Renal Biopsy: Clinical Correlations
November 7, 4:30–6:30 p.m.
Case 1 from Ibrahim Batal, MD – Columbia University

A 35-year-old Hispanic woman presented with elevated serum creatinine of 2.7 mg/dL (eGFR: 20 mL/min/1.73 m²) and urine protein/creatinine of 1.8 g/g. Her medical history was significant for hypertension and longstanding microscopic hematuria. There was no history of hearing loss or vision problems.

Family history was reportedly significant for microscopic hematuria in the patient’s 58-year-old mother, 16-year-old son, and 13-year-old daughter. Serologies, including C3 and C4, were normal. Ultrasound revealed mildly shrunken kidneys. Medications included Lisinopril, Paricalcitol, and Loestrin.

A renal biopsy showed a limited sample with three globally sclerotic glomeruli for light microscopy, no glomeruli for immunofluorescence, and one glomerulus for electron microscopy (Figure 1). Renal function continued to deteriorate.

Eight months later, the patient underwent a preemptive living related kidney transplant from her mother, who had microhematuria, normal renal function, normal blood pressure, and no proteinuria. Pre- and post-transplant crossmatches were negative. The patient received induction therapy with Thymoglobulin and was maintained on Tacrolimus, Mycophenolate Mofetil (MMF), and Prednisone.

Post-reperfusion biopsy, which was entirely submitted in formalin for light microscopy evaluation, showed mild tubulointerstitial scarring and mild vascular sclerosis.

Serum creatinine decreased to 1.1 mg/dL at day 2 after transplantation and then ranged between 1.1 and 1.4 mg/dL. Two months later, a biopsy was performed when serum creatinine reached 1.6 mg/dL (Figures 2 and 3). C4d staining was negative for peritubular capillaries. Immunohistochemical staining for SV40 was also negative.

1. The electron micrographic images of the native biopsy (Figure 1) shows:
   A. Mesangial and subendothelial electron dense deposits
   B. Attenuated glomerular basement membranes (GBMs)
   C. Lamellation and splitting of GBMs
   D. Subendothelial expansion by electron fluffy material and remodeling of GBMs
2. The aforementioned ultrastructural findings (native biopsy; Figure 1) are diagnostic for:
   A. Immune complex-mediated glomerulonephritis with membranoproliferative features
   B. Thin glomerular basement membrane nephropathy (TBMN)
   C. Hereditary nephritis/Alport’s syndrome
   D. Thrombotic microangiopathy

3. The combination of light and electron microscopy findings for the allograft biopsy (Figures 2 and 3) is most suggestive of:
   A. Acute T cell-mediated rejection and TBMN
   B. Antibody-mediated rejection and TBMN
   C. Acute T cell-mediated rejection and hereditary nephritis/Alport’s syndrome
   D. Antibody-mediated rejection and hereditary nephritis/Alport’s syndrome

4. The following conditions can be excluded based on the above findings:
   A. TBMN
   B. Hereditary nephritis/Alport’s syndrome
   C. Immune complex-mediated glomerulonephritis with membranoproliferative features

DISCUSSION

Inherited diseases of the GBM include TBMN, hereditary nephritis/Alport’s syndrome (AS), and rare and less-characterized forms of GBM abnormalities.1

TBMN is often characterized by isolated hematuria. The genetic basis of TBMN is heterogeneous and includes heterozygous mutations in the COL4A3 and COL4A4 genes, which encode for α3 and α4 chains of type IV collagen, respectively. The pathologic diagnosis relies on electron microscopy, which reveals an average GBM thickening of ≤250 nm as defined by the World Health Organization (WHO)2 in the absence of GBM lamellation. Monoclonal antibodies are commercially available for α1, α3, and α5 subunits of collagen IV. By immunofluorescence, biopsies from patients with TBMN show a normal pattern of staining manifested by continuous linear staining for α1 in all renal basement membranes, α3 in GBM and distal tubular basement membranes (TBMs), and α5 in GBM, distal TBMs, and Bowman’s capsule.3

Although TBMN is usually associated with a benign clinical course, some patients with TBMN may develop proteinuria, and a small fraction of such patients may develop ESRD.4,5 The outcome of patients with TBMN after kidney donation, as well as the recipients of allograft with TBMN, is still not well characterized. It is currently accepted that TBMN is not a contraindication for kidney donation provided that donors with TBMN have normal blood pressure, normal renal function, and no genetic evidence for AS.6

TBMN should be differentiated from AS. We will concentrate below on X-linked AS, which is the most common type of AS. X-linked AS is caused by mutations to COL4A5 gene, which encodes for α5 chains of type IV collagen.6 Compared with TBMN, AS is a more severe but less commonly encountered disease. Classical manifestations of AS include hematuria, hearing loss, anterior lenticonus, retinal flecks, and a high percentage of progression to ESRD. Typically, ultrastructural evaluation reveals GBM lamellation and splitting (basket weave appearance), although GBM thinning may be the only manifestation in a subset of patients with early disease. By immunofluorescence, affected males typically show lack of staining for the α5 and α3 subunits of collagen IV along the GBM. Carrier females usually show mosaic staining with segmental or patchy loss of α3 and α5 expression along the GBM.3 The outcome of kidney donors with AS or recipients of allograft from donors with AS is not well characterized. It is currently recommended to discourage mothers of affected males from donation to their sons because of their own risk of renal failure.6 If insisted, a predonation biopsy is recommended.6

References
Case 2 from Christine A. VanBeek, MD – Cedars-Sinai Medical Center

The patient is a 60-year-old Caucasian man with a history of well-controlled hypertension, cirrhosis due to hepatitis C, and hepatocellular carcinoma who is status post orthotopic liver transplant. His post-transplant course was complicated by recurrent hepatitis C (liver allograft biopsy 6 months after transplant), papillary stenosis requiring ERCP with biliary sphincterotomy (17 months after transplant), and post-transplant diabetes. His baseline serum creatinine at the time of transplant was 1.0 mg/dL. However, there was a progressive decline in renal function following transplantation. The serum creatinine reached 2.8 mg/dL at 5 months after transplant. After tacrolimus levels were lowered, the serum creatinine improved and plateaued at 2.0 mg/dL. At 7 months after transplant, renal biopsy 1 was performed. Following the biopsy, his immunosuppressive medications were adjusted again, and the serum creatinine improved to between 1.5 and 1.7 mg/dL for the next several months. However, the renal function began deteriorating again, and this time, the decline in renal function was accompanied by increasing proteinuria. A second renal biopsy was performed at 2 years after transplant.

Medications at time of biopsy 1:
- Tacrolimus (3 mg b.i.d.)
- Prednisone
- Amlodipine
- Acyclovir
- Insulin Lispro
- Nystatin
- Sulfamethoxazole/trimethoprim

Medications at time of biopsy 2:
- Tacrolimus (2 mg b.i.d.)
- Sirolimus
- Insulin glargine
- Amlodipine
- Lansoprazole

Laboratory data at time of biopsy 1:
- White blood cell count, 3900/UL; hemoglobin, 12.6 g/dL; platelets, 129,000/UL
- Na, 138 mmol/L; K, 5.1 mmol/L; Cl, 105 mmol/L; CO2, 22 mmol/L; BUN, 36 mg/dL; serum creatinine, 2.0 mg/dL; glucose, 295 mg/dL; HgbA1c, 6.8%
- Albumin, 4.6 g/dL; Tbili, 1.3 mg/dL; ALT, 169 U/L; AST, 21 U/L; AlkPhos, 240 U/L
- HCV RNA PCR 5,910,000 NTDT IU/mL
- Urinalysis: trace protein, 1+ urobilinogen, 13 hyaline casts, 6 granular casts, 1+ bacteria, 1 RBC, 1 WBC

Laboratory data at time of biopsy 2:
- White blood cell count, 2200/UL; hemoglobin, 9.6 g/dL; platelets, 119,000/UL
- Na, 141 mmol/L; K, 4.9 mmol/L; Cl, 108 mmol/L; CO2, 20 mmol/L; BUN, 40 mg/dL; serum creatinine, 3.0 mg/dL; glucose, 97 mg/dL; HgbA1C, 6.6 %
- Albumin, 4.3 g/dL; Tbili 0.6, mg/dL; ALT, 96 U/L; AST, 126 U/L; AlkPhos, 304 U/L
- HCV RNA PCR 6,850,000 NTDT IU/mL
- Urinalysis: 3+ protein, trace glucose, 13 hyaline casts, 10 granular casts
1. What features were noted in glomeruli by light microscopy in biopsy 2 that were not present in biopsy 1?
   A. Early crescent formation and ischemic capillary wall wrinkling
   B. Focal segmental glomerulosclerosis and capillary wall double contours
   C. Membranoproliferative glomerulonephritis
   D. Mesangial matrix expansion and capillary basement membrane thickening

2. What is the best explanation for the capillary wall abnormalities noted by light microscopy and electron microscopy in biopsy 2?
   A. Prior immune complex deposition due to hepatitis C with pale, largely resorbed deposits in subendothelial zones
   B. Chronic antibody-mediated rejection
   C. Hyperglycemia causing glomerular basement membrane thickening and duplication
   D. Persistent endothelial cell injury and endothelial cell detachment with formation of a new layer of glomerular basement membrane material

3. What finding was seen in biopsy 2 that could explain the rise in serum creatinine above baseline?
   A. Acute tubular injury
   B. Advanced diabetic nephropathy
   C. Crescentic glomerulonephritis
   D. Hypertensive nephrosclerosis

DISCUSSION

Biopsy 1: There are a total of 40 glomeruli in the biopsy, 10 of which are completely sclerotic (8 of these are in subcapsular scars). Few of the remainder display prominent ischemic capillary wall wrinkling, and the balance is structurally unremarkable. There is mild tubular atrophy with interstitial fibrosis; this is more pronounced in the subcapsular zone. The cells of some nonatrophied tubules are irregularly flattened. There is focal denudation of tubular basement membranes, and debris are in occasional tubular lumina. Artery walls are thickened by intimal fibrosis. Arteriolar walls display muscular hypertrophy. There was no evidence of a glomerular immune complex lesion by immunofluorescence or electron microscopy. Ultrastructural study reveals glomerular basement membranes with the normal trilaminar structure and mean thickness, as well as partial (estimated at approximately 50%) effacement of podocyte foot processes.

Biopsy 2: There are a total of 32 glomeruli in the biopsy, 10 of which are completely sclerotic (4 of these in subcapsular zone), and 5 have segmental sclerosis. In some sections, the segments of sclerosis are surrounded by enlarged podocytes with cytoplasmic vacuoles and protein reabsorption droplets. In many glomeruli, capillary walls exhibit segmental double contour formation and varying degrees of ischemic wrinkling. An endothelial cell mitosis is noted. Hypercellularity and crescents are not evident. There is mild interstitial fibrosis with tubular atrophy. Interstitial edema is present. Focal mononuclear cell inflammation is in the interstitium, primarily in areas of tubular atrophy. Tubules exhibit features of acute injury including irregular flattening of epithelial cells, denudation of tubular basement membranes, and tubular cell mitotic figures. Proximal tubular cell brush borders are diminished. Tubular cell cytoplasm focally exhibits protein reabsorption droplets and fine cytoplasmic vacuoles. Rare interstitial calcifications are identified. Artery walls are thickened by intimal fibrosis. Arterioles display muscular hypertrophy and few subendothelial insudates. There are no thrombi in arterioles or glomerular capillaries. There was no evidence of a glomerular immune complex lesion by immunofluorescence or electron microscopy. Fibrin stain was negative. Ultrastructural study reveals glomerular basement membranes with the normal trilaminar structure and mean thickness. Capillary walls are segmentally wrinkled in an ischemic pattern. There are several subendothelial lucent zones in ischemic and nonischemic loops. The subendothelial expansion is accompanied by duplication of glomerular basement membranes. There is considerable (estimated at approximately 60%–70%) effacement of podocyte foot processes with areas of microvillous transformation of podocyte cytoplasm.

In this case, proteinuria and renal dysfunction developed following a liver transplant. The second post-transplant native kidney biopsy documented focal segmental glomerulosclerosis (FSGS), chronic thrombotic microangiopathy (TMA) involving glomeruli, and acute tubular injury. After careful review of the clinical history, it was determined that immunosuppressive medications were the most likely etiology for the abnormalities. Both tacrolimus and sirolimus can be associated with a variety of patterns of renal injury; these histologic patterns will be briefly summarized in the following paragraphs.
Nephrotoxicity related to the mammalian target of rapamycin inhibitor (mTORI), sirolimus, was of particular interest because the FSGS and glomerular TMA were identified in biopsy 2 (taken after several months of exposure to sirolimus) but were not present before sirolimus exposure in biopsy 1. In addition, the introduction of sirolimus was associated with increasing proteinuria. Patients taking mTORI are at increased risk for proteinuria in renal allografts and native kidneys. FSGS lesions have been described in patients with sirolimus-associated proteinuria and are thought to be due to direct podocyte injury. Alterations in vascular endothelial growth factor (VEGF) and Akt pathways have been shown in cultured podocytes after sirolimus exposure, which could potentially interfere with podocyte survival, differentiation, and adhesion.

Sirolimus-induced TMA has been reported. In contrast to other types of TMA, loss of glomerular VEGF expression has been documented in renal biopsies with sirolimus-induced TMA. Because VEGF production is required for endothelial cell survival, mTORI-induced disruptions in the VEGF pathway could make endothelial cells more susceptible to endothelial injury and TMA. In biopsy 2, the chronic/smoldering TMA is manifested histologically by subendothelial expansion with glomerular basement membrane duplication, resulting from persistent endothelial cell injury. It is interesting to note that this pattern of glomerular injury is identical to what is seen in transplant glomerulopathy (TG) in renal allografts. TG is commonly due to chronic antibody-mediated rejection (AMR) in transplant kidneys. However, there is a subset of TG that cannot be explained by AMR, and hepatitis C patients in particular appear to be at increased risk for non-AMR forms of TG.

AKI is another side effect that can be seen in association with mTORI. Some studies have found that AKI is more common in patients who already have background moderate CKD or significant proteinuria before they are exposed to mTORI. In the rare descriptions of mTORI-associated AKI that include renal biopsies, acute tubular injury/necrosis is noted histologically. Sirolimus increases apoptosis and inhibits regeneration of cultured proximal tubular cells. There is evidence suggesting that mTORI impairs recovery from AKI due to other causes, presumably due to inhibition of tubular repair.

Calcineurin inhibitors (CNIs) are well known to cause acute and chronic nephrotoxicity. The CNI tacrolimus potentially contributed to the renal lesions seen in this patient’s biopsies. CNIs are capable of causing tubular injury and dysfunction either through direct tubular cell toxicity or functional toxicity due to vasoconstriction. Isometric cytoplasmic vacuolization of tubular cells is commonly noted in CNI-induced acute tubular injury. However, it is not specific for CNI toxicity and can be seen after exposure to other agents such as hyperosmotic solutions (e.g., radiocontrast, mannitol) and IVIG. There is even a case report of sirolimus toxicity with isometric vacuolization of tubular cell cytoplasm. Thus, it is not certain if the focal tubular cell vacuolization in biopsy 2 was related to CNI use, sirolimus use, or possibly heavy proteinuria. TMA is another potential manifestation of acute CNI toxicity. Intrarenal concentrations of CNI can increase when there is concurrent mTORI use, and patients on combined mTORI/CNI regimens could be at even greater risk for tubular injury and TMA than with either agent alone. Peripheral nodular hyalinization of arterioles, “striped” interstitial fibrosis, and FSGS are features of chronic CNI toxicity. The FSGS in chronic CNI toxicity is thought to be a secondary, adaptive form of FSGS related to glomerular hyperfiltration after significant nephron loss and not due to direct podocyte injury (unlike sirolimus-induced FSGS).

**References**

Case 3 from Evan A. Farkash, MD, PhD – University of Michigan

You are rounding on two of your patients who received kidney transplants from separate deceased donors. Both patients have delayed graft function. Postreperfusion biopsies are standard practice at your institution. You glance at the donor charts and before heading down to review the biopsies.

Clinical History
Case A: A 45-year-old woman with ADPKD received a renal allograft from a deceased donor. The donor is a 46-year-old Caucasian man with a body mass index (BMI) of 19 kg/m², admission creatinine of 0.7 mg/dL, and remote history of bulimia but otherwise limited prior medical care. The donor was found down at home in a pool of blood, imaging revealed a hemorrhagic stroke, and he was declared brain dead. The Kidney Donor Profile Index (KDPI) was calculated at 46%. The recipient’s postoperative course was notable for prolonged delayed graft function requiring intermittent hemodialysis, with a nadir creatinine of 2.8 mg/dL.

Case B: A 44-year-old man with diabetes mellitus (type 2) and hypertension received a renal allograft from a deceased donor. The donor is a 48-year-old African-American male prisoner (CDC high risk) with a BMI of 22 kg/m², admission creatinine of 1.5 mg/dL, and poorly controlled hypertension. The donor was found down in his cell in a pool of emesis, imaging revealed a hemorrhagic stroke, and he was declared brain dead. The KDPI was calculated at 91%. The recipient’s postoperative course was notable for slow graft functioning, requiring a short course of intermittent hemodialysis, with a nadir creatinine of 2.1 mg/dL.

Figure 1. Creatinine trend after transplantation for recipient A and recipient B.

Postreperfusion Renal Biopsy Findings
Case A: There are two cores of renal cortex with 19 glomeruli in multiple sections, of which 6 are globally sclerotic (32%). Nonsclerotic glomeruli show focal mild glomerulomegaly. There is widespread moderate mesangial expansion and mesangial hypercellularity, with focal nodularity best seen on hematoxylin and eosin (H&E) staining. Tubules show a diffuse loss of brush borders with epithelial flattening. There is prominent fine interstitial fibrosis, as well as segmental and subcapsular coarse fibrosis and tubular atrophy that involves about 30% of the cortex. Arteries have variable intimal fibrosis ranging from mild to severe.

Case B: There is a single core of renal cortex containing 53 glomeruli in a single Periodic acid-Schiff (PAS) section, of which 6 are globally sclerotic (11%). A single nonsclerotic glomerulus has ischemic type GBM wrinkling and collapse with periglomerular fibrosis; another has perihilar segmental sclerosis. Tubules have a patchy loss of brush borders and epithelial flattening. Fine interstitial fibrosis and tubular atrophy involves about 5% of the cortex. Arteries have severe intimal fibrosis. Arterioles have medial muscular hyperplasia and multifocal hyalinosis.
1. Which component of the donor history is NOT a factor in determining the KDPI?
   A. Cerebrovascular accident
   B. History of hypertension
   C. CDC high-risk status
   D. Creatinine
   E. All of these are components of the KDPI

   **Answer: C**

   Ten donor characteristics are each weighted and used to generate a score called the Kidney Donor Risk Index (KDRI). A donor’s KDRI is compared with all deceased donor KDRI scores in the previous year and assigned a percentile to obtain the KDPI. For example, a donor with a KDPI of 80% has a KDRI greater than 80% of the donors last year. The following factors are used to calculate the KDRI. CDC high-risk status may be a factor when considering whether or not to transplant an organ, but it is not a component of the KDRI.1

   1. Age     6. History of diabetes
   2. Height    7. Cause of death
   3. Weight    8. Serum creatinine
   4. Ethnicity  9. Hepatitis C virus status
   5. History of hypertension 10. Donation after circulatory death status

2. What is the dominant pathologic pattern in donor A’s biopsy?
   A. Arteriosclerosis
   B. Mesangial glomerular expansion
   C. Acute tubular injury
   D. Interstitial fibrosis and tubular atrophy
   E. All of the above

   **Answer: E**

   Donor A’s renal biopsy shows both acute and chronic changes, with diffuse acute tubular injury and moderate glomerular and tubulointerstitial scarring. Some of this may be attributed to moderate to severe renovascular disease. In addition to patchy global glomerulosclerosis, there is also mesangial expansion of unclear etiology. The differential diagnosis includes early diabetic glomerulosclerosis, idiopathic nodular glomerulosclerosis (smoking is a risk factor), and a primary glomerulopathy.

3. What is the dominant pathologic pattern in donor B’s biopsy?
   A. Arteriosclerosis
   B. Mesangial glomerular expansion
   C. Acute tubular injury
   D. Interstitial fibrosis and tubular atrophy
   E. All of the above

   **Answer: A**

   Donor B’s renal biopsy has arterial intimal fibrosis and arteriolar hyalinosis with hyperplasia diagnostic of severe renovascular disease. There is mild glomerular and interstitial scarring, and there is a single glomerulus with perihilar focal segmental sclerosis, but the dominant pathologic change is in the vascular compartment.
Questions for further thought: Both donors are men under the age of 50 who died of hemorrhagic stroke. One of the cases shows discordance between the KDPI and the pathology of the biopsy. Are there elements in the history that may cause the KDRI/KDPI to underestimate or overestimate the risk of graft failure for the recipient of this kidney? Are there features in the biopsy that might provide clues?

DISCUSSION

The current Kidney Allocation Scheme uses longevity matching in an attempt to optimize deceased donor allograft utilization. There are a multitude of immunologic, physiologic, and even sociologic factors that influence patient and graft longevity after transplantation. One important factor that contributes to the duration of renal allograft function is, in a broad sense, the quality of the organ at the time of transplantation. A kidney with inherent significant nephron loss due to donor renovascular or glomerular disease may lack the physiologic reserve to weather the “2K to 1K transition” for an extended period in the setting of a host immune response and immunosuppression-related toxicity.

A variety of studies has assessed the impact of donor factors on short- and long-term graft survival using histologic or clinical/demographic data. Some histologic features, such as glomerulosclerosis and arteriosclerosis, are closely associated with poor long-term graft survival, and composite histologic schemata such as CADI and MAPI have been developed to assess the risk of graft failure. The use of molecular methods to evaluate donors, such as expression profiling of postreperfusion biopsies, is an area of active interest.

However, histologic information may not always be available at the time of the decision to accept an organ, and when present, is typically obtained using a frozen section on a superficial wedge biopsy, which can be biased or unreliable. Therefore, a clinical and demographic assessment of donor risk factors is often a critical factor in organ allocation. The previous system for assessing deceased donor organ quality used a limited set of donor characteristics to create a separate classification of Expanded Criteria Donor (ECD) kidneys with a relative risk factor of >1.7 for early graft failure. ECD kidneys were defined as those from donors older than 60, or donors older than 50 with at least two of three other risk factors, including hypertension, stroke, and creatinine >1.5 mg/dL. The ECD classification expanded the potential donor pool; organs once turned down could be offered to patients further down the waitlist or with comorbidities limiting life expectancy.

However, the categorical ECD classification is somewhat artificial. There is no magical pathologic change in the renal parenchyma on one’s 50th or 60th birthday. Most renal pathologists can tell you stories of the pristine kidney from the 72-year-old donor or the chronically scarred kidney from the 30-year-old donor. The KDPI was developed as an alternative system that addressed donor risk factors using both continuous and categorical scoring. Using Cox regression on donor data from the Scientific Registry of Transplant Recipients on 69,440 recipients of deceased donor renal allografts over a 10-year period, 10 risk factors significantly associated with graft survival were identified. These risk factors were incorporated into a weighted model called the KDRI. By comparing the KDRI to a “median donor” and stratifying the results, the resulting KDPI percentile is a gestalt measure of the risk of early graft failure due to donor factors, where higher percentiles correlate with a higher risk of graft failure.

The strengths and limitations of the KDPI system are represented in the biopsies under comparison.

| Age | 0.077 | 0.102 |
| Height | -0.060 | -0.001 |
| Weight | 0.066 | 0.061 |
| Ethnicity | 0.000 | 0.179 |
| History of Hypertension | 0.039 | 0.126 |
| History of Diabetes | 0.013 | 0.000 |
| Cause of Death | 0.088 | 0.088 |
| Serum Creatinine | -0.066 | 0.110 |
| HCV Status | 0.000 | 0.000 |
| DCD Status | 0.000 | 0.000 |
| SUM | 0.157 | 0.666 |
| KDRI = (e^SUM)/1.22 | 0.96 | 1.59 |
| KDPI | 46% | 91% |

Table 1. An “under the hood” look at the impact of each of the donor demographic and clinical factors in the calculation of the KDPI/KDRI scores. Calculated values are summed, raised to a power of e, and normalized to the median donor score for the prior year (in this case, 1.22).

According to his medical record, Donor B has a long history of poorly controlled hypertension, which manifests in the postreperfusion biopsy as severe arteriosclerosis. Although there isn’t extensive scarring, it may be challenging for the recipient of this graft to maintain adequate perfusion of this graft at normal BPs, and the nadir creatinine of 2.1 mg/dL reflects that. Donor B’s KDRI increases for being older than 40, African American, less than 80 kg, having a history of hypertension, having elevated creatinine, and death from cerebral vascular accident (CVA). The corresponding KDPI score identifies this as a graft at high risk for early failure. As arteriosclerosis has been identified as a histologic risk factor for decreased long-term graft survival, the KDPI is probably an accurate assessment of graft quality in this case.
In contrast, donor A’s biopsy has comparatively more extensive chronic changes involving glomerular, tubular, interstitial, and vascular compartments, despite a much lower KDPI. Donor A’s KDRI increases for being older than 40, less than 80 kg, and death by CVA, but decreases for his height above 170 cm and low creatinine. There are three possible explanations for this clinicopathologic disconnect. First, although the biopsy shows extensive renovascular disease, because of his minimal prior medical care, he did not carry a history of chronic hypertension. Second, in the setting of alcoholism and bulimia, a BMI of 19 kg/m² is suggestive of decreased muscle mass, and his creatinine of 0.7 mg/dL may not be a true reflection of his GFR. Third, mesangial expansion raises the possibility of a primary glomerular disease, such as undocumented diabetes, idiopathic nodular glomerulosclerosis, or IgA nephropathy. The baseline nephron loss and renovascular disease in this kidney portend a poor prognosis, and the recipient’s nadir creatinine of about 3 mg/dL is consistent with the biopsy findings.

The KDPI is not a perfect predictor of graft survival. It has a c-statistic of 0.60, indicating moderate predictive value.1 Recipient and technical factors, such as cold ischemic time, HLA match, rejection, infection, and drug toxicity, can play a much larger role in determining graft longevity once the organ has been transplanted.10 In addition, it’s worthwhile noting that the KDPI is a wholesale predictor of graft failure, and all aggregate populations have outliers. Over the period from 2000 to 2007, about 15% of organs from donors with KDPI values similar to donor A failed within 3 years.10 Overall, the KDPI is an improvement over the prior ECD system. However, in donors with limited prior medical care, it has the potential to underestimate or slightly overestimate the risk of early graft failure due to its reliance on imputed values. In these cases, the histology of the graft may provide additional information. For both of the cases reviewed here, a postreperfusion biopsy was helpful in determining the cause of the patient’s delayed graft failure and provided prognostic value for the nephrologists, surgeons, and patient.

References
The patient is a 59-year-old man with a medical history of type II diabetes mellitus and hypertension, who progressed to ESRD at age 53. He initially commenced peritoneal dialysis, and then received a living-related kidney transplant (3/6 match) at age 54. He was negative for donor-specific antibodies (DSAs) at time of transplant. The graft had immediate function, and baseline serum creatinine was 60–80 µmol/L. Maintenance immunosuppression was tacrolimus, MMF, and prednisone.

Protocol allograft biopsies at 6 months and 2 years after transplant showed no acute rejection (Banff scores all 0).

At 3 years after transplant, the patient developed severe diarrhea, attributed to MMF, which was reduced in dose and ultimately switched to azathioprine. Due to ongoing diarrhea, the calcineurin inhibitor was switched from tacrolimus to cyclosporine A.

A routine screen for DSA at 5 years after transplant detected class II (DQ9) DSAs (MFI, 16,071). Retrospective testing of patient’s serum showed that he was negative for DSA at 3 years after transplant and had become positive for the DQ9 class II DSA at 4 years after transplant (MFI 12,236).

The serum creatinine had been stable at 80–90 µmol/L for the previous 3 years, and there was no significant proteinuria (24-hour urine total protein, 0.09 g).

Medications:
- CsA 75 mg b.i.d.
- Azathioprine 100 mg q.d.
- Prednisone 5 mg q.d.
- Insulin
- Rabeprazole
- Ramipril
- Metoprolol
- Aspirin
- Pravastatin

A renal allograft biopsy was performed on this clinically stable allograft at 5 years after transplant. Serum creatinine at time of biopsy was 86 µmol/L.

Renal biopsy images:
1. Features of acute rejection in this biopsy are found in:
   A. Glomeruli
   B. Tubulointerstitium
   C. Arteries only
   D. Arteries and peritubular capillaries
   E. All of the above

2. The clinicopathological features are consistent with:
   A. T cell-mediated rejection (TCMR)
   B. Antibody mediated rejection (ABMR)
   C. Mixed TCMR and ABMR
   D. Calcineurin inhibitor toxicity
   E. De novo vasculitis

3. Pathological features of acute/active ABMR include:
   A. Microvascular inflammation (glomerulitis and peritubular capillaritis)
   B. Peritubular capillary C4d deposition
   C. Intimal/transmural/necrotizing arteritis
   D. Thrombotic microangiopathy
   E. All of the above
Renal Biopsy Findings

Light Microscopy
There are two cores of renal cortex with up to 11 glomeruli per section, including 1 globally sclerosed. Glomeruli contain very occasional inflammatory cells but not amounting to significant transplant glomerulitis, and there is no evidence of chronic transplant glomerulopathy. The tubular epithelium shows no specific abnormality; in particular, no viral cytopathic changes are present. A very occasional intraepithelial lymphocyte within tubules is identified, but there is no significant lymphocytic tubulitis. There is an occasional focus of calcification. There is no interstitial edema or hemorrhage. There is patchy minimal chronic interstitial inflammation affecting approximately 5% of cortex. There is no significant tubular atrophy or interstitial fibrosis.

Peritubular capillaries show very focal (approximately 10%) mild-to-moderate accumulation of mostly mononuclear inflammatory cells, with occasional marginating neutrophil polymorphs. Immunocytochemical studies showed scattered CD3+ and CD68+ cells in the peritubular capillaries.

Up to four arteries are sampled, two showing no abnormality, one with a focus of mild intimal arteritis, and there is one artery with intimal fibrosis and superimposed intimal mononuclear cell infiltration, focal transmural inflammation, and possible early fibrinoid injury and scattered nuclear debris. The infiltrating intimal mononuclear cells are predominantly CD3+ T cells and scattered CD68+ cells. Arterioles show no hyaline thickening.

Banff Scores
Acute - g0, i0, t0, v3, ptc2
Chronic - cg0, mm0, ci0, ct0, cv1, ah0

C4d Staining
Indirect immunofluorescence and immunoperoxidase staining for C4d was negative in peritubular capillaries.

Renal Biopsy Diagnosis
1. Banff grade III acute rejection with isolated vasculitis (focal transmural and necrotizing arteritis).
2. Focal moderate peritubular capillaritis, C4d negative.

Comment
The presence of v3 vasculitis with early fibrinoid necrosis, focal moderate capillaritis (ptc2), together with the known positive class II DSA, is consistent with subclinical C4d negative acute antibody-mediated rejection.

Clinical Follow-Up
The patient was treated with three monthly doses of IVIG (2 g/kg) and pulse steroids with taper and was converted back to tacrolimus. Serum creatinine remained stable at 85 µmol/L. A post-treatment follow-up biopsy was performed at 5 years 4 months after transplant. The follow-up biopsy showed mild transplant glomerulitis, diffuse moderate peritubular capillaritis, borderline tubulointerstitial inflammation, and focal mild intimal arteritis superimposed on moderate intimal fibrosis. There was 10% interstitial fibrosis/tubular atrophy (IF/TA). There was no evidence of chronic transplant glomerulopathy.

Banff Scores
Acute - g1, i1, t1, v1, ptc2
Chronic - cg0, mm0, ci1, ct1, cv2, ah0

C4d Staining
Indirect immunofluorescence staining for C4d was negative in peritubular capillaries.

The features in the post-treatment biopsy are consistent with Banff grade IIA acute antibody-mediated rejection with concurrent borderline T cell-mediated rejection.

Tacrolimus was discontinued due to recurrence of diarrhea; cyclosporine was restarted. Prednisone was temporarily increased to 20 mg q.d., and then reduced to a maintenance dose of 7.5 mg q.d.

The patient maintained stable graft function over the following 2 years, with the last recorded serum creatinine of 100 µmol/L, and at 8 years after transplant, he died suddenly of cardiac complications, with a functioning allograft.
DISCUSSION

Diagnosis of C4d-Negative ABMR
The diagnosis of acute ABMR in this case is based on the Banff 2013 schema for renal allograft pathology. According to previous Banff schema for renal allograft rejection, deposition of complement split product C4d in peritubular capillary basement membranes, as immunohistologic evidence of antibody interaction with vascular endothelium was a requisite for a definitive diagnosis of ABMR. C4d deposition was required in association with morphologic evidence of acute graft injury (such as microvascular inflammation) and serologic evidence of circulating DSAs.

Both the initial and post-treatment biopsies in this case were negative for peritubular capillary C4d deposition, using both immunofluorescence and immunoperoxidase staining. A number of studies have recognized unequivocal C4d-negative ABMR. Gene expression microarray studies showed that the combination of DSA and endothelial activation, often associated with microvascular inflammation, significantly reduced graft survival, but up to 60% were C4d negative. Protocol biopsy studies in presensitized DSA-positive patients with histologic features of ABMR at 3 months after transplant showed significant progression to transplant glomerulopathy (TG) and IF/TA, even in the C4d-negative group. In a series of presensitized patients with acute ABMR, a minority (<20%) were C4d negative.

Although some of these studies showed a worse outcome in C4d-positive groups, a number of recent studies have shown that early microvascular inflammation (glomerulitis and peritubular capillaritis) is a more robust prognostic indicator than C4d status for later development of chronic ABMR (TG and chronic arteriopathy) and eventual graft loss, in both preformed and de novo DSA-positive patients. In a large retrospective study, diffuse peritubular capillaritis was found to be an independent risk factor for graft loss.

According to the Banff 2013 schema, a minimum threshold of moderate microvascular inflammation (Banff scores g + ptc ≥2) is sufficient evidence of current or recent antibody interaction with vascular endothelium, in the absence of peritubular capillary C4d deposition. In the presence of concurrent acute TCMR or borderline infiltrates, ptc2 alone is not sufficient to define moderate microvascular inflammation, and glomerulitis (g ≥1) must also be present. In the initial biopsy in this case, there was no glomerulitis (g0), and only focal moderate peritubular capillaritis (ptc2), but there was no evidence of concurrent TCMR. In the post-treatment biopsy, there was mild glomerulitis (g1) and diffuse moderate peritubular capillaritis (ptc2), along with borderline TCMR (i1,t1). Both the initial and post-treatment biopsies in this case meet the Banff 2013 criteria for the diagnosis of C4d negative ABMR.

The DSA in this case was a class II HLA antibody (DQ9). Many studies have made the observation that de novo DSA are predominantly directed at class II donor HLA mismatches; in our studies in Winnipeg, HLA mismatch (especially DR and DQ) was an independent predictor of de novo DSA.

Subclinical ABMR
This case of acute ABMR in a clinically stable allograft illustrates the reality of subclinical ABMR. Subclinical TCMR with tubulointerstitial infiltrates has long been recognized following the pioneer protocol biopsy studies of David Rush and colleagues. In recent years, subclinical ABMR has also been observed, in both presensitized patients and unsensitized transplants that develop de novo DSA. Temporal progression from subclinical ABMR to clinically evident ABMR has been documented.

Subclinical ABMR has been shown to significantly increase graft loss and warrants therapeutic intervention, although the best treatment strategies remain unclear. This highlights the importance of performing surveillance/protocol biopsies on clinically stable allografts, particularly in transplants at high risk of ABMR, or if DSA is detected on screening, to recognize early microvascular inflammation as a consequence of active antibody-mediated injury.

Vasculitis Lesions in ABMR
This case illustrates the presence of vasculitis in the clinicopathological setting of ABMR, with no histologic evidence of TCMR in the initial biopsy. One of the arteries in the initial biopsy in this case showed transmural arteritis with a possible focus of early fibrinoid necrosis (Banff score v3). Traditionally, intimal arteritis lesions in allograft biopsies were regarded as likely cellular rejection (TCMR), and vasculitis lesions had to show evidence of fibrinoid necrosis to be considered morphologic lesions of acute ABMR.

Intimal arteritis lesions are now being increasingly recognized as a manifestation of antibody-mediated graft injury, with a significantly higher rate of graft loss in ABMR cases that have intimal arteritis compared with ABMR without intimal arteritis. These arterial intimal lesions can show a predominance of infiltrating CD68-positive macrophages over CD3-positive T cells, which is also a feature of the microvascular inflammation in ABMR. Based on these observations, intimal arteritis/vasculitis lesions (Banff score v > 0) are now included in histologic evidence of acute/active ABMR according to the Banff 2013 schema. It is recognized that these intimal arteritis lesions may be indicative of ABMR, TCMR, mixed ABMR/TCMR, and in some cases may not represent definitive rejection.

Isolated Vasculitis Lesion
The initial biopsy in this case showed a severe vasculitis lesion (Banff score v3) in the absence of significant tubulointerstitial inflammation (Banff scores i0,t0), which meets the criteria for what has been referred to as “Isolated V Lesion” or “Isolated Endarteritis.” A recently published single centre study showed worse graft survival for transplants with isolated vasculitis compared with pure tubulointerstitial TCMR. A multicenter
In summary, this case shows that renal allograft pathology consistent with antibody-mediated injury can occur in patients with de novo DSA and stable graft function. Our longitudinal studies in Winnipeg\(^{10–12}\) have shown that the degree of injury can progress despite augmented immunosuppression, and when cellular rejection coincides with de novo DSA and antibody-mediated injury, it may accelerate the time to graft dysfunction and graft loss.

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Dr. C. Wiebe provided detailed clinical information.

**References**

Case 5 from Serena Bagnasco, MD – Johns Hopkins University

The patient is a 26-year-old white man with ESRD secondary to medullary cystic disease and juvenile nephronophthisis (no history of hypertension or diabetes as cause of ESRD).

He had two previous failed transplants: the first at age 12 from his mother in 2001, which lasted until 2010. Afterward, he was placed on hemodialysis for 4 years.

He received a second 6 antigen match transplant from a pediatric deceased donor in 2014 at an outside institution, complicated by primary nonfunction for unclear causes and requiring transplant nephrectomy after 5 weeks. By then, he had become highly sensitized and was enrolled in the incompatible transplant program at JHH. His PRA was 89%, and he had evidence of anti-angiotensin II type 1 receptor (AT1R) antibodies.

He received his third kidney transplant on December 5, 2014 from an ABO-incompatible living unrelated donor (he is type A and the donor was type AB), with mismatches for HLA Bw4, B55, B63, and Cw4. Complement-dependent cytotoxic (CDC) crossmatch was negative for donor T and B cells. Endothelial cell crossmatch was positive. There was very weak positivity for anti-B53 DSA at transplantation. Prior to transplant he had received two sessions of total plasma exchange (TPE) and rituximab. After transplant, he underwent six TPE procedures and received thymoglobulin and rituximab. His creatinine at discharge was 1.6 mg/dL.

Medications:
- Prednisone 20 mg/day
- Tacrolimus 2.5 mg/day
- mycophenolate 2,000 mg/day
- Labetalol 500 mg/day
- Clonidine 0.3 mg × 3/day for hypertension

There was never any issue with compliance.

On December 31, 2014 (4 weeks after transplant), his creatinine increased to 2.6 mg/dL (BUN, 32 mg/dL; K, 5.4 mEq/L; Na, 141 mEq/L; Hg, 10.3 g/dL; Ht, 32.5%; WBC, 4,880; tacrolimus within therapeutic range; urine protein 1+, urine culture negative, antibody screening pending.

A biopsy was performed.
1. Which is the least likely diagnosis in this biopsy?
   A. Acute T cell-mediated rejection Banff type IIA
   B. Acute rejection with mixed T cell-mediated and antibody-mediated components
   C. Acute rejection Banff type III
   D. Acute antibody-mediated rejection

2. When donor and recipient are incompatible for both ABO and HLA antigens, positive C4d in a kidney allograft is always a reliable indicator of antibody-mediated rejection.
   A. True
   B. False

3. If testing of a serum sample taken at the time of biopsy was to show the absence of detectable anti-ABO and anti-HLA donor-specific antibodies, it would rule out antibody-mediated injury in the graft.
   A. True
   B. False

DISCUSSION

Renal Biopsy Findings

Light Microscopy
The biopsy includes two cores of predominantly cortical renal parenchyma. There are approximately 18 glomeruli per section, none globally sclerosed. The glomeruli show sparse marginating leukocytes (g1) and no evidence of double contours. The tubules show focal apical blebbing, occasional mild vacuolization, focal dilatation, and flattening. There is no evidence of tubulitis (t0). The interstitium show diffuse interstitial edema and interstitial inflammation including mononuclear leukocytes and several eosinophils focally up to 30/HPF. Focal extravasated red blood cells are also noted. One small area shows fibrin, but it is not clear whether it is associated with any specific structure. There is minimal to mild interstitial fibrosis and tubular atrophy (ci0, ct0). The peritubular capillaries show diffuse mononuclear leukocyte margination highlighted by CD68 staining, and some show congestion (ptc3). Approximately five arteries per section are present; two are up to arcuate size. One shows few leukocytes under the endothelium, indicating very mild arteritis (v1), and the endothelium shows focal reactive changes. There is mild to moderate fibrointimal thickening (cv1-2), and no arteriolar hyalinosis is noted.
Immunofluorescence
Indirect immunofluorescence on frozen tissue shows the following:
C4d positive (up to 2+) linear staining in approximately 50% of the peritubular capillaries and 1–2+ staining in five glomeruli.
C3d does not stain the peritubular capillaries.

Electron Microscopy
Unremarkable

Diagnostic Interpretation
Interstitial inflammation (i1-3) without obvious tubulitis (t0), and mild intimal arteritis (v1): meets criteria for cell-mediated rejection IIA.
Several interstitial eosinophils (up to >30/ HPF): rule out possible drug reaction.
Sparse few marginating leukocytes in the glomerular capillaries (g0-1).
Diffuse margination in peritubular capillaries (ptc3), with focal positive C4d (C4d2), and negative C3d, concerning for antibody-mediated rejection: correlate with DSA.
mild to moderate arteriosclerosis (cv1-2) (g0-1, i2-3, t0, v1, ah0, cg0, ci0, ct0, mm0, cv1-2, C4d2, C3d0, ptc3, ti3).
Serum taken at the time of biopsy was negative for anti-HLA DSAs, and negative for anti-B (ABO) DSAs.
There was evidence of positive anti-AT1R antibodies: 37.6 U/mL (positive: >17; negative: <10 U/mL; borderline: >10 but <17 U/mL).

This patient was developing anti-AT1R antibody-mediated injury, associated with T cell-mediated rejection.
He was treated with a 3-day course of methylprednisolone with reduction of creatinine to 1.7 mg/dL. Subsequently, Losartan was added at 25 mg/day.

The clinical course after the biopsy is briefly summarized below:

At 8 weeks after transplant
- his creatinine increased to 2.7 mg/dL.
- a biopsy showed very mild interstitial inflammation (i0-1), no tubulitis (t0), no arteritis, no glomerulitis (g0), but persistent margination of CD68-positive cells in peritubular capillaries (ptc3) and diffusely positive C4d (C4d3, C3d0)
- serum was negative for anti-HLA DSAs but strongly positive for anti-AT1R (>40)
- TPE was started for AMR, resulting in a decline in creatinine to 2.1 mg/dL

At 11 weeks after transplant
- proteinuria was increased (UPCR: 0.26)
- a biopsy showed persistent margination (ptc3), C4d 2–3+, C3d0
- electron microscopy showed moderate, focally marked foot process effacement
- TPE continued

At 6 months after transplant
- creatinine was 3.7 mg/dL
- proteinuria was increased (UPCR: 0.35)
- Anti-AT1R was borderline 13 U/mL, on TPE, with no anti-HLA DSAs
- a biopsy showed glomerular focal deflation/consolidation (ischemic? FSGS?), mild glomerulitis (g1), double contours (cg2), (ptc2-3), negative C4d (C4d0, C3d0), and moderate to marked tubulointerstitial scarring (ci2-3, ct2-3), (cv2)
- electron microscopy: segmental widening of the subendothelial space. Podocyte partial foot process effacement ~40%–50%. Thickening and segmental multilayering of the basal lamina in the ptc.
- = changes suggestive of chronic active AMR, possible FSGS

At 7 months after transplant
- creatinine was 11 mg/dL
- proteinuria persisted
- anti-AT1R was increased to 17.7 U/mL, with no anti-HLA DSAs
- biopsy showed acute glomerular microangiopathic changes, chronic focally severe glomerular ischemic changes/collapse, transplant glomerulopathy (cg3); chronic transplant vasculopathy (cv3, v0, ah0), mild interstitial inflammation (i1, t0, ti1); moderate to severe tubulointerstitial scarring (ci2-3, ct2-3)
- IF: diffusely positive C4d in peritubular capillaries C4d3 (ptc0)
- electron microscopy: diffuse multilayered widening of the subendothelial space
- = severe acute and chronic endothelial injury, acute and chronic AMR likely resulting from persistent elevated level of anti-AT1R antibodies
This case is a good reminder that antibody-mediated injury in the renal allograft of sensitized patients is not always due to anti-HLA or anti-ABO DSAs.

The diagnostic interpretation of the biopsy presented is complex. Interstitial inflammation and mild arteritis meet criteria for cell-mediated rejection type IIa. In the kidney allograft, arteritis can occur in T cell-mediated rejection, in antibody-mediated rejection, or in the absence of other features of rejection as "isolated arteritis."1–4 The diffuse margination in PTC and the presence of anti-AT1R antibodies argue for a component of antibody-mediated rejection, despite very mild glomerulitis. This patient was ABO-incompatible with the donor, and positive C4d staining in the peritubular capillaries was not helpful because it is usually seen in ABO-incompatible kidney allograft biopsies in the absence of rejection.5 In this case, staining for C3d was negative in PTC, which is not supportive of complement deposition due to AMR. Although there was no evidence of anti-HLA/ABO DSAs, the high level of anti-AT1R antibody was consistent with the presence of antibody-mediated injury. The clinical course after this biopsy shows progression within 6 months with persistent microcirculation inflammation and development of early transplant glomerulopathy. The podocyte injury does not represent recurrent FSGS, but more likely represents a new lesion.

There is increasing evidence that antibodies toward non-HLA endothelial antigens may have a detrimental effect on graft survival,6,7 including those directed toward the angiotensin II type 1 receptor (AT1R).8 AT1R is expressed on multiple cell types including endothelial and immune cells and podocytes. Activating antibodies toward AT1R as mediators of refractory vascular rejection were first described by Dragun et al. in 2005.8 The mechanism of action of these antibodies is not mediated by complement cytotoxicity. The AT1R antibodies bind to the extracellular portion of the AT1 receptor and activate the Erk1/2 signaling pathway, leading to vascular endothelial dysfunction and pro-inflammatory cytokine production. Several independent studies confirmed a negative impact of anti-AT1R antibodies on graft survival. Pretransplant sensitization against AT1R and anti-AT1R antibody titer >10 U/mL were associated with increased risk of acute rejection and graft failure.9 De novo anti-AT1R antibodies were found to be an independent predictor of graft failure.10 Anti-AT1R antibodies can precipitate acute vascular rejection in the absence of anti-HLA DSA,11 Association of AT1R antibodies and accelerated rejection with renal allograft thrombosis has been described.12 A recent study reported C4d-negative antibody-mediated rejection in 11 patients with anti-AT1R but no anti-HLA DSA.13 In these patients, therapeutic resolution of the rejection episodes was accomplished mostly by plasma exchange and ARB treatment. Recently anti-AT1R antibodies have been implicated in the occurrence of post-transplant FSGS. Alachkar et al. reported one case of AMR and collapsing glomerulopathy in a kidney allograft protocol biopsy, accompanied by proteinuria, which resolved with plasmapheresis and losartan.14 In a series of 28 transplant recipients with a history of FSGS, pretransplant anti-AT1R antibodies were associated with an increased risk of FSGS recurrence.15

References