

Case 1 – Presented by David Cimabluk, MD and Melvin Schwartz, MD

CASE SUMMARY

Patient is a 31-year-old Caucasian man with no significant past medical history who presented with proteinuria of at least two years duration, nephrotic range at present. Notable laboratory data: BUN-17, Cr-1.6, Cholesterol-221, LDL-143, triglycerides 224
Urinalysis- SG 1:010, pH 5.0, 3+ protein, no abnormal sediment
Urine protein/creatinine ratio-8.6 g/g

The family history is significant for hypertension, heart disease, and hypercholesterolemia. His mother and maternal grandfather have polycystic kidney disease, and his mother has two brothers with polycystic kidney disease. The patient's mother also has a sister who is diagnosed with FSGS.

Renal biopsy showed an amorphous material occluding almost all glomerular capillaries. The material appears weakly eosinophilic on hematoxylin and eosin (H&E) stain, weakly periodic acid-Schiff (PAS) positive, and pale blue with Masson trichrome. The material stained positive for neutral lipids (positive oil red O stain), consistent with lipoprotein thrombi. At least half of the glomeruli contained small synechiae between the glomerular capillaries and Bowman's capsule. In addition, the Jones methenamine silver stain highlighted diffuse complexity of the peripheral glomerular capillary walls with wrinkling and double contouring. Interstitial fibrosis and tubular atrophy was widespread, involving 40% of the cortical tissue.

The immunofluorescence showed nondiagnostic glomerular segmental linear deposits of IgM (trace to 1+), kappa and lambda light chains (trace to 1+), C3 (2+), C1q (trace), and fibrin (trace). The glomerular capillary lumens were negative for all immunoglobulins, complement components, and fibrin. Electron microscopy showed lamellated thrombi in the capillary lumens. A diagnosis of lipoprotein glomerulopathy was rendered.

Apolipoprotein E (apoE) was sequenced with the use of genomic DNA from the patient and his available family members. The patient had a heterozygous C→T transition in exon 3 of apoE that changed the amino acid at position 25 of the mature protein from arginine to cysteine (apoE Kyoto mutation). Family genotyping showed that this mutation was transmitted through the maternal side of the family.

REVIEW

Lipoprotein glomerulopathy is a rare hereditary disorder that is associated with severe proteinuria and progression to renal failure. Lipoprotein glomerulopathy is characterized by the presence of lipoprotein thrombi in glomerular capillaries, an abnormal plasma lipoprotein profile that resembles type III hyperlipoproteinemia, and a marked increase in serum apolipoprotein E (apoE) concentrations. The condition was initially described in the review of renal lipidoses by Faraggiana and Churg (1987), and the designation “lipoprotein glomerulopathy” was later coined by Saito et al (1989) in their report of lipoprotein glomerulopathy affecting a 57-year-old woman.

GEOGRAPHIC DISTRIBUTION OF REPORTED CASES

Since the original report by Saito et al, lipoprotein glomerulopathy has been increasingly recognized, mainly affecting people of Japanese and Chinese origin. Very few cases have been reported in the United States and Europe. In a 2006 publication summarizing previously reported cases of lipoprotein glomerulopathy, a total of 51 cases involving 5 countries had been reported: Japan – 32 cases, China – 12 cases, Taiwan – 4 cases, France – 2 cases, United States – 1 case (patient was of Chinese ancestry). Additional cases continue to be reported, particularly in China at an accelerated rate (China – 16 cases, 2008; China – 13 cases, 2009; Hong Kong – 1 case, 2009; United States – 2 cases, 2007).

CLINICAL FEATURES

The usual presentation of lipoprotein glomerulopathy includes proteinuria or nephrotic syndrome, with moderate renal impairment and progression to chronic renal failure in most cases. Hematuria is rarely present. Age at onset varies widely, from 4 to 69 years (mean age 32 years), and male-female ratio is approximately 3:2.

Plasma levels of apoE and apoE-containing lipoproteins (total cholesterol, low-density lipoprotein cholesterol, triglycerides) are elevated, similar to familial type III hyperlipoproteinemia. However, lipoprotein glomerulopathy lacks the systemic manifestations of hyperlipidemia seen in type III hyperlipoproteinemia, such as cutaneous xanthomas and accelerated atherosclerosis. Furthermore, the genetic mutations differ in that lipoprotein glomerulopathy is associated with several novel

polymorphisms of the apoE gene (described below), while type III hyperlipoproteinemia is associated with homozygosity for the apoE-2 isoform.

PATHOLOGIC FINDINGS

Histopathologic examination provides the most important information for the diagnosis of lipoprotein glomerulopathy. The glomeruli are large and contain capillaries distended with lipoprotein thrombi, which have a vacuolated and laminated structure when viewed under high magnification. On hematoxylin and eosin (H&E) stain, the lipoprotein thrombi do not stain well and appear weakly eosinophilic. The lipoprotein thrombi are weakly periodic acid-Schiff (PAS) positive, pale blue with Masson trichrome, and stain positive for neutral lipids with oil red O stain. Immunoperoxidase stains show that the glomerular thrombi contain β -lipoprotein, apoB, and apoE. IgM, C1q, and fibrinogen often surround the thrombi by immunofluorescence. Electron microscopy shows the glomerular capillary lumina to be filled with partially lamellated, finely vacuolated lipoprotein thrombi.

The differential diagnosis includes other pale, eosinophilic, acellular material that can cause engorgement of the glomerular capillary lumina, including fibrin thrombi, immune deposits, or amyloid. Fibrin thrombi and immune deposits typically stain fuchsinophilic (red) with Masson trichrome stain, while the lipoprotein thrombi stain pale blue. The lack of congophilia excludes amyloid. The differential diagnosis also includes renal disease caused by type III hyperlipoproteinemia. Renal biopsy shows large numbers of foam cells in the glomerular mesangium and distending glomerular capillaries in patients with type III hyperlipoproteinemia, a histologic feature not described in lipoprotein glomerulopathy.

GENETICS

The mode of inheritance is not precisely known, but most reports suggest lipoprotein glomerulopathy is transmitted as autosomal dominant with incomplete penetrance. Mutations in the apoE gene have been shown to have a pathogenetic role in lipoprotein glomerulopathy. Reports have shown that patients can harbor mutations in the apoE gene and show no clinical evidence of lipoprotein glomerulopathy. Furthermore, one interesting case has been reported involving the aunt of a patient with lipoprotein glomerulopathy. The aunt was an unaffected heterozygous carrier of an apoE mutation, and the aunt's nephrectomy specimen which was removed for renal cell carcinoma showed amorphous material that was identical in appearance to lipoprotein thrombi in approximately 1% of glomeruli. On the basis of this difference in the extent of glomerular involvement between patients with lipoprotein glomerulopathy and an

unaffected carrier, it has been postulated that a “second hit” is required for lipoprotein glomerulopathy to develop.

At present, several apoE mutations have been discovered in patients with lipoprotein glomerulopathy. These include apoE Kyoto (Arg25Cys), apoE Sendai (Arg145Pro), apoE Tokyo (Leu141 to Lys143del), apoE1 (Gln156-Gly173→0), apoE Chicago (Arg147Pro), apoE Okayama (Arg150Gly), apoE Tsukuba (Arg114Cys), and apoE Hong Kong (Asp230Tyr).

PATHOGENESIS

There presently is no consensus on how mutated apoE causes deposition of lipids in the glomerulus, or how deposited apoE causes glomerular damage. It is hypothesized that mutations of the apoE gene produce changes in the apoE protein structure, which leads to aggregated deposits of lipoproteins that have high affinity or low clearance in glomerular capillaries. Furthermore, several of the apoE mutations in lipoprotein glomerulopathy have a point mutation in the apoE gene at a position that is in the LDL receptor-binding site. Research has shown that mutant apoE is defective in binding to the LDL receptor, but exhibits enhanced binding to the glomerulus, contributing to the development of lipoprotein glomerulopathy.

TREATMENT

There have been no randomized, controlled studies examining the different therapeutic approaches for lipoprotein glomerulopathy. The efficacy of different therapeutic agents has only been discussed in case reports. Regular treatments for patients with nephrotic syndrome, e.g., steroids, immunosuppressants, and anticoagulants, have been reported to be ineffective. However, recent reports have shown that intensive therapy using lipid-lowering agents resulted in clinical remission with histological resolution. In these studies, complete disappearance of lipoprotein thrombi was shown in serial renal biopsies, in addition to decreases in serum cholesterol, triglyceride, and apoE levels.

Half of reported patients of lipoprotein glomerulopathy have developed renal failure at 1 to 27 years after onset. Renal transplantation has been reported in 4 patients with lipoprotein glomerulopathy, and the disease recurred in all transplanted kidneys after an average time interval of 7 months. In two of the four cases, recurrent disease has caused graft loss. In the other two cases, recurrent disease has been associated with a steady decline in renal function associated with significant proteinuria.

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CNC Renal Biopsy: Clinical Correlation Sessions

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CASE PRESENTATION: HISTORY – CASE 2

The patient is a 42-year-old African-American female who presented initially in October 2003 (at age 37) with painful swelling in her knuckles, three miscarriages and a positive ANA, later diagnosed as SLE. She was treated with Plaquenil. In February 2004 she delivered a child prematurely with pre-eclampsia, having a creatinine level of 1.0mg/dl, serum albumin 2.4gm/dl and 3+ proteinuria. Patient did not follow up and was on Plaquenil 200mg/day and Hydrochlorothiazide for hypertension.

In February 2008 she developed progressive malaise, arthralgias and fever for 10 days following discontinuation of Plaquenil. She was hypertensive with creatinine of 4.4mg/dl. She has a strong family history of SLE. Physical exam revealed a relatively obese female – BP 113/78, pulse 74, weight 188lbs, focal alopecia, hyperpigmentation of face and neck and diffuse erythema across the trunk and both thighs. No changes in other systemic exam. The lab findings are as follows: BUN/Cr 81/4.0mg/dl, potassium 4.0, HCO₃ 22, glucose 155mg/dl, calcium 7.1mg/dl, albumin 2.4gm/dl, globulins 3.7gm/dl, CK 1540. CBC: Hgb 9.9gm/dl, Hct 28.3, nl WBC and platelet count. U/A: 4+ protein, 2-5 WBC, 2-5 RBC, occasional granular casts, unremarkable renal sonogram. Serology: ANA 1086, dsDNA 85, C3 69 (90-180), C4 22 (9-26), CH50 (22-60).

Differential diagnosis based on clinical data

This clinical presentation is typical of an active systemic lupus erythematosus with renal disease manifesting acute renal failure, nephrotic range proteinuria and microscopic hematuria. This suggests a range of lupus glomerular lesions, particularly a proliferative class of lupus with crescents, necrotizing features or vascular lesions. The clinical presentation can exclude thrombotic microangiopathy (or a TTP-like syndrome) or a florid vasculitic syndrome. Rarely other forms of renal disease or glomerular lesions have to be considered, which may or may not be related to SLE.

Pathological findings

Light Microscopy: The renal biopsy consisted of three cores of cortex containing up to forty-five (45) glomeruli in multiple levels of paraffin sections, two (2) of which were globally sclerosed. While nine (9) glomeruli showed segmental to partial capillary tuft collapse with sclerosis, focal capsular adhesion and hyperplastic epithelial cells in the Bowman space, six (6) displayed global collapse covered circumferentially by prominent epithelial cells with occasional vacuolization and protein droplets. The rest of the glomeruli showed no significant changes, except for mild mesangial hypercellularity and matrix increase. No proliferative, infiltrative or necrotizing lesions or crescents were identified. Only small areas of tubular atrophy with minimal interstitial inflammation and fibrosis were present, affecting about 15-20% of the cortical tissue examined. The

proximal tubular cells were engorged with numerous protein resorption droplets suggesting severe proteinuria. The small arteries and arterioles showed mild medial hypertrophy.

Immunofluorescence microscopy: Renal cortical tissue containing three to five (3-5) glomeruli revealed non-specific granular 2+ C3 staining, mainly in the glomerular mesangial areas and focally in the capillary walls. No staining for immunoglobulins (IgG, IgA, IgM), complement component C1q or light chains (lambda, kappa) was noted in the glomeruli or tubulo-interstitial compartment.

Electron microscopy: Tissue submitted for electron microscopy contained three (3) glomeruli, two (2) of which also showed segmental and global capillary tuft collapse. On EM of two (2) glomeruli, the capillary basement membranes in the preserved portions were of normal thickness and regular contour (350-400nm). The mesangial areas showed mild increase in matrix with a suggestion of occasional, small granular electron dense deposits. The other portions of the glomeruli had extensive wrinkling and collapse of the capillaries with focal separation of the overlying visceral epithelial cells. The foot processes were totally effaced and the visceral epithelial cells are large, vacuolated and increased in number in the Bowman space. The adjacent endothelial and mesangial cells disclose mild swelling and cytoplasmic vacuolization. Tubulo-reticular inclusions are not readily identified in the endothelial areas.

Pathological Diagnosis

- Focal segmental and global collapsing glomerulopathy.
- Evidence of tubular injury and simplification.
- Mild arteriosclerosis.
- No proliferative or significant immune-complex glomerular lesions seen.

Treatment and follow-up

The renal failure at this point was thought to be due to tubular injury secondary to prerenal hemodynamic factors in the setting of massive proteinuria. The patient received pulse steroids and Mycophenolate Mofetil along with supportive therapy and the creatinine decreased to 2.0mg in 2 weeks. She was later maintained on Plaquenil and a antihypertensive agent. The patient developed acute renal failure and anemia 4 months later with high ANA titer (1:1200), anti-dsDNA and other auto-antibodies. A repeat kidney biopsy at this time disclosed further progression of the renal parenchymal disease with increased globally collapsed glomeruli with typical features of collapsing glomerulopathy as well as globally sclerosed glomeruli, extensive tubular atrophy, simplification, microcystic change and interstitial fibrosis. This was superimposed by acute tubular injury of the residual tubules resulting in a creatinine level of 11.0mg/dl, most probably related to hemodynamic disturbances, but resolved to 1.5mg/dl. Due to onset of pericarditis, a month later she was given Prednisone and Immuran, however her creatinine progressively rose to 4.6mg and creatinine clearance declined to 21ml/min leading to irreversible renal failure 6 months later.

Discussion

In a young female with SLE, a range of immune complex mediated glomerular lesions as described in the ISN/RPS Classification of Lupus Nephritis (1) may be noted. In addition, concomitant or isolated active tubulo-interstitial disease and vascular lesions (lupus vasculopathy, thrombotic microangiopathy with or without phospholipid antibodies) and rarely necrotizing lupus vasculitis can cause renal insufficiency or failure (2, 3). The occurrence of other forms of clinically significant renal disease in SLE do not fall under the above entities. These morphologic/histologic renal lesions may or may not be pathogenetically related to SLE (4). Moreover, predisposition for other renal lesions, as in the general population, is not diminished in the setting of SLE. Such cases also expand the indications for performing renal biopsies in patients with history of SLE and emphasize its value of accurate diagnosis. Additionally, renal biopsies in SLE also yield prognostic information and help in appropriate therapeutic decision making.

In this patient with an established active systemic lupus erythematosus with acute renal insufficiency and nephrotic syndrome, instead of the expected finding of an immune complex mediated glomerular lesion (although the complement C3 level was not significantly low and urinalysis was generally bland), collapsing (segmental and global) glomerulopathy accompanied by acute tubular injury was noted. These findings are unusual and all other known causes of CG have been excluded in this case along with negative viral serologies (HIV, HCV, parvovirus).

Collapsing glomerulopathy (CG) is a distinctive morphologic glomerular lesion characterized by segmental and/or global wrinkling and collapse of the capillaries without significant increase in intraglomerular cellularity or matrix and conspicuous epithelial cell hyperplasia showing marked reactive changes, increased mitosis, vacuolization and varying numbers of lysosomal protein resorption droplets. These cells are often found capping the collapsed capillaries and filling the Bowman space, sometimes mistaken for cellular crescents (5-7).

CG has been regarded as a "malignant" or an aggressive form of focal segmental glomerulosclerosis (FSGS), affecting mainly African-American patients with massive proteinuria and renal insufficiency (8). Compared to the other variants of FSGS, the progression of CG to end stage renal disease (ESRD) is rapid, occurring within 12-18 months from the time of diagnosis. Most often, this is resistant to the usual forms of immunosuppressive therapy (9). This is due to a severe form of podocytic injury leading to a dysregulated phenotype with loss of differentiation antigens such as WT-1, podocytic proteins (synaptopodin, podocin), expression of PAX2, abnormal cell cycle regulation (P27, P57) and new expression of cell proliferative and macrophage markers as well as involvement of the parietal cells (5, 7, 10, 11). Varying degrees of CG is also a common feature in HIV associated nephropathy, particularly in the African-American population accompanied by microcystic tubular changes, active and chronic tubulo-interstitial changes (12).

Since then, a number of disease processes with diverse pathogenetic mechanisms causing podocytic injury that are associated with CG have emerged, suggesting this to be another unique pattern of glomerular injury with multiple categories of etiologic factors (5). A familial form of CG with a possible genetic predisposition has also been reported, perhaps with an environmental trigger (13). Other forms include medications,

autoimmune diseases, infections, ischemic insult, hematologic malignancies and specific genetic mutations (4, 5, 14).

Glomerular lesions described as focal segmental or global CG have been identified in patients with SLE and mixed connective tissue disease presenting with non-nephrotic to nephrotic range proteinuria and varying levels of renal insufficiency (4, 14-16). A review of the reported cases further showed that a majority of cases of CG associated with autoimmune disease states or merely have positive ANA, progressed to end stage despite immunosuppressive therapy (15), as is generally observed in idiopathic CG (5, 6). Even though a few responded initially to therapy as in this case for a few months, almost all of them entered chronic renal disease. Glomerular proteinuria secondary to a podocytopathy manifesting as minimal change disease or FSGS with extensive or substantial foot process effacement are not uncommon in some SLE patients, which often respond to steroid therapy (16, 17). In a proportion of these patients, the use of non-steroidal anti-inflammatory drugs have been implicated (17). This spectrum of podocytopathies in SLE demonstrate minimal or no glomerular immune-complex deposition and are limited to mild to moderate mesangial proliferation (13-17). A timely renal biopsy in SLE patients, based on the usual indications, can identify these lesions of podocytic injury as they carry prognostic and therapeutic significance.

Relationship to SLE

A commonly asked question is: are these glomerular lesions pathogenetically related to lupus or merely occur as coincidental renal lesions? Most of the reported patients with SLE or auto-immune diseases manifesting CG presented with a relatively high active SLE disease activity index, suggesting a direct role of active SLE. The clinicopathological features of both HIV and non-HIV associated CG due to other causes and idiopathic CG appear to be relatively similar except for a racial predilection in HIV+ and idiopathic CG and a somewhat better response to therapy or reversibility, at least initially in some etiologic groups (5). Additionally, African-American patients with SLE have a higher incidence of renal disease and, importantly, the more severe lesions. Thus with two different diseases having a higher incidence in an African-American patient (idiopathic collapsing glomerulopathy and SLE), it may not be entirely possible to predict a positive relationship. Apart from the temporal relationship of active SLE and development of CG, in the absence of other secondary causes, a potential link to immune-mediated podocytic injury and circulating inflammatory mediators further strengthen this notion. Kraft et al and Hertig et al raise the issue of concurrent T cell activation along with B cell mediated antibody formation and elaboration of cytokines in SLE as a means of podocytic injury, resulting in a spectrum of podocytopathies (18, 19). Previously, an immunohistochemical study of the detached podocytes in idiopathic collapsing glomerulopathy revealed loss of normal podocyte phenotype and acquiring macrophage differentiation antigens (20). More recently, in a review by Barisoni and Nelson, experimental evidence has shown that inflammatory and antibody mediated injury of podocytes as well as hyperplastic podocytes with macrophage epitopes elaborating inflammatory mediators, may all trigger capillary collapse and pseudocrescent formation in the Bowman space (21).

Glomerular podocytes have been the target of injury in various forms of glomerulonephritides including lupus nephritis in children (22). These podocytes sustaining immunological injury manifest a variety of morphologic and immunophenotypic changes including detachment from the basement membranes, frequently detected in urine sediments and suggesting that they could serve as markers of disease activity in lupus nephritis (23). The mechanism of podocytic injury causing collapsing glomerulopathy in the absence of significant immune-complex mediated glomerular lesions in SLE is unclear at the present time. It may be as a result of abnormal T-cell subsets and aberrant T cell function (24) indicating a dominant cell mediated immune response in selected SLE patients with underlying host susceptibility and specific antigenic or exogenous trigger. This may in turn translate into a different form of extracellular signaling pathway to induce podocytic injury (25). In summary, this case presented with active systemic lupus erythematosus along with nephrotic syndrome and acute renal failure, showing collapsing glomerulopathy as the renal histopathologic lesion with no evidence of immune complex mediated disease. A kidney biopsy in this situation is helpful to determine the accurate diagnosis, which has prognostic and therapeutic significance. The patient progressed to end stage renal disease and hemodialysis, 12 months after initial renal biopsy diagnosis of collapsing glomerulopathy.

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CASE 3: FROM LEIDEN UNIVERSITY MEDICAL CENTER, THE NETHERLANDS:

Pathologist: Ingeborg Bajema

Nephrologist: Stefan Berger

Biopsy numbers: H03-3454 and H04-741

CASE SUMMARY

This is a case of a 52-year old woman, with two renal biopsies. The first biopsy is from April, 2003, the second from January, 2004.

Clinical history and laboratory values at the timepoint of the first renal biopsy (H03-3454)

52 year old female, diagnosed with SLE 21 years ago. At that time-point she had a pericarditis, positive ANF, and positive anti-ds-DNA antibodies. She was treated with prednisone and azathioprine. Over the years, there were 4 episodes of relapsing SLE, at 6, 11, 17 and 18 years after the initial diagnosis. She developed hypertension and osteoporosis. She underwent *coronary artery bypass grafting* one year before the first biopsy, and received an aortic bifurcation prosthesis two years before the first renal biopsy. The renal biopsy was sent in with the question: lupus nephritis?

Laboratory values:

serum creatinine: 74 µmol/l

creatinine clearance: 88 ml/min

cholesterol: 5.1 mmol/l

serum albumin: 29 g/l

proteinuria: 3.3 g/24 hr

urinalysis: full of erythrocytes and leukocytes, some dysmorphic erythrocytes

urine culture: negative

ANA: strongly positive

Anti ds-DNA antibodies: negative

Anti-RNP antibodies: positive

blood pressure: 130/80; edema: negative

Clinical history and laboratory values at the timepoint of the second renal biopsy (H04-741)

Patient admitted to hospital because of progressive malaise with fatigue, loss of appetite and dyspnea during effort. Weight: stable. No fever. Progressive ankle edema. The renal biopsy was performed because of nephrotic syndrome.

Laboratory values:

Hb: 7.4 mmol/l

Leucocytes: 2.8 x 10.E.9/l

Thrombocytes: 403 x 10.E.9/l

BSE: 116

CRP 21 mg/l

Cholesterol: 10.8 mmol/l
HDL cholesterol: 1.0 mmol/l
serum albumin: 21 g/l
ureum: 9.6 mmol/l
serum creatinine: 81 μ mol/l
proteinuria: 9.4 g/24 hr
urine culture: negative
the urine contained both IgG, Lambda-M protein and kappa and lambda light chains.
blood pressure: 106/57; edema: ankle edema and pitting edema

DESCRIPTION OF HISTOPATHOLOGICAL FINDINGS IN THE BIOPSIES

First biopsy

The original glass slides from the first biopsy (H03-3454) showed cortical tissue with circa 10 glomeruli, without global glomerulosclerosis. All glomeruli were abnormal, showing varying amounts of mesangial extension, and intracapillary and extracapillary proliferation. Proliferative lesions were present in more than 50% of the glomeruli. Some glomeruli showed rather extensive influx of inflammatory cells. There was one glomerulus with a circumferential, cellular crescent; other glomeruli showed segmental crescent formation. There were subtle indications of wire-loop lesions, though not very outspoken. Breaks in the GBM were focally present. Glomeruli showed virtually no presence of chronic changes. Tubes contained very outspoken lysosomal granules, and casts were also present. There was slight indication of tubular atrophy and interstitial fibrosis. A large artery showed intimal fibrosis. Surrounding this artery and a few others, a non-specific lymphocytic infiltrate was present. Interstitial infiltrates around tubes were absent.

By immunofluorescence, relatively coarse deposits of IgG, IgM, C3 and C1q were found following the contours of the GBM. IgA was virtually negative. Kappa and lambda light chain stainings showed the same pattern as IgG. By EM, subendothelial deposits were found, consistent with the diagnosis lupus nephritis (class IVa (g))

Second biopsy

Slides from the second biopsy (H04-741) contained cortical tissue with circa 7 glomeruli. There was no global glomerulosclerosis. All glomeruli were abnormal, showing areas with eosinophilic material which stained positively in the Congo Red stain and showed typical apple green birefringence by polarization microscopy, and was thus recognized as amyloid. By immunohistochemical staining, this appeared to be AA-amyloid. In glomerular areas not affected by amyloid, proliferative lesions as encountered in the first biopsy, were absent. Some glomeruli showed small adhesions from the glomerular tuft to Bowman's capsule. Tubular atrophy and interstitial fibrosis were increased in comparison to the first biopsy. Tubular epithelium showed presence of some lysosomal granules. A large artery contained intimal fibrosis. Interstitial infiltrates were mostly present in areas of tubular atrophy and interstitial fibrosis.

By immunofluorescence, showed a weakly positive, granular patterns of deposits in the mesangium with continuation along the GBM of IgG, IgA, IgM, C3, C1q and kappa and lambda light chains. By EM, deposits of amyloid were confirmed.

DIAGNOSIS AND CONSIDERATIONS

At the time of the first biopsy, differential diagnostic considerations were few. This was a patient known with systemic lupus erythematosus for over 20 years whose presentation with renal disease became very late in the course of the disease. At the time of biopsy, she was going through her fifth relapse. She had positive ANF and positive anti-ds-DNA antibodies at the time of biopsy. The immunofluorescent findings were virtually diagnostic for lupus nephritis with a full-house pattern, even though IgA was almost negative. The histological lesions showed practically no chronic changes in the presence of extensive acute cellular lesions, therefore classified as class IVa (g). Within a year, a second renal biopsy was performed, because of persistent nephrotic syndrome. This biopsy showed massive amyloid deposits, of the AA-type.

After the finding of amyloid in the second biopsy, slides from the first biopsy were re-evaluated, and amyloid appeared not to be present. However, deeper cuts through the biopsy showed one glomerulus in which amyloid was present. Therefore, the amyloid was missed in the first biopsy due to sampling error. The first biopsy contained 10 glomeruli. The class IVa (g) lupus nephritis was in line with what was clinically expected.

DISCUSSION

AA amyloidosis can occur in the context of chronic systemic inflammatory and infectious diseases. Retrospectively reviewing 68 cases with renal AA amyloidosis, Verine et al. found that this condition was related to chronic infection in 41%, chronic inflammation in 38%, a tumor in 10%, and hereditary disease in 10% (1). Only in 1%, associated disease remained undetermined. Nephrotic syndrome and renal insufficiency were present in the majority of cases with renal AA amyloidosis. Amyloid in glomeruli was indicative of worse clinical outcome than amyloid only present in vessels.

Amyloidosis in lupus nephritis is a very rare finding. A review which appeared in 2007, reported that by that time, only 24 patients had previously been described in the literature (2). Most cases reported before 1986 did not report on the type of amyloid involved. More recent cases always show depositis of AA-amyloid. The amyloid fibril protein found in AA amyloid is derived from the related acute phase reactant serum amyloid A. A persistent elevation of the serum precursor protein SAA could be a requirement for the formation of AA amyloid. It is interesting that patient with SLE usually have only modest elevations of SAA, but in some in which AA amyloid was found, elevated levels were reported (3).

It is mentioned that in some of cases with lupus nephritis and AA amyloidosis, associated diseases such as infectious diseases could have contributed to the development of AA amyloidosis. Another suggestion is that many cases had SLE for many years before developing amyloid depositis. However, reviewing 15 cases reported until 1995, Orellana et al. show large variability in time between SLE diagnosis and onset of symptoms of amyloidosis, ranging from one year to 35 years (3). They also mention a fairly high number of male patients in the reported cases, six out of 15.

Although most case-reports describe a combination of lupus nephritis and amyloid deposits in the renal biopsy, some patients with SLE have been described who underwent renal biopsy because of nephrotic syndrome, and whose biopsies only show amyloidosis, without overt lupus nephritis (3,4,5). An interesting case was reported by Gomez-Puerta et al. of a 60 year-old patient known with lupus nephritis for 13 years before the onset of amyloidosis (6). First renal biopsy, performed because of overt proteinuria, showed amyloid in the absence of lupus nephritis (no light microscopic findings indicative of lupus nephritis, immunofluorescence studies showed no deposits of immunoglobulins or complement). Patient was treated with cyclophosphamide, and remained in remission for some years with low doses prednisone and hydroxychloroquine. Three years later, a second biopsy was performed because of recurrent nephrotic syndrome. Amyloid deposits were still present, but additionally, lupus nephritis class III with depositis of C3, C1q, IgA and IgM was found. This is the first report of a patient with SLE and amyloidosis who develops lupus nephritis in a kidney affected by amyloid deposits.

In contrast to SLE where AA-amyloid is rarely found, it frequently occurs in, for instance, rheumatoid arthritis. Two types of AA amyloidosis in the kidney have been distinguished: type 1, characterized by glomerular involvement, and type 2, characterized by vascular involvement only. In 53 patients with rheumatoid arthritis and AA amyloid deposits in the kidney, rapid deterioration of renal function was found in patients with type 1 amyloidosis whereas type 2 patients, renal function did not deteriorate significantly in a follow-up period of 5 years (7).

CONCLUSION

Although it is a rare finding, AA amyloidosis should be considered in patients with SLE, in particular those with nephrotic syndrome who do not improve after immunosuppressive therapy. Case-reports show that renal amyloidosis can either precede, follow, or appear together with various subclasses of lupus nephritis.

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CASE 4

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CLINICAL HISTORY

A 41 year old African-American man with history of nasopharyngeal carcinoma, 5 years prior to this admission presents with rapidly progressive acute renal failure requiring dialysis. He also has hypothyroidism and anemia of chronic disease. There is no history of smoking, alcohol or drug abuse. While on dialysis, he developed an infection of the hemodialysis catheter. He was taken off dialysis and the catheter was removed. He also had a Port-A catheter in place for five years originally used to treat nasopharyngeal carcinoma. The patient had no complaints except tenderness in the base of the left neck where the old infected dialysis catheter was. On admission creatinine was 1.72, improved to 1.53, but worsened to 2.6 and went up to 7.4mg/dL. He developed anasarca and edema of the upper and lower extremities. Solu Medrol 100mg IV x3 doses and Cytoxan was given but the patient did not seem to improve at all. He was also treated with levothyroxine, labetalol, hydralazine, IV vancomycin, rifampin and Mucomyt. Creatinine dropped to 2.14. The patient was discharged but returned to the emergency room a week later complaining with fever and chills. He was found to have drainage from the previous catheter exit site. Patient was admitted, was given IV Cytoxan x1 dose in this admission. A pan-CT showed no abscess and TEE showed no intra-atrial thrombi. Laboratory values in this admission included a white cell count of 10.5, hematocrit 29, hb 9.9grs, Platelet: 183, BUN: 20, Glucose 97, Na 136, cl 10, ALT/AST:31/19, Calcium:7.7, Bilirubin:36, Plasma protein 4,6, Albumin 1.5, normal coagulation values, potassium: 4 and creatinine 1.55mg/dL, blood pressure: 142/90mmHg.

Chest X-ray negative; Urine culture negative; Blood cultures 2/2 grew Staph aureus.

Clinical concerns at this admission were:

1. Staph aureus bacteremia
2. Chest abscess
3. Propable port-A-Cath infection
4. Acute renal failure to rule out glomerulonephritis
5. History of nasopharyngyal

A renal consult was sought. Physical exam by consulting nephrologist is notable for 1+ pitting edema. Lungs, heart, abdomen, neurologic evaluation and neck were unremarkable. Nephrologist' impression was,

1. Rapidly progressive glomerulonephritis with acute renal failure
2. Exit site infection of the previous dialysis catheter
3. Bacteremia
4. Hypertension
5. Anemia of chronic disease

Patient was kept on low dose prednisone, Cytoxan was discontinued, Epogen was described

A renal biopsy was performed.

RENAL BIOPSY FINDINGS

Light Microscopy

Twenty four glomeruli were present in the biopsy sample; 5 were globally sclerosed (20%). Non-sclerotic glomeruli appeared slightly enlarged, but there were no crescents, inflammatory infiltrates or fibrinoid necrosis. Silver stain show mild and focal mesangial hypercellularity, but to capillary wall thickening or spikes. Tubular epithelial cells were disrupted with loss of brush boarder; focally dilated tubules with RBCs and or protein were noted. Focal lymphocytes were present in the interstitium but there was no significant interstitial fibrosis. Arteries were intact. Overall findings were mild and excluded vasculitis.

Immunofluorescence

Twelve intact glomeruli were evaluated. There was diffuse mesangial and capillary loop granular deposits with IgA, kappa and lambda and C3. The remaining immune globulins were unremarkable.

Electron microscopy

Two glomeruli were evaluated. Extensive foot process effacement and focally thick capillary loops were found. There were abundant subendothelial and paramesangial electron dense deposits. Rare, bell shaped subepithelial deposits (humps) were found as well.

Diagnosis: IgA dominant post-infectious glomerulonephritis

DISCUSSION

The findings in this biopsy raise the differential of IgA nephropathy and post-infectious glomerulonephritis (PIGN) presenting with IgA deposits. Classic acute PIGN clinical presentation is characterized by low serum complements; renal biopsy reveals neutrophils in the glomeruli and “lumpy-bumpy” granular deposits of predominantly IgG and C3 on capillary loops identified by immunofluorescence. The characteristic ultrastructural finding is large subepithelial bell shaped deposits often called “humps”. Atypical PIGN can present with mild mesangial hypercellularity, no inflammatory infiltrate in the glomeruli or interstitium and absence of IgG immune deposits (1). Exclusively IgM or C3 only deposits may be found in chronic PIGN making diagnosis very difficult, unless typical “humps” are found on electron microscopy and laboratory studies confirm infection. Other immune globulins C4 and C1q may also be present predominantly in the mesangium, but predominantly IgA deposits are a rare finding in PIGN. In 2003, Nasr et al reported IgA dominant post-staphylococcal glomerulonephritis occurring in five patients with diabetes. Pathologic findings included acute endocapillary proliferative and exudative glomerulonephritis and intense deposits of IgA mimicking

IgA nephropathy (2). A report of 13 cases of IgA dominant PIGN was reported by Haas *et al* in 2008 (3); all patients presented with acute renal failure similar to this patient (mean creatinine value was 4.4 mg/dL), hematuria which was macroscopic in 3 patients and proteinuria (nephrotic range in 6). Six patients in the study by Haas had recent staphylococcus infection (3 methicillin resistant: MRSA), 5 were diabetic, (3 with diabetic nephropathy). Complement levels were below normal in four patients, but normal in the remaining 9 patients. Wound infection was the source of bacteria in 2 patients; others had pneumonia, or abscess adjacent to old an orthopedic hardware. Two additional patients had HIV and 1 had hepatitis C. There was no history of documented infection in 4 patients. Four patients developed ESRD 10+/-13 months after biopsy.

Histopathologic findings of the study by Haas *et al* included diffuse proliferative GN and exudative lesions, but 3 biopsies showed only focal mesangial hypercellularity and 4 had exclusively diffuse mesangial hypercellularity. All biopsies showed interstitial mononuclear cell inflammation (except those with exudative lesions).

Immunofluorescence showed IgA deposits in all 13 cases in the mesangium or the capillary loops and “granular” C3 staining, similar to the biopsy presented here. Some biopsies had weak IgG deposits and almost all had absent C1q. Electron microscopy revealed large subepithelial deposits (humps) in all cases, more frequent in some biopsies compared to others; at least five subepithelial or intra-membranous deposits were found. In some biopsies, humps were located in “notch” regions instead of subepithelial locations. Subendothelial deposits were present in about half of the cases. This rare pattern of PIGN is important to recognize so that patients are not erroneously labeled as having IgA nephropathy. Notably, it occurs even in the absence of clear history of infection. Clinical symptoms may be mild and infection may be subclinical at the time of renal biopsy.

Previous series by Nasr and also by Satoskar *et al* (2,4) who reported mesangial IgA nephropathy secondary to MRSA may differ from that by Haas *et al* because their cases did not have “humps” on electron microscopy, therefore the diagnosis of IgA nephropathy secondary to infection may have been justified.

The case presented here is similar to the cases described by Haas and is interpreted as IgA dominant PIGN and not as IgA nephropathy.

Interestingly, a study by Koyama *et al* reported that a Japanese population with IgA nephropathy have Staph Aureus membrane antigen deposition in glomeruli: 68% (79/116) of renal biopsies from patients with IgA nephropathy and 6/10 with Henoch-Schonlein Purpura (5,6). The latter studies raised important issues in the immune pathogenesis of IgA nephropathy and implicate bacterial infection at least in some patients with IgA.

MRSA infection is currently on the rise. Clinical symptoms are not infrequently perplexing. Renal biopsy is frequently performed for unexplained renal failure, histopathologic findings vary as is response to therapy (7).

CLINICAL FOLLOW UP

This patient was prescribed Vancomycin 1gm per 24 hours x 4 weeks, recovered the acute episode of renal failure and is reported to have stable renal function a year later.

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CASE #5

Guillermo Herrera, MD

CLINICAL SUMMARY:

76 y/o female with progressive renal dysfunction and proteinuria was referred for renal evaluation after laboratory testing indicated an elevated serum creatinine (2.9 mg/dl) and sub-nephrotic range proteinuria (1.2 grams/day). Estimated glomerular filtration rate was 17 cc/minute.

When initially evaluated the patient was asymptomatic and denied taking any medications or over the counter drugs, including non-steroidal anti-inflammatory drugs. Her past medical history included hyperlipidemia for which she had been treated with lovastatin, but she had stopped taking this drug a year prior. She also related a history of breast carcinoma 16 years prior treated with lumpectomy and tamoxifen without recurrence.

Physical examination revealed a slightly elevated blood pressure (142/90 mm Hg) and no other findings.

Patient had normal serum albumin, normal blood glucose, and mild hypocalcemia (8.5 mg/dl). Blood cell counts were all within normal limits; the patient was not anemic (Hgb 13.5 mg/dl).

U/A revealed a pH=6, glucosuria (1+), proteinuria (3+), hematuria (11-20 red blood cells/HPF) with normal morphology, and granular casts. The patient also had phosphaturia (fractional excretion of phosphate 31%.

Serological tests including ANA, ANCA, and anti-GBM antibody were normal. Complement levels were also normal.

PATHOLOGY FINDINGS:

Renal biopsy contained approximately 30 glomeruli. Approximately 30% of the glomeruli were globally sclerosed. The viable glomeruli were unremarkable. The main findings were in the tubular interstitial compartment. There was marked swelling of the cytoplasm of proximal tubular cells. The cytoplasm of the proximal tubular cells was granular and eosinophilic on the hematoxylin and eosin stained sections, bright red with the trichrome stain, and did not stain at all with the PAS stain. The swelling to the proximal tubular cells was of such magnitude that in many instances the tubular lumina were almost totally or completely obliterated. Scattered mononuclear inflammatory cells (lymphocytes and plasma cells) were identified in a moderately fibrotic interstitium. No tubulitis was apparent. Mild to focally moderate vascular sclerosis was also present.

FINAL DIAGNOSIS:

PROXIMAL TUBULOPATHY, KAPPA LIGHT CHAIN-RELATED, WITH MARKED PROMINENCE OF LYSOSOMAL COMPARTMENT AND ABUNDANT INTRACYTOPLASMIC NON-CRYSTALLINE “INCLUSIONS”.

PATHOLOGY AND CLINICAL FINDINGS:

Plasma cell dyscrasias are often associated with renal manifestations. Renal dysfunction is often the first clinical manifestation of an occult plasma cell dyscrasia. Recognizing the early and subtle renal manifestations associated with an underlying neoplastic plasma cell process is very important for the nephropathologists. Early intervention in these cases, when the plasma cell load is still small and damage to other organs has not occurred, is important. Aggressive clinical management aiming at “curing” or controlling effectively the underlying malignant process is the recommended treatment of choice. Circulating light and heavy chains produced by the clone/s of neoplastic plasma cells can be nephrotoxic. Any of the three renal compartments can be affected, and although often only one compartment reveals pathological alterations, more than one can be involved in some instances. There are several factors that determine the degree and type of renal damage that may occur. Among these factors one of the crucial ones is related to the physicochemical characteristics of the pathologic light chains. The present case illustrates the effects of nephrotoxic light chains in proximal tubules. Arguably of all of the renal manifestations of plasma cell dyscrasias, proximal tubular damage- when it occurs by itself- is the least recognized and the association of proximal tubular damage with an underlying plasma cell dyscrasia is frequently missed. Proximal tubular damage is only seen in association with monoclonal light chain disorders, and not in heavy chain-related renal diseases, as the metabolism of light chains typically occurs in the proximal tubules. There are specific changes in the proximal tubules that should provide a clue to the diagnosis.

Because the light microscopic changes associated with proximal tubular injury can be subtle and, at times difficult to assess due to poor fixation, autolysis, and/ or staining artifacts, ultrastructural evaluation of proximal tubular morphology becomes crucial in making the correct diagnosis.

Before addressing the pathological findings in this condition, it is important that the catabolism of normal light chains be reviewed, in order to fully understand why the pathological alterations take place. Circulating light chains due to their low molecular weight are freely filtered through the capillary walls and delivered to the proximal tubules where they are metabolized. Keep in mind that other low molecular weight proteins such as myoglobin and beta-2 microglobulin, as well as some drugs-including illicit drugs- are also metabolized in a similar fashion. Light chains are endocytosed into the proximal tubules via the cubilin/megalyn receptor located in the brush borders. In normal individuals an excess of light chains is often found in the circulation; these become catabolized in the proximal tubules by lysosomal action. If the light chains are physicochemically normal and the amounts of filtered light chains are small, this is a very effective process that does not alter tubular homeostasis. Aminoacids resulting from this process are returned to the circulation. In contrast, in the case of pathological light chains exhibiting certain physicochemical structural characteristics, the process of

light chain catabolism is altered and the lysosomes are unable to destroy them. As a consequence, the lysosomal system becomes overloaded and non-functional. The lysosomes get enlarged and acquire atypical shapes. Once they extrude their hydrolytic enzymes into the cytoplasm of the tubular cells, cell damage with apical blebbing, vacuolization, desquamation, and loss of microvillous borders occurs.

There are two main morphologic expressions of proximal tubular damage. The best known manifestation is acute tubular damage with intracytoplasmic crystalline like inclusions representing the typical finding in what is generally recognized as renal Fanconi syndrome. The intracytoplasmic inclusions in the proximal tubules provide a relatively easy to identify marker for the pathologists to make a definitive diagnosis. This combined with monotypical staining in proximal tubular cytoplasm for one of the light chains and not the other (almost universally kappa- only a few lambda cases reported in the literature) makes possible an unequivocal. Unfortunately, routine immunofluorescence staining using frozen tissues may not depict the abnormal light chain in the cytoplasm of the tubular cells, likely because the light chains show certain structural abnormalities that result in an inability of commercially available polyclonal antibodies to label them. If the fluorescence staining is performed using paraffin embedded sections, after pronase digestion, the chances of identifying the crystalline-like inclusions increase considerably. Another very effective method to demonstrate monoclonality of light chains in association with the crystalline-like cytoplasmic tubular inclusions is ultrastructural immunolabeling.

The second pattern of proximal tubular injury is the most difficult one to recognize. By light microscopy changes of acute tubular damage may be overt or subtle and rather non-specific. Ultrastructural evaluation in these cases reveals a prominence of the lysosomal system with large and atypical lysosomes. Again demonstrating monoclonality for light chains is crucial in making the diagnosis. Although the majority of these cases also appear to be kappa light chain-related, some cases show lambda monotypicality. The same problems with routine immunofluorescence in frozen tissue may be found in demonstrating light chain monoclonality. Ultrastructural labeling may also be very important in making and documenting the diagnosis.

The present case falls in the second category. This patient had tubules filled with angulated and enlarged lysosomes to the point where virtually no other cytoplasmic organelles were seen in the proximal tubular cells. These "constipated" lysosomes were packed with kappa light chains.

DIFFERENTIAL DIAGNOSIS:

Proximal tubular damage is the classical lesion seen in association with nephrotoxic acute tubular necrosis. Therefore, finding changes consistent with acute tubular necrosis at the light microscopic level provides a diagnosis but does not indicate a specific cause. There are many nephrotoxins that are associated with acute tubular necrosis. The pathologists should go further than making a morphologic diagnosis of acute tubular necrosis if at all possible, especially if the cause of the proximal tubular damage is not clear from a clinical perspective.

The proximal tubular alterations noted are not specific for monotypic light chain-related lesions, unless it can be demonstrated that the monotypic light chains are present within the engorged and enlarged lysosomes. Prominence of the lysosomal compartment can also be seen in other toxic proximal tubular nephropathies such as is seen for example with cocaine use, cyclosporin intake or myoglobinuric acute renal failure. The pathologists must be very careful in identifying the proximal tubular alterations and considering the possibility of an associated underlying light chain plasma cell dyscrasia. Furthermore, it is important to point out that intracytoplasmic inclusions in proximal tubules that may be confused with those seen in these patients may be found in association with other etiologies, such as drugs and toxins. This is why demonstration of the content of the inclusions is crucial in making a precise diagnosis. Results of serum and urine protein electrophoresis, and most importantly, detection of free light chains in the serum become important pieces of information that may aid in correctly interpreting the findings. There should be no hesitation to repeat the immunofluorescence evaluation if initially negative, use paraffin embedded tissue, and/or ultrastructural immunolabeling to confirm the diagnosis.

CLINICO-PATHOLOGIC CORRELATION:

The selective alterations in the proximal tubules result in proximal tubular dysfunction as the initial presenting problem and renal failure in advanced cases with much damage. Some cases slowly evolve into renal failure as the progressive damage to the tubules continues without treatment of the underlying disorder.

The patient presented showed clear evidence of proximal tubular dysfunction resulting in proteinuria, phosphaturia, and glucosuria. These patients also often have aminoaciduria, but this is more difficult to test clinically. So in terms of clinical and pathologic findings this case is a typical example of an early presentation of an underlying plasma cell dyscrasia with proximal tubular damage which required careful pathologic assessment of proximal tubular morphology to make a definitive accurate diagnosis.

Additional laboratory evaluation revealed a SPEP with a monoclonal band in the IgG kappa region (0.23 g/dL). UPEP with immunofixation revealed a monoclonal kappa light chain (304 mg/dl). A serum free light chain assay showed a marked predominance of kappa light chains with a kappa to lambda ratio of 1390:1. Bone marrow aspirate and biopsy showed 9% plasma cells with no plasma cell aggregates and no atypia. The plasma cells expressed CD38, CD138, and kappa light chains by flow cytometry.

It is not uncommon for patients with either the crystalline tubular cytoplasmic inclusions or the type of proximal injury seen in this case, to show a subdued plasma cell proliferative process in the bone marrow. It is incorrect to assume that there is not a plasma cell dyscrasia because the number of plasma cells is within normal limits, or there are no atypical plasma cells, or aggregates of plasma cells are missing. Evaluation of the plasma cells by either immunohistochemistry (tricky because of the decalcification that is routinely used in bone marrow specimens may give a false negative result or too much background staining) or flow cytometry after careful isolation of the plasma cells.

An important point to remember is that once damage in the kidney is demonstrated, the plasma cell process with a monoclonal paraprotein band in the serum is no longer an MGUS (monoclonal gammopathy of unknown significance), but a plasma cell dyscrasia with end organ damage that requires consideration for treatment. Treatment of these patients early in the course of their disease, which is often when the renal biopsy identifies them, may result in a “cure” or at least significant control of the neoplastic process. There is an “old” school of hematologists who still view this disease as a slow and not very harmful process that does not merit an aggressive treatment approach, justifying their position by alluding to the fact that the treatment is inherently toxic. There is much disagreement with this view at this time.

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