assuming a standard deviation for the DBP of 10 mm Hg with protection against type I error of 5% and 80% of power, and determined that a study required 200 subjects. We therefore adopted studies with about 200 or more subjects. Evaluated genes were ACE, AGT, AT1, AT2, renin (REN) and ACE2. The search identified 40 reports, 11 studies were excluded and put together to a new systematic review.

Conclusions: From the results, we were able to draw conclusions with nearly consistent findings that the conventional variants (ACE ID, AGT M235T, AT1 A1166C and AT2 variant) are not associated with antihypertensive effects by RAS blockade, at least by one individual SNP. By contrast, significant associations have been reported (by one report each) for AGT rs7079, AT1 haplotype, REN, and ACE2. For these variants, further evaluations and confirmation are anticipated.

PUB387

The Impact of Endothelin Receptor Antagonists on Predictors of Mortality in Patients with Heart Failure Natalia Maroz, Amir Kazory. Nephrology, Hypertension, and Renal Transplantation, University of Florida, Gainesville, FL.

Background: Elevated blood urea nitrogen (BUN) and decreased level of serum sodium have been consistently shown to predict adverse outcomes in patients with heart failure (HF). Endothelin-1 receptor antagonism (ERA) represent an emerging therapeutic option for HF but their impact on mortality is not yet well-known. This study was designed to explore the currently available data on the impact of these agents on predictors of mortality in HF patients.

Methods: Articles cited in Pub Med database from 1980 to 2010 using key words: “endothelin receptor antagonist” and “heart failure” were searched. Those clinical randomized controlled trials that exclusively included HF population were identified, and relevant articles were selected. The results of these studies were then reviewed and compared with regards to impact on levels of BUN and serum sodium.

Results: A total of 40 relevant articles were identified that used four different ERA agents (tezosanatan, erastanetan, bosantan, and darusentan.). Twelve randomized, placebo-controlled trials were selected to be included in this study. While 10 studies could not find any significant change in the levels of BUN and serum sodium prior to and after therapy, 2 studies did not report them.

Conclusions: Although there is a promising theoretical basis for the use of ERA in patients with HF, currently available data does not support any beneficial impact on predictor of mortality. Future large-sized trials are needed to further evaluate long-term impact of ERA and their potential impact on patient’s outcome.

PUB388

Resistant Hypertension and Vitamin D Deficiency in a Tertiary Care Clinic Alexis Payette, Jean-Philippe Lafrance, Michel Vallee. Department of Nephrology, HMR, University of Montreal, Montreal, QC, Canada.

Background: Vitamin D deficiency is thought to play a significant role in cardiovascular disease and hypertension. Several studies have been used to establish the link between vitamin D and hypertension, although the precise mechanisms underlying this association are incompletely understood. The goal of this study is to evaluate the possible association between vitamin D deficiency and resistant or difficult-to-control hypertension.

Methods: We conducted a retrospective study among patients referred for resistant or difficult-to-control hypertension in a tertiary center in Montreal. We collected the demographic, clinical and laboratory data of 49 patients via review of their medical records.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.
Background: Ambulatory blood pressure monitoring has traditionally been used to diagnose white coat hypertension and only recently has had a more widespread use. The loss of circadian blood pressure rhythm on ABPM correlates with the presence of obstructive sleep apnea and renal insufficiency and may be a prognostic marker. Our study aimed to determine the prevalence of the different types of hypertension in our clinic population, and if OBP is reliable in HTN diagnostics in patients with diabetes.

Methods: In 24 Caucasian diabetic patients we compared tonometric (OBP) to cuff-based (Takeda TM2421) BPs. Patients were seen twice—2 weeks. At visit 1, 15 minutes rest was followed by 3 Takeda BPs and 2 minutes continuous cuff-based BPO. At both visits AMBP was recorded with BPO.

Results: No significant difference in BP control was noted. 42% of subjects with HBPM had controlled BP compared to 45% of non HBPM subjects. No difference in education existed between the groups.

Conclusions: We did not observe a significant relationship between circulating levels of 25(OH) vitamin D and blood pressure levels in our population of patients with resistant or difficult-to-control hypertension. These results suggest that vitamin D deficiency is not a significant factor in the severity of resistant or difficult-to-control hypertension.

PUB390
Role of Ambulatory Blood Pressure Monitoring as a Predictor of Renal and Cardio-Vascular Endpoints Mihail Ion Soare, Dianne T. Sandy, Mauro Braun, Rute C. Paixao, Umbala Pasupala. Nephrology and Hypertension, Cleveland Clinic Florida, Weston, FL.

Background: Ambulatory blood pressure monitoring has been used to diagnose hypertension and cardiovascular risks better than office blood pressure (OBP). Limited information is available about the relationship between use of HBPM and blood pressure control in a population with Chronic Kidney Disease (CKD). We hypothesized that HBPM use in a CKD population would be associated with better blood pressure control as measured by OBP. We also wanted to establish the sample size of 112 subjects would be needed to show a higher rate of BP control in the HBPM group (one-sided difference) with a power of 80% and a p value of <0.05. A significant difference was defined as 20% or more subjects at higher rate of BP control in the HBPM group (yes or no) as well as other demographic and medical questions. BP control was determined by clinic BP at time of enrollment.

Results: Preliminary results follow enrollment of 70 subjects (44% male and 56% female). This included 26 HBPM and 44 non HBPM users from 2 Nephrology clinics at Cooper University Hospital. No significant difference in BP control was noted. 42% of subjects with HBPM had controlled BP compared to 45% of non HBPM subjects. No significant difference in mean age (65 years in both groups) or CKD stage between HBPM and non HBPM groups existed. Men were more likely to use HBPM (54%) then women (23%). 50% of Caucasians used HBPM compared to 37% of enrolled African Americans and 27% Hispanics. No difference in education existed between the groups.

Conclusions: A preliminary analysis of our study shows no clear difference between HBPM use or non-use in BP control in this population with CKD. The study has not reached target enrollment. Preliminary enrollment and more severe HTN in the HBPM group are potential explanations for a possible type 2 error. The study is ongoing. A difference in frequency of HBPM use between Caucasians, African-Americans, and Hispanics may exist.
Results: The equilibrium interaction studies all demonstrated that the interaction of albumin with the polyanion was dominated by size exclusion and that charge interactions were small and would not have a major influence on transglomerular transport. Sedimentation analysis of albumin transport in polyanion solutions with induced-charge separation at hydraulic conductivity levels equivalent to physiological GFRs again demonstrated that albumin transport is governed by size exclusion effects and that the electrical effects of induced charge separation were small if not negligible. Conclusions: The lack of significant charge selectivity and electrical effects clearly reflects the influence of the relatively high ionic strength and screening of charge interactions under physiological conditions. These results also support recent direct measurements of the glomerular sieving coefficient of albumin by 2-photon microscopy to be in the range of 0.01-0.03 as predicted by purely size selectivity transport studies.

Funding: Government Support - Non-U.S.

PUB396

Methods: Plasma elimination rates measured over 24h were determined for tritium-labeled Ficolls (radii range 3.5-8.5nm), dextrans (5.0-10.5nm), albumin and IgG in healthy control and nephrotic (induced by pyrouronic amnionucleouside (PA)) Sprague Dawley rats. Nephrotic state resulted in a 1000-fold increase in the urinary excretion of intact albumin and 500- fold increase in intact IgG. Tissue uptake was also measured. Results: Plasma elimination rate of albumin (t=15) (3.6nm radius) and IgG (t=6) (5.5nm radius) in control rats were coupled as their elimination rates were identical at 0.019±0.003 h^-1 independent of their size. Their elimination rate was far enhanced as compared to the elimination rates of inert transport markers of equivalent hydrodynamic radius; their elimination rate corresponded to the elimination of a 7.5nm radius Ficoc (n=5) and >10.5nm dextran (n=5). The renal centric mechanism of the enhanced plasma elimination was demonstrated in nephrotic states where the increase in the plasma elimination rate for albumin and IgG was equal to the increased quantities of material excreted in the urine. In nephrotic states plasma elimination was uncoupled and enhanced destroyed as the plasma elimination of both albumin and IgG was identical to Ficolls with radii of 3.6nm and 5.5nm respectively.

Conclusions: Given recent studies that the glomerular sieving coefficient of albumin is in the range of 0.01-0.03, the present studies indicate that the renal centric mechanism is the albumin retrieval pathway in proximal tubular cells and the retrieval pathway participates in retrieving filtered IgG.

Funding: Government Support - Non-U.S.
**PUB399**

**Arterial Stiffness Is an Independent Determinant of Compensatory Hyperfiltration after Kidney Donation**

Pierre Fesler, Jean Ribstein, Guilhem Daugas, Claude Bernard, Paris, France; 4 INSERM U773, Centre de Recherche de Lille, Villeneuve d'Ascq, France; 1 Willy Morelle, 2 Unité d'inflammation et d'immunologie 1, Centre Hospitalier Universitaire, Lille, France.

**Background:** After kidney donation, the remaining kidney tends to hyperfiltrate, thus limiting the initial loss of renal function. However, the potential determinants of this compensatory hyperfiltration (CHF) and the possible influence of arterial function are unknown.

**Methods:** In 26 normotensive healthy donors (51 ± 9 years [mean ± SD], 22 females), glomerular filtration rate (GFR) was measured by the clearance of continuously infused Technetium-DTPA and timed urine collections at baseline -i.e.- before donation and 1 year after donation. CHF was computed as post-donation GFR minus half of baseline GFR. Arterial function was assessed at baseline through carotid-femoral pulse wave velocity (PWV) and carotid augmentation index (AIx).

**Results:** After kidney donation, there were no significant changes in blood pressure (BP), but subjects became hypertensive. GFR decreased from 104 ± 17 mL/min/1.73 m² at baseline to 87 ± 17 mL/min/1.73 m² at 1 year post donation. In univariate analysis, CHF was inversely correlated to PWV (r² = 0.23, p = 0.012), but not mean BP or AIx. In multivariate analysis, CHF remained inversely correlated to PWV (p = 0.020), independent of baseline age and mean BP (model r² = 0.34, p = 0.002).

**Conclusions:** In healthy subjects, increased arterial stiffness seems to be associated with a limited magnitude of post-donation hyperfiltration. This could reflect an influence of arterial function on renal reserve, providing further insights into the relationship between macrocirculation and renal microcirculation.

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**PUB400**

**Evaluation of Vascular Function in Hemodialysis Patients: Small Artery Elasticity Index (SAE) Correlates with Pulse Wave Velocity (PWV) and Flow-Directed Dilatation (FMD)**

William D. Paulson, 2 John White, 1 David M. Pollock, 2 Jennifer S. Pollock, 2 Allison Dubner, 2 Gaston K. Kapuku, 2 Charlie Norwood VA Medical Center, Augusta, GA; 4 Georgia Health Sciences University, Augusta, GA.

**Background:** Cardiovascular disease is the most important cause of mortality in dialysis patients, and contributes to vascular access failure. Tests of vascular function are important in evaluating and treating these problems, but standard methods such as PWV and FMD may not be fast and easy enough for widespread use. Measurement of arterial elasticity may be limited, but its increased stiffness is believed to be a potential cause of interdialytic hypertension. This study evaluated the simple, rapid, and inexpensive measurement of arterial elasticity using the small artery elasticity index (SAE) as a potential cause of interdialytic hypertension.

**Methods:** Sixteen hemodialysis patients underwent measurement of arterial blood pressure, SAE (HDI/PulseWave System), carotid-femoral PWV (SphygmoCor System), and FMD by standard protocol. SAE is measured by a tonometer that is applied noninvasively to the skin over the radial artery. The HDI device performs pulse contour analysis of the radial artery waveform. We plan to study a total of 60 dialysis patients.

**Results:** Mean values were generally consistent with arterial dysfunction (high systolic and pulse pressures, low SAE, high PWV, low FMD):

<table>
<thead>
<tr>
<th>Systolic BP</th>
<th>Pulse Pressure</th>
<th>SAE</th>
<th>PWV</th>
<th>FMD</th>
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<tbody>
<tr>
<td>138.7 mmHg</td>
<td>60.8 mmHg</td>
<td>0.03 mmHg/m/sec x100</td>
<td>9.48 m/sec</td>
<td>53.1%</td>
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In simple regression analysis, SAE negatively correlated with systolic blood pressure (R² = 0.27, P = 0.038) and PWV (R² = 0.35, P = 0.027), but did not reach significance with FMD (R² = 0.10) in this small initial sample. In multiple regression analysis, SAE correlated with PWV, FMD, and mean arterial pressure (R² = 0.80, all P < 0.014), indicating that 80% of SAE variation was due to its relation with these 3 variables.

**Conclusions:** SAE closely correlated with standard measures of vascular function and independently correlated to PWV and FMD. Further study with a larger number of patients is needed to confirm these promising results.

**Funding:** Clinical Research Support
Conclusions: To conclude, abnormally O-glycosylated IgA1, soluble CD89-IgA and IgA antibodies in pNeph--FLAG-hHO1 transgenic rats with GEC targeted HO-1 overexpression revealed an improvement in the intracellular killing of HD-PMNs and RTR-PMNs against C. glabrata at MIC values. Drug-free controls were included. These data provide confirmation that caspofungin in addition to its antifungal activity can modulate the immune system and potentiate its effects, which may be relevant in the setting of an impaired phagocytic host defense. In summary, our study demonstrated that RTR-PMNs were more susceptible to caspofungin than HD-PMNs, supporting the hypothesis that caspofungin may be a potential therapeutic agent in patients with RTR and to confirm the drug interaction between CsA and EVL in rats with uninephrectomy. Further studies are needed to elucidate the mechanisms underlying these observations.

PUB405

Involvement of Anaphylatoxins C3a, C5a in Tubulointerstitial Fibrosis in Rats with Unilateral Urinary Obstruction Fang Liu, Ping Fu, Department of Nephrology, West China Hospital of Sichuan University, Chengdu, Sichuan Province, China.

Background: To investigate the role of anaphylatoxins C3a, C5a in tubulointerstitial fibrosis in rats with unilateral ureteral obstruction (UUO) and to clarify the possible protective effects of EVL.

Methods: In vivo study, the expressions of C3a, C5a and their receptors C3AR, C5AR were detected by immunohistochemistry staining, real time PCR and Western blot were used to detect the expressions of α-SMA, E-cad, C3AR, CD88, CTGF and TGF-β1.

Results: In vivo study, the expressions of C3a/C5a and C3a/C5a were detected in tubular epithelial cells 7 days after UUO established, especially in dilated tubules. In vitro study, after HK-2 cells were cultured with C3a and C5a for 72 hr, many cells had strong surface expression of E-cad, suggesting the forming of E-cad, and showed a slender shape and loss of microvilli on the cell surface. The expressions of α-SMA, E-cad, C3a, C5a, CD88, TGF-β1 and CTGF mRNA expressions induced by C3a and C5a were blocked by TGF-β1R antagonist (TGF-β1RA) (P < 0.05).

Conclusions: Anaphylatoxins C3a, C5a and their receptors were involved in the early pathophysiologic process of tubulointerstitial fibrosis in rats with UUO by inducing TGF via the up-regulations of C3AR and CD88 and the activation of TGF/CTGF signaling pathway.
Conclusions: EVL aggravates CsA-induced organ injury but pharmacologic interaction between EVL and CsA at the organ level that of SRL in vitro level. This finding provides a better understanding of difference of EVL and SRL in combined treatment with CsA.

Funding: Government Support - Non-U.S.

Conclusion: Plasma Induces Non-Muscle Myosin Type IIA-Mediated Changes in Neutrophil Morphology

Conclusions: Variable glycosylation level of total IgG was significantly lower than that of purified anti-MPO antibodies IgG (1.021±0.201 vs. 1.43±0.134, P=0.004, expressed by absorbance value-405nm). Variable glycosylation level of total IgG was significantly higher than that of purified anti-GBM antibodies IgG (1.034±0.340 vs. 0.73±0.333, P=0.007). The antigen binding level of non-SNA-binding IgG was significantly lower than that of SNA-binding IgG for anti-MPO antibodies (0.572±0.590 vs. 0.96±0.670, P=0.001) and anti-PR3 antibodies (0.362±0.330 vs. 0.560±0.531, P=0.003), while significantly higher for anti-GBM antibodies (1.301±0.594 vs. 1.172±0.583, P=0.044). SNA-binding fraction of anti-MPO and anti-PR3 antibodies-containing IgG could induce higher level of neutrophil respiratory burst than non-SNA-binding fraction. Variable region glycosylation levels were different among different antigen-specific IgG, and could influence the antigen-binding ability of antigen-specific IgG, which might be dependent on the type of target antigens.

Funding: Government Support - Non-U.S.

Conclusions: Novel transcription factors in regulation of M2 macrophage phenotype

Conclusions: Our data provide an insight into the cellular and molecular mechanisms of transfusion-related injury

Key Words: neutrophils, migration, MYH9, Nfx

Funding: Private Foundation Support

Conclusions: Activation Markers of Thrombogenesis and Endothelial Dysfunction in End Stage Renal Disease

Conclusions: Our data provide an insight into the cellular and molecular mechanisms of transfusion-related injury

Key Words: neutrophils, migration, MYH9, Nfx

Funding: Private Foundation Support

Conclusions: Mouse Glomerular Epithelial Cell Line with HO-1 Overexpression

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Funding: Private Foundation Support

Conclusions: Plasma Induces Non-Muscle Myosin Type IIA-Mediated Changes in Neutrophil Migration and Morphology during Phagocytosis

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Conclusions: Plasma Induces Non-Muscle Myosin Type IIA-Mediated Changes in Neutrophil Migration and Morphology during Phagocytosis

Key Words: neutrophils, migration, MYH9, Nfx

Funding: Private Foundation Support
87% FLAG+ in FACS assay. The homogenous GEC cells are capable to be replicated in limited passage, thus making them clone homogenous and preserved physiological characteristics, with transplating,-FRAG-hGHD1, constantly expressed.

Conclusions: This novel GEC cells, with hhO1 over expression will assist future mechanistic studies, supplemental to animal model, of the renoprotective role of HO-1 in GEC injury.

Funding: Other NIH Support - AHA-SDG

PUB413
Imaging Activation of Complement
Sarah De Freitas, Adam Badar, Richard Smith, James Clark, Greg Mullen, Steven H. Sacks.
MRC Centre for Transplantation and Imaging, King’s College London, London, United Kingdom.

Background: Ischaemia reperfusion injury (IRI) is an important result of cardiovascular disease as well as impacting on organ transplantation. Complement activation contributes to inflammation and the degree of injury in IRI.

We characterised the first in vitro radio pharmacological targeting of activated complement. The SPECT tracer, based on the N-terminal binding domain of endogenous complement receptor 2 (CR2) binds CR2 and can be a product of complement activation that remains bound to IRI tissue.

Preliminary imaging studies evaluating the radiotracer in the setting of IRI were done using a mouse myocaridal IRI model.

Methods: CR2 was radio labelled with [99mTc(CO)3]+ via the engineered C-terminal hexahistidine tag. Serum stability was assessed at 37°C in human and mouse serum and analysed using instant thin layer chromatography and gamma detection via radio TLC scanning. The tracer was then assessed using SPECT/CT system. Controls included sham operated mice, and injection of a radiolabelled inactive mutant of CR2 (K41E CR2). Myocardiac slices were sectioned for autoradiography and histology (H&E and C3d staining).

Results: After 8hrs serum stability was over 94%. Emission tomography revealed significant uptake in the hearts of mice (n=3) induced with MIRI compared with controls. Autoradiography of heart sections suggested that this was localised to the left ventricle. No activity was detected in control hearts. Histological staining confirmed that CR2 was present in areas of tissue damage on myocardiac sections, and absent in hearts of control mice.

Conclusions: Initial in vivo evaluation of the CR2-[99mTc(CO)3]+ SPECT tracer indicates that this has potential as an imaging ligand for delineating the footprint of complement activation in organs affected by IRI.

Adam Badar is joint first author.

PUB414
Repression of Heme Oxygenase(HO)-1 Expression in Glomerular Epithelial Cells (GEC) Maria Detsika, Pu Duann, Elias A. Lianos. 1 Medicine, RWJ Medical School, New Brunswick, NJ; 2Thorax Foundation, University of Athens, Medical School, Athens, Greece.

Background: Of the glomerular cells, the least capable of upregulating HO-1 expression in response to the natural HO-1 substrate/inducer, heme, or to various forms of injury are the GEC (J Lab Clin Med. 147, 150, 2006). This may be a disadvantage given the well-established cytoprotective effects of heme degradation products, it may also be justified because production of catalytically active Ferrous iron also occurs following given the well-established cytoprotective effects of heme degradation products, it may also be justified because production of catalytically active Ferrous iron also occurs following... 

Results: After 8hrs serum stability was over 94%. Emission tomography revealed significant uptake in the hearts of mice (n=3) induced with MIRI compared with controls. Autoradiography of heart sections suggested that this was localised to the left ventricle. No activity was detected in control hearts. Histological staining confirmed that CR2 was present in areas of tissue damage on myocardiac sections, and absent in hearts of control mice.

Conclusions: Initial in vivo evaluation of the CR2-[99mTc(CO)3]+ SPECT tracer indicates that this has potential as an imaging ligand for delineating the footprint of complement activation in organs affected by IRI.

Adam Badar is joint first author.

PUB415
TNF-α and Tunicamycin Interfered with Glycosylation of Nephrin Protein, Then Resulted in Endoplasmic Reticulum (ER) Stress
Shokichi Naito, Tomoko Okamoto, Togo Aoyama, Mariko Kamata, Chikako Okina, Yasuo Takeuchi, Kouji Kamata. Department of Internal Medicine, Kitasato University School of Medicine, sugamihara, Kanagawa, Japan.

Background: Accumulation of deglycosylated proteins in ER induces unfolded protein response. However, it is difficult to directly identify these deglycosylated proteins. We have successfully generated antibody against deglycosylated nephrin protein. (Naito et al; Clin Exp Nephrol, 2011, in press) In this study, we investigate ER stress by deglycosylated nephrin protein.

Methods: HEK293-NW cells producing nephrin protein were cultured with 0-10 µg/ml of tunicamycin, an N-linked glycosylation inhibitor, or 10-30 ng/ml of TNF-α for 2hrs. Cell survivals were evaluated by using Trypan blue dye exclusion test. Then, production of deglycosylated nephrin proteins and expression of GRP78 and calreticulin were analyzed using immunoprecipitation method and western blotting.

Results: Cell death in HEK293-NW cells increased in more than 1 µg/ml of tunicamycin, while did not increase in any dose of TNF-α. Tunicamycin with more than 0.05 µg/ml and TNF-α with more than 0.1 ng/ml induced deglycosylated nephrin proteins in HEK293-NW cells. Tunicamycin with more than 0.5 µg/ml increased GRP78 and calreticulin in the cells. TNF-α with more than 10 ng/ml increased GRP78 in the cells, and did not increase calreticulin in any dose.

Conclusions: Deglycosylated nephrin proteins induced unfolded protein response in the cells. TNF-α showed a different response compared with it in tunicamycin.

PUB416
Novel Method for Simultaneous Determination of P-cresylsulphate and P-cresylglucuronide: Clinical Data and Pathophysiological Implications Eva Schepers, Natalie Meert, Griet L.R.L. Glorieux, Nathalie Neirynck, Annemieke Dhondt, Raymond C. Vanholder. Internal Medicine, Nephrology, University Hospital Gent, Gent, Belgium.

Background: The uremic retention solutes p-cresylsulphate and p-cresylglucuronide, two conjugates of p-cresol, were never determined simultaneously. In the present paper an HPLC method was developed and used to quantify both compounds in parallel in an in vivo observational study and their in vitro effect was evaluated by flow cytometry.

Methods: P-cresylsulphate and p-cresylglucuronide were determined in serum. For the validation specificity, linearity, recovery, precision and the quantification limit were evaluated. In vitro, concentrations of both compounds were determined in 15 controls and 77 hemodialysis patients, as well as protein binding in the dialoyzed group and the reduction ratios during hemodialfiltration. In addition, in the in vitro effect of the solutes on leukocyte free radical production at measured concentrations was assessed.

Results: A fast and accurate HPLC method was developed to simultaneously quantify p-cresylsulphate and p-cresylglucuronide. Both conjugates are retained in uremia with a substantially higher total serum p-cresylsulphate in comparison to p-cresylglucuronide (31.4 ± 15.8 vs 7.3 ± 6.5 mg/L) but also a substantial difference in protein binding (92.4 ± 7.1 vs 78.6 ± 6.4 %). P-cresylglucuronide per se has no effect on leukocyte oxidative burst activity whereas in combination with p-cresylsulphate a synergistic activating effect was found.

Conclusions: Serum concentrations of p-cresylsulphate and p-cresylglucuronide are elevated in uremia. Both conjugates show a different protein binding, resulting in a different dialytic behavior. Biologically, both conjugates are synergistic in activating leukocytes.

PUB417
Preeclampsia, Hemopexin and Extracellular ATP. Pro-Inflammatory Activation by Hemopexin and Hemopexin-ATP Complexes Floor Spaans, Marijke M. Faas, Chwian Chiang, Theo Borghuis, Harry Van Goor, Winston W. Bakker. Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, Netherlands.

Introduction: Preeclampsia is a PE and (to a lesser extent) after Hx alone (P<0.05) as compared with saline stimulation. ATP alone was negative in this respect. In endothelial...
cells, ICAM-1 expression was upregulated after stimulation with either Hx or with Hx-ATP. Cells were also confluent with serum stimulation (p<0.05). Again ATP alone did not affect ICAM-1 expression.

Conclusions: Endothelial cells are activated by both Hx or Hx-ATP complexes equally, whereas monocytes are activated by Hx-ATP complexes and to a lesser extent by Hx. This may reflect the in vivo situation, i.e. enhanced pro-inflammatory stimulation in PE (due to inactivated Hx and enhanced plasma ATP), and moderate stimulation in healthy pregnancy associated with increased plasma Hx activity.

PUB418
Renal Lymphangiogenesis in Experimental Proteinuric Nephropathy Parallels Fibrosis-Development over Time Saleh Yazdani,1 Menno Hovingh,1 Maartje C.J. Slagman,1 Andrea B. Kramar,1 Klaas A. Sjollema,1 Gerjan Navis,2 Harry Van Goor,3 Jacob Van den Born.1 1Nephrology, University Medical Center; Groningen, Netherlands; 2Pathology, University Medical Center, Groningen, Netherlands; 3Microscopy Center, University Medical Center, Groningen, Netherlands.

Background: Renal lymphangiogenesis was reported in transplantation and in nephropathies with interstitial fibrosis, mostly cross-sectionally. Proteinuria (UP) is one important cause of progressive renal fibrosis. Whether UP in itself could trigger a renal lymphangiogenic response has not been established, moreover, the temporal relationship between development of fibrosis and lymphangiogenesis is unknown.

Methods: To evaluate the time course of lymph vessel (LV) formation related to UP and morphological damage we studied unilateral adriamycin nephropathy (1.5 mg/kg; left kidney clamped for 12 minutes), UP was measured and sacrificed at 6, 12, 18, 24, and 30 weeks after nephrosis induction, and kidneys were harvested (n=6 time point). LVs were quantified by tissue FAXIS/Image J using podoplanin/VEGF-R3 double staining. Myofibroblasts (α-SMA), collagen III, macrophages (ED1); lymphangiogenic factors: VEGF-C and -D and focal glomerulosclerosis were also quantified.

Results: After 6 weeks UP (100-200 mg/24h) was established however without influx of interstitial macrophages, myofibroblasts, collagen III deposition and LV formation. LV density gradually increased over time (18.6 ± 3.4 LV/mm² at 30 wks vs. 4.6 ± 1.5 at 6 wks; p<0.0001), especially in fibrotic regions. Increase in LV density was associated with macrophage and myofibroblast numbers, focal glomerulosclerosis and proteinuria (all p<0.05). Besides, increase in LV size was observed over time. VEGF-C was expressed by interstitial cells, VEGF-D by tubular epithelium. All parameters remained low in non-adriamycin exposed kidneys.

Conclusions: UP up to week 6 is not associated with renal lymphangiogenesis. However, subsequent chronic proteinuria induces lymphangiogenesis in temporal correlation with interstitial fibrosis, myofibroblasts and myofibroblasts and development of interstitial fibrosis. Whether modulation of lymphangiogenesis may modulate fibrosis development should be subject of future studies.

PUB419
The Prognostic Significance of Crescentic Lesions in IgA Nephropathy Abdulkareem Alsuwaida,1 Mohammed A. Al-Ghonaim,1 Hala M. Foury,2 and Saleh Yazdani.1 1Medicine, King Saud University, Riyadh, Saudi Arabia; 2Pathology, King Saud University, Riyadh, Saudi Arabia.

Background: Oxford classification of IgA Nephropathy has been developed to predict renal outcome. However, this classification system does not include crescentic lesions. The objectives is of this study is to assess the prognostic significance of crescentic lesion in predicting renal outcome.

Methods: A retrospective review of all biopsied cases of IgA nephropathy from May 1998 to May 2011 was undertaken done at the King Khalid University Hospital, Saudi Arabia. The renal biopsy routine slides were reexamined and clinical and laboratory parameters were also collected. Predictors of worsening of renal function (WRF), which was defined as 50% increment in baseline serum creatinine, were examined.

Results: Among 59 patients included in the study 17 (28.3%) had histological evidence of crescentic lesion. Baseline levels of serum creatinine, but not proteinuria, were significantly higher (p=0.01) in the patients with crescentic lesions, versus patients without crescent (310 umol/l vs. 137 umol/l, respectively). Worsening of renal function was observed in 11 patients (18.3%). The odd ratio of WRF among those with any crescent were significantly higher (p = 0.01) in the patients with crescentic, versus patients without crescent (6.08 ± 0.04 vs 5.67 ± 0.13, p = 0.02) and 1 day (6.01 ± 0.23 vs 5.57 ± 0.02, p = 0.00004) and to the latex test at 6 (5.95 ± 0.06 vs 5.68 ± 0.05, p = 0.01) than in artificial urine albumin. P. aeruginosa adherence was more to the latex test at 2 days (6.7 ± 0.03 vs 5.57 ± 0.01, P = 0.0003) in the artificial urine with THP versus the artificial urine alone.

Conclusions: TIP facilitates the binding of E. coli to both silicone and latex catheters, and the binding of P. aeruginosa to latex catheters. THP may play a role in the pathogenesis of CAUTI.

Funding: NIDDK Support, Veterans Administration Support

PUB421
Effect of Pioglitazone in Controlling Proteinuria and Renal Failure in Focal Segmental Glomerulosclerosis Induced by Adriamycin in Male Wistar Rats Pratik Das. Nephrology, Majlaja Ben Kidney Hospital, Kolkata, India.

Background: Focal Segmental Glomerulosclerosis (FSGS) results in impaired renal function and proteinuria. Peroxisome proliferator-activated receptor-gamma (PPAR-γ) modulate angiogenesis and PPAR-γ agonists have been shown to be protective in non-diabetic glomerulosclerosis. This study compares the effects of Pioglitazone and Losartan in controlling progressive renal damage in Oxurorin-induced FSGS.

Methods: Progressive renal damage was induced in I.V. Doxorubicin (5mg/kg) and baseline serum creatinine and 24-hour urinary protein were estimated. The animals were randomized into 4 treatment groups, each comprising of six animals after proteinuria development at Day-7. Oral treatment was distilled water (control-C), Losartan 9mg/kg/day(L), Pioglitazone 4mg/kg/day(P) or a combination of L and P(L+P), for 8 weeks. 24 hour urinary protein was estimated at 14-day intervals, serum creatinine was estimated at Day-63 and excisional renal biopsy obtained. The pathological changes were estimated using a semi-quantitative histological score.

Results: Proteinuria continued to progress in all groups but the degree of proteinuria varied. At Day-21, 35 and 49, and 63, L and P+L treated rats had significantly reduced in proteinuria.

Funding: Government Support - Non-U.S.

PUB420
Tamm Horsfall Protein Promotes Adherence of Uropathogenic Bacteria to Urinary Catheters James M. Bates, Hajamohideen S. Raffi, Satish Kumar. Medicine/Nephrology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

Background: Catheter associated urinary tract infection (CAUTI) is a common problem. Tamm-Horsfall Protein (THP) is urine’s most abundant protein and has been shown to bind to uropathogenic bacteria. We hypothesized that Tamm-Horsfall Protein (THP) may adsorb to the surface of urinary catheters and induce bacterial binding. In this study, we determined the effect of adherence of Escherichia coli and Pseudomonas aeruginosa (P. aeruginosa) to silicone and latex urinary catheters.

Methods: E. coli strain UTI89 and P. aeruginosa (ATCC 27314) were grown in tryptose broth and brain heart infusion broth, respectively for 24 hours at 37°C with 5% carbon dioxide-5% H2-Thymidine (64 Ci/mmol specific activity). The radio-labelled bacteria were washed in Dulbecco’s phosphate buffered saline (DPBS) and resuspended in artificial urine with or without THP. The bacterial solutions were incubated with 1-cm sections of the two types of catheter at 37°C. Three sections were removed from each tube at 1, 6 and 24 hours and washed with DPBS. Each segment was measured by scintillation counting. On days 2, 4 and 7, three sections were removed from each tube, and sonicated in DPBS to dislodge the bacteria from the catheters. The bacteria were quantitated by serial dilution and culture on agar. The data were expressed as the mean ± SE and analyzed with Student’s t-test.

Results: E. coli adherence (expressed as Log CFU/cm² catheter), in the artificial urine with THP, was greater to the silicone catheter at 1 hr (6.25 ± 0.17 vs 5.73 ± 0.01, P = 0.02), 6 hr (6.08 ± 0.04 vs 5.67 ± 0.13, P = 0.02) and 1 day (6.01 ± 0.23 vs 5.57 ± 0.02, P = 0.00004) and to the latex catheter at 6 (5.95 ± 0.06 vs 5.68 ± 0.05, P = 0.01) than in artificial urine albumin. P. aeruginosa adherence was more to the latex catheter at 2 days (6.7 ± 0.03 vs 5.57 ± 0.01, P = 0.0003) in the artificial urine with THP versus the artificial urine alone.

Conclusions: TIP facilitates the binding of E. coli to both silicone and latex catheters, and the binding of P. aeruginosa to latex catheters. THP may play a role in the pathogenesis of CAUTI.

Funding: NIDDK Support, Veterans Administration Support

PUB422
Calcium Oxalate Crystal Deposition Is Associated with Production of More Urinary Osteopontin and Decreased Proteinuria in the Hyperoxaluric Rat Model Saeed R. Khan, Aslam Khan. Pathology, University of Florida, Gainesville, FL.

Background: Calcium oxalate (CaOx) crystal deposition in the kidneys is associated with renal injury. The cause of injury, oxalate (Ox) or CaOx crystals, has been argued. Since short term experimental hyperoxaluria does not produce CaOx crystal deposition in animals, we decided to test the effect of CaOx on adenohypophysis and renal damage in hyperoxaluric rats with and without renal CaOx deposits.

Methods: Male Sprague-Dawley rats were fed a diet containing 5% HLP for 28 days. 24 hour urine was collected on days 7, 14, 21 and 28. After that rats were sacrificed and their kidneys processed for various microscopic and molecular investigations. The rats were divided into 8 groups. All rats, with and without renal CaOx crystals on 28th day, significantly increased their urinary Ox by day 7. Those with crystals excreted more Ox. With time however, differences in urinary Ox between the two groups decreased. It was highly significant (p<0.001) on day 7, less significant (P<0.01) on day 14 and even lesser (p<0.05)
on day 21. No statistically significant was seen on day 28, the last day. Urinary citrate and calcium were not significantly affected by hypoxuria. Significant increases were seen in urine excretion of hydrogen peroxide (HP), kidney injury molecule-1 (KIM1), monocytic chemoattractant protein-1 (MCP1) and osteopontin (OPN) by rats with renal crystals than control rats and those without crystals. There was a highly significant increase in renal expression of ED2 and KIM1 as determined by densitometric analyses of western blots.

Conclusions: Increase in urinary excretion of MCP-1 starts as early as day 7 and only by rats with crystal deposits while HP and KIM-1 are also increased by day 7 but in both group of rats. Increase in urinary OPN is seen by day 14 in rats with crystals but by 28 days by both group of rats as evaluated by densitometric analyses of western blots.

Shoji Kagami. 1

Background: Angiotensin II (ang II) is a key mediator for development of glomerular hypertrophy and glomerulosclerosis. Glomerular endothelial cell (GEC) plays a crucial role in the initiation and progression of glomerulonephritis (GN). However, little is known about gene expression for ang II-induced GEC injury and its contribution to progression of GN.

Methods: Profiling gene expression of RNAs from immortalized human GECs stimulated by ang II (10⁻⁵M) for 0, 4, 12, and 24 hours was performed. Progressive GN was induced in rats by rage shots showed significant increase in OPN expression in ODN excretion. Results were presented here show that both high oxalate as well as CaOx crystals are injurious to the kidneys. There are, however subtle differences indicating the possibility that oxalate and CaOx crystals trigger different pathways.

Funding: NIDDK Support

PUB425

Lack of Flow in Dilated Renal Tubules in Murine Model of HIV-Associated Nephropathy

Date: 2021-06-14

Background: HIV-associated nephropathy (HIVAN) is the most common cause of end stage renal disease in HIV-infected patients. Histopathological findings in HIVAN include collapsing glomerulosclerosis and severe tubulointerstitial disease with microsclerotic dilatation of the renal tubules. Severity of tubulointerstitial disease is the best histologic predictor of progression to ESRD in most forms of chronic kidney disease. We therefore hypothesize that in kidney, tubular obstruction leads to increased intratubular pressure and dilatation resulting in decreased glomerular filtration and glomerular collapse. In these studies, we tested the hypothesis that dilated renal tubules in kidneys of HIV-transgenic mice, which develop a renal phenotype identical to HIVAN, do not have flow.

Methods: We injected HIV-transgenic mice and wild-type mice with 3kDa Texas Red-conjugated dextran which is freely filtered across the glomerulus and large, non-filterable, 500kDa fluoresceinated dextran. Kidneys were then analyzed for presence of fluorescent dextran in tubules, glomeruli and blood vessels. Tubules with preserved flow were determined by the presence of red fluorescence and tubules without red fluorescence were presumed to have no flow. Green fluorescence was used to identify patent blood vessels.

Results: High molecular weight dextran was detected within glomeruli and peritubular capillaries but not within tubules, confirming that it was not filtered by glomeruli. Low molecular weight dextran was detected in cells lining normal-appearing tubules in wild type and HIV-Tg mice but not in dilated tubules in HIV-Tg mice. The pattern of punctate sub-apical fluorescence suggests that the red dextran was localized to endocytic vesicles.

Conclusions: These results suggest that in HIVAN, as tubules become dilated, they lose flow. However, we have not ruled out the possibility that the lack of fluorescence in dilated tubules reflects impaired endocytosis in these cells. Further studies are needed to determine the role of loss of tubular patency in the progression of HIVAN and other chronic kidney diseases.

Funding: NIDDK Support

PUB426

In-Dwelling Urinary Catheter Reduces the Protective Effect of Tamm-Horsfall Protein Against Urinary Tract Infection

Date: 2021-06-14

Background: Tamm-Horsfall protein (THP) is critical to the host defense against urinary tract infection (UTI). However, little is known about the protective effect of THP against UTI in the presence of an indwelling urinary catheter.

Methods: Mouse urinary catheters were made from 2 cm. long segments of polyethylene tubing with a spiral portion at one end and a straight portion on the other end. The catheters were inserted into the bladder and the ends of the catheters were connected to a solenoid pump. The catheters were used for the study of UTI in vitro and in vivo.

Results: The results showed that the protective effect of THP against UTI was significantly reduced in the presence of an indwelling urinary catheter.

Conclusions: These findings suggest that the protective effect of THP against UTI is lost in the presence of an indwelling urinary catheter. This finding may have important implications for the treatment of UTI in patients with indwelling urinary catheters.

Funding: NIDDK Support, Veterans Administration Support

PUB427

Cardiorenal Syndrome Type I May Be Immunologically Mediated

Date: 2021-06-14

Background: Cardiorenal syndrome type 1 (CRS1) is characterized by acute worsening of cardiac function leading to AKI. CRS1 pathophysiology is complex and unclear.

An alteration of immune response has been postulated as a potential mechanism involved in CRS1, but has not been demonstrated. The aim of this pilot study was to demonstrate that plasma of pts with CRS1 was able to trigger a response in a mouse model of CRS1. In fact, monocytes which result in apoptosis. In fact, monocytes have a central role in initiation, development and outcome of the immune response.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PQ - Poster; PUB - Publication Only

Underline represents presenting author.

941A
Obesity Is Associated with Increase in Glomerular Size in Renal Transplant Recipients.
Sangeev Akking, 1 Vishal K. Varma, 1 Grace Chabala Chibesakunda, 1 Suman Setty 1 Medicine, University of Illinois at Chicago, IL. 2Pathology, University of Illinois at Chicago, IL.

Background: Obesity-related glomerulomegaly has been well described but little is known about the progression of this disease. We investigated renal allograft recipients with various body mass index (BMI) and serial protocol biopsies provide a unique opportunity to examine the progression of glomerulomegaly.

Methods: Renal transplant recipients in the first year after transplant with at least 2 protocol biopsies (K/2y; mean age 47±12y) were included. Recipient BMI at the time of transplant was noted and individuals were grouped as non-obese (BMI ≤30, n=14) or obese (BMI >30, n=23). A subset was categorized as normal (<25, n=8) or morbidly obese (BMI >35, n=15). Surface area of three to nineteen glomeruli per biopsy was measured by electron microscopy and image analysis. The mRNA expression of nephrin, podocin, CDA2P, podocalyxin and podoplanin in microdissected glomerular foot process were measured by electron microscopy and image analysis. The mRNA expression of nephrin, podocin, CD2AP, podocalyxin and podoplanin in microdissected glomerulus was determined by real time RT-PCR.

Conclusions: This is the first study to investigate whether aristolochic acid can damage glomerular podocytes and, if so, what are its manifestations?

Eighteen male SD rats were equally divided into the following 2 groups: model group in which the rats received the extract of Aristolochia manshuriensis Kom Am (15 mg/kg, i.p., n=9); control group received tap water only by gavage. 24hr urinary protein excretion was measured and urinary protein composition was analyzed with SDS-PAGE electrophoresis at the end of the 1st and 4th week, respectively. At the end of the 4th week, all the rats were sacrificed and their kidney tissue was collected for electron microscopic analysis and immunohistochemical staining. The least change of glomerular size and cellular morphology was observed in the control group. The mRNA expression of nephrin, podocin, CD2AP, podocalyxin and podoplanin in glomerulus was significantly down-regulated in the model group compared with the control group. Their mRNA expression was reduced 34%, 62%, 56%, 50% (P<0.01) and 27% (P<0.05), respectively.

Conclusions: Aristolochic acid can damage the glomerular podocytes, leading to decrease in the regulation of nephrin, podocin, CD2AP, podocalyxin and podoplanin mRNA expression in podocytes, the segmental effacement of foot process, and proteinuria.

Generation of Heme Oxygenase (HO-1) Deficient Rats Using Zinc Finger Nuclease (ZFN)-Mediated HO-1 Gene Disruption Pu Duan, Elias A. Lianos. Medicine, RWJ Medical School, New Brunswick, NJ.

Background: We have previously shown that, compared to renal tubules, the ability of rat glomeruli to upregulate the cytoprotective enzyme, HO-1, in response to natural H2O2 inures or injury is limited, the last capable cell being the terminal differentiated GEC (1 Lab Clin Med, 147[3], 150, 2006). Since the role of GEC in regulating glomerular apoptosis is critical, we investigated whether HO-1 expression in the obese vs. non-obese group, while as not striking, showed the same trend.

Funding: NIDDK Support

PUB430

Atypical Case of Thrombotic Thrombocytopenic Purpura Badria M. AlGaithi, 1 Nasreen H. Mohamed, 1 Christoph Lichti. 1Division of Nephrology, The Hospital for Sick Children; 2Pathology, Toronto General Hospital, Toronto, ON, Canada.

Background: Thrombotic thrombocytopenic purpura (TTP) is a rare thrombotic microangiopathy disorder (TMA), characterized by thrombocytopenia, microangiopathic haemolytic anemia, neurologic abnormalities, renal impairment, and fever. TTP is caused by deficiency of the von Willebrand factor cleaving protease (VWFPCP), ADAMTS-13, which cleaves the VWF multimers produced by endothelial cells. In the absence or abnormal ADAMTS-13, ultralarge VWF multimers occur, which bind platelets and promote clot formation. Different from the typical presentation of TTP, we report a case with ADAMTS-13 deficiency who presented with malignant hypertension and progressive kidney failure.

Methods: Retrospective review of the medical record.

Results: A 15 year old girl, presented with a hypertensive crisis (BP 190/110 mmHg) with features of chronic hypertension (i.e. exudative hypertensive retinopathy). Investigations revealed creatinine 191 umol/L, hemoglobin 94 g/L, platelets 113x10^9/L, and signs of hemolysis. Ultrasound demonstrated normal kidneys with no renal artery stenosis. Our impression was atypical hemolytic urmic syndrome (aHUS) and/or TTP. Renal function rapidly deteriorated. Thus, a renal biopsy was performed demonstrating TMA a diagnosis confirmed via repeat biopsy 6 months later. Hence, the patient was commenced on plasmapheresis (TPE) and maintained on intermittent hemodialysis (IHD). While complement work up was normal, ADAMTS-13 activity level was <5% with no mutations or antibodies detected. Recovery of renal function was noted at 6 months; therefore, IHD and TPE were slowly weaned off. Three months later, the patient had a mild disease recurrence coinciding with severe hypertension which was controlled by 6 sessions of plasma infusions.

Conclusions: This case is relevant as a hypertensive crisis was the first manifestation of TTP in this female adolescent. Furthermore, ADAMTS-13 activity was only initially decreased but returned to normal upon TPE and remained normal even at the first disease recurrence. While the patient is currently stable off hemodialysis further TTP crises have to be anticipated – a scenario for which we may consider rituximab treatment.
Ho-1 in nephron physiology. Apoptosis and its possible mechanism as well as to examine whether candesartan has direct effects on the TLR4 expression on MC. However, evidence for the role of TLR4 in the Ang II immune events and present in the kidney. It is reported recently that Ang II contributes significantly, and causes intercellular ROS accumulation, and subsequently apoptosis on MCs. Candesartan inhibits the above effects dramatically.

Ang II upregulates TLR4/MyD88 mRNA expression as well as TLR4 protein synthesis in a reaction. TLR4 protein was evaluated by western blotting. MyD88 mRNA expression were determined by reverse transcription-polymerase chain reaction previously reported in HO-1 knock-out mouse (PNAS, 94[20], 10919, 1997; J Clin Invest. 103[8], R23, 1999).

Conclusions: The HO-1 deficient rat can reinforce our understanding on the role of HO-1 in nephron physiology.

Funding: Other NIH Support - AHA-SDG

Pub433

Angiotensin II Induces Mesangial Cell Apoptosis Via TLR4/MyD88 Pathway Jinlei Lv,1 Hong-Bo Xiao,1 Qinkai Chen,1 Guohua Ding.2 1Division of Nephrology, First Affiliated Hospital of NanChang University, NanChang, JiangXi Province, China; 2Division of Nephrology, Renmin Hospital of Wuhan University, WuHan, HuBei Province, China.

Background: Angiotensin II (Ang II) could induce mesangial cell (MC) apoptosis both in vivo and in vitro, but the precise mechanisms are far from well understood. Toll-like receptors (TLRs) have been identified to be functionally receptors for response to innate immune events and present in the kidney. It is reported recently that Ang II contributes to the TLR4 expression on MC. However, evidence for the role of TLR4 in the Ang II mediated MCs apoptosis and the direct effect of candesartan on TLR4 expression in MCs is paucity. The aim of this study is to investigate the involvement of TLR4 and its proximal adaptor myd88 in Ang II-induced MC apoptosis and its possible mechanism as well as to examine whether candesartan has direct effects through this pathway.

Methods: MC was cultured in DMEM medium and treated with candesartan and/or Ang II. Apoptosis was determined by Hoechst staining and flow cytometry with annexinV FITC and propidium iodide. The intracellular formation of reactive oxygen species (ROS) was detected by confocal microscopy with fluorescent probe CM-H2DCFDA. TLR4 and MyD88 mRNA expression were determined by reverse transcription-polymerase chain reaction. TLR4 protein was evaluated by western blotting.

Results: Ang II induces MC oxidative stress and apoptosis in a time-dependent manner. Ang II upregulates TLR4/MyD88 mRNA expression as well as TLR4 protein synthesis significantly, and causes intercellular ROS accumulation, and subsequently apoptosis on MCs. Candesartan inhibits the above effects dramatically.

Conclusions: These results support our hypothesis firstly that TLR4/MyD88 pathway is involved in the process of Ang II-induced apoptosis through the up-regulation of intracellular ROS formation, candesartan suppresses MC apoptosis via a direct action that depends on this pathway.

Funding: Government Support - Non-U.S.

Pub434

Localization of the G-Protein-Coupled Receptor 40 (GPR40) in the Kidney Seong Kwon Ma,1,2 Jianchuan Chen,1 Raymond C. Harris,1 Jian-Kang Chen.1 1Medicine, Vanderbilt University, Nashville, TN; 2Internal Medicine, Chonnam National University School of Medicine, Gwangju, Korea.

Background: GPR40 is a seven transmembrane G protein coupled receptor that is activated by long chain fatty acids. Although GPR40 has been shown to play a role in insulin secretion in pancreatic islets, its role in other organs, including the kidney, is still undetermined. As a first step in understanding potential roles in the kidney, our initial studies were directed at determining the expression of GPR40 in the kidney.

Methods: Expression of GPR40 was determined in male BalB/c mice by semi-quantitative RT-PCR, immunoblotting, and immunohistochemical and double- or triple-labeling immunofluorescent staining with nephron-segment specific markers

Results: Initial experiments detected GPR40 mRNA expression in the kidney. Immunoblotting analysis was used to screen for an antibody specifically recognizing for a single band of GPR40 in the homogenates of renal cortex/outer stripe of the outer medulla (OSOM). Immunohistochemical staining with the identified specific GPR40 antibody revealed the highest labeling of GPR40 in the majority of renal tubules in OSOM as well as in a subset of cortical tubules. Double- or triple-labeling immunofluorescent staining confirmed that in the cortex, GPR40 was expressed in the THP-, DBA-, and AQP2-positive tubules, with minimal expression in the LTA-positive tubules. However, in the medulla, GPR40 staining was the highest in the LTA-positive tubules of the OSOM.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents presenting author.

Pub435

HIV-Induced Reduction of Regulation of Vitamin D Receptor (VDR) Increases Activation of Renin Angiotensin System in Tubular Cells Divya Salhan, Shabina Rehman, Ashwani Malhotra, Mohammad Husain, Pravin C. Singhal.

Medicine, North Shore LIJ Hofstra Medical School, Great Neck, NY.

Background: Renin angiotensin system (RAS) has been demonstrated to play an important role in the development of HIV-associated nephropathy (HIVAN). Vitamin D receptor (VDR) has been reported to be a negative regulator of renin transcription. Since the activation of the RAS plays an important role in the progression of HIVAN, we hypothesized that HIV infection may be activating renal cell RAS by downregulating renal cell VDR expression.

Methods: To have a model of tubular cell HIV infection, mouse tubular cells (MCT) were transduced with either vector only or HIV (NL4-3) constructs. Vector/MCTs and HIV/MCTs were incubated in serum-free media (SFM) for 24 hours and then followed by protein and RNA extraction. Immuno blotting and real time PCR studies were conducted for protein and mRNA expression for VDR, angiotensinogen (Agt) and renin. Ang II ELISA was carried out on culture medium prepared under similar conditions. To establish a causal relationship between VDR and the RAS, MCTs were silenced for VDR by transfection of siRNA-VDR and then evaluated for the RAS activation. To confirm relationship between VDR and the RAS, vector/MCCs and HIV/MCTs were treated with vitamin D2 analogue (calcitriol, 25 nM) for 24 hours and evaluated for VDR expression and the activation of the RAS as mentioned above.

Results: HIV/MCTs showed attenuated (P>0.05) expression of VDR when compared to control and vector/MCTs. HIV/MCTs also displayed 2-fold enhanced expression of renin. Moreover, HIV/MCTs showed 2.5 fold increase in Ang II (intracellular) when compared to vector/MCTs. Similarly, siRNA-VDR/MCTs displayed activation of the RAS. On the other hand, Vitamin D treated HIV/MCTs not only showed upregulation (P<0.01) of VDR but also displayed attenuated (P<0.01) expression of renin and reduction (P<0.01) in intracellular Ang II production.

Conclusions: These findings indicated HIV-induced VDR down regulation promotes RAS activation in tubular cells. The present study provides a mechanistic insight into the RAS activation in HIVAN patients.

Funding: NIDDK Support

Pub436

Calcineurin Correlates with Endoplasmic Reticulum (ER) Stress in Podocyte In Vivo and In Vitro Jianing Tao, Rongrong Hu, Xue-Wei Li, Hang Li, Xue-Wang Li. Renal Division, Peking Union Medical College Hospital, Beijing, China.

Background: Podocyte injury is an early phenomenon in diabetic nephropathy. Saturated free fatty acid palmitate was key in causing insulin resistance by inducing ER stress in pancreatic cells. Our previous in vitro work showed it can also induce podocyte apoptosis via ER stress. If ER stress with altered calcium homeostasis could activate calcineurin, which mediates dephosphorylation of synaptopodin and disrupts the stabilization of cytoskeleton in podocytes, was explored.

Methods: Six, nine, twelve-week-old male C57BLKs/j db/db mice (n=7) and age-matched db/+ control mice (n=5) were studied. Twenty-four hour urine protein output (24UP) were assayed. The intensity of Glucose-regulated protein 78(GRP78), calcineurin was co-localized with synaptopodin in renal samples by immunofluorescence(IF) stain.

Results: In podocyte treated with palmitate with or without ursodeoxycholic acid, the changes in protein and mRNA expressions of calcineurin were assayed by western blot and real-time PCR. Calcineurin and synaptopodin were co-localized by IF stain.

Conclusions: 24UP of all studied db/db mice were significantly higher than each age matched control (p<0.05), and it was significantly higher in 9w and 12w db/db mice compared with that of 6w db/+ control mice (p<0.05). Confocal microscopy showed steady increased expression of calcineurin and GRP78 in podocytes only in db/db mice with age increase. Palmitate significantly increased cultured murine podocytes calcineurin protein and mRNA expressions time-dependently when loading 0.5 mM (3h, 5h compared with 0h, p<0.05) and dose-dependently when loading 0.25-0.5 mM for 5h (compared with control, p<0.05). Addition of 10μM ursodeoxycholic acid inhibited calcineurin increase (compared with palmitate treatment group, p<0.05).

Conclusions: There has calcineurin activation at the onset of albuminuria in DN animal model. Calcineurin correlates with ER stress in podocytes induced by palmitate in vitro. The inhibitory effect of ursodeoxycholic acid suggests activation of calcineurin, podocyte injury and consequent proteinuria could be ameliorated by relief of ER stress.

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493A
Clinicopathological Features and Treatment Discussion on HBV-Associated Endocapillary Proliferative Glomerulonephritis

Jian Chen, Yinghao Yu. Dept. of Nephrology, Dong Fang Hospital, Fuzhou, Fujian, China.

Background: To discuss the clinicopathological features and treatment on HBV-associated endocapillary proliferative glomerulonephritis.

Methods: A total of 29387 biopsies were included from Jan. 2001 to Oct. 2009. 25 cases were diagnosed as HBV-associated endocapillary proliferative glomerulonephritis by renal biopsy. 10 cases of relatively comprehensive information have been done a retrospective analysis.

Results: 9 cases of acute nephritic syndrome, 4 cases of gross hematuria and 3 of hypertensive disease. In the 9 cases, 5 cases of Hypoproteinemia, 1 case of NS, 4 of oliguria, 3 of acute kidney injury(AKI), 1 of abnormal liver function. Diffuse endocapillary proliferative and neutrophilic leucocyte infiltration could be seen in the diseased glomeruli;Immunogoldenulin like IgG, IgM, IgA and C3, C4, C1q, Fb deposited in many parts, in which commonly the “horseshoe” phenomenon itself is full in the renal capsula. Urine of HBsAg deposition was improved without further need for RRT and remains stable at 3 years of follow up without evidence of MM. Patient’s AKI got worse requiring Renal Replacement Therapy (RRT). Simultaneous treatment with bortezomib and dexahemazine was initiated. Patient’s renal function subsequently improved without further need for RRT and remains stable at 3 years of follow up without evidence of MM.

Conclusions: 1. Both cases of renal limited MIDD demonstrate reasonable response in terms of renal parameters and future development of MM after treatment with bortezomib.

2. Given the favorable side effect profile, bortezomib may be used as a first line of treatment in such patients.

Funding: Clinical Research Support - Non-U.S.

PUB438

Association of Podocyteopathy and Proteinuria in Membranous Lupus Nephritis

Nirai B. Desai,1 David J. Cimbalku,2 William Whittier,1 Edmund J. Lewis.1

1Department of Pathology, University of Michigan Medical School, Ann Arbor, MI; 2Transplant Immunology, Medical City Dallas, Dallas, TX.

Background: In patients (pts) with lupus nephritis, a wide range of proteinuria exists irrespective of the presence of peripheral or mesangial immune aggregate deposition. Biopsies from pts with membranous lupus nephritis (MLN) often exhibit evidence of glomerular involvement overlapping multiple pathogenetic mechanisms. Podocytopathy in pts with MLN may represent an independent pathogenic process underlying the development of nephrotic range proteinuria.

Methods: We conducted a retrospective clinicopathologic study of pts with a histologic diagnosis of MLN (WHO Va and Vb) and proteinuria. The degree of immune aggregate deposition in the capillary walls and mesangium was detailed using immunoelectron microscopy (EM) and electron microscopy (EM). The degree of glomerular epithelial cell effacement and average foot process width (FPW) was detailed using EM. Baseline and follow-up (FM) and electron microscopy (EM). The degree of glomerular epithelial cell effacement was quantitated in EM. Five patients (pts) were biopsied at baseline, 12 were biopsied at follow up (FM).

Results: Nine patients had subnephrotic range proteinuria (<3 gms proteinuria/gm creatinine [g/g]) and eleven demonstrated nephrotic range proteinuria (>5 gms proteinuria/gm creatinine [g/g]). Mean creatinine was 1.63 +/- 0.83 g/dl (p=0.001) and foot process effacement (88.6 % +/- 11% vs. 48.3 % +/- 36.1%, p=0.002) and average FPW (1798 +/- 736 nm vs. 1000 +/- 333 nm, p=0.008) was greater in the nephrotic group compared to subnephrotic.

All biopsies from nephrotic pts demonstrated at least 75% foot process effacement. There were no other significant histopathologic differences between the groups. The nephrotic pts were younger (31.9 +/- 10.8 vs. 44.2 +/- 11.4 years, p=0.002) and demonstrated a shorter time from diagnosis of lupus to time of biopsy (28.6 +/- 26.2 vs. 106.7 +/- 62.8 months, p= 0.001).

Conclusions: The single distinguishing morphologic feature in pts with MLN and nephrotic range proteinuria was diffuse visceral epithelial cell foot process effacement. No association between immune aggregate burden and proteinuria was observed. We conclude that nephrotic range proteinuria in patients with MLN is likely a manifestation of concomitant podocytopathy.

PUB439

Monoclonal Immunoglobulin Deposition Disease without Evidence of Multiple Myeloma or Extra-Renal Involvement Treated with Bortezomib

Two Pilot Cases and Literature Review

Kumar Gaurav, Manish Gera, Raj Jeevan. Nephrology, Internal Medicine Nephrology Inc., Terre Haute, IN.

Background: Monoclonal Immunoglobulin Deposition Disease (MIDD) can present pathologically as two broad forms: AL/AH amyloidosis, which usually presents with both renal (nephrotic syndrome) and extrarenal (neuropathy, hyperviscosity) manifestations. Secondly, it may present in form of Light/Heavy Chain Deposition Disease (L/HCD) which may further manifest as AKI, CKD, tubulointerstitial or nephrotic syndrome and less commonly with extra renal features. Incidence of MM in this patient population is about 10-15%. Diagnosis of Incidence of MM in this patient population is about 10-15%. Diagnosis of MIDD in renal biopsy is challenging and can be diagnosed by inconspicuous deposits of monoclonal Ig or κ/λ paraprotein in Banff criteria. In this report we describe two such patients with MIDD which manifested as nephrotic syndrome and were treated with bortezomib.

Methods: In 2019, 2 of our patients were diagnosed with MIDD. Only case 1 presented with nephrotic syndrome. Both the patients had proteinuria with 15-20 g/day. Light Chain Deposition Disease was diagnosed in both patients and bortezomib was started. Both the patients were monitored at 4 monthly interval. Case 1 underwent renal biopsy which demonstrated deposits of monoclonal IgM kappa light chain and case 2 showed deposits of lambda light chain.

Results: Patient 1 was treated with bortezomib and dexamethasone. At 12 months follow up, proteinuria reduced from 20 g/day to less than 2 g/day. Patient 2 was treated with bortezomib alone. At 12 months follow up, proteinuria reduced from 15 g/day to 1 g/day.

Conclusions: This report describes 2 such cases with MIDD treated with bortezomib in a pilot study. Further studies are required to assess the long term outcome of MIDD with bortezomib.
patients with acute cellular rejection. Level of OPG was increased in all 8 patients with acute cellular rejection. The level of 2 chemokines IP-10 and OPG was increased in the one patient with BK Nephropathy.

Conclusions: Glomerular involution is a special pattern of glomerular injury (Kidney Int 2007;71:144). It is distinct from global glomerulosclerosis, and characterized by a reduction in size, the presence of vital podocytes, parietal epithelial cells and the notable absence of periglomerular fibrosis. Involved glomeruli were detected in children with frequently relapsing minimal change nephrotic syndrome (MCNS). The percentage ranged from 0-33% and depended on the interval between disease onset and renal biopsy. In the present study, we show that glomerular involution is present in renal biopsies of patients with several types of proteinuric disorders.

Methods: We identified 4 patients with proteinuria and biopsy reports that suggested the presence of glomerular involution. All slides were reviewed, and evaluated for the presence of involved glomeruli, based on the abovementioned morphologic criteria.

Results: Patient 1 is a child with a compound heterozygous NPHS1 mutation, with a spontaneous partial remission, and a 2nd biopsy during relapse. Patient 2 has a membranous nephropathy, with 2nd biopsy during relapse. Patient 3 has a micronodular nephropathy with FSGS. Patient 4 has a mesangiocapillary glomerulonephritis, with 2nd biopsy during relapse. The characteristics are in the table. The percentage involved glomeruli ranged from 5-30%. The identification of involved glomeruli in adults was hampered by the presence of globally sclerosed glomeruli of other types, with surrounding fibrosis.

Conclusions: Glomerular involution is not limited to children with frequently relapsing MCNS. Typically, involved glomeruli were seen in patients with longstanding, limited proteinuria. Additional studies are needed to determine if involution is a significant cause of nephron depletion in adult proteinuric patients.

ANCA-Associated Systemic Vasculitis Newly Onset in Maintenance Hemodialysis Patients Huijuan Mao, Changying Xing. Department of Nephrology, First Affiliated Hospital of Nanjing Medical University, Nanjing, Jiangsu Province, China.

Background: ANCA-associated systemic vasculitis (AASV), newly onset in uremic patients maintaining renal replacement therapy for several years, has not been reported before. Here we reported two cases in order to cause the clinical concerntration. Here we reported two cases in order to cause the clinical concerntration. The two cases, both of male, aged 76 and 65 years old respectively, were patients with renal replacement therapy for more than 3 years. Both cases showed severe proteinuria, hematuria, hypertension and pericardial effusion. Here we reported two cases in order to cause the clinical concerntration.

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Mesangial Hypercellular Infantile Nephrotic Syndrome Olivia Marchese, Ajla T. Vasti, Zhongxin Yu, Kevin Couloures, Dwayne D. Henry, Martin A. Turman. Pediatric Nephrology, Oklahoma University Health Sciences Center, Oklahoma City, OK.

Background: Infantile nephrotic syndrome (NS) is mainly caused by genetic mutations and has poor treatment response and prognosis. Idiopathic diffuse mesangial hypercellularity (DMMH) has a good response to treatment and prognosis. DMMH is seen in young children with NS, but has not been reported as cause of infantile NS.

Methods: A nine-month-old girl presented with gross hematuria, edema, hypertension, large proteinuria, decreased renal function and hypoalbuminemia. Tests for secondary (infectious and autoimmune causes, malignancy) and primary etiology were obtained. Renal biopsy was performed.

Results: Investigations for secondary NS were negative. Karyotype was 46 XX. Genetic tests for genes in PLCE1, LAMB2, WT1, NPHS1, and NPHS2 genes were negative for known disease-causing mutations. Histologic testing showed mesangial expansion primarily by increased mesangial cellularity, and mild mesangial sclerosis. Immunofluorescence was negative. Electron microscopy showed expanded mesangial areas by increased cell membrane thickness and extracellular accumulation of foot processes, podocyte hyper trophy and microvillar transformation of the podocyte cytoplasm. No immune type electron dense deposits were seen. She was treated with steroids and supportive treatments. Her renal function normalized and proteinuria improved over two weeks, with gross hematuria lasting 3 weeks. Steroids did not achieve full remission, and Tacrolimus was initiated. Full remission was then achieved with normal renal function, normal blood pressure and no recurrence during 5-month follow-up period.

Conclusions: We suggest that DMMH be included in differential diagnosis of infantile NS. It has good prognosis with good response to immunosuppressive treatment and therefore its recognition is important in order to start treatment. This is contrary to NS due to genetic mutations that comprises majorly of cases of NS in first year of life, is resistant to immunosuppressive treatment and treatment attempts are not recommended.
of non-drug induced severe LN with negative dsDNA antibodies. Our patient had no evidence of drug induced LN. On (hydralazine) LN have been described in the literature to have negative dsDNA and anti-Sm antibodies on serological screening. Our patient had no evidence of drug induced LN. On diagnosis of MCD, mesangial proliferative glomerulonephritis or FSGS.

Conclusions: Extra medullary haematopoiesis (EMH) in the kidney represents an interesting “speculative challenge” in terms of differential diagnosis in renal masses, tissue biopsy is nullifying. Furthermore the localization of hemopoietic tissue in the kidney, that is not a customary district, raises controversial questions: 1) Does the kidney possess a niche for haematopoietic stem cells (HSCs)? 2) Is the heterotopic haematopoietic tissue a true “station” of hemopoisis or just an aberrant tissue that must be cyto-reduced?

PUB447

Negative Double Stranded DNA and Anti-Smith Antibodies in Severe Lupus Nephritis

Gagangeet S. Sandhu,1 Anip Bansal,1 Aditi Ranade,2 James P. Jones.1 Nephrology, St. Luke’s - Roosevelt Hospital Center, Columbia University College of Physicians & Surgeons, New York, NY; 2Pathology, St. Luke’s - Roosevelt Hospital Center, Columbia University College of Physicians & Surgeons, New York, NY.

Background: In Systemic lupus erythematosus (SLE) auto-antibodies are generated against a variety of intracellular antigens. Anti-Smith (Sm) and anti-double stranded DNA (dsDNA) antibodies are particularly considered to be nephritogenic. In addition, severe lupus nephritis (LN) may also be a part of the process of anti-myeloperoxidase (MPO) antibody (p-ANCA) formation by promoting neutrophil degranulation. However, apart from mere seropositivity, ANCs may have a possible pathogenetic role in LN. We have considered the role of Anti-Myeloperoxidase (MPO) auto-antibody in renal tissue.

Methods: Review of PubMed literature for cases of lupus nephritis with negative dsDNA antibodies.

Results: Although extremely rare, a few subsets of patients with drug-induced (hydralazine) LN have been described in the literature to have negative dsDNA and anti Sm antibodies on serological screening. Our patient had no evidence of drug induced LN. On further review, and similar to our case, we found only 6 additional well documented cases of non-drug induced severe LN with negative dsDNA antibodies.

Conclusions: Although considered nephritogenic, dsDNA and anti-Sm antibodies may be negative even in patients with severe proliferative LN.

PUB448

Association of Glomerular Podocyteopathy and Systemic Lupus Erythematosus

Roberto Savio Silva Santos, Daniela Loss Mattedi, Liliany P. Repizo, Lecticia Jorge, Rui Toledo Barros, Viktoria Woronik. Department of Nephrology, University of Sao Paulo, SP, Brazil.

Background: The aim of this study was to evaluate the clinicopathologic features of patients with systemic lupus erythematosus and glomerular podocyteopathy.

Methods: We performed a retrospective study of 17 patients with SLE diagnosis according to the American Rheumatologic Association, proteinuria and a histologic biopsy was nullifying. Furthermore the localization of haemopoietic tissue in the kidney, that is not a customary district, raises contradictory questions: 1) Does the kidney possess a niche for haematopoietic stem cells (HSCs)? 2) Is the heterotopic haematopoietic tissue a true “station” of hemopoisis or just an aberrant tissue that must be cyto-reduced?

Conclusions: A total of 17 patients with SLE and glomerular podocyteopathy, that was characterized by nephrotic syndrome, good response to steroid therapy and seems to entail low risk of progression to ESRD. Funding: Government Support - Non-U.S.
Before the study, all the mice were divided into the sham-operation group, the UUO model group, and the treatment group. The body weight of all the mice in the three groups was measured daily. All the mice were sacrificed at the 7th day after the operation. The left kidney was collected and stored at −80°C for further examination.

### Methods

#### Wnt-7α Counteracts Epithelial to Mesenchymal Transition in Mice of Unilateral Ureteral Obstruction Model

**Model**

- **UO Model** group and treatment group: the body weight of mice was measured everyday. All mice were sacrificed at the 7th day after the operation. The left kidney was collected and stored at −80°C for further examination.

**Methods**

- Eighteen male C57BL/6 mice were randomly divided into three groups: sham-operation group, the UO model group, and treatment group, the body weight of mice was measured everyday. All mice were sacrificed at the 7th day after the operation. The left kidney was collected and stored at −80°C for further examination.

**Results**

- Compared with sham-operation group, the body weight of the model group was significantly lower (P<0.05). The relative area of interstitial fibrosis was significantly larger (P<0.05). Furthermore, the expressions of vimentin and α-SMA were significantly up-regulated (P<0.05), and E-cadherin were significantly down-regulated (P<0.05). Compared with model group, all the above-mentioned abnormalities were restored to some extent and showed significant difference (P<0.05) in treatment group.

**Conclusions**

- Wnt protein could decrease the interstitial fibrosis by counteracting epithelial to mesenchymal transition in UUO mice.

**Funding**

- Government Support - Non-U.S.
He received two cycles of therapy with velcade, dexamethasone, and dexamethasone, consolidation with cytotoxan, and an autologous stem cell transplantation. The patient’s hematuria and proteinuria resolved after the stem cell transplant. Subsequent SPECT, quantitative immunoglobulins, and bone marrow biopsy have shown no signs of recurrence of IgA myeloma.

**Patient Data**

<table>
<thead>
<tr>
<th>Bone Marrow Biopsy</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercellular normo (60-70%), 20% atypical plasma cells consistent with plasma cell myeloma</td>
<td>Normalcy normo (60-70%, 20% atypical plasma cells consistent with plasma cell myeloma)</td>
</tr>
<tr>
<td>IGA level</td>
<td>2040 (6-243)</td>
</tr>
<tr>
<td>Urine Analysis</td>
<td>Total protein 3397 (50-100), RBCs 4261 (0-4)</td>
</tr>
</tbody>
</table>

*normal values in parentheses*

The patient has had no more flares of HSP over the last three years.

**Conclusions:** Resolution of both HSP and nephropathy are documented after treatment of IgA myeloma with simultaneous reduction in IGA levels. The resolution of nephropathy with treatment of this myeloma argues for overproduction of the IGA subtype as the main pathogenic factor in this case.

**PUB455**

Lack of Awareness in Future Medical Professionals of Risk of Consuming Hidden Phosphate-Containing Diet

Yoshiko Shutto,1 Michiko Shimada, 1 Hideaki Yamabe,1 Mohammed S. Razzaque,2 1Nephrology, Hiroaki University School of Medicine, Hiroaki, Japan; 2Oral Medicine, Harvard School of Dental Medicine, Boston.

**Background:** Hyperphosphatemia is the single most important determinant of mortality in chronic kidney disease (CKD) patients receiving hemodialysis. CKD patients are advised to take low phosphate diet with phosphate lowering drugs. However, phosphate-containing ingredients are widely used as preservative in processed foods/soda drinks, and thereby can affect to total phosphate intake. As patients seek advice from medical professionals on phosphate-containing diet, we conducted a survey to determine the level of awareness in future medical professionals of diet containing artificial phosphate ingredients.

**Methods:** We randomly selected 190 medical and nursing students (average age: 21.6 years) at Hiroaki University School of Medicine in Japan and asked them to fill out a questionnaire.

**Results:** While 99% of students are aware of increased sugar-content in soda drinks, only 7% are aware of presence of phosphate (phosphoric acid) in such drinks. Similarly, only 12% of students are aware of presence of phosphate-containing ingredients in processed foods, including burgers/pizzas, etc. More importantly, 68% of the surveyed students are unaware of possible harmful effects of unrestricted consumption of phosphate-containing foods/drinks. Furthermore, 28% of surveyed students consume “fast food” at least once/week, while another 36% take such food once/month. However, after realizing long-term risks of consuming excessive phosphate, 41% of students want to reduce their phosphate-intake by minimizing consumption of fast foods/soda drinks, while another 48% showed interest in getting more information.

**Conclusions:** The survey highlights two important points: medical/nursing students, the future medical professionals, who will soon assume the role of patient management 1) are not thoroughly aware of the risk related to prolonged high-phosphate intake; 2) are not fully aware of the foods/drinks that contain hidden phosphate ingredients. This survey exposes the need for an education initiative to raise the awareness of risk posed by diet with hidden phosphate-containing ingredients.

**Funding:** NIDDK Support RO1-DK077276

**PUB456**

Interdialytic Weight Gain and Food Intake in Hemodialysis Patients, Users of a Private Clinic in Sao Paulo, Brazil Carmen B. Tzanno-Martins,12 Camila Machado de Barros,1 Bárbara Margareth Menardi Biavo,1 Jacqueline Santos,1 Elzo R. Junior,2 1Nephrology, Home Dialysis Center, São Paulo, SP; Brazil; 2Nephrology, Integrated Center of Nephrology, Guarulhos, São Paulo, Brazil.

**Background:** Individuals with chronic kidney disease (CKD) on dialysis treatment can present disorders of nutritional status and inadequate dietary intake. The purpose of this study was to evaluate interdialytic weight gain and food intake in hemodialysis patients, users of a private clinic in Sao Paulo.

**Methods:** This is a cross-sectional study which evaluated 97 patients. Clinical and socio-demographic data were collected, weight and height were measured and food intake was evaluated by three 24-hour-recalls. Study variables were: pre and post-dialysis weight, interdialytic weight gain, body mass index (BMI), energy intake, carbohydrates, proteins, lipids, sodium, phosphorus and potassium. It was also conducted qualitative analysis of the diet through the consumption of different food groups.

**Results:** According to BMI, the majority of elderly and adult had normal weight, but the elderly had a higher prevalence of malnutrition (25.0%) compared to younger patients (5.3%). The analysis of interdialytic weight gain showed that 48.5% had adequate weight gain (3% to 5% of “dry weight”), and only 15.5% of subjects had excessive weight gain. The intake of oils, sugar, beans and meat, fish and eggs proved to be excessive. Energy intake, as well as the intake of carbohydrates and lipids, presented below requirements values for most individuals. The consumption of protein and sodium was high for most individuals. Potassium intake was below the recommended values and phosphorus proved to be inadequate.

**Conclusions:** The interdialytic weight gain was adequate in most of the population studied but it was identified inadequate food consumption, characterized by an imbalance of intake of macro and micronutrients. Therefore, it is important for individuals with CKD the adoption of proper diet to maintain their health.

**PUB457**

Treatment of Late Antibody Mediated Rejection in Kidney Transplant Recipients: The Johns Hopkins Experience Bassam G. Abu Jameleh,1 Gaurav Gupta,1 Robert Avery Montgomery,2 Brandon L. Trolinger,1 Edward S. Kruai,1 Niraj Desai,1 Nada Alahakkar,1 1Division of Nephrology, Johns Hopkins University; 2Division of Transplant Surgery, Johns Hopkins University; 3Department of Pharmacy, Johns Hopkins University, Baltimore, MD.

**Background:** Several strategies for treating early antibody mediated rejection (AMR) have been investigated, however, evidence is sparse on the utilization and success of these therapies in late AMR. In this study, we present data from 13 patients who were treated for late AMR at our institution.

**Methods:** We collected data from 13 patients who developed biopsy-proven AMR after 6 months from the time of transplantation. AMR was defined as having at least two of the following pathologic changes: glomerulitis (g-score), peritubular capillary margination (ptc-score) and C4d staining (C4d-score). All patients had class I and/or class II donor specific antibodies (DSA) and were treated with plasmapheresis followed by low dose Cyogam +/- Rituximab +/- Bortezomib +/- high dose IVIg. 11 patients had at least one follow up alloraft biopsy. Parameters followed through and post-treatment were serum creatinine (SCr), estimated GFR (eGFR), DSA levels and histopathologic scores.

**Results:** The median follow up from the time of proven AMR was 4 months (range: 1 to 15 months). The mean rate of change in SCr was -0.07 mg/dl/month (range: -0.24 to +0.11 mg/dl/month). The mean change in eGFR since diagnosis of AMR was +0.55 ml/min/1.73m2 (range: -1.71 to +3.33 ml/min/1.73m2). DSA levels of 8 patients, all with class II, did not decrease with treatment; the other 5 patients responded partially, however continued to have low level DSA. Only 2 patients had reduction in their g-scores, 2 in their ptc-scores and 5 in their C4d-scores.

**Conclusions:** We infer that treatment of late AMR is not associated with significant improvement in SCr, eGFR, DSA levels or histopathologic scores.

**PUB458**

Nested Case Control Study with Follow up Survey for Polymavirus among Kidney Transplant Recipients Ahmed G. Adam,1 Nagwa Farouk,1 Mona Salem,2 Doaa Hashad,1 Hala S. Elwakil.1 1Internal Medicine - Nephrology, Dialysis & Transplantation Unit, Faculty of Medicine; 2Pathology, Faculty of Medicine; 3Clinical Pathology, Faculty of Medicine, University of Alexandria, Alexandria, Egypt.

**Background:** Polymavirus associated nephropathy is an increasingly recognized cause of graft dysfunction among kidney transplant recipients. It is related somehow to modern potent immunosuppression aimed at reducing acute rejection. Asymptomatic viremia and/or nephritis with or without worsening renal function may be present. Biopsy remains the gold standard for diagnosis. Untreated cases lead to allograft dysfunction or loss. There is no safe and effective antiviral therapy, so prevention based upon a screening and preemptive strategy is superior to an approach that relies upon therapy of established disease.

**Methods:** 74 transplant recipients in University of Alexandria, Egypt were included. Urine decoy cells, PCR for BKV and Biopsy were done. Positive cases of cytology or PCR are followed up within 1-3 months.
Results: 7+ve for decoy cells (10%), 3 viremia by PCR (4%), only one nephropathy (1.4%) presented with tubulo-interstitial nephritis with intranuclear inclusions of Polymoma JC virus rather than BK, CMV or Herpes. Cases with stable renal function and viremia with or without viremia cleared the virus spontaneously during the period of follow up without the need of intervention. A biopsy proven nephropathy and deteriorating graft function lost the graft and was suggestive of JC Nephropathy (the 11th reported so far) rather than BK.

Case II

Decoy cells | PCR | Renal Biopsy
---|---|---
1 | +/+ | ITN-Inclusion
2 | -/-/- | 500/50/0
3 | +/+ | 100
4 | +/+ | -/-
5 | +/+ | -/-
6 | +/+ | -/-
7 | +/+ | -/-
8 | +/+ | -/-
9 | +/+ | Infarctive 45 Rejection

Follow up of Decoy, PCR based on Therapy

Conclusions: The trend in quantitative rather than qualitative PCR for BKV is more important than single value especially when considering therapeutic plane. JC as a cause of nephropathy post transplantation do happen and must be considered as part of Polyoma related nephropathy; however more rare than BKV nephropathy, and possibly more aggressive. Decoy cell, viremia and viremia are neither synonymous nor interchangeable and all are necessary for proper management.

PUB459
Therapeutic Options for Recurrent Focal Segmental Glomerulosclerosis after Kidney Transplantation- The Need for Multicenter Trials

Vaqar Ahmed, Sarat C. Kuppachi, Beje S. Thomas, Anita Sultan, Maria F. Egidii, Nephrology, Medical University of South Carolina, Charleston, SC.

Background: No controlled trials have been performed to address the management of recurrent FSGS post KTX. Most studies reported single center experience with enrollment of few patients and discrepant results. Purpose of the study is to compare our management of recurrent FSGS with other experiences (not shown) and to address the need for collaborative approach.

Methods: Analysis of 7 KTX recipients with recurrent FSGS who received plasmapheresis (PP). Additionally 5 patients received 3 doses of rituximab (RTX) at 375 mg/meter square. Two living donor recipients received PP and RTX pre-KTX.

Results: One patient had complete remission while 3 had partial remission. Mean follow up 463 days. No graft losses or initiation of dialysis.

Table 1: Summarizes patients demographics, treatment of recurrent FSGS and outcomes in our institution.

<table>
<thead>
<tr>
<th>Induction post Tx</th>
<th>Maintenance IS</th>
<th>Serum at diagnosis of FSGS recurrence</th>
<th>Proteinuria [in grams] before PP</th>
<th>Proteinuria [in grams] after last PP</th>
<th>Duration of follow up (days)</th>
<th>Outcome</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymoglobulin</td>
<td>Prednisone, MMF, Tacrolimus</td>
<td>3.8</td>
<td>PP</td>
<td>9.4</td>
<td>2.4</td>
<td>240</td>
<td>Ser 2.8, PCR 5.71</td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>Prednisone, MMF, Simultus</td>
<td>4.3</td>
<td>PP and RTX</td>
<td>5.4</td>
<td>0.79</td>
<td>285</td>
<td>Ser 2.9, PCR 0.8</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Prednisone, MMF, Tacrolimus, Everolimus</td>
<td>4.9</td>
<td>PP and RTX</td>
<td>20</td>
<td>5.8</td>
<td>365</td>
<td>Ser 1.3, PCR 8.6</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Prednisone, MMF, Tacrolimus</td>
<td>1.8</td>
<td>PP and RTX</td>
<td>3.7</td>
<td>3.7</td>
<td>1380</td>
<td>Ser 2.1, PCR 4.8</td>
</tr>
<tr>
<td>Thymoglobulin</td>
<td>Prednisone, MMF, Everolimus</td>
<td>1.2</td>
<td>PP and RTX</td>
<td>7.73</td>
<td>3.8</td>
<td>150</td>
<td>Ser 1.4, PCR 4.6</td>
</tr>
<tr>
<td>Basiliximab</td>
<td>Prednisone, MMF, Tacrolimus</td>
<td>4.7</td>
<td>PP</td>
<td>4.8</td>
<td>6.7</td>
<td>90</td>
<td>Ser 2.1, PCR 4.7</td>
</tr>
<tr>
<td>Daclizumab</td>
<td>Prednisone, MMF, Tacrolimus</td>
<td>1.1</td>
<td>PP and RTX</td>
<td>7.4</td>
<td>4.4</td>
<td>730</td>
<td>Ser 2.1, PCR 4.1</td>
</tr>
</tbody>
</table>

RTX= Rituximab, PP= Plasmapheresis

Conclusions: PP was effective only in temporary reduction of proteinuria. Whether RTX had significant outcomes could not be established. Multicenter trials are warranted to assess optimal strategies for recurrent FSGS. As RTX seems to have a role in FSGS treatment, question should be arisen about its consideration for an early introduction or perhaps to be used as an induction agent in this patient population.

PUB460
Urinary Tract Infections in Renal Transplant Patients: The Emergence of ESBL

Mamdouh N. Albaqumi, Lutfi Alkorbi, Saad Alghamdi. KFSHRC, Riyadh, Saudi Arabia.

Background: UTI affects 5%-36% of kidney transplant patients. The typical organisms causing post-transplant UTI are the enteric gram negative bacilli and enterococci. However, there is an increased rate of ESBL infections among renal transplant patients which might carry a significant morbidity. We report our center experience with UTI and ESBL- UTI impact in renal transplantation.

Methods: We retrospectively reviewed electronic data and medical charts of 165 renal transplant patients followed up by nephrology team of KFSH & RC from 2003 until 2007

Results: A total of 165 patients with renal transplant were included in the analysis. 65 out of 165 (39%) patients develop UTI. Median time interval of developing UTI was 8 months. Females tend to develop more UTI as compared to males, 38(62.3%) vs. 23(37.7%) p<0.05. The episodes of acute rejection at 1 year were 11 (17.0%) in the UTI group as compared to 22 (20.0%) in the group without UTI with no statistical difference. There was no difference in graft and patient survival between the two groups at 1 and 2 years follow up. Both groups received similar immunosuppression. Among the UTI group, 9 patients (13.8%) were found to have ESBL positive UTI. Upon subgroup analysis of those developing ESBL, we found that the most frequent β-lactamase mediated resistant gram negative enteric bacilli was E. coli 14 (87.5%) followed by Klebsiella pneumonia 2 (12.5%). All patients in ESBL, group 9 (100%) had documented recurrent UTI as compared to 40 (71.4%) in the non ESBL UTI group. Univariate analysis of the risk factors for β-lactamase-mediated resistant gram negative bacilli infection, showed that living related donor, recurrent UTI and number of UTI (>3 episodes) were significantly associated with ESBL UTI.

Conclusions: We found that UTI has no significant impact on renal function, graft function, and patient survival in the first two years. In our study 13.8% developed one or more infections due to ESBL producing gram negative bacilli. We found that recurrent UTI and number of UTI (>3 episode) are the most important risk factors for development of ESBL UTI. Efforts should be made to prevent first episode of UTI in this population of patients which might have a positive impact on long term outcome.

PUB461
Transplant Biopsy for the Early Diagnosis of Graft Dysfunction Secondary to Haemolytic Uraemic Syndrome

Marsoor N. Ali, Sunil Bhandari. Renal Medicine, Hull and East Yorkshire Hospitals NHS Trust, Hull, East Yorkshire, United Kingdom.

Background: Haemolytic uraemic syndrome, either recurrent or de novo is uncommon post transplantation and is usually diagnosed from haematological changes including thrombocytopenia, anaemia and fragments on blood film. Confirmation with the transplant biopsy is helpful.

Methods: We highlight a series of three patients with acute transplant dysfunction which presented at clinic or haematological clues to the aetiology. Subsequent transplant biopsy revealed Haemolytic uraemic syndrome (HUS); two of which had HUS as primary renal disease and one had de novo HUS secondary to possible use of tacrolimus therapy.

Results: Early transplant biopsy prior to development of classical haematological features of low platelets and fragments should be undertaken to confirm the diagnosis. In this series biopsies were performed early prior to the fall in platelets and appearance of fragments.

Treatment, using plasma exchange and fresh frozen plasma in combination, intravenous immunoglobulin and switching immunosuppression, led to recovery of haematological parameters but salvage of only one graft function. Screening for complement and ADAMT13 mutations were negative.

De novo and recurrent HUS may occur post transplantation. Classical haematological changes of thrombocytopenia, anaemia (microangiopathic haemolytic anaemia) are a late phenomenon, occurring in only 50% of patients.

Conclusions: Transplant biopsy should be considered early to institute early therapy and recovery of haematological parameters and potentially transplant function thereby avoiding toxic therapy and complications.

PUB462
Cutaneous Calciniphylaxis in a Renal Allograft Recipient

Amarpal Brar, Carmencita Yudis, Nabil Sumrani, Fasika M. Tedla. SUNY Downstate Medical Center, Brooklyn, NY.

Background: Calcific uremic arteriopathy is a disease first described in patients on dialysis. It involves calcium deposition in the walls of small and medium sized arteries with consequent ischemic necrosis and gangrene.

Methods: We describe a case of cutaneous calciphylaxis in a patient with renal allograft transplant.

Results: A 52-year-old African American woman with end stage renal disease due to lupus nephritis who was on hemodialysis for 16 years received a deceased donor kidney transplant in 2002. She received thymoglobulin induction; and tacrolimus and prednisone for maintenance immunosuppression. Both pre and post transplant serum calcium and phosphorus values were normal.

Eighty years after transplantation, patient was admitted with skin lesions bilaterally on the medial aspect of her thighs. She then had 6 cm X 3cm lesion in left lower extremity transplanted in 2002. She received thymoglobulin induction; and tacrolimus and prednisone for maintenance immunosuppression, led to recovery of haematological parameters but salvage of only one graft function. Screening for complement and ADAMT13 mutations were negative.

Conclusions: Transplant biopsy should be considered early to institute early therapy and recovery of haematological parameters and potentially transplant function thereby avoiding toxic therapy and complications.

Wound swabs grew Pseudomonas aeruginosa. Intravenous antibiotics were started. A skin biopsy of the lesion confirmed the diagnosis of calciphylaxis.

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Later treatment included sodium thiosulfate 25 grams intravenously every other day and cinacalcet 60 mg twice a day.

She was lost to follow up and then returned four months later with worsened leg lesions with purulent discharge. Despite aggressive intervention, patient had multiple episodes of infection at the site of lesions and died due to complications of sepsis.

**Conclusions:** Calcific uremic arteriolopathy is a rare entity in patients with renal transplant, it is associated with high mortality and an evidence based strategy for management needs to be evaluated.

**PUB463**

**Risk Factors and Outcomes in Renal Transplant Recipients with Hyperuricemia**

**Amarpal Brar, Mary C. Mallappallil, Candace D. Grant, Faisika M. Tedia, Nabil Sumrani, Melissa Rampal, Moro O. Salifu.** SUNY Downstate Medical Center, Brooklyn, NY.

**Background:** Hyperuricemia is a common finding post-transplant. However risk factors for development of hyperuricemia and its impact on allograft function remain unclear.

**Methods:** To determine risk factors for our predominantly African American kidney transplant patients, we analyzed 208 renal transplant recipients in our center from 1998 to 2007, whose data set for serum uric acid was complete.

**Results:** During this period, immunosuppression consisted of antithymocyte globulin induction plus tacrolimus, mycophenolic acid and steroids maintenance immunosuppression. Only 7 patients received cyclosporine. Hyperuricemia was defined as uric acid level over 7 mg/dL. Patients were divided into 2 groups based on their uric acid levels; hyperuricemia (>7mg/dL, 61.5%) and normal (<7mg/dL, 38.5%). Mean age (48.8 ± 13.1 vs. 46.6 ± 14), black race (70.3% vs. 60%), BMI (25.9 ± 5.7 vs. 24.8 ± 4.9), HLA mismatch, tacrolimus levels, cholesterol, donor age, diabetes and race were not significantly different between the two groups. Male gender (63.3% vs. 36.3%, p=0.001) and DGF (18.9% vs. 7.6%, p=0.026) were significantly higher in hyperuricemic patients. Patients with hyperuricemia had significantly higher discharge creatinine (2.32 (2.31) vs. 1.51 (1.25), p<0.001), 6 weeks creatinine (1.95 (1.15) vs. 1.49 (0.91), p=0.22), 6 months creatinine (1.60 (1.30) vs. 1.00 (0.01) and 1 year creatinine (1.60 (1.03) vs. 1.20 (0.3), p<0.001) compared to recipients with uric acid level less than 7. However using logistic regression, we did not identify any independent predictors of hyperuricemia in this population. There was also no difference in acute rejection or overall graft survival between the two groups.

**Conclusions:** Demographics and clinical characteristics did not predict the development of hyperuricemia in this population. Hyperuricemia may be secondary to the high cell turnover in this patient population. This study may be limited by sample size and future studies are needed to elucidate the mechanism of hyperuricemia posttransplant.

**PUB464**

**Mycophenolate Mofetil Induced Duodenal Villous Atrophy in a Renal-Transplant Recipient and Its Effect on Tacrolimus and Lipid Levels**

Monique E. Cho

Transplant Recipient and Its Effect on Tacrolimus and Lipid Levels

**Methods:** A 28 year-old kidney transplant recipient, starting after 41 months of maintenance therapy with tacrolimus (FK) and MMF. Infectious etiologies were excluded and the anti-motility agents failed to improve his symptoms. He developed sharp increase in FK levels from 5 to 19 ng/mL, despite having his dose reduced. His LDL decreased from 79 to 16 mg/dL in a few days, his diarrhea improved and completely resolved within 4 weeks. His FK level, lipid profile and transaminases returned to baseline values within 8 weeks. Genotyping revealed that the patient is a homozygous variant for CYP3A5 6986A>G and ABCB1 1236C>T, 2677G>T/A, and 3435C>T, indicating that CYP3A4 is the enzyme primarily involved in drug bioavailability and metabolism. While few case reports have described duodenal villous atrophy with MMF use recently, it is important to consider the effect of intestinal barrier disruption on drug absorption and consequent renal and hepatic toxicities. Possible underlying mechanisms include reduced CYP3A4 expression/activity due to duodenal villi shortening, complex interplay between p-glycoprotein and CYP3A genotype and phenotype, and potential FK-statin interaction.

**Funding:** NIDDK Support

**PUB465**

**Clinical Usefulness of BK Virus Monitoring by Plasma BKV Quantitative PCR in Renal Transplant Recipients**

Byung Ha Chung, Yu Ah Hong, Hyun Gyu Kim, In O Sun, Bumsoon Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. Department of Internal Medicine, Seoul St. Mary’s Hospital, The Catholic University of Korea, Seoul, Korea.

**Background:** Because of the lack of effective therapy for BK virus associated nephropathy (BKVAN), early diagnosis of BKV replication is the basis for the prevention of BKVAN. Present study analyzed the clinical usefulness of BK viremia monitoring by plasma real time PCR to prevent the development of BKVAN.

**Methods:** First, we compared plasma PCR with urine decoy cell and urine BKV real time PCR (urine PCR) for the diagnosis of BKVAN in comparison. Second, we prospectively monitored plasma PCR at 1, 3, 6, 9, 12 months after kidney transplantation (KT). We analyzed the kinetics of BKV replication and investigated the effectiveness and safety of preemptive immune suppressant (IS) reduction in BKV viremia.

**Results:** From Oct. 2006 to Oct. 2008, the prevalence of BKVAN was 3.0 % in the study population (6 / 200). The sensitivity and negative predictive value for BKVAN by decay cell, urine and plasma BKV real time PCR was 100 % respectively. However, plasma BKV real time PCR was superior to urine real time PCR and urine decoy cell in specificity and positive predictive value. In the prospective monitoring of BKV real time PCR, BK viremia developed in 8.3 % (12 / 145) within 1 year after transplantation and the median interval from KT to the development of viremia was 163 days (29 – 685). After reduction of immune suppressant, viremia was successfully cleared out in 91.6 % (11 / 12) and it took 103 days (25 – 254). BKVAN developed in only one patient, who took intensive desensitization before KT because of high grade sensitization and ABO mismatch to donor. In comparison between patients with viremia and without viremia, allograft function and the frequency of acute rejection did not differ significantly up till post-transplant 1 year (P > 0.05, respectively).

**Conclusions:** Plasma PCR is reliable method for the diagnosis of presumptive BKVAN and regular monitoring of it is useful to prevent the development of BKVAN without the risk for acute rejection.

**PUB466**

**Sirolimus-Induced Granulomatous Lung Disease in a Kidney Transplant Recipient**

Abdelaziz A. Elsanjak, Rishi Raj, Kamonpun Ussavarungsi, Vineet Sood, Melvin E. Laski. Internal Medicine, Texas Tech University Health Sciences Center, Lubbock, TX.

**Background:** Pulmonary complications of sirolimus immunosuppression include pneumonitis, bronchoalveolitis obliterans, organizing pneumonia, and alveolar hemorrhage. We here present a case of granulomatous lung disease, a rare side effect of sirolimus treatment.

**Methods:** Clinical case report and literature review

**Results:** The patient is 54 year old Hispanic female s/p DDKT for ESRD due to ADPKD. The patient presented with dyspnea, dry cough, fatigue and low grade fever: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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for two weeks. Her initial regimen included mycophenolate mofetil 750 mg BID, and tacrolimus 3 mg daily, which had been switched to sirolimus 3 mg daily at the ICU because of post transplant DM. CXR showed bilateral infiltrates; WBC was normal. She was treated initially with cephalaxine, azithromycin, baricitinib, and dexamethasone, but five days later she nonetheless became hypoxic. Blood culture, sputum culture, and fungal and viral screening were negative. CT showed a bilateral lower lobe ground glass infiltrate. Bronchoscopy with bronchial lavage and transbronchial biopsy revealed granulomatous lung disease. Tissue was sent for cytology, AFB, PCP, and fungal and viral culture. No infectious cause was found. Videoadsorbed thorascopic and wedge lung biopsy again revealed granulomatous lung disease. Tissue for cytology, AFB, CMV, and PCP came back negative. Sirolimus induced granulomatous lung disease was suspected. Sirolimus was therefore discontinued and prednisone 60 mg daily was started. The patient’s symptoms subsequently rapidly resolved. A follow up spiral chest CT in two months showed complete resolution of lung infiltrates.

**Conclusions:** Sirolimus was the cause of the granulomatous lung disease in our patient. We report the case of a 27-year-old woman, with past history of one abortion, which underwent a kidney biopsy for end-stage renal failure.

**Funding:** Clinical Revenue Support

**PUB467**

De Novo Glomerulonephritis (GN) in Renal Transplants (Tx): Are We Spuriously Counting the Number of Cases of De Novo GN Post Transplant by Presuming Diabetic Nephropathy (DN) as the Cause of End Stage Renal Failure (ESRD)?

**Background:** De novo and recurrent GN are important causes of renal allograft failure that account for as many as 5-10% of allograft failures. Presumed DN as the cause for ESRD in patients with long standing uncontrolled diabetes mellitus is commonplace. Without a proper diagnosis, recurrent or de novo disease other than DN might either be under or over diagnosed in grafts.

**Objectives:** To determine whether the incidence of presumed DN as the cause of ESRD without a native kidney biopsy prior to renal transplant inadvertently exaggerates or underestimates the diagnosis of de novo GN cases in renal allografts.

**Methods:** We retrospectively reviewed the charts ofTx patients undergoing 225 renal allograft biopsies performed over a 12 year period at our center from January 1997 to December 2009. The review included determining the biopsy diagnosis of the failing renal allograft, the etiology of ESRD based upon Form CMS-2728-U3 and whether a native renal biopsy was performed to confirm the cause of ESRD. Presumed DN as the cause of allograft failure, the etiology of ESRD based upon Form CMS-2728-U3 and whether a native renal biopsy was performed to confirm the cause of ESRD. Recurrent DN is the most common form of recurrent GN.

**Results:** Among 225 post Tx biopsies reviewed, only 33.3% (n=75) had a native renal biopsy and 66.6% (n=150) had no prior renal biopsy. Of the 150 patients reviewed, 33.3% (n=50) had the diagnosis of presumed DN and were included in analysis. Only 6% (n=3) of those 50 patients had de novo GN diagnosed as the cause of allograft failure without features of DN, whereas 22% (n=11) had DN alone in their post biopsy specimen. The remaining 36 patients had other diagnoses including acute cellular(1%), antibody mediated(0.6%) rejection, chronic transplant glomerulopathy(0.8%), acute TIN(12%), and CNI toxicity(14%) along others.

**Conclusions:** De novo GN is an infrequent cause of renal allograft failure in patients presumed to have DN as the cause of ESRD. Recurrent DN is the most common form of recurrent disease in transplanted kidneys in our center. The diagnosis of DN as the presumed cause of ESRD does not significantly increase the number of de novo GN cases and thus we would not over estimate the immunosuppressive approach for these transplant recipients because of the risk for recurrent GN.

**PUB469**

Eculizumab in Acute Recurrence of Thrombotic Microangiopathy Associated with Anti-Phospholipid Antibodies after Renal Transplantation

**Carine Hadaya, Pierre-Yves F. Martin. Nephrology, University Hospital of Geneva, Switzerland.**

**Background:** Renal thrombotic microangiopathy (TMA) is a severe complication of systemic lupus erythematosus (SLE), associated with the presence of anti-phospholipid antibodies (aPL). In its most fulminant form, TMA leads to a rapid and irreversible end-stage renal failure. Eculizumab, an anti-C5 monoclonal antibody, is the therapy of choice for patients with paroxysmal nocturnal hemoglobinuria and a promising therapy to cure and prevent recurrences of atypical hemolytic uraemic syndrome (aHUS).

**Methods:** We report the case of a 27-year-old woman, with past history of one abortion, which underwent a kidney biopsy for end-stage renal failure.

**Results:** Severe TMA, complete glomerular scarring and diffuse tubulo-interstitial fibrosis were diagnosed. The presence of aPL antibodies (lupus anticoagulant, IgG anti-cardiolipin and IgG anti B2 glycoprotein type I), anti-nuclear and anti-nucleosome antibodies at high titer and a reduced level of C3 level was compatible with the diagnosis of fulminant TMA in a SLE patient in presence of aPL. No evidence of genetic or biological abnormalities, similar to those described in aHUS, were detectable. After 10 months of dialysis, the patient underwent living related kidney transplantation. Immunosuppressive therapy was based on rabbit-anti-thymocytes globulin induction, mycophenolate mofetil, methylprednisolone and rituximab. The graft produced urine immediately but as serum creatinine remained at 172µmol/L at day 6, a graft biopsy was performed. Isolated diffuse glomerular and arteriolar TMA, C4d negative, was detected. Despite daily plasma exchange, performed from day 7 to 10, the patient developed oligoanuria leading to the administration of weekly eculizumab perfusion under penicillin prophylaxis. Three months post transplant, serum creatinine is 100µmol/L without proteinuria, C3 level is within the normal range and aPL antibodies are undetectable. Graft biopsy revealed complete resolution of TMA without sequel.

**Conclusions:** This case report demonstrates for the first time the benefit of eculizumab therapy in a fulminant recurrence of TMA related to aPL antibodies and resistant to classical therapy after kidney transplantation.

**PUB470**

The Effect of Changes of Body Composition on Graft Function after Kidney Transplantation

**Seong Seok Han,1 Curie Ahn,1 Jin Suk Han,2 Suhngwong Kim,1 Yong Su Kim.1 1 Internal Medicine, Seoul National University College of Medicine, Seoul, Korea; 2Clinical Research Center for End Stage Renal Disease, Seoul, Korea.**

**Background:** Kidney transplantation and accompanying medical conditions including immunosuppressive agents may result in the changes of body composition. But the changes and its effects on graft function are not well delineated, especially in Asian recipients. In this study, we prospectively observed the changes of body composition in 50 consecutive kidney recipients and evaluated its effect on graft function.

**Methods:** A total of 50 Korean recipients (ages, 47 ± 11.2 years; living donor, 60%; body mass index, 21.8 ± 2.64 kg/m²) were enrolled as a prospective cohort. Body composition (muscle and fat mass) was assessed 2 weeks, 1, 3, 6, 9, and 12 months after kidney transplantation by the bioelectrical impedance analysis. Body composition in 2 weeks of transplantation was used as baseline for reducing the effect of excessive water after operation.

**Results:** All the patients had good graft function during the study period (last serum creatinine (SCR), 1.15 ± 0.28 mg/dL). The muscle mass decreased within 6 months (41.4 to 38.9 kg, P = 0.056), but was regained after 6 months. The fat mass continuously increased over time (13.2 to 15.2 kg, P = 0.004). The waist circumference showed a similar trend to the muscle mass (81.7 to 78.9 cm in 6 months, P = 0.043; to 80.5 cm in 1 year, P = 0.313).

**Conclusions:** Body composition in the Asian transplant recipients changes over time and this change is associated with graft function.

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**Underline represents presenting author.**

**951A**
Proteinuria and the Risk of End Stage Renal Disease in Kidney Transplant Recipients Allyson Hart,1 Liangxing Zou,2 James Hodges,2 Hassan N. Ibrahim.1 1Medicine - Division of Renal Diseases and Hypertension, University of Minnesota, Minneapolis, MN; 2Coordinating Center for Biometric Research - Biostatistics, University of Minnesota, Minneapolis, MN.

Background: Proteinuria is associated with increased risk of progression to end stage renal disease (ESRD), decreased graft survival, and death. We describe the association between proteinuria, graft survival and all-cause death in a prospective cohort of 153 kidney transplant recipients.

Methods: 153 kidney transplant recipients were followed prospectively with annual 24-hour urine protein and albumin measurements, with the first measurement occurring on average 58 days after transplantation. Analyses of time to death, ESRD, or the first of death or ESRD used Cox regression and Kaplan-Meier estimates.

Results: 19 (12%) of the 153 subjects died and 19 (12%) progressed to ESRD. Median baseline 24-hour urine protein and albumin were 0.34 g/g and 26 mg/g, respectively. Higher urine albumin was associated with higher risk of ESRD, but also with lower risk of death. Results were similar for proteinuria. The combined outcome of death or ESRD was not associated with baseline albuminuria or proteinuria. The results did not change notably when adjusted for age, sex, systolic blood pressure, donor source, or presence of acute rejection in the first 3 months after transplantation.

Results: Complement C5b blockade by eculizumab is highly effective in reversing recurrent aHUS in kidney transplant allograft. Pharmacodynamic monitoring of C5b-9 may guide adaptation of eculizumab dose and interval. As novel treatment option, eculizumab may facilitate access to transplantation for patients with aHUS.

Acute Rejections (ARs) Associated with Subsequent or Concurrent Chronic Graft Dysfunction (CGD) in Kidney Transplants (Tx) Ajay K. Israni,1,4 Robert Leduc,2 David P. Schlach,2 William S. Oetting,3 Pamala A. Jacobson.2 1Medicine, HCMC, Univ. of MN, Minneapolis, MN; 2Biostatistics, Univ. of MN, Minneapolis, MN; 3School of Pharmacy, Univ. of MN, Minneapolis, MN; 4DeKAF Investigators.

Background: AR is a risk factor for CGD and allograft failure. However not all ARs lead to CGD. Therefore, we examined the severity of AR and response to AR treatments, for ARs that are associated with CGD.

Methods: We enrolled 2,366 at the time of tx between 2006-2010. CGD was defined as ≥25% rise in serum creatinine (Scr) after 3 months post-tx, that resulted in a biopsy. The rise in Scr is defined relative to a baseline Scr that was initially set at 3 months post-tx and re-set after AR.

Results: CGD occurred in 366 recipients at 509 ± 387 days from the 3 month baseline. All CGD biopsies had chronic changes. AR was more likely among recipients with CGD than without CGD (p < 0.0001).

Conclusions: Complement C5b blockade by eculizumab is highly effective in reversing recurrent aHUS in kidney transplant allograft. Pharmacodynamic monitoring of C5b-9 may guide adaptation of eculizumab dose and interval. As novel treatment option, eculizumab may facilitate access to transplantation for patients with aHUS.

Terminal Complement Blockade by Eculizumab Effectively Reverses Recurrent Atypical Hemolytic Uremic Syndrome after Kidney Transplantation Nils Hevey,1 Marcus Weitz,2 Martina Guthoff,3 Mark Dominik Alschner,1 Hans-Ulrich Haering,1 Silvio Nadalin,1 1Dept. of Diabetes and Endocrinology, Angiology, Nephrology and Clinical Chemistry, University of Tuebingen, Germany; 2Dept. of Pediatrics, University of Tuebingen, Germany; 1Robert-Bosch Hospital, Stuttgart, Germany; 3Dept. of General, Visceral and Transplantation Surgery, University of Tuebingen, Germany.

Background: Recurrence of atypical hemolytic uremic syndrome (aHUS) is frequent after kidney transplantation, limiting transplant options for these patients. The reported incidence of 15 - 90% is largely dependent upon the underlying dysfunction of the complement system. Plasmapheresis is current standard therapy, yet limited efficacy. The humanized C5b-antibody eculizumab is a novel therapeutic option, blocking terminal complement activation. We report eculizumab to effectively reverse recurrent aHUS in kidney transplantation.

Methods: A 43 year old patient with a history of postpatral aHUS presented for second kidney transplantation. Mutation of complement factor H had been ruled out. On day 7 after transplantation, the patient developed severe recurrent aHUS with systemic hemolysis, thrombopenia and acute kidney injury under calciuminhibitor-free immunosuppression. Complement C5b-9 (membrane attack complex, MAC) was highly detectable.

Results: Eculizumab was administered classical weekly, with subsequent prolongation of intervals. Hemolysis and thrombopenia ceased quickly and allograft function fully recovered. No unwanted side effects were observed. Under continuous monitoring of SCr, dosing intervals of eculizumab were successively tapered to every other month, currently 6 months after transplantation, allograft function is excellent with an eGFR of 41 ml/min/1.73 m² (serum creatinine 1.4 mg/dl) and C5b-9 within reference range.

Conclusions: Higher level of albuminuria and proteinuria were associated with higher risk of ESRD but lower risk of death in kidney transplant recipients. This result differs from what has been described in other populations, and needs to be confirmed.

Funding: NIDDK Support

Prophylactic Ganciclovir for Gastrointestinal Cytomegalovirus Infection in Renal Transplant Recipients Hyun Chul Kim, Eun-Ah Hwang, Sung Bae Park, Seung Yeup Han. Internal Medicine, Keimyung University School of Medicine, Daegu, Korea.

Background: Cytomegalovirus (CMV) can cause morbidity in renal transplant recipients, and the gastrointestinal (GI) tract is a major target for CMV disease. Currently, there is no report concerning CMV prophylaxis to prevent GI CMV infection in CMV intermediate-risk patients(R+) patients). The aim of this study was to evaluate the benefit of ganciclovir prophylaxis on CMV infection in R+ patients.

Methods: In 41 patients who received renal transplantation after January 2009, intravenous ganciclovir (5mg/kg, twice daily) was started for 14 days just after transplantation. The historical control group consisted of 45 patients received renal transplantation between January 2007 and December 2008. For evaluating effect of prophylaxis on CMV infection, routine endoscopic examination with mucosal biopsy was performed at the time of pretransplantation and then 1, 3 and 6 months posttransplant.

Results: The average age of 86 studied patients was 43.7±10.6 (14-63) years and male to female ratio was 11.13. 43 (50%) patients received deceased donor transplantation and 34 (77.7%) patients were seropositive for CMV IgG at the time of transplantation. The incidence of GI CMV infection was significantly lower in prophylaxis group than in historical control group (24.4% vs. 48.9%, p < 0.026). The patient age, numbers of deceased donor and trough level of tacrolimus at 1, 3 months posttransplant were significantly lower in prophylaxis group than those of historical control group. In multivariate analysis for risk factors associated with GI CMV infection, ganciclovir prophylaxis was the only significant risk factor.

Conclusions: Prophylactic treatment with ganciclovir decreased the incidence GI CMV infection in seropositive renal transplant recipients.

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
AKI from pyelonephritis with dehydration; serum creatinine quickly reached 5.16 mg/dL on January 6, 2011, requiring RRT for uremic symptoms, acidosis, oliguria and volume overload. Kidney allograft biopsy revealed acute tubular necrosis. She remains on HD for ESRD; serum creatinine in May 2011 was 3.94 mg/dL.

Conclusions: This is the first report of SORO-ESRD among renal transplant recipients. Multicenter studies are mandated to accurately identify the extent this syndrome contributes to renal allograft losses. More research intoreno-prevention strategies in RTR is warranted. This may well call for critical process re-engineering of several current accepted paradigms and norms in transplant nephrology practice.

PUB478
Incidence, Risk Factors and Clinical Characteristics of Delayed Graft Function in Living Donor Renal Transplantation
Hoon Suk Park, Yu Ah Hong, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Busnoum Choi, Cheel Whee Park, Yong-Soo Kim, Chul Woo Yang. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: Delayed graft function (DGF) is the need for dialysis during the first week even after renal transplantation. DGF can be seen in deceased donor renal transplantation (DDRT) with relatively longer ischemic time. But DGF in living donor transplantation (LDRT) is rare, so its incidence and risk factors have not yet been established. We investigated the incidence, risk factors and clinical characteristics of DGF of LDRT for the last 10 years.

Methods: The 429 cases of LDRT for the last 10 years were analyzed. We compared recipient and donor’s characteristics, HLA mismatch numbers, the frequency of non-related donors, gender matching status, total ischemic time and graft weight between DGF and non DGF group. We also reviewed the biopsy findings and clinical outcomes in the cases with DGF.

Results: The incidence of DGF in LDRT is 7/429(1.6%) and is significantly lower than the incidence of DGF in DDRT (13.7%) during the same period (P<0.05). In univariate analysis, numbers in HLA mismatch were significantly increased in DGF group (4.4±2.88, P=0.05). The frequencies of female recipients and non-related donors were also increased in DGF group (85.7±36.5%, P=0.05). The biopsy findings during DGF were available in six out of seven cases except the one in which exploratory laparotomy was done. Two cases were related to rejection, one case was related to acute pyelonephritis and the other three cases were related to ATN. The shortest duration of dialysis in DGF is just 1 day and the longest one is 97 days, whereas in DDRT, the shortest duration of dialysis in DGF is 1 day and the longest one is 55 days and most of them were within 7 days. The two cases related to rejection resulted in graft failure within three years after transplantation. But the other cases not related to rejection have been followed up with favorable graft function.

Conclusions: We conclude that the strenuous strategies including biopsy should be done when DGF developed in LDRT because DGF related to rejection in LDRT shows poor prognosis.

PUB479
Incidence, Risk Factors and Clinical Characteristics of Recurrent Focal Segmental Glomerular Sclerosis in Adult Renal Transplantation
Hoon Suk Park, Yu Ah Hong, Hyun Gyeong Kim, Sun Ryoung Choi, In O Sun, Byung Ha Chung, Busnoum Choi, Cheel Whee Park, Yong-Soo Kim, Chul Woo Yang. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: Recurrence of Focal segmental glomerular sclerosis (FSGS) is known as being relatively common compared with the recurrence of the other primary diseases in renal transplantation. Furthermore, it’s recurrence tends to result in graft failure. We investigated the incidence, risk factors and clinical characteristics of recurrent FSGS in renal transplantation for the last 20 years.

Methods: The cases of which the primary renal diseases were FSGS in renal transplantation were 25 for the last 20 years. We investigated the subtypes of recurrent FSGS (early recurrence or late recurrence) and compared the possible factors that may cause recurrence between the group with recurrence and the other group without recurrence.

Incidence, Risk Factors and Clinical Characteristics of Recurrent Focal Segmental Glomerular Sclerosis in Adult Renal Transplantation

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only
Underline represents presenting author.

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Results: The rate of recurrence in FSGS was 40% (10/25). The eight cases reached early and the other two cases reached late. There was no variable significantly different between the group with recurrence and the other group without recurrence. Five year graft survival rate (38%) in the group with recurrence was significantly lower than in the other group without recurrence (p=0.01). Univariate Cox regression according to the subgroups showed that the early recurrence influenced the graft survival significantly (HR = 3.16, 95%) (1.9–5.34). There were 7 cases in 25 cases with FSGS, who underwent plasmapheresis before transplantation to prevent the recurrence of FSGS or rejection. FSGS recurred in 2 cases out of them. Eight out of 10 patients with the recurrence of FSGS were treated with plasmapheresis. Six cases resulted in graft failure and the one case with the amount of proteinuria less than 1 g/day had been closely monitored and the other case with the amount of proteinuria more than 1 g/day has been treated with the subsequent plasmaphereses.

Conclusions: We conclude the graft survival in FSGS with recurrence was poor, so the more intensive protocol of plasmapheresis should be done.

PUB480
Successful Use of Plasma Exchange To Prevent Recurrence of Type I Membranoproliferative Glomerulonephritis after Kidney Transplantation: A Case Report
Department of Nephrology and Hypertension, Universitair Ziekenhuis Brussel, Brussels, Belgium; Immunohematology Laboratory - Immunohematology Clinic, Erasme Hospital, Brussels, Belgium; Department of Pathology, Universitair Ziekenhuis Brussel, Brussels, Belgium.

Background: Membranoproliferative glomerulonephritis (MPGN) with constitutional activation of the alternative complement pathway relapses in about 80% of patients with a history of disease recurrence on a previous graft. There is so far no efficient strategy to prevent recurrence.

Methods: Case report on the use of intermitent plasma exchange to prevent recurrence of MPGN in a second kidney transplant, after loss of a previous graft due to disease recurrence.

Results: The 26 year-old patient with end stage renal disease due to MPGN received a first renal transplant in 2007. She developed aggressive disease recurrence with strong mesangial C3 positivity, and electron dense deposits causing rapid graft failure. Activation of the alternative complement pathway (low C3, high C4 and normal C4b) was persistent before, during and after transplantation, although the underlying pathogenic mechanism could not be documented by extensive evaluation. After a second kidney transplantation, in January 2011, standard immunosuppression was combined with plasma exchange (40 mL/kg weight per treatment) at decreasing frequency, with a maintenance schedule of 1 exchange every two weeks after the first month. The patient maintained good graft function (creatinine 1.3 mg/dL) without signs of complement activation, microhematuria and proteinuria during the whole follow-up. A protocol biopsy 3 months after transplantation showed no signs of disease recurrence on immunofluorescence and electron microscopy.

Conclusions: Prophylactic plasma exchange might correct the underlying pathogenic mechanism in patients with idiopathic MPGN, thereby preventing complement activation and recurrence of disease after renal transplantation.

PUB481
Erythropoietin Requirements for Anemia in Renal Transplant Recipients Compared to Chronic Kidney Disease Patients
Ashish V. Regulagadda, Shirley Shwu-Shiow Chang, Rocco C. Venuto. Department of Nephrology, University at Buffalo, Buffalo, NY.

Background: The risk of developing post transplant anemia (PTA) increases as renal function declines. Immunosuppression, infections, and inflammation purportedly increase the risk for PTA and may impair response to erythropoiesis-stimulating agents (ESA).

Methods: We compared 39 renal transplant (RT) recipients (26 deceased and 13 living) with PTA to 41 anemic chronic kidney disease (CKD) patients receiving ESAs. All patients had hemoglobin (Hb) less than 11 g/dL at time of initiation. Exclusion criteria were recent hospitalization and active bleeding. Data collected included age, gender, race, and medication use, specifically renin-angiotensin-aldosterone system (RAAS) blockers, immunosuppressants, prophylactic anti-viral and anti-bacterial agents.

Results: The mean Hb at ESA initiation was similar (9.08 ± 0.79 g/dL in transplant vs. 9.5 ± 0.81 g/dL in CKD, p=.35). Deceased RT recipients (Hb 8.95 ± 0.89 g/dL) tend to be more anemic at baseline than living donor RT recipients (Hb 9.34 ± 0.47 g/dL, p=.09). The 30% of RT recipients who were not Caucasian (Hb 8.73 ± 0.75 g/dL) were more anemic than their Caucasian counterparts (9.23 ± 0.77 g/dL, p=0.15) despite similar eGFR (p=.86). Transplant recipients appear to need larger doses of darbepoeitin (5945.7 ± 1424 mcg) than CKD patients (517 ± 502 mcg, p=.08) but did not take longer to reach the target 11 g/dL (mean 2.66 ± 2.57 months). This was true even though transplant recipients (31.4 L/min) had eGFRs that were at least as high as their p=.94 CKD counterparts (33.4 L/min). There was no difference in the rate of iron deficiency (defined as Transferrin<20% and/or Ferritin<200 ng/ml) before ESA initiation. Use of RAAS blockers was lower in RT vs. CKD patients (23% vs. 39%, p=.01-XX- b>) respectively.

Conclusions: Renal transplant recipients developed anemia requiring ESA when their eGFRs were at least as high as race and gender matched CKD patients. Transplant recipients did not require significantly higher doses of ESA or take longer to reach target compared to CKD patients.

PUB482
Successful Treatment of Pseudallescheria boydii Brain Abscesses in a Renal Transplant Recipient
Dawinder S. Sohal, Melvin E. Laski, Vinjeta Sood. Internal Medicine/Nephrology, Texas Tech University Health Science Center, Lubbock, TX.

Background: Pseudallescheria boydii (Scedosporium apiospermum), is a ubiquitous, saprophytic fungus found in still waters. It usually causes skin infections. It can cause CNS infections with high mortality even if diagnosed and treated promptly and may be fatal in immunosuppressed patients. We present this case demonstrating successful treatment of Pseudallescheria boydii lung infection and brain abscesses in a renal transplant recipient.

Methods: Case report and literature review.

Results: A 63 year old woman was hospitalized for altered mental status 6 weeks after kidney transplant. Her post transplant course had been marked by poor diabetes control but no episodes of rejection. Past medical history was positive for DM2, HTN, and Bell’s palsy. Immunosuppression included tacrolimus, everolimus, and prednisone. She also took prophylactic TMP/SMX and valganciclovir. After admission, mental status rapidly worsened and respiratory failure developed requiring mechanical ventilation. Brain MRI revealed multiple hypodense ring enhancing lesions with surrounding edema. Chest CT demonstrated a right lower lobe infiltrate. Lab: WBC 7,400, Hct 34%, platelets 134,000, creatinine 0.4 mg/dL. Tacrolimus level was 15.6 ng/mL. Transbronchial biopsy and brain biopsy were both performed within 48 hours.

Brain biopsy revealed fungal abscess, and lung and brain biopsy cultures grew Pseudallescheria boydii. Three brain abscesses were each surgically drained and voriconazole was administered with resulting clinical improvement. The patient was eventually discharged to a rehabilitation facility without neurological impairment.

Conclusions: Pseudallescheria boydii is a neurotrophic pathogen which has been rarely reported to infect immunosuppressed individuals. It is sometimes confused with Aspergillus sp and Fusarium sp on pathology, which may lead to inappropriate treatment with amphotericin. As shown by this case, modification of immune suppression, aggressive drainage of abscesses, and voriconazole therapy may result in cure. Early, accurate diagnosis, aggressive surgical drainage, and specific treatment are necessary.

Funding: Clinical Revenue Support

PUB483
Clinical Characteristics of Renal Cell Carcinoma of Native Kidney in Renal Transplant Recipients
In O Sun, Hoon Suk Park, Sun Ryoung Choi, Yu Ah Hong, Hyun Gyu Kim, Byung Ha Chung, Bumsoo Choi, Cheol Whee Park, Yong-Soo Kim, Chul Woo Yang. Division of Nephrology, Department of Internal Medicine, Catholic University of Korea, Seoul, Republic of Korea.

Background: The aim of this study is to investigate the incidence and clinical characteristics of renal cell carcinoma (RCC) in native kidney of renal transplant recipients.

Methods: Between 1991 and 2010, 1425 patients underwent kidney transplantation at our institution. We retrospectively evaluated the clinical features and outcomes of five patients of RCC in the native kidney following transplantation.

Results: The patients included three men and two women, with a mean age of 63 years (range, 52-74). The incidence of RCC was 0.35%. The median interval between renal transplantation and occurrence of RCC was 16.2 years (range, 9 to 20). In our center, all patients with RCC had acquired renal cysts before (N=3) and after renal transplantation (N=2). The mean duration of dialysis was 12 months (range, 2 to 39), and most patients had duration of dialysis within 8 months except one. Four patients were asymptomatic and one patient presented a vague abdominal discomfort. And all patients received standard immunosuppressive treatment at the time of RCC diagnosis. Of the 5 patients, 4 received therapy with cyclosporine and prednisolone. And one patient received triple immunosuppression including cyclosporine, azathioprine and prednisolone. The mean tumor size was 3.2 cm (range, 0.5-5.1), and the stage of all RCCs was low grade at diagnosis. Two patients had clear cell carcinoma, another two papillary RCC and the last one multilocular cyst RCC. Radical nephrectomy was performed in all case except one who refused operation. Four patients who received radical nephrectomy showed no evidence of local recurrence or distant metastasis during median follow-up of 2.9 years. However, the patient who didn’t have surgical treatment experienced the spinal metastasis of RCC 6 years later.

Conclusions: Follow-up period seems to be an important factor for development of RCC in renal transplant recipients.

PUB484
Delayed Graft Function Due to Early Onset Renal Transplant Artery Stenosis
Si-Yen Tan, David Cumberland, Shahin Merican, Heinz Regelle, Mohan Rao, T, Chau Keng. 1 Nephrology, Prince Court Medical Centre, Kuala Lumpur, WP; Malaysia; 2 Department of Pathology, Medical University of Vienna, Austria; 3 Department of Surgery, Queen Elizabeth Hospital, Adelaide, Australia.

Background: Transplant renal artery stenosis (TRAS) is a late transplant complication which characteristically presents with impaired graft function and hypertension. TRAS is however a rare case of delayed graft function (DGF). The aim of this study was to investigate the incidence, clinical characteristics and outcomes of TRAS and evaluate risk factors for TRAS. In this study, we report the incidence and outcomes of TRAS.

Methods: We report here 2 cases of DGF due to TRAS.

Results: The first patient had a living related transplant with donor renal artery anastomosed end to side into the internal iliac artery. This was complicated by DGF with...
trials. TRAS is associated with tissue injury during surgical anastomosis. The pathophysiology of early TRAS is likely to be different from the more common later onset as a potential cause of DGF which is amenable to early intervention angiographically. The alpha-interferon secondary to CNI therapy; however, larger studies along with alpha- and CNI as a part of their immunosuppressive medication, and four of them had HCV infection.

Biopsies from the other recipients showed no evidence of acute cellular transplantation. All recipients were biopsied to evaluate for rejection. Borderline cellular disease processes are associated with TRI expression in transplant kidney biopsies.

TRIs arise from the membranes of the rough endoplasmic reticulum of endothelial cells. TRIs have been associated with viral infections and autoimmune diseases, particularly human immunodeficiency virus (HIV) infection and lupus nephritis, as well as administration of exogenous interferon. However, it is not clear whether some other underlying illnesses or diseases precipitate TRIs or if they are concomitant with TRI expression in transplant kidney biopsies.

Methods: During the three-year period (2008 to 2011), TRIs were observed in five post-transplant kidney biopsies on ultrastructural examination. Donor and recipient records were reviewed for demographics, underlying diseases, and laboratory data including pertinent serological tests as well as kidney biopsy findings.

Results: All donors and recipients were HIV-negative and had no clinical or serologic evidence of autoimmune diseases. Four of five recipients had hepatitis C virus (HCV) infection. Three out of these four recipients had never received any specific treatment for HCV infection; the other one received pegylated interferon 2 years prior to the kidney infection. Three out of these four recipients had never received any specific treatment for evidence of autoimmune diseases. Four of five recipients had hepatitis C virus (HCV) were reviewed for demographics, underlying diseases, and laboratory data including prostate-specific antigen (PSA).

Conclusion: In conclusion, these results suggest over-immunosuppression as a contributing factor of BK viremia. Our results also suggest that adjustment of immunosuppression therapy towards a target ATP level >256.5 ng/ml may reduce the risk of developing BK viremia. Further prospective studies are, however, required to validate the above findings.

Funding: Private Foundation Support

Screening of BK Viraemia and Nephropathy in Kidney Transplant Recipients Vivian W. Yiu, Rui Gao, Miriam Rose Berry, Yisu Yisu Gu, Afzal N. Chaudhry, Sharon Mulfrey, Nephrology, Addenbrookes Hospital, Cambridge, United Kingdom.

Background: BK viremia affects 15% of renal transplant recipients (85% of whom are developed nephropathy). Protocol screening to treat and BK viremia was set up in our hospital. We aim to assess the effectiveness of the protocol on BK nephrology and graft outcome.

Methods: A database was set up from electronic and paper records on patients receiving a kidney transplant or simultaneous kidney-pancreas(SPK) graft from May 2009 to January 2011.

Results: Of 263 transplant recipients, 39 (14.8%) developed BK viremia. Baseline demographics of those with viraemia(Gp 1) and those without(Gp 2) were similar. Compliance with screening was 86%. Onset of viremia was mostly within 5-20 wks. Risk factors for viremia include Campath induction, a deceased donor, SPK graft, acute rejection(AR) and Mycophenolate(MMF) use. Urine testing was performed in 28% patients and biopsy in 40% patients with a high BK viral load(>10,000 copies/ml). Immunosuppression reduction occurred in 50.0% of Gp 1. The only additional treatment was Ciprofloxacin, used in 10.2%. 75% of Gp1 patients had more intense screening for 6 months after the resolution of viremia. No patients died or lost their grafts as a result of BKV. Investigations beyond protocol requirements were performed in 19.0%.

Baseline characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gp 1 n (%)</th>
<th>Gp 2 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Age (yrs)</td>
<td>52.0</td>
<td>48.8</td>
</tr>
<tr>
<td>Gender (M:F)</td>
<td>30:9</td>
<td>143:81</td>
</tr>
<tr>
<td>Mean follow up (mths)</td>
<td>12.1</td>
<td>7.8</td>
</tr>
<tr>
<td>Kidney graft</td>
<td>31</td>
<td>188</td>
</tr>
<tr>
<td>SPK graft</td>
<td>8</td>
<td>34</td>
</tr>
<tr>
<td>Deceased donor</td>
<td>31</td>
<td>162</td>
</tr>
<tr>
<td>Liver donor</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>0 AR</td>
<td>23</td>
<td>187</td>
</tr>
<tr>
<td>≥1 AR</td>
<td>16</td>
<td>37</td>
</tr>
<tr>
<td>Kidney recipients only:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMFimmunosuppression</td>
<td>20</td>
<td>96</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>11</td>
<td>92</td>
</tr>
</tbody>
</table>

In conclusion, these results suggest over-immunosuppression as a contributing factor of BK viremia. Our results also suggest that adjustment of immunosuppression therapy towards a target ATP level >256.5 ng/ml may reduce the risk of developing BK viremia. Further prospective studies are, however, required to validate the above findings.

Funding: Private Foundation Support
Conclusions: Outcomes for patients with BK viroemia were good. The protocol was refined by reducing excess screening, starting intense screening for patients treated for AR and highlighting need for biopsy in those with high viral loads. Risk stratification can tailor screening for individual patients to improve efficiency and cost.

PUB489

Lower Dose of Mycophenolate Mofetil Is Enough for Rituximab Treated Renal Transplant Patients

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Background: Rituximab, anti-CD 20 antibody, enabled HLA-sensitized and ABO incompatible renal transplantations possible without splenectomy. As rituximab has a profound suppressive effect on B lymphocytes, we planned to know the differences of the conventional immunosuppressive regimen without any harmful effect on graft in rituximab-treated patients compared to usual patients.

Methods: We investigated 69 patients who underwent rituximab treated (200 mg or 500 mg, then every 6 weeks for 3 months and then every 6 months for 2 years) and historic transplantation between January 2009 and March 2011 (group 1). The outcomes of seventy-two renal transplant recipients who did not require rituximab were compared as controls (group 2). All patients except 11 patients were treated with a combination of tacrolimus (FK506), mycophenolate mofetil (MMF) and methylprednisolone (MPD) after two doses of basiliximab for induction therapy in group 1.

Results: Graft survival was 98.3% in group 1 and 100% in group 2 (p=0.446). Renal function and incidence of infection including cytomegalovirus and BK virus were not significantly different between the two groups. Acute cellular rejection episodes occurred in 5.2% in group 1 and 9.7% in group 2 (p=0.511). Hyperacute rejection and antibody-mediated rejection episode was absent. The drug levels of FK506 and the doses of MPD after 1 year and 2 years after transplantation showed no difference between the two groups (p=0.257 and 0.625 at 1 year, p<1.000 and 0.667 at 2 years). The required dose of MMF (g/day, mean ± S.D.) was lower in rituximab-treated group post operative 1 month, 3 months, 6 months and 1 year (1.25 ± 0.45 g vs. 1.42 ± 0.39 g at 1 month, p=0.035, 1.15 ± 0.50 g vs. 1.38 ± 0.34 g at 3 months, p=0.006, 1.07 ± 0.51 g vs. 1.30 ± 0.42 g at 6 months, p<0.015, 0.92 ± 0.57 g vs. 1.22 ± 0.42 g at 1 year, p<0.007).

Conclusions: These results suggest that lower dose of MMF is enough for successful immunosuppressive effect in rituximab treated renal transplantation.

PUB492

Women Are Extremely Disproportionately Affected by High Panel Reactive Antibody Levels Prior to Kidney Transplantation

Antony J. Bleyer, Patricia L. Adams, Gregory B. Russell. Nephrology, Wake Forest Medical School, Winston-Salem, NC.

Background: panel reactive antibody (PRA) levels are obstacles to kidney transplantation. The epidemiology of high PRA levels has not been well studied.

Methods: Data from the United Network of Organ Sharing on individuals placed on the transplant list for a first kidney transplant were analyzed from 1990 until 2010. A total of 240,938 individuals between 18 and 60 yrs were placed on the waiting list. Women were 7.5 times (95% CI 6.99-7.94) more likely to have a high PRA (>85%) than men. Of 7,721 individuals on the waiting list with high PRA levels, 6,384 (82.3%) were women. Of 20,180 individuals with a medium PRA (15 to 85%), 13,253 (66%) were women. Table 1 shows the effect of pregnancy on PRA. Findings remained consistent across all ages younger than 60 yrs. Table 1 shows the effect of pregnancy on PRA.

Conclusions: Women were 7.5 times (95% CI 6.99-7.94) more likely to have a high PRA (>85%) than men. Of 7,721 individuals on the waiting list with high PRA levels, 6,384 (82.3%) were women. Of 20,180 individuals with a medium PRA (15 to 85%), 13,253 (66%) were women. Table 1 shows the effect of pregnancy on PRA. Findings remained consistent across all ages younger than 60 yrs. Table 1 shows the effect of pregnancy on PRA. Findings remained consistent across all ages younger than 60 yrs.

PUB493

Early Versus Late Conversion to Sirolimus from Calcineurin Inhibitor Based Immunosuppression in Adult Kidney Transplant Recipients: A Single Center Retrospective Study

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Background: To compare the efficacy and safety of conversion from calcineurin inhibitors in adult kidney transplant recipients to sirolimus as a component of their immunosuppression therapy.

Methods: We conducted a single center retrospective study of adult kidney transplant recipients who received initial immunosuppressive therapy with either cyclosporine (CYA) or tacrolimus (TAC) and were then switched to sirolimus (SIR) after 3-6 months (early conversion) or >6 months (late conversion) or remained on CYA or TAC. Patient

Key: TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

Underline represents author's name.
records were reviewed for patient demographics, medications, adverse reactions, graft rejection in addition to serum creatinine, urine protein and triglycerides for up to 5 years post transplant.

Results: We identified 220 adult kidney transplant recipients: CYA (n=73), TAC (n=70) and SIR (n=77) and mycophenolate as part of their immunosuppressive regimen. There was no difference in overall demographics between the three treatment groups. Patients receiving SIR were more likely to also receive HMGR Co Reductase Inhibitor (statin) compared to CYA and TAC patients (p < 0.0001). There was not a statistical difference in graft survival between patients converted from calcineurin inhibitors to SIR either early versus late (p = 0.83). The incidence of transplant rejection was not statistically different between the three groups (p = 0.74) nor were the number of transplant failures between the groups (p = 0.88). The adverse events included hypertriglyceridemia (p = 0.12) and proteinuria (p = 0.004) in groups (p = 0.74) nor were the number of transplant failures between the groups (p = 0.88).

Conclusions: From our single center experience we did not observe a statistical difference in the occurrence of rejection or graft failure in our adult kidney transplant recipients on a calcineurin inhibitor compared with those converted to sirolimus.

PUB496

Affective Disorders among Patients on Different Renal Replacement Therapy Modalities: A Systematic Review 1Julio Lamprea, 1Priscilla Auguste, 1Patti Ephraim, 1Deidra C. Crews, 1Johanna Sheu, 1Temitope Olufade, 1Tanjala S. Purnell, 1Raquel Greer, 1Neil R. Powe, 1Hamid Rabb, 1L. Ebony Boulware.

Background: The relation between patient’s use of various renal replacement therapies (RRTs) and their development of affective disorders, has been poorly explored. We performed a systematic review of published studies to identify differences in rates of depressive and anxiety disorders among patients treated with different RRTs.

Methods: We searched PubMed and performed a hand-bibliographic search to identify studies comparing rates of affective disorders among patients receiving different RRTs (hemodialysis-HD, peritoneal dialysis-PD and renal transplant-TX). Two reviewers abstracted outcome data and assessed article quality. We calculated standardized effect size estimates (cohen’s d) of outcomes among patients on different RRTs. We considered effects with a p-value of less than 0.05 statistically significant.

Results: Among 91 studies identified as potentially relevant, 11 studies met the eligibility criteria. All studies had observational (cross-sectional) designs. Most studies reported outcomes based on validated questionnaires. However, no studies reported differences in outcomes while adjusting for potential confounders. A majority of studies (8 of 9) comparing patients on HD to patients on PD demonstrated no significant differences in rates of disorders, while most (5 of 7) studies comparing patients on HD to patients with a TX reported lower rates of disorders among patients on TX.

PUB497

Successful Renal Transplantation Despite Preexisting HLA-DQ or -DP Donor Specific Antibodies (DSA) 1Trung T. Luong, 3Chantale Lacelle, 1Bhavna A. Lavingia, 1Meleie Debroy, 1Juan Arenas, 1Christopher Y. Lu. 1Division of Nephrology; 2Department of Pathology; 3Division of Surgical Transplantation, all from UT Southwestern Medical Center, Dallas, TX.

Background: The clinical significance of HLA-DQ and -DP DSA detected by single antigen Lumineux beads remains unclear. The ability to transplant in the presence of preformed -DQ and -DP DSA may be advantageous for highly sensitized patients, however the risk of rejection versus the increased morbidity and mortality of remaining on the waitlist must be considered.

Methods: We followed 5 patients who received a kidney transplant in the presence of preformed HLA-DQ or -DP DSA and a positive B cell (pronase) flow cytometry crossmatch (FCXM) are shown in Table 1. No episodes of rejection were observed and all patients have had good allograft function.

Results: Cross-sectional studies suggest patients receiving transplants may have lower rates of affective disorders compared to patients on hemodialysis. However, the evidence is of variable quality, limiting definitive inferences. Prospective studies with well-balanced treatment groups are needed.

Table 1: Studies comparing clinical outcomes between RRT treatments

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Comparison Number of studies</th>
<th>Treatmentordova</th>
<th>Symptons of anxiety</th>
<th>Symptons of depression</th>
<th>Diagnosis of depression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HD vs PD TX</td>
<td>HD vs TX</td>
<td>HD vs TX</td>
<td>HD vs TX</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 1st RRT</td>
<td>1 1st RRT</td>
<td>1 1st RRT</td>
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<td></td>
<td></td>
<td>1 1st RRT</td>
<td>1 1st RRT</td>
<td>1 1st RRT</td>
<td>1 1st RRT</td>
</tr>
</tbody>
</table>

Conclusions: Despite being dialysis dependant ESRD, kidneys continue to produce renin contributing to blood pressure. After removal of both kidneys, this patient developed hypertension from renin deficiency. His blood pressure was so low that long term vascular access was unsustainable. After receiving a kidney allograft, his blood pressure rose even prior to initiation of calcineurin inhibitors or any other agents known to cause elevated blood pressure. Normal blood pressure maintenance was reestablished after transplanting a new source of renin production.
TABLE 1: PATIENT DEMOGRAPHIC AND CHARACTERISTICS

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>Cause of ESRD</th>
<th>Prior transplant</th>
<th>dTFCXM</th>
<th>dMESP (cutoff value)</th>
<th>B cell FCMX (cutoff value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>F</td>
<td>Black</td>
<td>HTN</td>
<td>No</td>
<td>1050</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>2</td>
<td>55</td>
<td>F</td>
<td>White</td>
<td>HTN</td>
<td>No</td>
<td>1050</td>
<td>0.001</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* Age at transplant * Cause of ESRD: HTN = hypertension, nephrosclerosis, IgA = IgA nephropathy, GN = proliferative glomerulonephritis. * Immediate pre-transplant, dMESP = delta molecule of equivalent soluble fluorochrome * dTFCM = delta median fluorescence intensity, pre-transplant * dTFCM with PTD = post-transplant day * C = creatinine in mg/dL.

Conclusions: Renal transplantation in the presence of preexisting HLA-DQ or -DP DSA may be considered as an option for highly sensitized patients. Funding: Other NH Care Support - UT Southwestern O'Brien Kidney Research Core (NIH P30DK079328) NIH UT Southwestern Clinical Translational Service Award (NIH U1L-RR240822)

PUB498
Factors Predicting Graft and Patient Survival in Living Related Kidney Transplantation, Pinaki Mukhopadhyay, 1 Manish Rath, 2 Harbir Singh Kohli, 2 Vivekanand Jha, 2 Vinay Sakhuja. 2

Background: About 554 transplant recipient from Jan 2002 to Dec 2006 were studied retrospectively. Outcomes measures were overall patient and graft survival. Demographic laboratory and perioperative variables were analyzed. Multivariate statistical analysis was undertaken using log-rank test and Cox’s proportional hazards model.

Methods: Among 554 transplant recipient from Jan 2002 to Dec 2006 who were studied retrospectively. Outcome measures were overall patient and graft survival. Demographic, laboratory and perioperative variables were analyzed. Multivariate statistical analysis was undertaken using log-rank test and Cox’s proportional hazards model.

Results: Of the 554 recipient 86.1% were male and 13.9% were female. The average recipient and donor age was 33.6 ± 10.3 and 42.36 ± 11.27 years. Recipient transplanted right pre-transplant, dMESP = delta molecule of equivalent soluble fluorochrome * dTFCM = delta median fluorescence intensity, pre-transplant * dTFCM with PTD = post-transplant day * C = creatinine in mg/dL.

Conclusions: Renal transplantation in the presence of preexisting HLA-DQ or -DP DSA may be considered as an option for highly sensitized patients. Funding: Other NH Care Support - UT Southwestern O'Brien Kidney Research Core (NIH P30DK079328) NIH UT Southwestern Clinical Translational Service Award (NIH U1L-RR240822)

PUB500
Expanded Criteria Donor Kidney Transplant Recipients: One Year Analyses from the Mycophenolic Acid Observational Renal Transplant Registry, K. Ram Peddi, 1 Kimi Udeta Stevenson, 1 Kevin M. McCague, 2 Anne Wiland. 2 (California Pacific Medical Center; *Novartis.*

Background: The Mycophenolic Acid Observational Renal Transplant (MORE) Registry, a prospective study of new renal transplant recipients (RTRs) receiving mycophenolic acid (MPA) therapy, is designed to determine effectiveness, tolerability and safety of enteric-coated mycophenolate sodium (EC-MPS) versus mycophenolate motefil (MMF) regimens. The objective of this analysis was to compare 12-month outcomes in RTRs who received Expanded Criteria Donor (ECD) kidneys to RTRs who received non-ECD kidneys.

Methods: Based on local practices at 40 US sites, outcomes analyzed included: graft survival (GS), patient survival (PS), first biopsy-proven acute rejection (BPAR), adverse event (AE) rates, serum creatinine (SCr) and proportion of RTRs maintained on at least full recommended MPA dose (1.44/2.0 g/day, EC-MPS/MMF). A total of 102 ECD (73 EC-MPS/29 MMF) and 832 non-ECD (557 EC-MPS/275 MMF) RTRs were included.

Results: Donor (60 v. 39 yrs, p<0.01) and recipient ages were higher (61.6 v. 58.1, p<0.03) in the ECD group. More African American RTRs received ECD kidneys (32.3 v. 23.6%, p=0.07). The majority of RTRs received induction therapy (99% ECD, 97% non-ECD), tacrolimus (97% ECD, 96% non-ECD) and steroids (69% ECD, 66% non-ECD) for maintenance therapy. At 1, 3, and 6 months, more non-ECD RTRs received full MPA doses (non-ECD: ECD: 78.2/71.0%, p<0.01; 62.7/64.3%; p<0.01; 51.9/42.3%, p=0.14; 45.7/41.0%, p<0.50). Comparable 12-month effectiveness, tolerability and safety outcomes were achieved in both groups whether they received EC-MPS or MMF. Comparing 12-month outcomes in ECD to non-ECD RTRs regardless of MPA type, BPAR (10.0/9.7%, p=0.91), biopsy-proven acute rejection (BPAR) (5.9/4.5%, p=0.08) and death censored graft survival (DCGS) (97% non-ECD, 90% ECD) were similar whereas mean serum creatinine (SCr) (1.75 v. 2.6%, p=0.07) was higher in the ECD RTRs. There were no differences in reported AEs (infections including BK and CMV, diabetes, gastrointestinal, neoplasms, hemoglobin) among the groups.

Conclusions: Graft survival and BPAR were similar between RTRs who received ECD and non-ECD kidneys in the short-term. However, as expected, RTRs who received ECD kidneys exhibited higher mean SCr.

Funding: Pharmaceutical Company Support

PUB501
Impact of Substance Abuse on Access to Renal Transplantation, Gurprataap Singh Sandhu, 1 Muhammad W. Khattak, 2 Bhanu K. Patibandla, 1 Akshita Narra, 1 Martha Pavlakis, 1 Noelle C. Dimitri, 1 Alexander S. Goldfarb-Rumyantsev, 1 (Division of Nephrology, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; 2Transplant Institute, The Cleveland Clinic, Cleveland, Ohio; 3Transplant Institute, Beth Israel Deaconess Medical Center, Boston, MA).

Background: With an ever-increasing demand for kidneys and limited supply pool, it is essential to understand the balance between utility and equality in access to transplantation. The goal of this project was to evaluate the association between recipient’s substance abuse and renal transplant access in patients with end-stage renal disease (ESRD).

Methods: We used data from the United States Renal Data System. The primary variables of interest were abuse of alcohol, tobacco, or illicit drugs based on information from Centers for Medicare & Medicaid Services form 2728. We analyzed three outcomes in Cox model: (1) being placed on the waiting list for renal transplantation or transplanted (which ever occurred first); (2) first transplant in patients who were placed on the waiting list; and (3) graft loss or mortality after transplant. In addition, we performed subgroup analysis based on age, race, sex, diabetic status, and donor type.

Results: We analyzed 1,077,699 patients (age of ESRD onset 62.9±15.5 years, 54.1% males, 66% black, and 29% African American). When compared those with no substance abuse, abusing all three substances was associated with reduced transplant
access (hazard ratio 0.39, P=0.001 for wait listing/transplant; hazard ratio 0.67, P=0.019 for transplant). This trend was similar in most subgroups studied.

Conclusions: We demonstrated that patients with ESRD abusing or dependent on tobacco, alcohol, or illicit drugs are less likely to be placed on the waiting list for kidney transplant, and once on the list are less likely to be transplanted. The possible utility justifications for such disparity and potential interventions are discussed.

PUB502
Renal Transplantation from HBs Antigen Positive Donor to Antigen Negative Recipient Immunized with Intradermal Hepatitis B Vaccine: First American Experience
Girumkeshwar Singh, Andrea C. Hsta, Daniel Sklarov, Michael J. Gareno, Gregory Lee Braden. Baystate Medical Center, Tufts University School of Medicine, Springfield, MA.

Background: The US renal transplant waiting list has 88,972 patients & grows by 4200 every year. Donor pool expansion is imperative to meet the demand-supply mismatch. Inclusion of HBs antigen positive (HBsAg+) donors has been recommended in endemic countries. We present the first American case of a successful HBsAg+ renal transplant into a newly immunized recipient.

Methods: May 2000: A 58 year-old man with mesangiocapillary glomerulonephritis needed pre-emptive renal transplant. His wife was a living unrelated donor match. She had chronic hepatitis B, was HBsAg+ with anti-HBs antibody. She had normal liver function & was HBsAg negative & had undetectable Hepatitis B DNA (HBV DNA).

The recipient (negative for all hepatitis antigens & antibodies) did not mount an antibody response to 3 doses of monthly intramuscular recombinant hepatitis B vaccine (40 mcg). Intra-dural vaccine was administered (5 mcg) every 2 weeks for 6 months. By the 4th dose, anti-HBs antibodies titers reached immune levels (>10 mIU/mL). After the intradural course, his antibody titer was 160 mIU/mL. This tapered to <10 mIU/mL at 14 months. An intramuscular vaccine booster (40mcg) resulted in an anamnestic response with titer >150 mIU/mL in a month. The patient underwent a successful living-unrelated donor transplant from his wife in August 2002 utilizing prednisone, mycophenolate mofetil & tacrolimus.

Results: Over 9 years, the patient had no evidence of hepatitis B infection. Anti-HBs antibodies slowly declined to 5.8 mIU/mL in May 2011. He has continued to be HBsAg negative with undetectable HBV DNA. Allograft function has been well preserved with a 24 hour creatinine clearance of 78.4 mL/min in December 2010. Immunosuppression was switched to prednisone+ sirolimus 3 years post-transplant.

Conclusions: Our successful long-term experience, using intradermal Hepatitis B vaccination with booster doses led to sustained anti-HBs antibody response allowing transplantation from a HBsAg+ donor. Larger studies are warranted given this strategy's possible utility justifications for such disparity and potential interventions are discussed.

PUB503
Transient Elastography To Assess Hepatic Fibrosis in Renal Transplant Recipients
Claudia Sommerger, Christoph Seitz, Michael Schaur, Gunta Millonig, Martin G. Zeier, Sebastian Mueller.

Background: Transient elastography (TE) is a noninvasive, reliable and valid tool for the assessment of hepatic fibrosis. There are some limitations in certain patient groups, for example in patients with ascites or increased central venous pressure. Until now, TE has not been frequently used in renal allograft recipients. We evaluated TE in renal allograft recipients, a patient cohort who often shows hypervolemia and high central venous pressure especially in the early posttransplant period.

Methods: In 109 renal transplant patients liver stiffness measurement (LSM) was performed by transient elastography (TE). The severity of hepatic fibrosis was staged by Forns-Index.

Results: In 91/109 patients (n=29 women, age 47±8 years, 68±59 months post transplantation, BMI 25.5±3.7 kg/m²) it was possible to assess hepatic fibrosis by TE. The mean stiffness was 7.2±3.3 kPa with a mean interquartile range (IQR) of 0.9 kPa. TE and Forns-Indices correlated significantly (p=0.002) with an area under the ROC curve of 0.722. The sensitivity and specificity for detection of fibrosis was 34% and 92% with a cut-off of 8 kPa. Failure of LSM occurred in 61% of the patients with BMI > 28 kg/m². Liver stiffness was increased yearly in the early post transplant to mean 10.05 indicating significant influence of hypervolemia on the assessment of liver stiffness by TE.

Conclusions: TE might be a rapid, non-invasive tool to assess and identify renal allograft recipients with liver fibrosis. However, several confounders including obesity and hypervolemia leading to increased central venous pressure have to be considered.

PUB504
Management of Transplanted Patients Stage 3B and 4 in France. ANTICIPE Study

Background: The French society of nephrologists recently published guidelines for the management of renal transplant patients stage 3b and 4 in France. The present study evaluates the clinical characteristics, the outcomes of patients and the adherence to guidelines.

Methods: During one week of consultations for grafted patients, in a national, prospective and observational study conducted among nephrologists, we recruited 1497 patients. After exclusion of incomplete records or improperly included, we studied 1446 patients. 546 patients with renal insufficiency were on stages 3b and 4 (GFR Cockcroft-Gault).

Results: Age 52.8 ± 13.8, 60% men, 40% women, weight 70.9 kg ± 14.4, height 167.9 cm ± 9.6, BMI <25kg/m²: 53.8% between 25 and 30. 33.6%, Blood pressure 138/78.9 ± 18/ +11.3. Initial nephropathy was: Glomerular 1st 26%, glomerular 2nd 5.4%, diabetis7.4%, tubulointerstitial 12.4%, vascular 10.1%, polycystic 14.4, other 11.3% and 12.4% undetermined. Time between transplantation and the study was 1 year to 14.7%, from 2 to 3 years for 24.5%, 4 to 5 years 16.7% higher than 10 years to 21.9%.

Before the graft: 85.7% were hypertensive, 15.7% diabetics and 69.8 never smokers. In the history, we found 13.4% of ischemic heart disease, 7% heart failure, 3.3% for stroke. 92.1% of patients were on dialysis (HD 85.5%) before transplantation. The average time between first dialysis and transplantation was 3.9 years. The graft is 94.5% of deceased donors, living 4.4%, 1% cœur arrested. Only 0.9% of bi-transplantation. HLA mismatch was: 0.26%, 1.8%, 2.8%, 2.3%, 4: 25.8%, 5: 66%, 5: 6.7%, Cold ischemia was <6 hours: 4.4%, <24 hours: 21%, 23% of patients were diazyzed after transplantation. 24.1% had a rejection and 1.9% had more than 2. Among the complications related to transplantation, we have: 33% of hospitalized patients over 7 days for infections. The treatment is 65.9% corticosteroids, 87.5%, calcineurin inhibitors 10.8% azathioprine, 73.4%, mycophenolate mofetil, 9.7%, inhibitor of mTOR: another immunosuppressives 1.3%.

Other treatments are: 80% antihypertensive non RAS blockers and 59.5%, blockers, 29.9% ESA, 59.7%, statins, 64.1% vitamin D.

Conclusions: Nephrological monitoring of 147 investigators was used to develop this photograph of the transplanted patients stage 3b and 4.

Funding: Pharmaceutical Company Support

PUB505
Three-Year Graft Function Is Preserved in the Absence of Rejection or Delayed Graft Function
Pasikla M. Tedla, Amanpali Brar, Angella Brown, Thin Maw, Subodh J. Saggi, Moto O. Sallif. Renal Diseases Division, Department of Medicine, SUNY Downstate Medical Center, Brooklyn, NY.

Background: It is generally accepted that hyperfiltration results in progressive renal damage in native kidneys. Although graft function generally deteriorates over time, the natural course of progression in renal transplant recipients (RTRs) at different levels of renal function has not been studied longitudinally in patients free of acute rejection (AR) or delayed graft function (DGF). We conducted a single-center study of graft function over a three-year period in patients that did not have AR or DGF.

Methods: 275 patients were classified into stages of chronic kidney disease (CKD) based on serum creatinine at six weeks after transplant. Glomerular filtration rate (GFR) was estimated using the abbreviated MDRD formula at 6 weeks (6wk), 6 months (6m), 1, 2, and 3 years (1yr, 2yr, 3yr). Temporal change in mean eGFR across and within CKD stages was analyzed using repeated-measures ANOVA. CKD stages were compared for other variables by ANOVA, t-test or χ² as appropriate.

Results: 123 of 275 RTRs had complete data at 3yr. The mean eGFR of stages 1-4 CKD at 6 weeks were 111, 74, 46 and 21 ml/min. The groups were similar in mean age, hemoglobin, albumin, blood pressure at baseline (6wks) and tacrolimus levels at all time points. There was no significant difference in number of total or HLA DR mismatch, or proportion of African-Americans, retransplants, diabetics, or positive flow-cytometry crossmatches. Female RTRs had higher eGFR than male RTRs at all time points (mean difference 17.21 ml/min, p<0.001).

There was no significant change in mean eGFR in each CKD stage over 3 years (p=0.34)

Conclusions: In the absence of AR or DGR, graft function remained stable over three years irrespective of baseline eGFR.
**PUB506**

**Treatment Strategy for BK Nephropathy in Renal Transplant Recipients: Five Years Follow Up**  
Ying Wang, Mona Razavian, Kate Wyburn, Josette M. Eris, Steven J. Chadban.  
Medhat Haleem, Osama Gheith, Praasad Nair.

**Background:** BK nephropathy (BKN) is a significant cause of graft loss. There is no single defined treatment algorithm established and usually management consists of a substantial reduction in immunosuppression.

**Methods:** We retrospectively investigated renal transplant recipients with biopsy proven BKN in our centre from 2003 to 2011. They were followed up over 1-8 years after treatment with a protocol of reduction in immunosuppression and intravenous cidofovir, with or without ciprofloxacin or leflunomide.

**Results:** 7 cases of BKN were diagnosed with incidence of 1%. Demographic characteristics of the patients with BKN

<table>
<thead>
<tr>
<th>Age &amp; Gender</th>
<th>Months post-Tx</th>
<th>Additional BKN Therapy</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>60M</td>
<td>9</td>
<td>Cipro</td>
<td>Recovery</td>
</tr>
<tr>
<td>40M</td>
<td>17</td>
<td>Cipro</td>
<td>Graft loss</td>
</tr>
<tr>
<td>81M</td>
<td>16</td>
<td>Cipro</td>
<td>Graft loss</td>
</tr>
<tr>
<td>35M</td>
<td>7</td>
<td>No</td>
<td>Recovery</td>
</tr>
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<td>62M</td>
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<td>LEF &amp; IVIG</td>
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</tr>
<tr>
<td>65M</td>
<td>3</td>
<td>Cipro</td>
<td>Recovery</td>
</tr>
</tbody>
</table>

All were male and 6 had received treatment for acute rejection prior to the diagnosis. The median time to diagnosis was 10 months post transplantation. A reduction in immunosuppression and commencement of cidofovir as an initial treatment strategy were applied to all cases. In addition, 4 patients received ciprofloxacin 250 mg daily concurrently. 2 out of 7 patients were switched from mycophenolate to leflunomide after failure to respond to the initial therapy. 1 patient received intravenous immunoglobulin. The median follow-up was 5.1 years after the treatment. Of 7 patients, 4 showed sustained clinical and virological improvement while 3 incurred graft loss, one due to ongoing BK nephropathy. Two graft losses occurred after a reduction in viral load and initial improvement in graft function: one due to transplant artery thrombosis and one due to rejection following non-adherence to immunosuppression. An overall BK nephropathy related graft loss was 14.3%.

**Conclusions:** BK nephropathy in renal transplant setting remains a serious complication. Reducing immunosuppression and administration of cidofovir produced sustained remissions for the majority of our cases, however, the optimal therapeutic strategy remains unclear and warrants prospective randomized studies.

**PUB507**

**A Positive Flow Cross Match in HIV Patients May Not Necessitate Desensitization**  
Sana R. Akbar, Sadanand S. Palkar, Prakash N. Rao, Cecil L. Rhodes.

**Background:** Renal transplantation in HIV positive patients is becoming common place. Advances in the patho-physiology and treatment of HIV have made it possible for these individuals with end stage renal disease, to undergo successful renal transplantation. Solid phase antibody testing, and donor specific cross-matching by complement dependent cytotoxicity (CDC) and flow cytometry (FCXM) enable the assignment of risk, and determination of the necessity for desensitization therapy with intravenous immunoglobulin or plasmapheresis.

**Methods:** We would like to report on a series of three HIV positive recipients being evaluated for possible living donor renal transplants.

**Results:** All three patients had a PRA of < 20%. Solid phase antibody testing revealed the presence of a few HLA antibodies, none of which were donor specific. Upon testing all recipients demonstrated positive T and B cell flow cross-matches with their donors. Positive Flow cytometric auto cross-match testing indicated the presence of auto-antibodies. These results would indicate that the initial donor specific positive flow cytometry cross-matches were caused by auto-antibodies.

**Conclusions:** Therefore, though the preliminary FCXM made these 3 patients appear to be incompatible with their donors, the auto-crossmatch revealed otherwise. Our study would suggest that the presence of positive donor specific flow cross-match in HIV positive recipients should not automatically impute a high risk situation requiring desensitization protocols. These patients can indeed be successfully transplanted without the need for desensitization.

**PUB508**

**Effective Therapy for Resistant Acute Antibody-Mediated Rejection: Case Report and Review of Literature**  
Narayanan Nampoori, Torkii Al-Otaibi, Osama Gheith, Medhat Haleem, Praasad Nair.

**Background:** In an effort to reduce the long-term toxicities of immunosuppressant drugs, corticosteroid and calcineurin inhibitor (CNI) sparing immunosuppression protocols have become increasingly popular in managing kidney transplant recipients. The most vexing clinical condition caused by antibodies in organ transplants is antibody-mediated rejection. Limitations of the current AMR therapies include (1) AMR reversal tends to be gradual than prompt, (2) expense, (3) rejection reversal rates below 80%, (4) common appearance of chronic rejection after AMR treatment, and (5) long-term persistence of donor specific antibodies after therapy. Since these limitations may be due to the lack of effects on mature plasma cells, BKN's effects on mature plasma cells may represent a quantum advance in anti-humoral therapy.

The initial experience described herein represented the first clinical use of bortezomib as an anti-humoral agent in renal allograft recipients in Kuwait. We aimed to present 2 cases with resistant acute antibody mediated rejection to the standard therapies, and were managed successfully with bortezomib therapy.

**Methods:** Therefore, we were confronted by two cases of resistant acute antibody mediated rejection with mild chronic changes. The available therapies for antibody mediated rejection lack direct effects on the major antibody producing cell (the mature plasma cell).

**Results:** Although B lymphocyte specificity has been provided with rituximab, yet it does not deplete plasmablasts or mature plasma cells, which represent the major source of antibody production. Each case was managed by one cycle of bortezomib with partial response and satisfactory 1-year graft survival in spite of sustained anti-HLA antibodies.

**Conclusions:** Immunosuppression minimization carried risk of resistant acute antibody mediated rejection and renal grafts can be rescued by earlier bortezomib use especially in the absence of chronic changes.

**Key:** TH - Thursday; FR - Friday; SA - Saturday; OR - Oral Abstract; PO - Poster; PUB - Publication Only

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cell biology and structure

\[ \text{cell signaling} \]

\[ \text{cell activation} \]

\[ \text{cell adhesion} \]

\[ \text{cell biology and structure} \]

\[ \text{cell signaling (continued)} \]

\[ \text{cell volume} \]

\[ \text{cell-matrix interactions} \]

\[ \text{chemotherapy} \]

\[ \text{children} \]

\[ \text{chronic allograft failure} \]

\[ \text{chronic allograft nephropathy} \]

\[ \text{chronic allograft rejection} \]

\[ \text{chronic diabetic complications} \]

\[ \text{chronic dialysis} \]

\[ \text{chronic glomerulonephritis} \]

\[ \text{chronic graft deterioration} \]

\[ \text{chronic hemodialysis} \]

\[ \text{chronic inflammation} \]

\[ \text{chronic kidney disease} \]

\[ \text{cell volume} \]

\[ \text{cell transfer} \]

\[ \text{cell survival} \]

\[ \text{chemokine receptor} \]

\[ \text{chemokine} \]

\[ \text{children} \]

\[ \text{chronic allograft failure} \]

\[ \text{chronic allograft nephropathy} \]

\[ \text{chronic allograft rejection} \]

\[ \text{chronic diabetic complications} \]

\[ \text{chronic dialysis} \]

\[ \text{chronic glomerulonephritis} \]

\[ \text{chronic graft deterioration} \]

\[ \text{chronic hemodialysis} \]

\[ \text{chronic inflammation} \]

\[ \text{chronic kidney disease} \]
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end stage kidney disease

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ethnic minority

expression

extracellular matrix

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familial nephropathy

fibrinolysis

fibrinolytic system

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fibroceptin

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GFR

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TH‑PO083, TH‑PO087, TH‑PO217,
TH‑PO240, TH‑PO241, TH‑PO245,
TH‑PO287, TH‑PO288, TH‑PO296,
TH‑PO306, TH‑PO308, TH‑PO341,
TH‑PO345, TH‑PO384, TH‑PO388,
TH‑PO398, TH‑PO507, TH‑PO568,
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TH‑PO639, TH‑PO684, TH‑PO685,

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outcomes (continued).... TH‑PO687, TH‑PO691,
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FR‑PO1409, FR‑PO1412, FR‑PO1414,
FR‑PO1419, FR‑PO1434, FR‑PO1435,
FR‑PO1450, FR‑PO1608, FR‑PO1609,
FR‑PO1615, FR‑PO1664, FR‑PO1687,
FR‑PO1881, FR‑PO1889, FR‑PO1906,
FR‑PO1914, FR‑PO1935, FR‑PO1939,
FR‑PO1942, FR‑PO1974, FR‑PO2062,
FR‑PO2064, FR‑PO2084, FR‑PO2100,
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SA‑PO2136, SA‑PO2186, SA‑PO2459,
SA‑PO2520, SA‑PO2655, SA‑PO2668,
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SA‑PO2949, SA‑PO2957, SA‑PO2976,
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PUB502
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TH‑PO533, TH‑PO536, TH‑PO546,
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FR‑PO1532, FR‑PO1540, FR‑PO1555,
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SA‑PO2833, SA‑PO2839, SA‑PO2840,
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systemic lupus erythematosus
systolic blood pressure
TGF-beta
tocrolimus
tubular epithelium
tubule cells
vascular access

ureteric bud

urea modeling

urea

vascular access

vascular calcification (continued)

vascular disease

vascular endothelial growth factor

vascular

vasculitis

vasopressin

vessel-ureteral reflux

vitamin A

disease

vitamin D

von Willebrand factor

water channels

water-electrolyte balance

vesico-ureteral reflux

virology

vitamin A

vitamin B12

vitamin C

vascular disease

vascular

vascular access

vascular calcification

vascular
disease

vesico-ureteral reflux

disease