KIDNEY WEEK 2022

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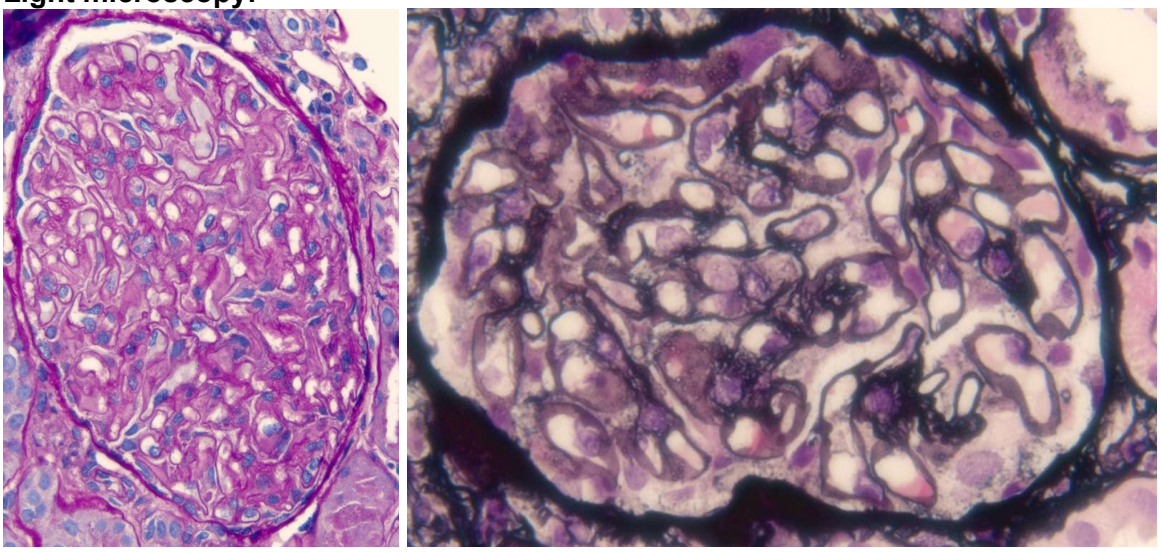
Renal Biopsy: Clinical Correlations

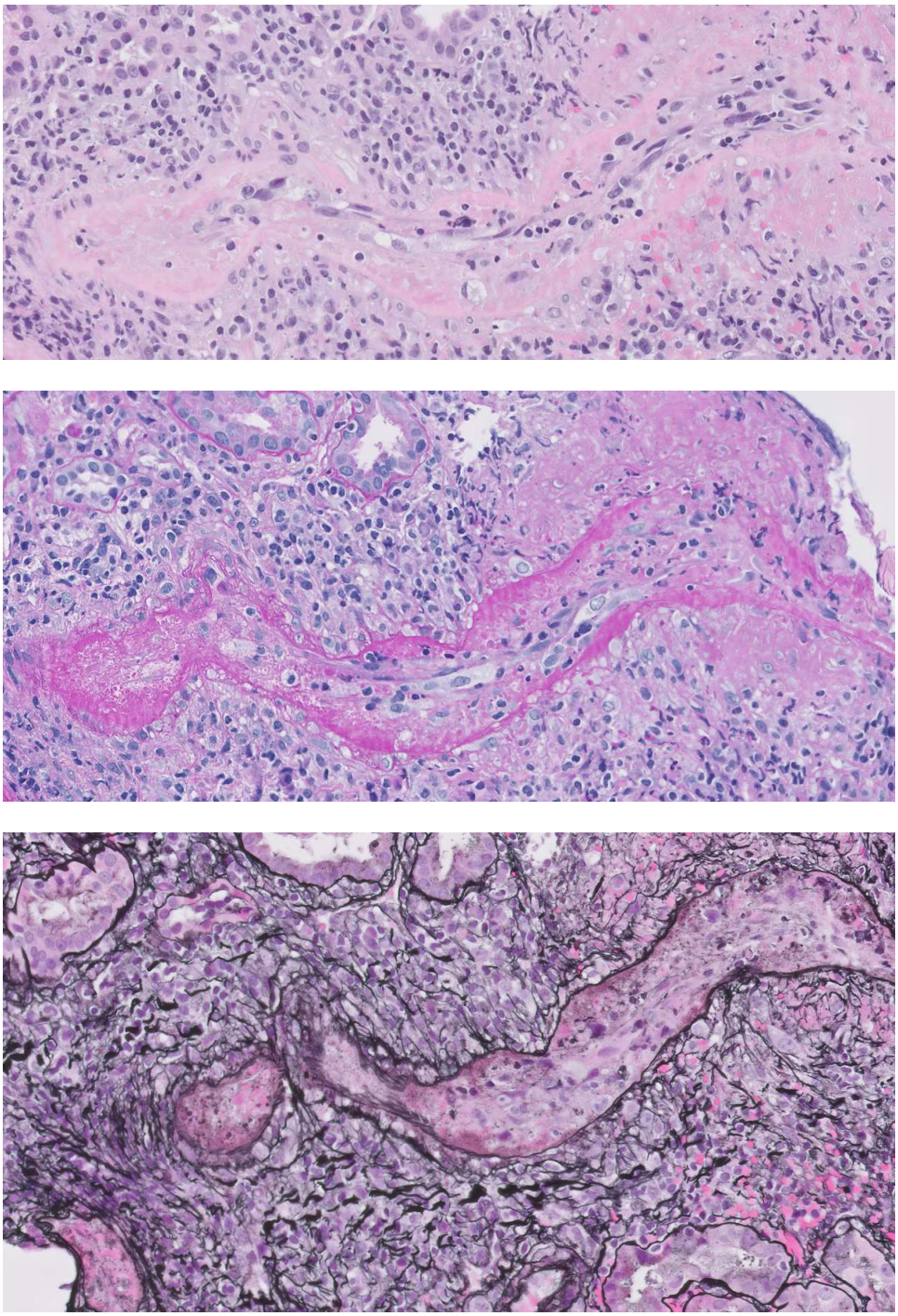
Case 1 from Vivek Charu, MD, PhD - Stanford University

A 40-year-old man with a past medical history of systemic lupus erythematosus (SLE, 9-years duration), anti-phospholipid antibody syndrome (with prior stroke, on anticoagulation), and psoriasis with psoriatic arthritis presents with increasing proteinuria and mild renal insufficiency. His SLE and psoriasis were managed with prednisone (10 mg BID) and ustekinumab (45 mg every 12 weeks). His serum creatinine was 1.2 mg/dL, elevated from a baseline of 0.75-0.95 mg/dL, and his urine protein-to-creatinine ratio was 1.96 g/g. Urinalysis demonstrated 100 protein and large hemoglobin, with 36 RBCs seen per HPF on urine microscopy. His complements were low (C3-36, C4<8.0), ANA was positive (1:1280, speckled pattern), and anti-dsDNA was mildly elevated at 9. His albumin was 2.9 g/dL. He was normotensive (BP-122/83), and physical exam was notable for a mild rash on the back of the patient's calf, attributed to psoriasis. Additional laboratory data demonstrated a hemoglobin of 13.5 g/dL, a WBC count of 3.6 K/uL, a platelet count of 224 K/uL, an LDH of 183 (normal), a total bilirubin of 0.3 mg/dL, and a direct bilirubin of 0.1 mg/dL. A renal biopsy was performed.

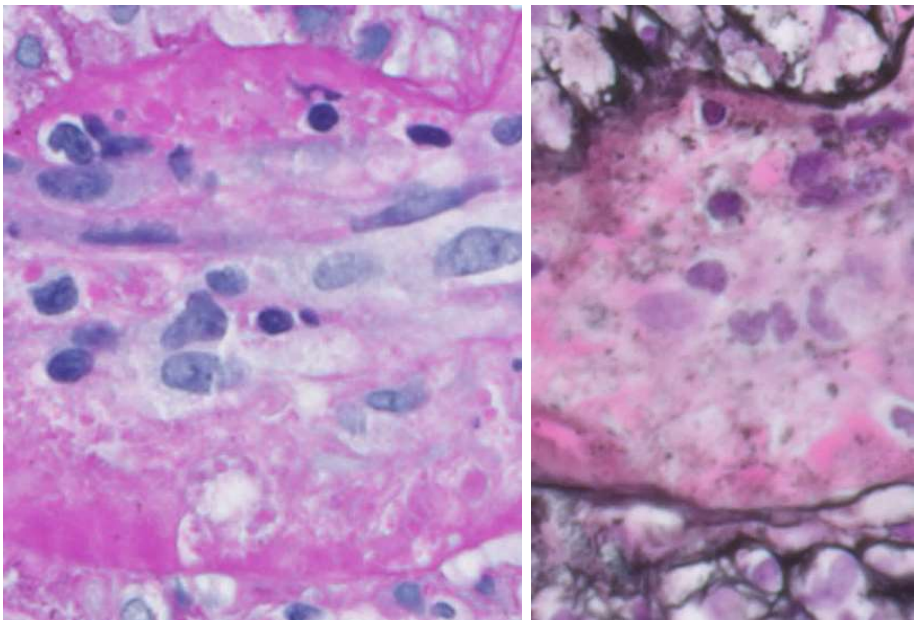
Light microscopy:



Representative PAS (left) and silver-stained (right) images of the glomeruli.

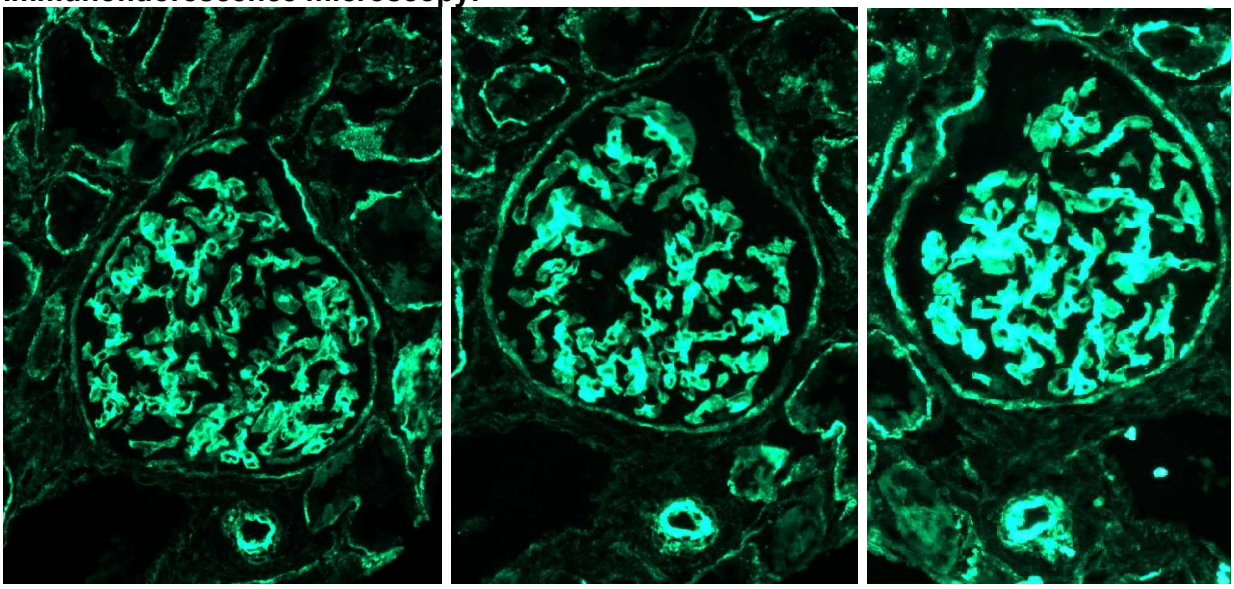


H&E (top), PAS (middle), and silver-stained (bottom) images of a small interlobular artery (40x).

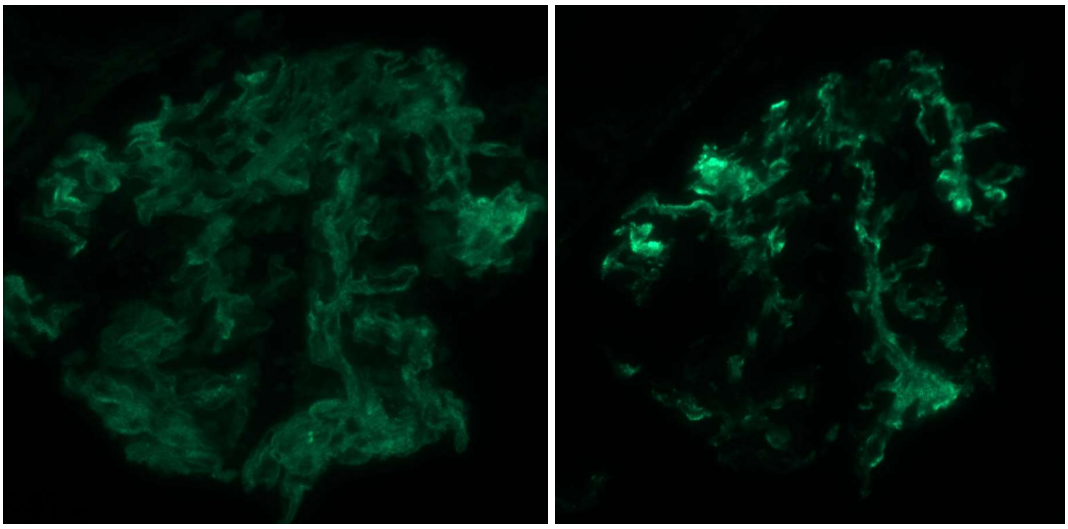


High-power magnification of the vascular lesion on PAS (left) and silver-stained (right) sections.

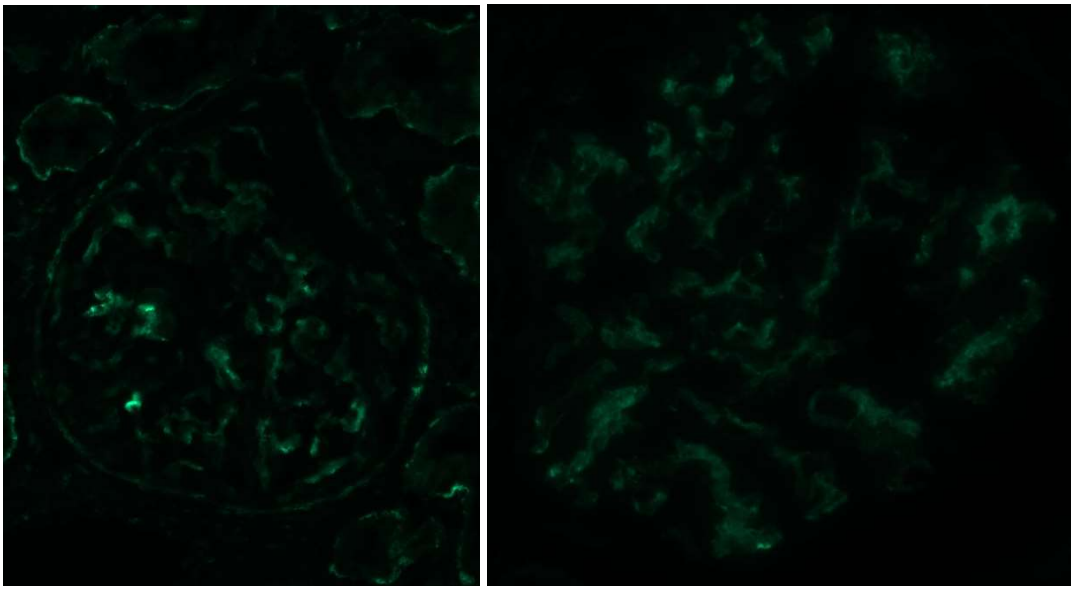
Immunofluorescence microscopy:



IgG (left), kappa (middle), and lambda (right) light chains.

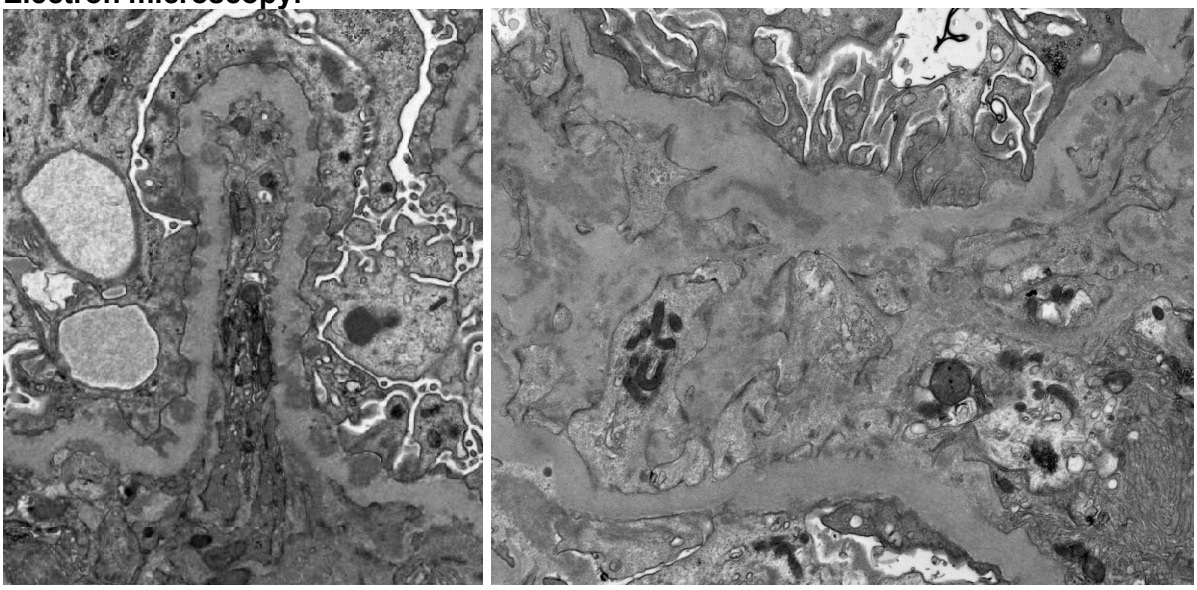


IgA (left) and IgM (right)



C1q (left) and C3 (right)

Electron microscopy:



Representative electron microscopy images.

Pathologic Findings

Light microscopy: A total of 27 glomeruli was sampled in the biopsy, with 2 showing global glomerulosclerosis (7%). The glomeruli demonstrated segmental mesangial expansion, but there was no evidence of endocapillary proliferation, bulky immune complexes (hyaline deposits/thrombi), neutrophils/karyorrhexis, fibrinoid necrosis or crescents. The capillary loops were mildly thickened, and silver-stained sections demonstrated numerous capillary loop "vacuolizations." Interstitial fibrosis and tubular atrophy were minimal, affecting 5-10% of the cortex sampled. Two small interlobular arteries demonstrated near complete disruption of the vessel walls by an inflammatory cell infiltrate and associated fibrinoid necrosis. Prominent endothelialitis was seen, and brightly eosinophilic material was seen in the intima, suggestive of immune complex type deposits.

Immunofluorescence microscopy: Standard immunofluorescence on the frozen tissue (scale 0-3+) demonstrated 3+ diffuse granular glomerular mesangial and capillary wall staining for IgG and kappa and lambda light chains. IgG, kappa, and lambda light chains also stained tubular basement membrane deposits and focal arteriolar walls. There was 1+ predominantly granular capillary wall staining for IgA, 2+ predominantly granular mesangial staining for IgM, and 1+ segmental granular mesangial staining for C3 and C1q.

Electron microscopy: Ultrastructural examination of the glomeruli demonstrated abundant mesangial electron dense deposits, rare subendothelial deposits, and numerous subepithelial deposits. Tubuloreticular inclusions were seen in glomerular endothelial cells and peritubular capillary endothelial cells. Small tubular basement membrane electron dense deposits were seen.

Clinical Follow-Up

Following the kidney biopsy, additional testing for ANCA and cryoglobulins were negative. The patient was treated with high-dose prednisone and started on mycophenolate mofetil. His serum creatinine normalized to his baseline (0.75-0.95 mg/dL) rapidly. He continued to have 1-2 grams of proteinuria for approximately 1-1.5 years, which subsequently reduced to 0.5-1 gram at 2 years. At 2 years of follow-up, his prednisone had been tapered to 2.5 mg daily, and he is maintained on mycophenolate mofetil at a dose of 3000 mg daily. He has preserved renal function (latest serum creatinine at baseline of 0.75-0.95 mg/dL).

Questions

1. **What is the main pattern of glomerular injury in this case?**
    A. Mesangial proliferative lupus nephritis (ISN/RPS class II)
    B. Focal proliferative lupus glomerulonephritis (ISN/RPS class III)
    C. Diffuse proliferative lupus nephritis (ISN/RPS class IV)
    D. Membranous lupus nephritis (ISN/RPS class V)

The primary glomerular abnormality in the biopsy is thickening of the glomerular basement membranes with capillary wall staining for IgG, IgA, and kappa and lambda light chains by immunofluorescence microscopy and subepithelial electron dense deposits seen on ultrastructural examination. These findings are diagnostic of membranous nephropathy. No active/proliferative lesions (including endocapillary hypercellularity, neutrophils/karyorrhexis, bulky immune complexes, fibrinoid necrosis, or crescents) are seen.

2. **Which pattern of vascular injury best characterizes the biopsy findings in this case?**
    A. Arteriosclerosis
    B. Lupus vasculopathy (non-inflammatory)
    C. Lupus vasculitis/true renal vasculitis
    D. Thrombotic microangiopathy
    E. Uncomplicated vascular immune-complex deposits

The biopsy demonstrates a necrotizing arteritis, characterized by destruction of the vessel wall by an inflammatory cell infiltrate and associated fibrinoid necrosis. These findings are diagnostic of small-vessel vasculitis affecting the kidney, and in the setting of SLE, would be consistent with lupus vasculitis, or true renal vasculitis. See the discussion below for additional details on vascular lesions in SLE.

3. **Which entities should be included in the broader differential diagnosis for the illustrated vascular findings in this case? (select all that apply)**
    A. ANCA-associated small-vessel vasculitis
    B. Anti-glomerular basement membrane nephritis
    C. Cryoglobulinemic vasculitis
    D. Giant-cell arteritis
    E. Takayasu arteritis

The biopsy demonstrates a small vessel vasculitis, and as such, the differential diagnosis would include ANCA-associated small vessel vasculitis and cryoglobulinemic vasculitis. Serologic testing for ANCA and cryoglobulins is helpful to exclude these possibilities. See the discussion below for additional details on the differential diagnosis for vascular lesions in SLE.

Diagnosis

Lupus vasculitis
Membranous lupus nephritis (ISN/RPS class V)

Discussion

This biopsy demonstrates true renal vasculitis in the setting of SLE and concomitant membranous lupus nephritis (ISN/RPS class V). Vascular lesions in SLE are divided into 5 categories, described in the table below. Importantly, patients may have multiple renal vascular lesions in the same biopsy.

Vascular lesion	Histologic description
Arteriosclerosis	• Fibrointimal thickening of arteries (e.g., as seen in the absence of lupus nephritis). • No thrombosis, necrosis, or inflammatory infiltration of the vessel wall is seen.
Uncomplicated vascular immune complexes	• Vascular immune complexes seen within the vessel walls by immunofluorescence microscopy. • No thrombosis, necrosis, or inflammatory infiltration of the vessel wall is seen. • The vessel lumen is not compromised.
Lupus vasculopathy (non-inflammatory)	• Abundant immune complexes affecting preglomerular arterioles (typically), causing significant luminal narrowing or occlusion. Deposits may extend into the media. • No necrosis or inflammatory infiltration of the vessel wall is seen.
True renal vasculitis/ lupus vasculitis	• True inflammatory infiltration of the arterial intima and media by neutrophils and mononuclear leukocytes, often accompanied by fibrinoid necrosis and rupture of elastic lamellae
Thrombotic microangiopathy	• Arterioles show swelling of the endothelial cells and subendothelial space. The arteriolar lumen may be severely narrowed, and fibrinoid necrosis may occur (without a significant inflammatory component). Fragmented and/or hemolyzed erythrocytes may be seen. • Interlobular renal arteries may show swelling of the intima, which may be accompanied by mucoid intimal hyperplasia. The cellular intimal proliferation may be seen and give rise to an “onion skin” pattern lesion. • Discrete immune-completes deposits are not seen. There is no leukocyte infiltration of the vessel wall.

The prevalence of vasculitis (at any site) in the setting of SLE is reported to be between 11 and 36%.[1] While cutaneous lesions are most frequent, medium and large vessel vasculitis can affect any of the visceral organs. True renal vasculitis is rare and has been infrequently reported in the literature. Among patients with SLE undergoing renal biopsy, the prevalence of true renal

vasculitis has been reported to be between 0.6% and 2.8%.[2,3] True renal vasculitis is histologically indistinguishable from the vasculitides seen in polyarteritis nodosa (when large vessels are affected) or microscopic polyangiitis (when smaller vessels are affected), and the exact pathogenesis of renal vasculitis in SLE is unclear. *Moeckel et al* reported a case of true renal vasculitis with electron dense immune-complex type deposits seen by immunofluorescence and electron microscopy, suggesting immune-complex mediated vasculitis may underly the pathogenesis of this lesion in at least a subset of cases.[4]

The differential diagnosis for renal vasculitis in a patient with SLE must include ANCA-associated vasculitis and cryoglobulin-associated vasculitis; the finding of vasculitis on kidney biopsy should prompt laboratory studies for ANCA and cryoglobulins. The presence of immune-complexes within the vessel walls would suggest a pathogenesis distinct from ANCA-mediated vasculitis, but often vasculitic lesions are focal and not well-represented across tissue seen by light, immunofluorescence, and electron microscopy. Patients with SLE can have circulating ANCAs, and some studies suggest that patients with SLE and positive ANCA serologies may have more active renal disease. [5] Similarly, patients with SLE may also have detectable cryoglobulins, and immune-complex mediated vasculitis in the setting of cryoglobulins should be excluded. In patients who lack circulating ANCAs and cryoglobulins, a diagnosis of true renal vasculitis in the setting of SLE can be made. Due to the rarity of this lesion, systematic studies are lacking, and specific treatment approaches have yet to be established.

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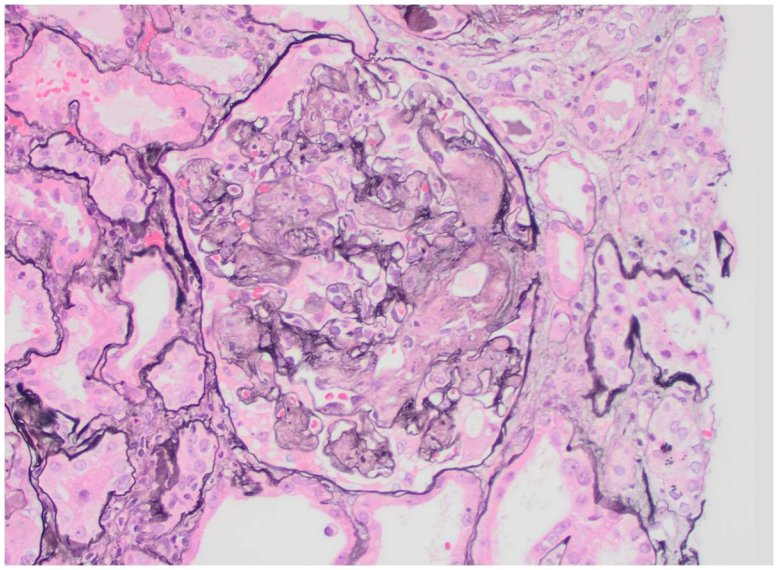
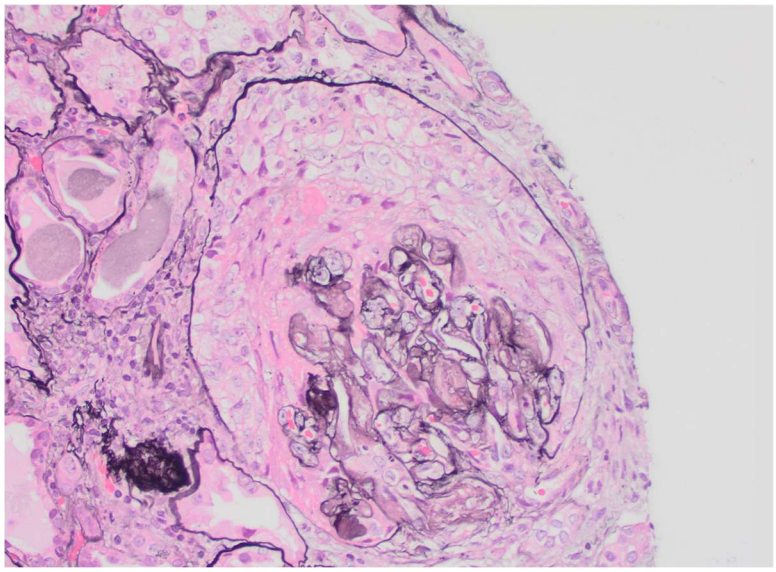
Case 2 from Nicole K. Andeen, MD – Oregon Health & Science University

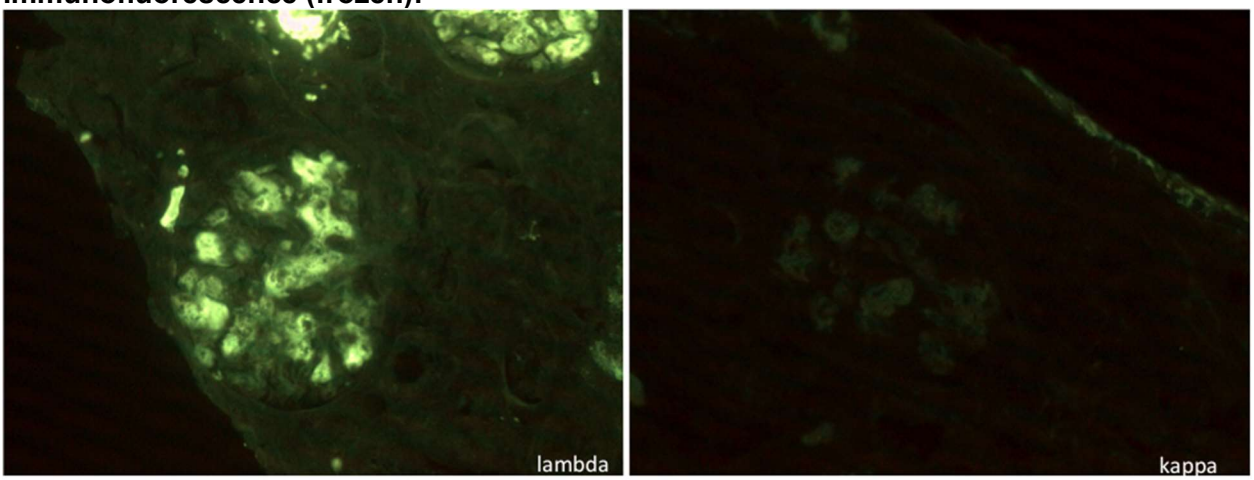
A 75-year-old relatively healthy woman with a history hypertension and aortic valve repair presented with massive proteinuria (26 g) and AKI with a creatinine of 3.8 mg/dL, up from a baseline of 0.5 mg/dL. She became oliguric and was hospitalized with a creatinine which continued to rise rapidly to 6.7 mg/dL. At the time of inpatient kidney biopsy, she had no other systemic symptoms and no known history of a rheumatologic disorder, infection, or malignancy. Laboratory studies for ANA, ANCA, anti-GBM, anti-PLA2R, hepatitis B and C, HIV, SARS-Co-V2 PCR, blood cultures, and complement levels were negative/normal.

Jones silver stain:

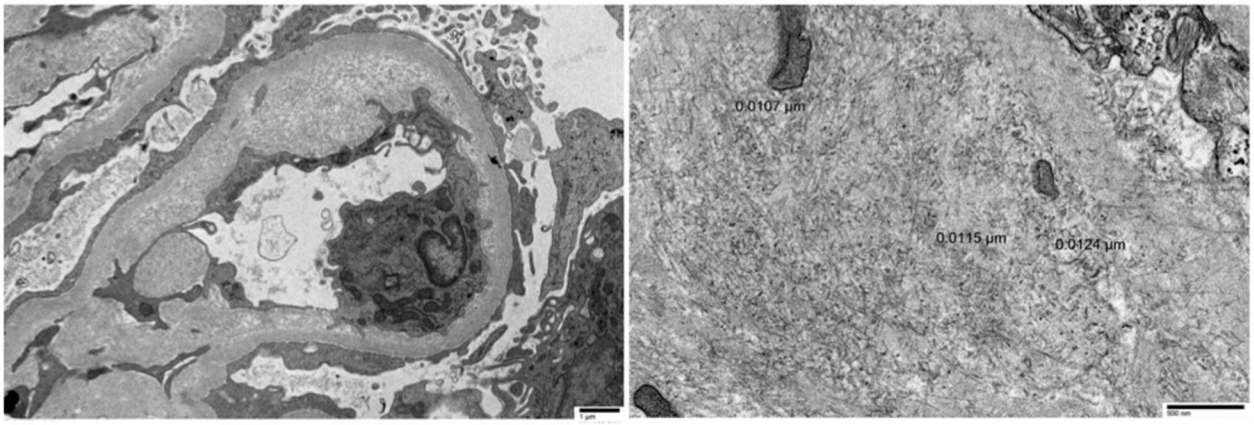




Immunofluorescence (frozen):



Electron microscopy:



Pathologic Findings

Light microscopy: Kidney biopsy consisted of renal cortex containing 35 glomeruli, 3 of which (9%) were globally sclerosed and 17 of which (49%) had cellular to focally fibrocellular crescents. Glomeruli had mesangial and segmental peripheral capillary wall involvement by acellular matrix with a pale staining quality on PAS and Jones stain. No subepithelial “spicules” were identified. Occasional neutrophilic influx was seen in glomerular capillaries. The tubulointerstitium had acute tubular injury and a mild interstitial inflammatory infiltrates composed of mononuclear leukocytes and a few neutrophils. There was mild tubular atrophy and interstitial fibrosis and mild arteriosclerosis. A Congo red stain for amyloid was positive, highlighting glomerular amyloid, but no tubulointerstitial or vascular amyloid. DNAJB9 stain was negative.

Immunofluorescence microscopy: Standard frozen immunofluorescence on frozen tissue (scale 0-4+) demonstrated bright, smudgy, mesangial predominant glomerular staining for lambda light chain (4+), with dull highlighting for IgG (1+), IgM (1+), C3 (1+), and C1q (trace to 1+); there was no significant glomerular staining for kappa light chain or fibrin/fibrinogen. Tubular casts and tubular resorption droplets stained for kappa and lambda light chains; kappa and lambda had similar staining intensity and distribution. There was no significant staining of

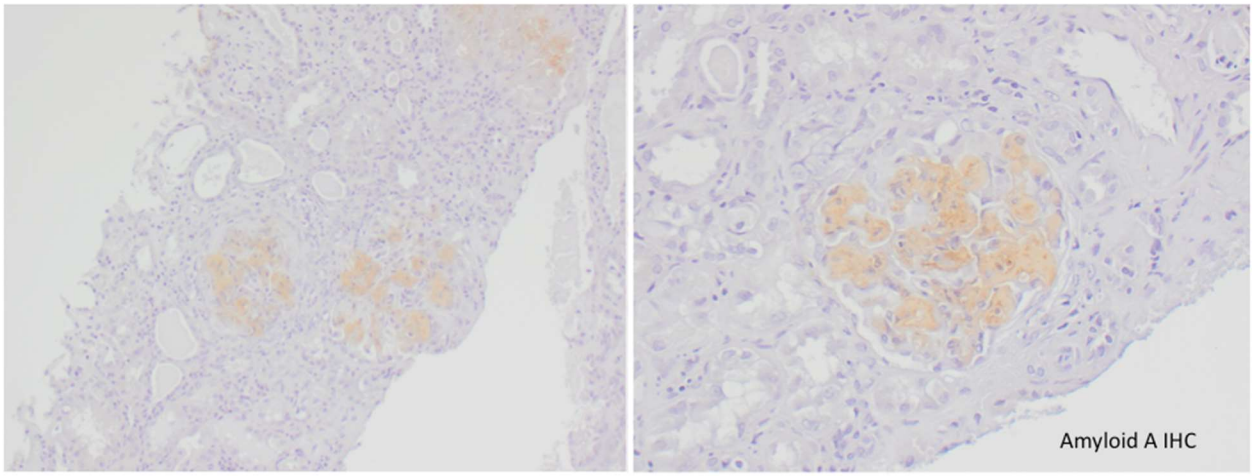
the tubular basement membranes, interstitium, or vasculature for any of the tested immunoreactants.

Electron microscopy: Glomeruli showed prominent mesangial infiltration by haphazardly arranged fibrils with a diameter which ranged from 8-12 nm. There was segmental subendothelial space infiltration by fibrils, with focal infiltration of the glomerular basement membrane and outward projections toward podocytes. Podocyte foot processes were diffusely effaced. No endothelial tubuloreticular inclusions were seen. No conventional immune deposits were present.

Initial Diagnosis
Crescentic AL amyloidosis, lambda light chain type.

Clinical Follow-Up
Based on the initial diagnosis, presence of ~50% crescents, and impending dialysis requirements, the patient received an initial dose of cyclophosphamide, bortezomib, and dexamethasone (CyBorD).

However, subsequent laboratory studies and bone marrow biopsy resulted within the next couple days while in the hospital revealed no detectable paraprotein in blood or urine. Bone marrow biopsy showed no evidence of a B-cell or plasma cell neoplasm. Based on this information, additional studies were performed on the kidney biopsy, which revealed staining for serum amyloid A protein by immunohistochemistry. Mass spectrometry performed at Mayo also confirmed AA rather than AL amyloidosis.



Revised Biopsy Diagnosis
Crescentic AA amyloidosis, false positive lambda chain staining.

Clinical Follow-Up Part 2
In the ensuing months, the patient underwent extensive testing in attempt to determine the etiology of AA amyloid, including consultations with two rheumatologists and repeat serologic evaluations, which were unrevealing. CT of the head, chest, abdomen, and pelvis showed no evidence of malignancy, abscesses, or other occult infection. She is clinically well with no fever, and infectious workup including blood cultures and transesophageal echocardiogram to evaluate for the possibility of vegetations on the prosthetic aortic valve have been negative. She has not undergone genetic testing but has no family history of kidney disease. She was

evaluated at 2 referral amyloid clinics, and the etiology of, and optimal treatment for, her AA amyloid remain unknown.

Remarkably, despite receiving the wrong initial diagnosis and arguably wrong treatment, the patient clinically responded well to cyclophosphamide and prednisone without adverse effects. Proteinuria improved from 26 grams to 4 grams, and she remained off dialysis with a creatinine which had improved from 6.7 mg/dL to 2.0 mg/dL at last available follow-up (~6 months).

Questions

1. **What is the most common type of amyloidosis to be associated with crescents?**
    A. AL amyloid, kappa light chain type
    B. AL amyloid, lambda light chain type
    C. AA amyloid
    D. ALECT2 amyloid
    E. APOIV amyloid

Crescents are uncommon in amyloidosis. Of reported cases, the large majority are associated with AA amyloid (1, 2), although a few cases of AL amyloid with crescents (1, 3, 4) have been described.

2. **Based on available data – which is currently guided by a report from a national referral center – what is the proposed "false-positive" rate for amyloid subtyping by immunofluorescence microscopy compared with mass spectrometry?**
    A. 0.5%
    B. 2.9%
    C. 6.2%
    D. 8.0%
    E. 12%

Five of 170 samples (2.9%) sent for mass spectrometry at Mayo showed false-positive staining, all of which appeared to be lambda light chain AL amyloidosis but were AA amyloidosis (5). These selected cases represent those identified for mass spectrometry, and no further clinical or histologic evaluation was available for integration. Additional studies from other institutions, particularly using amyloid A IHC routinely, and incorporating other clinical and pathologic features, are needed to further characterize this phenomenon and false positive rate in daily practice.

3. **What are the common clinical associations with AA amyloidosis?**
    A. Chronic infections, particularly in setting of injection drug use with "skin popping" or "muscling"
    B. Rheumatoid arthritis
    C. Malignancy
    D. Genetic/familial
    E. All of the above

All of the above are associated with AA amyloidosis.

Discussion

This case highlights false-positive staining for lambda light chain in a patient with crescentic AA amyloid. In addition to documenting some atypical morphologic features which may have been clues to the correct diagnosis initially, it highlights a rare example of a pitfall in relying on clinical associations when suspecting AA amyloid. Finally, it provides a small data point regarding the potential utility of immunosuppression in the treatment of crescentic AA amyloid without a known etiology.

AL amyloid is generally the most common form of amyloidosis seen on kidney biopsy, although depending on location, other types including AA amyloid or ALECT2 amyloid may dominate (6). AL amyloid occurs in older adults with a circulating monoclonal protein and plasma cell clone, and those with renal involvement commonly present with nephrotic syndrome (6). In up to 1.9% of cases of AL amyloidosis, a paraprotein may not be detected by conventional assays (7). Thus, some patients may come to kidney biopsy with a negative paraprotein evaluation, or prior to testing for a monoclonal protein, as occurred in this case.

Crescentic amyloidosis is rare. Case series examining the prevalence of crescents in amyloid are relatively limited, although one series of biopsies and autopsies describes crescents in up to 13% of renal amyloidoses (1). In review of this literature, 16 of 21 (76%) recently reported cases of amyloid with crescents have been associated with AA amyloid, most commonly in patients with rheumatoid arthritis or underlying malignancies; less commonly, AL amyloid may have crescents (1-4, 8-10). It has been suggested that the mechanism of crescent formation is due to glomerular basement membrane rupture in area of amyloid deposition (1) but is likely multifactorial. Thus, by light microscopy, the presence of crescents, in addition to the inflammatory background and lack of vascular involvement were atypical for AL amyloid.

Like any test, there are known pitfalls of relying entirely on immunofluorescence for a diagnosis. A mass spectrometry based study performed on 170 cases at Mayo revealed 5 cases (2.9%), all of which appeared to be lambda light chain AL amyloidosis but were AA amyloidosis (5). Due to the nature of a national reference center, these cases represented those selected for mass spectrometry, and limited additional clinical or histologic evaluation was available to better understand other clinicopathologic features associated with unreliable immunofluorescence staining. The reason for this false positivity is not entirely clear; AA amyloid is known to be “sticky” and can also show false positive reactivity for DNAJB9, a protein considered otherwise sensitive and specific for fibrillary glomerulonephritis. Additional studies from other institutions, particularly with inclusion of clinical and pathologic features use of amyloid A IHC routinely are needed to further characterize this phenomenon and determine false positive rate in daily practice.

AA amyloid is most commonly associated with injection drug use with “skin popping” and “muscling” and associated chronic infections in our region of the US (11). It is well-described in patients with rheumatoid arthritis (12), inflammatory bowel disease, and Familial Mediterranean Fever (13, 14), some solid organ malignancies (2), and others. Recently, a mutation in the *SAA1* promoter was identified as a cause of hereditary AA amyloidosis in one family (15). This mutation led to a doubling of the basal *SAA1* promoter activity with sustained elevation of serum amyloid A levels (15). Despite an extensive clinical, radiographic, and serologic evaluation, our patient has no identified reason for AA amyloid. Genetic testing has not been perused, although at age 75, she is above most reported ages of familial AA amyloid, and she has no family history of kidney disease. In the absence of a known driver, genetics and her known prosthetic aortic valve (which has been without complications) represent possible contributors to AA amyloid. In our experience, ~95% of cases of AA amyloid have a known, longstanding clinical

association for AA amyloid at the time of kidney biopsy. Thus, this rare case represents a pitfall in relying on the presence of these clinical associations when suspecting AA amyloid.

Finally, Tocilizumab, a monoclonal antibody against the IL-6 receptor, may be beneficial when prescribed early in familial AA amyloid (15). Other studies have demonstrated efficacy of anti-inflammatory or immunosuppressive agents in select etiologies of AA amyloid (16), including cyclophosphamide for AA amyloidosis in the setting of rheumatoid arthritis (17, 18). The mechanism for clinical response to cyclophosphamide in this patient is not known, but given quick rate of improvement, presumably represents treatment response to the acute crescentic injury. The mechanistic connection between AA amyloid and crescents, and whether continued immunosuppression could also help ameliorate the amyloid deposition itself in this patient, remain important questions. The clinical response to cyclophosphamide and prednisone in this case provides an additional data point when considering therapeutic options for a select subset of patients with AA amyloidosis.

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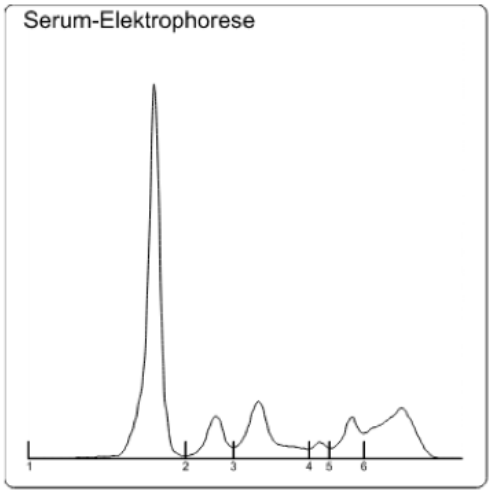
Case 3 from Renate Kain, MD, PhD – Medical University Vienna

A 66-year-old man presented to the orthopedic outpatient clinic with pain and signs of inflammation in his left knee (an endoprosthesis had been implanted 3 years previously). There was ulceration on both feet (toes 2 and 3 on the left and 4 and 5 on the right) which was interpreted as gangrene due to peripheral vascular disease because he had a clinical history of cardiac insufficiency (NYHA II). He was admitted to the orthopedic ward, antibiotic treatment with Ampicillin/Sulbactam was initiated, and lavage of the knee was performed 2 days later. Microbiology confirmed infection with *Staphylococcus aureus*, and further pre-operative investigations revealed deep vein thromboses in the left popliteal and superficial femoral veins.

After surgery, the patient was admitted to ICU and required treatment with catecholamines. A post-operative serum creatinine (176 umol/l, 2.31 mg/dL) was interpreted as prerenal failure since it returned to normal within 10 days; he was transferred back to the orthopedic ward. A dermatological consultation initially suggested the ulcers on both feet could be the result of septic emboli. Since the patient was certain that they had only been present for 2-3 weeks and had originated from blisters after a long hike that became hemorrhagic and ruptured, and as there was no evidence for peripheral arteriopathy, this was dismissed. TTE had revealed a small perimembranous VSD, a small ASD, both without hemodynamic relevance. Consequently, the skin ulcers were assumed to be most likely the source of the infection of the knee endoprosthesis.

However, over the next 4 days, he developed leukocyturia, hematuria, and proteinuria. His serum creatinine increased again to 140.8 umol/l (2.31 mg/dL), eGFR (MDRD) was 56 ml/min/1.73 m^2, albuminuria peaked at 18 g in 24 hours with an albumin:creatinine ratio in urine at 11750m and serum albumin fell to 18.8 g/dL. The leukocyte count was 20.91, and the CRP was 25.1 mg/dL. On day 16 after admission, the knee endoprosthesis was removed (and subsequently proved to be microbiologically negative). The patient was transferred back to ICU where, 4 days later, nephrological consultation was requested. By that time, he had nephrotic range proteinuria, leukocyturia, and microscopic hematuria with 95% dysmorphic erythrocytes. Autoimmune (ANCA, ANA, dsDNA with subsets, anti-GBM, anti-PLA2R) and Hepatitis serology were negative.



Serum electrophoresis relative				
1. Albumin Elpho	-	49	%	55.8 – 66.1
2. Alpha-1-Globulin	+	7.9	%	2.9 – 4.9
3. Alpha-2-Globulin	+	14.9	%	7.1 – 11.8
4. Beta-1-Globulin	-	2.3	%	4.7 – 7.2
5. Beta-2-Globulin	+	7.4	%	3.2 – 6.5
6. Gamma-Globulin		18.1	%	11.1 – 18.8

Serum electrophoresis showed reduced Albumin and beta-2 globulin but raised alpha-1-, alpha-2- and beta-2-globulin. Immune fixation excluded presence of paraproteinaemia.

Normal range		
0,90 - 1,80 g/L	C3c	0.94
0,10 - 0,40 g/L	C4	0.15
3,3 - 19,4 mg/	Free Kappa LC	108.33
5,71 - 26,3 mg/L	Free Lambda LC	96.10
0,26 - 1,65	Ratio Free Lambda:Kappa LC	1.13
40 – 230 mg/dl	IgM	31
70 – 400 mg/dl	IgA	247
700 – 1600 mg/dl	IgG	734
405 - 1011 mg/dL	IgG 1	660
169 - 786 mg/dL	IgG 2	215
11 - 85 mg/dL	IgG 3	17.1
3 - 201 mg/dL	IgG 4	67.1

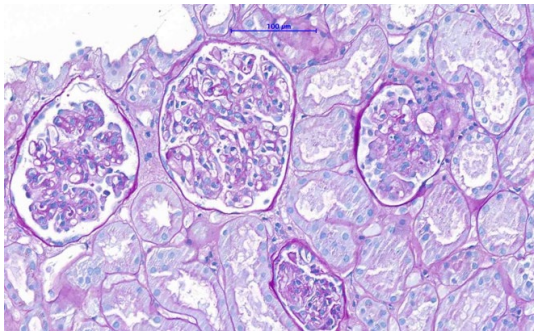
Complement and Ig levels were, except for IgM that was decreased, within the normal range. Free kappa and lambda light chains were raised however the ratio was normal.

Antibiotic treatment with Cefozolin and Fosfomycin was continued, and he received albumin infusions since he was hemodynamically unstable. A CT-guided renal biopsy was performed with a presumptive differential diagnosis of postinfectious glomerulonephritis or necrotizing and crescentic glomerulonephritis. Secondary focal segmental sclerosis because of the high degree of proteinuria and hypoalbuminaemia was also considered.

Pathological Findings

Light microscopy and immunohistochemistry: Renal biopsy showed a diffuse, segmentally accentuated mesangial and endocapillary proliferative glomerulonephritis. The endocapillary infiltrate was dominated by neutrophilic granulocytes. Mesangial immune complexes contained IgA and C3. Apparent were both large subendothelial and scanty subepithelial immune complexes containing IgA and C3. IgG was negative, and discreet mesangial staining was seen with antibodies to IgM and C1q. Focal necrosis with reactivity of Bowman's epithelial cells (small crescents) could be seen in 2 glomeruli. The provisional diagnosis before electron microscopy was that of an IgA-Nephritis with unusual features – as mesangial IgA was segmentally accentuated; postinfectious IgA-dominant nephritis, and IgA-Nephritis exacerbated by infection were also considered. A MEST Score of M1/E1/S0/T0/C1 was applied.

Electron microscopy: EM confirmed the presence of electron-dense deposits in the mesangium, sub-endothelially, together with occasional subepithelial "hump"-like deposits. EM also confirmed the presence of neutrophils within capillary lumen; effacement of podocytic foot processes was about 50%.



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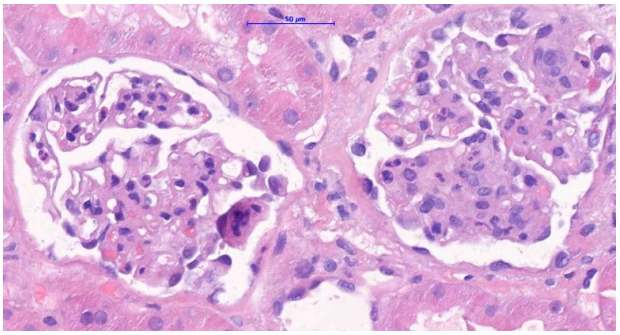
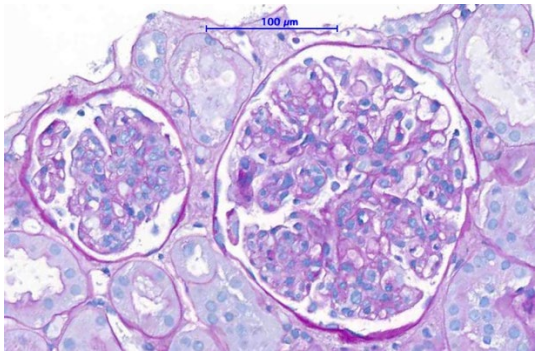


H&E



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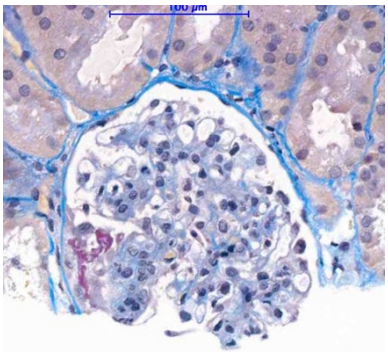
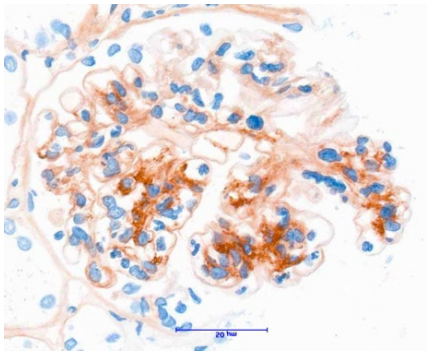
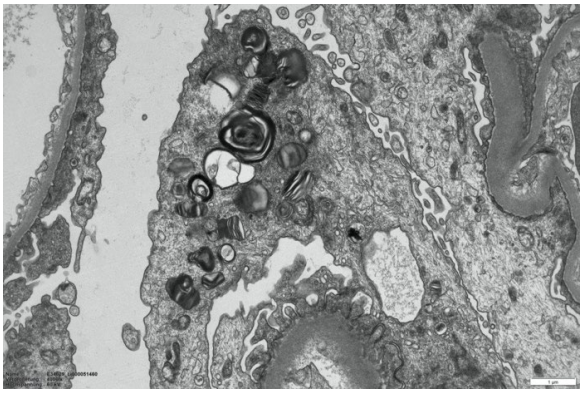
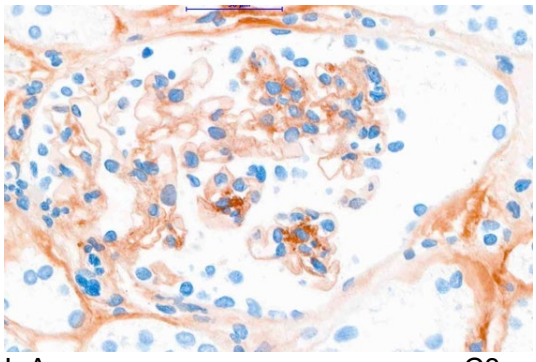


AFOG

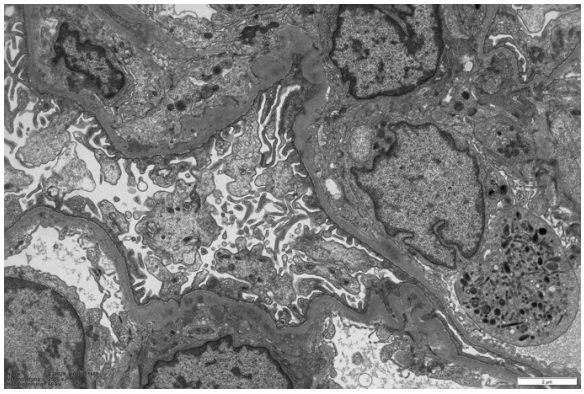
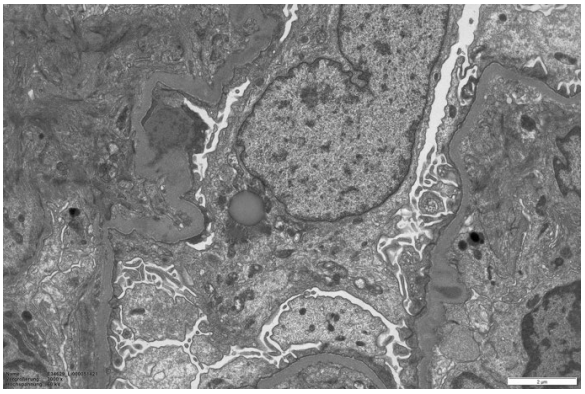






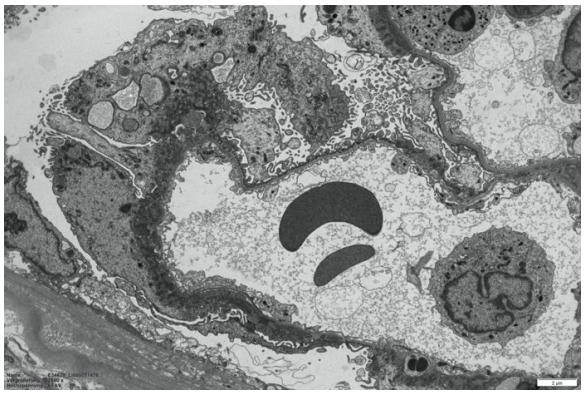
IgA

C3c



TEM

Clinical Follow-Up

The day after the biopsy the patient was transferred to the renal ward; the antibiotic regimen was changed to Dalbavancin and Cefalexin; and antiproteinuric treatment was initiated with RAAS blockade, spironolactone, SGLT2 inhibitors, and diuretics; neither corticosteroids nor other immunosuppressive treatment were given. Lavage of the knee was continued, anticoagulation, which had been stopped immediately before the renal biopsy, was not reinitiated. During the following period, the patient lost 15 kg in weight, CRP declined to 161.9 nmol/l, and his renal function returned to normal with an eGFR (MDRD) of 93 ml/min (normal range >60). However, his proteinuria persisted albeit a decreased level with albuminuria at 35 umol/l and an albumin:creatinine ratio of 4653.



Questions

1. **What is the primary morphological glomerular pattern by light microscopy?**
    A. Mesangial proliferative glomerulonephritis
    B. Endocapillary proliferative glomerulonephritis
    C. Mesangial and endocapillary proliferative glomerulonephritis
    D. Focal segmental glomerulosclerosis
    E. Focal necrotizing and crescentic glomerulonephritis

The morphological pattern clearly shows both, endocapillary and mesangial proliferation. Mesangial expansion was mainly due to cell and not matrix increase. Typical features of segmental sclerosis were not seen. Although Fibrin was observed in 2 of 16 glomeruli in the Bowman's space with small extracellular proliferations, it cannot be considered a true focal necrotizing and crescentic glomerulonephritis.

2. **What do you consider the most likely diagnosis, taken into account clinical history and morphological presentation (LM, IHC, TEM)?**
    A. Acute IgA-Nephritis
    B. Acute IgA-Vasculitis
    C. IgA-Nephritis with atypical features
    D. Acute Post-infectious glomerulonephritis
    E. Acute IgA-dominant post-infectious glomerulonephritis

Despite some unusual features, the morphology in the context of clinical history of Staph. aureus infection clearly points towards an IgA-dominant APIGN. There was severe proteinuria but no hypocomplementemia which raised the question about concurrent minimal change disease or (secondary) FSGS, which were excluded by TEM. The IgA was segmentally accentuated as sometimes seen in IgA-Vasculitis, and C3 was not stronger as has been described.

3. **Knowing the pathophysiology of this patient's kidney disease, what was an unexpected finding in the TEM?**
    A. Subepithelial "humps"
    B. Thinning of the basement membrane
    C. Neutrophilic granulocytes
    D. "Zebra" bodies
    E. Mesangial and subendothelial electron dense deposits

While all other features seen can be considered consistent with IgA-dominant APIGN, only the "Zebra" bodies do not fit the picture and can be considered unusual.

Discussion

IgA-dominant APIGN was described more than 25 years ago as defined entity in the context of *Staph. aureus* infection (1), and several subsequent cases have been described in the literature (reviewed in 2), and a distinction in *Staphylococcus* infection-associated glomerulonephritis (SAGN) and IgA-dominant deposition infection-related glomerulonephritis (IgA-IRGN) has been proposed (3). The pathogenesis of IgA-dominant APIGN following *Staph. aureus* infection is not fully understood as the features after methicillin resistant (MRSA) and responsive *Staph. aureus* infections appear similar and may involve interactions of the host with antigens common to both (2). The clinical picture is mostly characterized by acute onset renal failure, proteinuria, and hematuria following *Staphylococcus* infection, most commonly of the skin (2). Clinically patients can present with hypocomplementemia with decreased levels of C3 or C4 and raised polyclonal serum IgA.

Pathohistological features and immunohistochemistry of IgA-dominant APIGN characteristically can include both, changes seen in IgA-Nephritis (mesangial and endocapillary proliferation, crescent formation, and mesangial deposition of IgA and C3) but also those of APIGN (like marked granulocytic infiltrate and subepithelial "humps" by TEM; 4). Subendothelial deposits have been reported as small and rare (2), however were seen prominently in our patient – a finding also described in cases with methicillin sensitive *Staph. aureus* infection (SAGN; 3) which may represent a distinguishing feature.

The differential diagnosis includes underlying IgA-Nephritis exacerbated by infection and IgA-Vasculitis (HSP). In IgA-dominant APIGN, IgA can be detected also in the deposits along the capillary wall, although C3 staining is generally stronger with a typical granular or "starry sky" pattern than IgA, and there should be greater mesangial staining for kappa LC, while in primary IgA-Nephritis, a dominance of lambda LC has been reported (2,5). As aberrantly *O*-linked glycosylated IgA1 and antiglycan autoantibodies are now considered to play a major role in primary IgA-Nephritis, the detection of galactose-deficient IgA1 (Gd-

IgA1) in circulation and/or tissue may distinguish primary IgA-Nephritis from other forms (6). However, conclusive evidence is missing and would require larger studies for confirmation. In contrast, IgA-Vasculitis – although also seen in older patients – can be excluded on clinical grounds if purpura and gut manifestation are absent. Crescents by themselves are not a distinguishing feature, as they can be seen in both IgA-Nephritis and IgA-Vasculitis but are also a feature of APIGN (7).

In this context, it is noteworthy that, while predictive classification schemes, like the Oxford Classification of IgA-Nephritis (8), are firmly established, their application to IgA-dominant APIGN is not recommended. Indeed, its use may be futile and merely deflect from the fundamental difference in disease pathogenesis and subsequent treatment which is in principle that of the underlying infection in IgA-dominant APIGN following *Staph. aureus* infection (3).

Morphological features that may obscure the primary diagnosis include foot process effacement and, as seen here, “Zebra” bodies in podocytes. Both are related to heavy proteinuria, such as occurs in minimal change disease and focal segmental sclerosis and, also in rarer situations, such Fabry’s disease. Exclusion of the former pathologies may be difficult in the presence of immune complexes, except when foot process effacement is present in less than 50% of capillaries, or when it occurs only in capillary loops where electron dense deposits are seen. Extensive proteinuria has been described in IgA-dominant APIGN (3); however, its persistence may indicate another or additional glomerular pathology that may require a re-biopsy at a later stage.

The occurrence of lamellar inclusions or so called “Zebra” or “Zebroid” bodies is a characteristic feature of Fabry’s disease (9); however, they can be seen in the context of numerous other mainly cationic amphiphilic drugs drugs, among them but not exclusively, (hydroxy)-chloroquine (10). However, “Zebra” bodies are not uncommon in renal biopsies (10) and a recent report suggests that in their presence Fabry’s disease should still be considered (11).

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Case 4 from Benjamin A. Adam, MD - University of Alberta

A 42-year-old man presented with a 3-month history of nephrotic range proteinuria. His past medical history was significant for bipolar I disorder requiring frequent hospital admissions, nephrogenic diabetes insipidus secondary to lithium treatment for 5 years, and a 6-year history of untreated chronic lymphocytic leukemia (CLL).

Laboratory findings:

Serum	**Value**	**Reference Range**
Creatinine	102	50-120 (umol/L)
eGFR	78	>59 (mL/min/1.73m2)
Urea	5.0	2.5-8.0 (mmol/L)
Sodium	139	133-146 (mmol/L)
Potassium	4.6	3.5-5.0 (mmol/L)
Chloride	110	96-109 (mmol/L)
CO2	22	23-31 (mmol/L)
Calcium	2.12	2.10-2.60 (mmol/L)
Magnesium	0.85	0.70-1.00 (mmol/L)
Phosphate	1.27	0.80-1.45 (mmol/L)
Albumin	**32**	35-50 (g/L)
Total Protein	**51**	64-84 (g/L)
Triglyceride	**4.42**	0.00-1.70 (mmol/L)
Fasting Glucose	5.5	3.3-6.0 (mmol/L)
Creatine Kinase	22	<250 (U/L)
ALT	20	<50 (U/L)
Hemoglobin	142	135-175 (g/L)
Platelets	**118**	140-450 (10**9/L)
Leukocytes	**152.3**	4.0-11.0 (10**9/L)
Neutrophils	6.1	1.8-7.5 (10**9/L)
Lymphocytes	**146.2**	1.0-4.5 (10**9/L)
Ferritin	34	12-300 (ug/L)
IgA	**4.54**	6.94-16.18 (g/L)
IgG	**0.59**	0.70-4.00 (g/L)
IgM	**<0.10**	0.60-3.00 (g/L)
Complement C3	1.12	0.80-2.10 (g/L)
Complement C4	0.26	0.15-0.50 (g/L)
ANA	Negative	Negative
anti-dsDNA	6	<120 (MFU)
ANCA Screen	Negative	Negative
Anti-GBM	2.2	<20 U/L
Cryoglobulin Screen	Negative	Negative
Serum Protein Electrophoresis	No evidence of monoclonal protein	
Urine	**Value**	**Reference Range**
Protein/Creatinine Ratio	**350.37**	<13 (mg/mmol)
pH	7.0	4.5-8.0
Specific Gravity	1.016	1.005-1.030
Protein	**3+**	Negative
Hemoglobin	**1+**	Negative

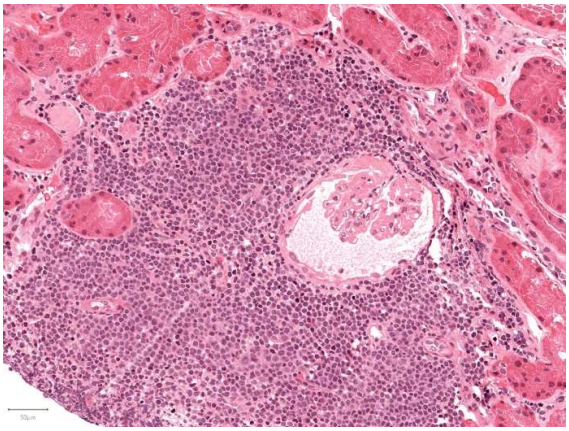
Glucose	Negative	Negative
Ketones	Negative	Negative
Leukocytes	Negative	Negative
Urine Protein Electrophoresis	**Significant amount of albumin, moderate amount of alpha, beta and gamma globulins; suggestive of combined glomerular and tubular proteinuria consistent with glomerular and tubular damage.**	

A kidney biopsy was performed; representative images are below.

Light microscopy:

H&E

H&E



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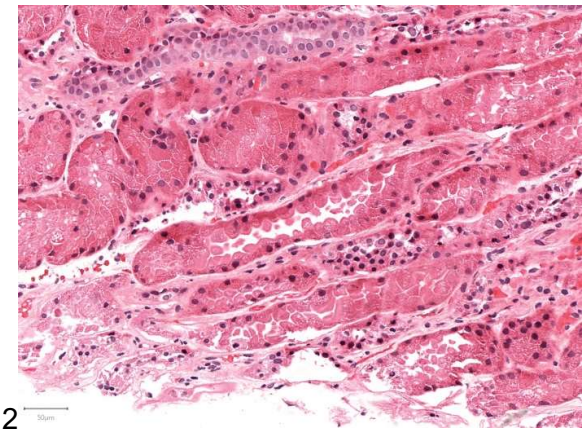

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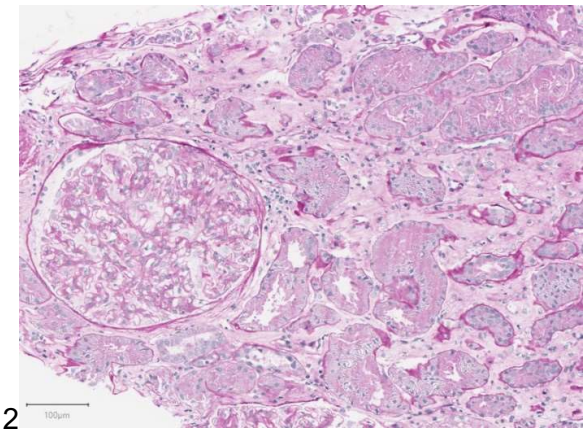

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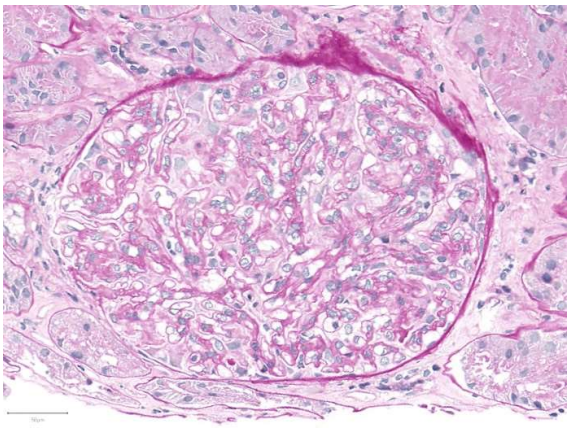
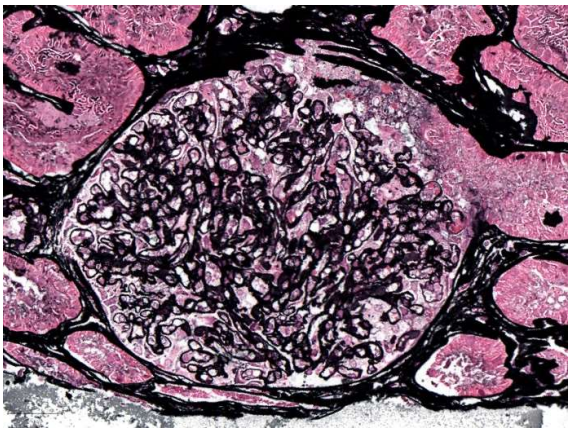
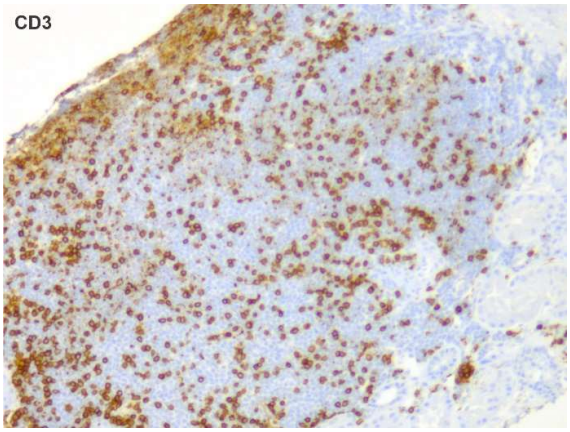




Immunohistochemistry:

CD3



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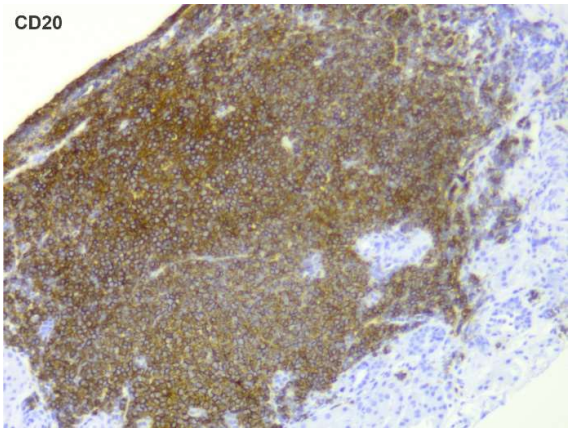

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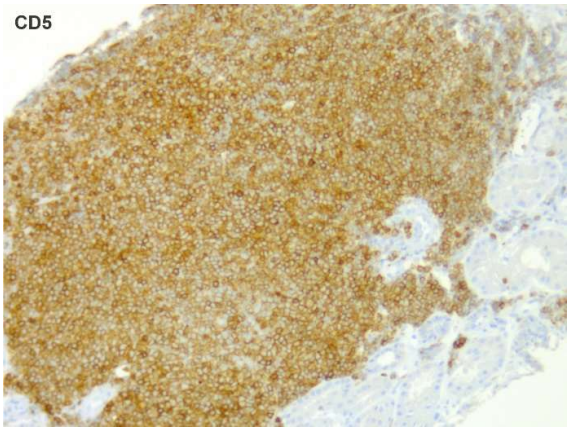

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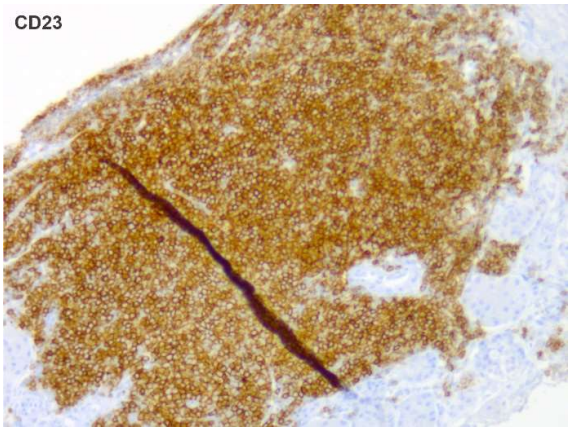
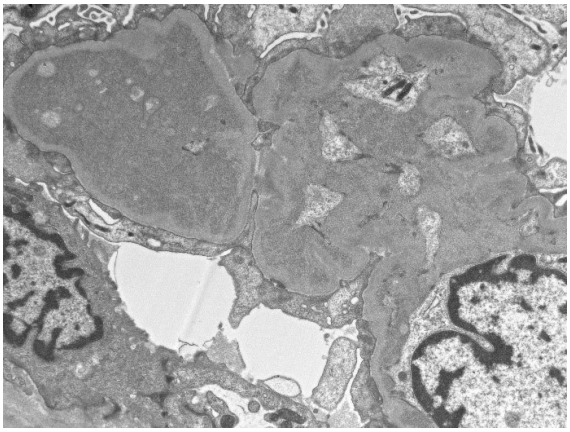
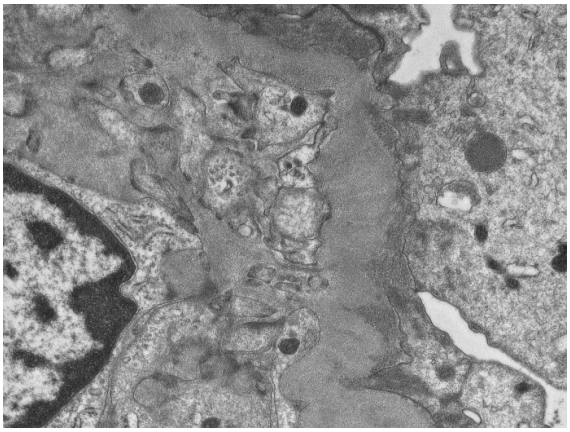


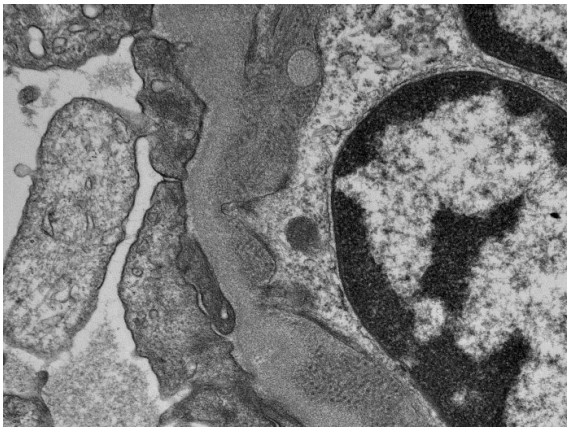
Immunofluorescence:

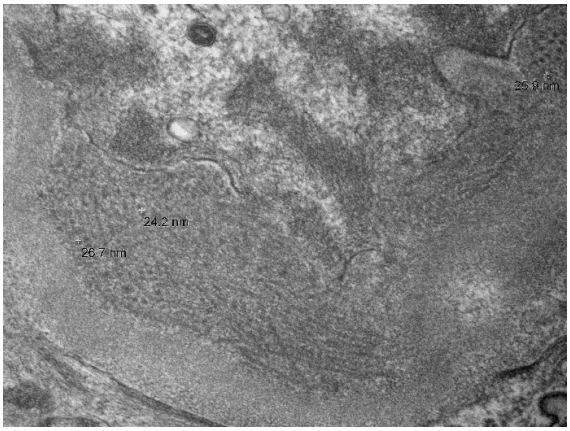
Stain	Interpretation
IgG	Strong granular mesangial and peripheral capillary loop staining
IgA	Negative
IgM	Negative
C3	Strong granular mesangial and peripheral capillary loop staining
C1q	Moderate granular mesangial and peripheral capillary loop staining
Kappa	Mild granular mesangial and peripheral capillary loop staining
Lambda	Negative
Albumin	Moderate tubular epithelial staining

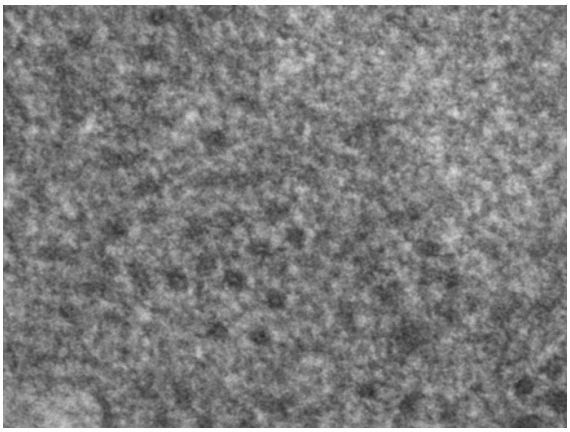
Electron microscopy:

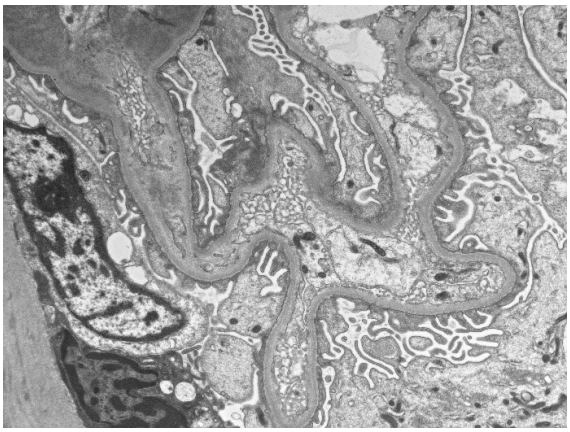












Pathologic Findings

Light microscopy: The kidney biopsy contained a total of 15 glomeruli, 5 of which were globally sclerotic. Three of the non-sclerotic glomeruli were hypertrophic. The non-sclerotic glomeruli also showed focal mild mesangial matrix expansion and hypercellularity and focal ischemic capillary loop wrinkling. There was no significant endocapillary hypercellularity, crescents, necrosis, thrombi, or segmental sclerosis. The glomerular basement membranes appeared within normal limits. The background renal parenchyma showed mild to moderate interstitial fibrosis and tubular atrophy involving approximately 20-30% of the cortex. There was patchy dense tubulointerstitial inflammation consisting predominantly of small monotonous lymphoid cells. A few glomeruli were surrounded by this lymphoid infiltrate. There was also patchy mild acute tubular injury characterized by tubular epithelial cell attenuation with brush border loss and hobnail change. The biopsy included three arteries with mild fibrous intimal thickening. The arterioles showed moderate hyalinosis. Congo red histochemical stain was negative for amyloid.

Immunohistochemistry: The dense tubulointerstitial lymphoid infiltrate consisted predominantly of CD20-positive B-cells with co-expression of CD5 and CD23. A smaller number of background CD3-positive T-cells were also noted. DNAJB9 immunohistochemistry was negative.

Immunofluorescence: Direct immunofluorescence performed on frozen tissue demonstrated six glomeruli with granular mesangial and peripheral capillary loop staining for IgG (3+), C3 (3+), C1q (2+), and kappa (1+). IgA, IgM, and lambda were negative.

Electron microscopy: Transmission electron microscopy demonstrated numerous subendothelial, mesangial, and subepithelial microtubular deposits organized in parallel arrays. Some of the subepithelial deposits exhibited a membranous pattern. The microtubular structures displayed electron-lucent cores and had an average diameter of 24 nm. No immune-complex type deposits were identified. There was also segmental podocyte foot process effacement, mesangial matrix expansion,n and subendothelial space widening. The background renal parenchyma showed patchy interstitial fibrosis and tubular atrophy with associated dense tubulointerstitial lymphocytic inflammation.

Clinical Follow Up

Following the biopsy, kidney specific treatment was not immediately initiated due to hospitalization for psychiatric illness. Two months post-biopsy, his urine protein/creatinine ratio was found to have worsened to 1627 mg/mmol (from 350), after which he was started on a CLL chemotherapy regimen (fludarabine, cyclophosphamide, rituximab). After 5 cycles of chemotherapy, his urine/protein creatinine ratio remained elevated at >1000 mg/mmol, until approximately 1-year post-biopsy when it started to drop to 477 mg/mmol. Three years post-biopsy, his urine/protein creatinine ratio has stabilized at approximately 100 mg/mmol. His serum creatinine remained stable throughout the post-biopsy period at approximately 100 umol/L (eGFR 75 mL/min/1.73m2). He has also since remained stable from a CLL perspective, with no evidence of relapsed lymphocytosis.

Questions

1. **What is the predominant light microscopic pattern of injury in this biopsy?**
    A. Crescentic glomerulonephritis
    B. Focal segmental glomerulosclerosis
    C. Tubulointerstitial nephritis
    D. Nodular glomerulopathy
    E. Thrombotic microangiopathy

The primary abnormality by light microscopy is tubulointerstitial nephritis, characterized by patchy dense interstitial and tubular lymphocytic infiltration consistent with renal parenchymal involvement by CLL. The glomeruli showed minimal light microscopic changes (focal glomerulomegaly, focal mild mesangial matrix expansion and hypercellularity, and focal ischemic changes) but no evidence of significant endocapillary hypercellularity, crescents, necrosis, thrombi, or segmental sclerosis. There were no glomerular or vascular abnormalities suggestive of thrombotic microangiopathy.

2. **Based on the immunofluorescence findings, what glomerular disease is in the differential diagnosis?**
    A. Amyloidosis
    B. Fibrillary glomerulopathy
    C. Cryoglobulinemic glomerulopathy
    D. Immunotactoid glomerulopathy
    E. All of the above

Immunofluorescence demonstrated a nonspecific pattern of 3+ IgG and C3 glomerular staining (with 1-2+ kappa and C1q staining), which can potentially be seen in all of the above entities.

3. **The immunohistochemistry findings indicate direct renal parenchymal involvement by which neoplastic entity?**
    A. Chronic lymphocytic leukemia
    B. Follicular lymphoma
    C. Myeloma/plasmacytoma
    D. Small cell carcinoma
    E. Rhabdomyosarcoma

Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is an indolent B-cell lymphoproliferative disorder composed of small lymphocytes with characteristic positivity for CD20, CD5, and CD23. This unique staining pattern helps distinguish it from other neoplasms

that can involve the kidney, including other indolent lymphoproliferative disorders (e.g., follicular lymphoma and myeloma/plasmacytoma, positive for CD10 and CD138, respectively) as well as potentially similar appearing primary or metastatic carcinomas (e.g., small cell carcinoma, positive for pankeratin and neuroendocrine markers) and sarcomas (e.g., rhabdomyosarcoma, positive for desmin and myogenin).

4. **Which disease is associated with deposits that do NOT have a characteristic substructure on electron microscopy?**
    A. Amyloidosis
    B. Fibrillary glomerulopathy
    C. Cryoglobulinemic glomerulopathy
    D. Immunotactoid glomerulopathy
    E. IgA nephropathy

Amyloidosis is characterized by randomly oriented fibrils with an average diameter of 7-12 nm. These fibrils are congophilic and display apple green birefringence under polarized light. The fibrils in fibrillary glomerulopathy are also randomly oriented but have a slightly thicker diameter of 9-25 nm. Most fibrillary glomerulopathy deposits are negative for Congo red and positive for DNAJB9. Cryoglobulinemic glomerulopathy can have short and curved microtubules ranging from 10-30 nm, although not every case has deposits with clear substructure. Immunotactoid glomerulopathy has parallel microtubular deposits with hollow cores that are often >30 nm in diameter but can range from 20-90 nm. IgA nephropathy shows nonspecific granular immune-complex type deposits without a characteristic substructure.

Diagnosis

Immunotactoid glomerulopathy with concurrent renal parenchymal involvement by chronic lymphocytic leukemia (CLL).

Discussion

This is a complicated biopsy with multiple findings. The most likely explanation for the clinical presentation of nephrotic range proteinuria is immunotactoid glomerulopathy. The additional presence of direct renal involvement by CLL and features suggestive of lithium toxicity are extraordinary but of uncertain clinical significance.

Immunotactoid glomerulopathy is a rare glomerular disease seen in less than 0.1% of adult native kidney biopsies. It is most often observed in patients over 50 years of age and almost always presents with nephrotic range proteinuria. The pathogenesis of this entity is unknown, but many cases are associated with an underlying plasma cell dyscrasia or lymphoproliferative disorder, such as CLL, as in this patient.

Immunotactoid glomerulopathy is characterized pathologically by the presence of microtubular deposits of IgG ranging from 20-90 nm in diameter, organized in parallel arrays and Congo red and DNAJB9 negative. Various light microscopy patterns can be seen, including mesangioproliferative (present in a mild form in this case), membranoproliferative, membranous, endocapillary proliferative, and focal crescentic. Immunofluorescence generally shows predominant IgG staining along with C3, C1q, and light chain restriction in most cases. Electron microscopy shows characteristic microtubular deposits in subendothelial and mesangial areas, although subepithelial and intramembranous deposits can also be seen.

The differential diagnosis for immunotactoid glomerulopathy includes other glomerular diseases with organized deposits, including amyloidosis (which is Congo red positive), fibrillary

glomerulopathy (which is DNAJB9 positive), and cryoglobulinemic glomerulopathy (in which glomerular pseudothrombi are often present and serum studies are positive for cryoglobulins). Treatment for immunotactoid glomerulopathy usually targets the underlying associated condition, such as chemotherapy for hematologic malignancy, as in this case. The clinical course depends on the underlying disorder, with approximately 50% achieving partial remission and 17% progressing to end-stage kidney disease.

Direct renal parenchymal involvement by lymphoid malignancies, including CLL, is a relatively uncommon finding on kidney biopsy, although it has been reported in up to 60-90% of patients with lymphoma/leukemia in several autopsy studies. Indeed, the kidneys are believed to represent the most frequent extramedullary site of leukemic infiltration. Although the clinical significance of this finding is unclear, with only 23% of patients with pathologic infiltration demonstrating kidney dysfunction, improvement with CLL treatment has been documented in patients with infiltrative disease on biopsy. Various glomerular diseases have been reported in association with CLL, including AL and AA amyloidosis, minimal change disease, membranous nephropathy, thrombotic microangiopathy, fibrillary glomerulopathy, immunotactoid glomerulopathy, C3 glomerulopathy, focal segmental glomerulosclerosis, and proliferative glomerulonephritis with immune complex deposits.

Lithium therapy, which has primarily been used for the treatment of bipolar disorder, is a well-documented nephrotoxin that damages the collecting ducts with long term use (generally >10 years). Even after discontinuation, CKD can develop up to 10 years after lithium treatment has stopped. The clinical manifestations of lithium nephrotoxicity include nephrogenic diabetes insipidus (40%), ESKD (0.5-1%), and rarely nephrotic syndrome. Characteristic pathologic findings include tubular dilation and microcyst formation, a hobnail appearance of the tubular epithelial cells, glomerulomegaly, segmental and global glomerulosclerosis, and interstitial fibrosis and tubular atrophy. The clinical management of lithium nephrotoxicity includes reduction or discontinuation of lithium therapy and amiloride, which may reduce the nephrotoxic effects through inhibition of the epithelial sodium channel. In the current case, lithium therapy was discontinued prior to the biopsy, when clinical evidence of kidney disease was identified; however, the presence of histologic features compatible with lithium toxicity (i.e., tubular epithelial hobnail change, glomerulomegaly, global glomerulosclerosis, and mild to moderate interstitial fibrosis and tubular atrophy), although nonspecific, are suggestive of chronic sequelae of the patient’s 5-year history of lithium treatment.

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