Renal Biopsy: Clinical Correlations
November 4, 4:30-6:30 p.m.
Case 1 from Volker Nickeleit, MD—University of North Carolina, Chapel Hill

The patient is a 33-year-old white woman with a reported history of hypertension and obesity and no other significant medical history. At the time of presentation, the patient was treated with an ACE inhibitor for control of hypertension. There was no evidence of peripheral edema, arthritis or arthralgias, skin lesions, fever, or abdominal pain. The BP at presentation was 160/100 mmHg; a physical examination did not include diagnostic observations.

Laboratory data (as provided on the biopsy requisition form) included the following:

<table>
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<tr>
<td>Serum creatinine</td>
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<tr>
<td>Blood urea nitrogen</td>
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<tr>
<td>eGFR</td>
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<tr>
<td>Serum albumin</td>
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<tr>
<td>Glucose</td>
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<tr>
<td>Hemoglobin A1c</td>
<td>5.5</td>
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Urinalysis: Hematuria 1+ (no red blood cell casts); proteinuria 1.8 g/24 h.

Serum complement levels, antinuclear antibody, anti-DNA, and hepatitis panel results; and ANCA titers were within normal limits, and there was no clinical evidence of an infection. The renal biopsy was performed to evaluate the underlying cause for the proteinuria.

Biopsy Findings

Light microscopy: Nine glomeruli were detected in 20 levels of section evaluated with hematoxylin and eosin, periodic acid-Schiff, trichrome, and Jones silver stains. Three of nine glomeruli were globally sclerosed or showed severe atrophy. Six of nine glomeruli were enlarged with focal and segmental tuft sclerosis, small foci of hyalinosis, and adhesion formation between sclerosed tufts and the Bowman capsule. Endocapillary proliferations and crescent formation were not present; mesangial zones did not show significant changes. The tubulo-interstitial compartment showed moderate (to severe) fibrosis and tubular atrophy, associated (nondiagnostic) lymphocytic infiltrates, and compensatory hypertrophy of some nonatrophic tubules. Intratubular red blood cell casts were not present. Minimal to mild sclerosis was seen in arterioles and arteries.
1. **The depicted glomerular changes are most suggestive of:**
   A. Minimal change disease
   B. Proliferative glomerulonephritis with crescents
   C. Nodular glomerulosclerosis
   **D. FSGS**
   E. Amyloidosis

   The glomerulus shows segmental tuft scarring with hyalinosis and adhesion formation to Bowman's capsule without evidence of endocapillary proliferations in perfused capillaries. Characteristic and diagnostic Kimmelstiel-Wilson type mesangial nodules are lacking.

**Immunofluorescence microscopy:** “coarsely granular” mesangial and very minor segmental glomerular capillary wall deposits for IgG: 3+ (on a scale of 0–4; IgG subclass staining: IgG1: 2+, IgG2–IgG4: 0); complement factor C3: 2+; κ light chain: 2+; and no significant intraglomerular staining for IgA, IgM, complement factor C1q, λ light chain, and fibrinogen. No significant extraglomerular staining.

   **Immunofluorescence:** IgG1 deposits in mesangial zones (2+); no staining for IgG2 through IgG4; no staining for IgA, IgM.

   **Immunofluorescence:** κ light chain deposits in mesangial zones (2+); no staining for λ light chains.

2. **Monoclonal staining for IgG κ can be seen in all except:**
   A. Immunotactoid glomerulonephritis
   B. Amyloidosis
   C. Monoclonal immunoglobulin deposition disease
   D. Proliferative glomerulonephritis with monoclonal IgG deposits
   **E. Lupus glomerulonephritis**
In Lupus glomerulonephritis, the immune complex deposits are typically IgG dominant, however, they are polyclonal with both kappa and lambda light chain components.

Electron microscopy: One glomerulus was examined. Podocytes were segmentally mildly activated with minor microvillus transformation of the cytoplasm and minimal segmental foot process effacement. Glomerular capillary walls were within normal limits with only minor activation of endothelial cells; tubuloreticular inclusions were not present. Most mesangial zones showed accumulation of abundant granular electron dense immune complex–type deposits, some extending into the overlying GBM. The deposits did not show a specific substructure.

3. The digital electron photomicrographs show:
   A. Typical deposits seen in amyloidosis
   B. Typical deposits seen in immunotactoid glomerulopathy
   C. Subepithelial hump-like immune complex deposits
   D. Mesangial and paramesangial immune complex–type electron dense deposits

   The deposits shown demonstrate a typical finely granular structure. There is no evidence of a fibrillar or microtubular substructure seen in cases of Amyloidosis or Immunotactoid glomerulopathy. Subepithelial deposits characteristic for humps are not present.

Diagnosis
- Glomerulomegaly with focal and segmental tuft sclerosis; moderate to severe interstitial fibrosis
- Mesangiopathic glomerulopathy with monoclonal IgG1/k light chain deposits

Discussion
The observed findings are unusual. In an obese patient presenting with subnephrotic range proteinuria, moderate to marked chronic renal damage, glomerulomegaly, FSGS, and minor podocyte injury detected by electron microscopy, a secondary variant FSGS has to be considered. The current biopsy findings can support such interpretation. However, glomerular IgG1/k light chain deposits are not part of “FSGS” and indicate another underlying glomerular disease process.

In general, monoclonal immunoglobulin deposits in a renal biopsy can be seen in different settings, often due to the accumulation of light chains and less frequently due to heavy chain accumulation:

a) Monoclonal immunoglobulin deposition disease (MIDD: light chain [LCDD], heavy chain [HCDD], light and heavy chain [LHCD]); type of deposit seen ultrastructurally: powdery
b) Myeloma cast nephropathy; type of deposit seen ultrastructurally: intratubular, dense, chunky cracked casts
c) Amyloidosis (light chain [AL], heavy chain [AH], light and heavy chain [AHL]); type of deposit seen ultrastructurally: nonbranching randomly arranged fibrils
d) Light chain proximal tubulopathy with or without crystal formation and with or without Fanconi syndrome; type of deposit seen ultrastructurally: intracellular, crystallloid, or dense intralysosomal

e) Monoclonal immunotactoid glomerulopathy or glomerulonephritis; type of deposit seen ultrastructurally: microtubular

f) Cryoglobulinemic glomerulonephritis (type I); type of deposit seen ultrastructurally: microtubular or mottled

These cases are characteristically tightly associated with paraproteins in the serum and/or urine, lymphoproliferative disorders, plasmacytomas, and multiple myeloma. A renal biopsy can be the first diagnostic step leading to further workup and the detection of an underlying hematologic disorder.

g) Membranoproliferative glomerulonephritis (MPGN) type 1, which one case series reports for 22% of cases showing monoclonal deposits; type of deposit seen ultrastructurally: electron dense immune complexes

Cases of MPGN and monoclonal immunoglobulin deposits by immunofluorescence (reportedly mainly IgM [36%] or IgG [46%], κ light chain restricted) were associated with monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma, or lymphomas. MPGN as a morphologic pattern of glomerular injury is linked to a variety of etiologies, including hematologic disorders. MPGN with monoclonal immunoglobulin deposits by renal biopsy (and monoclonal spike [M-spike] or MGUS clinically) is equivalent to a “monoclonal gammopathy of renal significance” and requires careful patient management and workup. MPGN with monoclonal immunoglobulin deposits shows overlap with (h):

h) Proliferative glomerulonephritis with monoclonal IgG deposits; type of deposit seen ultrastructurally: electron dense immune complexes

Proliferative GN with monoclonal IgG deposits (PGNMIGD) was initially characterized by Nasr and colleagues. Biopsies mostly show IgG 3 (less often IgG 1, IgG 2) heavy chain and usually κ light chain restriction typically accompanied by complement factor C3 and often also C1q accumulation in mesangial regions and along glomerular capillary walls. By light microscopy, an MPGN or endocapillary proliferative pattern was noted in 92% of patients (32% accompanied by crescent formation) and a membranous GN with only segmental limited proliferative changes in 5% of patients. At time of presentation, 98% of patients had proteinuria (>1 g/24 h), hematuria (77%), renal insufficiency (68%), low serum complement levels (16%), edema (62%), dysproteinemia (30%), and multiple myeloma (2%). During follow-up, none of the patients with or without an M-spike developed a lymphoproliferative disorder or plasma cell dyscrasia. Most patients receiving various treatment regimens (mainly immunomodulatory therapy) showed full or partial recovery, with <25% progressing to ESRD. Similar observations were made among patients with monocytic IgA deposits, lacking evidence of underlying hematologic disorders or plasma cell dyscrasias in the vast majority of these patients (65%), but often revealing subtle monoclonal plasma cell proliferations in targeted assays. In this latter series of 14 patients, only one progressed to ESRD, and none was reported to have developed an overt hematologic malignancy during follow-up. PGNMIGD can flare during follow-up. It has also been observed as recurrent or de novo disease after kidney transplantation even in the absence of M-spike before transplantation, underscoring the pathogenic potential of the detected intraglomerular monoclonal immunoglobulin deposits. Reported therapeutic interventions were mainly based on prednisone, calcineurin inhibitors, mycophenolate-mofetil, or cyclophosphamide; in few patients, rituximab or bortezomib were administered.

The etiology of PGNMIGD remains elusive; the vast majority of patients do not develop overt hematologic disorders or dysproteinemia. Whether specific phenotypes of PGNMIGD, i.e., proliferative vs. nonproliferative, or different heavy chain or γ subclass deposits carry pathophysiologic and clinical significance remains to be determined in future studies. Here is the take-home message:

MGUS and M-spike do not necessarily reflect disease; however, the detection of corresponding clonal deposits in a renal biopsy indicates “monoclonal gammopathy of renal significance.”
PGNMIGD often lacks dysproteinemia or M-spike and underlying lymphoproliferative disorders; however, close patient monitoring and management is necessary to exclude the possibility of hematologic malignancies.

IgG subclass staining is required to properly diagnose PGNMIGD.  

In this patient, findings of PGNMIGD are atypical because proliferative glomerular lesions are absent, and IgG1 deposits (rather than the more frequently reported IgG3 deposits) are largely limited to normal-appearing mesangial zones. Hematologic workup showed normal serum and urine protein electrophoresis results, no M-spike, and no underlying hematologic disorder. The patient was treated with an ARB and showed stable or unchanged renal function over 12 months of follow-up (subsequent loss to further follow-up). Likely, the “secondary FSGS,” presumably due to hyperfiltration injury in the setting of nephron loss and obesity, is driving the clinical presentation. Whether potentially a nonproliferative glomerulopathy with MIGD of the IgG1 subclass represents a clinically inconspicuous variant of “PGNMIGD”—as suggested in this anecdotal case—is currently undetermined.

References
Case 2 from Vivette D. D'Agati, MD—Columbia University Medical Center

A 70-year-old white man (5'4", 184 lb) with a longstanding history of hypertension and poorly controlled type 2 diabetes mellitus (both for 20 years) and known diabetic proliferative retinopathy presents with worsening nephrotic syndrome and AKI on CKD. His baseline serum creatinine concentration was 1.5 mg/dL in September 2014.

The patient's other medical history includes coronary artery disease, for which he underwent a coronary artery bypass graft procedure and percutaneous coronary intervention with stents. He is a retired hairdresser and a prior smoker who has no family history of disease.

In December 2016, the patient developed influenza and was noted to have a serum creatinine concentration of 2.1 mg/dL, with nephrotic syndrome, 24-hour urine protein 10.0 g, and serum albumin 1.7 g/dL.

In February 2017, he was admitted to a local hospital with shortness of breath, cough, weakness, anasarca, left lower lobe pneumonia with pleural effusion, and sepsis with positive blood cultures for *Streptococcus pneumoniae*. Vital signs on admission included a BP of 99/44 mmHg, respiratory rate 16/min, pulse 95/min, and temperature 98.2°F. His medications included insulin, sitagliptin, amlodipine, metoprolol, bumetanide, atorvastatin, and levofloxacin.

Laboratory findings on admission included the following:

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
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<td>White blood cell count</td>
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<td>Hematocrit</td>
<td>29.6%</td>
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<tr>
<td>Platelets</td>
<td>528,000/µL</td>
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<td>Serum creatinine</td>
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<td>Urine protein-creatinine ratio</td>
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<td>Serum albumin</td>
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<td>Cholesterol</td>
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<td>Electrolytes</td>
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<td>Potassium</td>
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<td>Chloride</td>
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<td>CO₂</td>
<td>24 mEq/L</td>
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<td>Anion gap</td>
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Urinalysis: 4+ protein, large blood (30–50 red blood cells per high-power field), few leukocytes, and 1+ glucose.

The following serology results were negative or normal: C3, C4, antinuclear antibody, hepatitis B antigen, hepatitis C virus antibody, ANCA, and anti-GBM antibody. Serum and urine protein electrophoresis findings were negative for monoclonal protein. Prostate-specific antigen level was elevated at 16 ng/mL. The kidneys measured 12.0 cm and 10.3 cm by ultrasound. CT scan of the abdomen and pelvis showed kidney stones in the right kidney with no evidence of hydronephrosis, and extensive diverticulosis without diverticulitis. An infection was responding to levofloxacin, with creatinine level decreasing from 3.5 to 2.7 mg/dL, when a renal biopsy was performed.
1. The most likely biopsy finding is:
   A. Diabetic nephropathy alone
   B. Diabetic nephropathy plus nondiabetic renal disease
   C. Nondiabetic renal disease alone
2. What is the most likely cause of the glomerular hypercellularity?
   A. Cryoglobulinemic glomerulonephritis
   B. Acute infection-related glomerulonephritis
   C. Sepsis with leukocytosis
   D. Leukocyte margination due to renal vein thrombosis

3. The immunofluorescence pattern is most typical of which disease?
   A. Membranous glomerulopathy
   B. Acute infection-related glomerulonephritis
   C. Cryoglobulinemic glomerulonephritis
   D. Fibrillary glomerulonephritis

4. What electron microscopic feature is key to the final diagnoses?
   A. Subendothelial and subepithelial deposits
   B. Mesangial and subepithelial deposits
   C. Two types of subepithelial deposits
   D. Organized subepithelial deposits

Discussion
This case exhibits the coexistence of three apparently unrelated glomerular diseases. The underlying diffuse mesangial sclerosis with mild nodularity is consistent with preexisting diabetic nephropathy (DN) (renal biopsy diagnosis No. 1), which was expected based on the history of known CKD, poorly controlled diabetes mellitus for >20 years, and diabetic retinopathy. The additional findings of diffuse glomerular capillary wall thickening by subepithelial deposits and intervening basement membrane spikes accompanied by diffuse subepithelial granular staining for IgG (3+) and C3 (3+) are diagnostic of superimposed membranous glomerulopathy (MGN) (renal biopsy diagnosis No. 2). Immunostaining for phospholipase A2 receptor (PLA2R) was performed and showed positive findings in the distribution of the subepithelial IgG, consistent with primary, anti-PLA2R mediated MGN. These findings correlate with the development of nephrotic syndrome several months prior to the onset of pneumonia. In addition, the numerous glomerular intracapillary neutrophils and presence of scattered larger hump-shaped subepithelial deposits (visible by both light and electron microscopy) supported a third diagnosis of acute infection-related glomerulonephritis (IRGN) (renal biopsy diagnosis No. 3), correlating with the development of pneumonia and sepsis due to Streptococcus pneumoniae, AKI, hematuria, and the improvement in renal function after antibiotic therapy. Some of the humps appeared to be superimposed on the membranous changes (which were distinguished by their more regular, smaller subepithelial deposits separated by basement membrane spikes), suggesting a possible temporal sequence. The unusually intense staining for C3 (3+) in a subepithelial distribution exceeded that typically seen in primary MGN, further supporting superimposed IRGN. There were focal red blood cell casts and acute tubular injury, but only 25% of the cortex had tubular atrophy and interstitial fibrosis.

Renal biopsy in patients with diabetes is challenging, both clinically and pathologically. This is a common scenario in nephrology practice given the high prevalence of diabetes in the general population and the reluctance to perform kidney biopsy unless the patient exhibits an atypical clinical course or laboratory finding. We examined this issue in a cohort of renal biopsy patients at Columbia University Medical Center. Among 2642 native kidney biopsies interpreted at Columbia in 2011, an astounding 620 (23.5%) were from diabetic patients. The cohort was 61% male with a median age of 62 years. On biopsy, 37% of patients had DN alone, 36% had nondiabetic renal disease (NDRD) alone, and 27% had DN plus NDRD. The major diagnoses in those with DN plus NDRD were acute tubular necrosis, arteriomegaloathrofia, FSGS, IgA nephropathy, MGN, acute interstitial nephritis, pauci-immune GN, and IRGN. Our patient manifested two of these superimposed conditions, a combination that to our knowledge has not yet been reported. Longer duration of diabetes mellitus was associated with a greater likelihood of DN and a lower likelihood of NDRD, and each added year of DN reduced the odds of NDRD by 5%. Diabetes mellitus duration of ≥12 years was the best predictor (58% sensitivity, 73% specificity) of DN alone. Of the 384 patients with NDRD (220 alone, 164 with concomitant DN), 186 revealed lesions that in general would alter treatment decisions. Thus,
nephrologists should maintain a low threshold for renal biopsy diagnosis in diabetic patients with an atypical clinical course.

A similar retrospective biopsy-based study was performed at Kaiser Permanente from 1995–2005.(2) Among 233 patients with diabetes mellitus, the mean age was 58 years and 53% were male. In this group, 27.5% had DN alone, 53.2% had NDRD alone, and 19.3% had DN plus NDRD. In the last group, the major causes of superimposed NDRD were IgA nephropathy (16%), MGN (13%), arterionephrosclerosis (13%), IRGN (11%), other immune complex GN (11%), and acute tubular necrosis (9%). Patients with NDRD tended to be younger than patients with DN and had significantly less diabetic retinopathy. These findings again underscore the need for judicious use of renal biopsy in those patients with atypical clinical course.

IRGN is increasing common in elderly patients. In a report of 109 cases of IRGN in patients ≥65 years, an immunocompromised background was present in 61%, most commonly diabetes or malignancy.(6) The most common site of infection was skin, followed by pneumonia and urinary tract. The most common causative pathogens in the age group were staphylococcus (46%) followed by streptococcus (16%) and other gram-negative organisms. IRGN in elderly patients has a worse prognosis than IRGN in the pediatric age group, with only 22% achieving complete remission, 44% having persistent renal dysfunction, and 33% progressing to ESRD (after mean 29 months of follow-up). Predictors of ESRD included the presence of diabetes, higher creatinine levels at biopsy, dialysis at presentation, the presence of diabetic glomerulosclerosis, and greater tubular atrophy and interstitial fibrosis.

Although diabetes and DN are generally poor prognostic indicators in patients with IRGN, the presence of relatively mild diabetic changes and low tubulointerstitial chronicity in our patient may have been mitigating factors. Importantly, reduced serum C3 level (not detectable in our patient) was reported in only 69% of elderly patients with IRGN.(6) Based on the changing epidemiology of bacterial infection–related GN in adults, with a shift towards older men and patients with diabetes and other comorbidities, renal biopsy has an increasingly important role in this population.(7)

There is a paucity of recent data on MGN occurring superimposed on DN.(8) Most reports predate or lack PLA2R testing.(9) Our case was PLA2R-positive; more PLA2R testing is needed in such cases to determine the prevalence of anti-PLA2R mediated MGN in the setting of established DN.(10) As for other anti-PLA2R mediated MGN, the titers of anti-PLA2R antibody can be useful to monitor immunologic activity and guide management.(11,12)

**Clinical Follow-up:**

In this case, the finding of two different diseases superimposed on DN altered prognosis and management. Serum PLA2R titer obtained after biopsy was 1:10,240. Following treatment with levofloxacin initiated in February 2017, serum creatinine concentration decreased to 1.93 mg/dL by March 2, 2017, with serum albumin level increasing to 2.3 g/dL. The creatinine value was 2.18 mg/L on March 17, 2017. The patient was referred to Columbia for second opinion on April 13, 2017. His serum albumin level had improved to 2.7 g/dL, and edema had resolved. The nephrology consultant felt that the AKI was mostly due to IRGN, which was resolving and would not require specific treatment beyond antibiotic therapy. On the other hand, it was felt that MGN with this degree of proteinuria usually requires immunosuppression but that immunosuppression in this age group with diabetic and infectious comorbidities would be a major intervention to be undertaken with caution. Given the increasing level of serum albumin, it was reasoned that a negative PLA2R result might indicate potential spontaneous remission not requiring immunosuppression. Therefore, a repeat PLA2R titer was obtained on May 1, 2017; it was strongly positive (1:5120). To minimize risk of complications from immunosuppression, the patient began taking Rituxan (rituximab) 1 g, IV with two doses given 2 weeks apart. One month later (June 2017), he had a serum creatinine concentration of 2.3 mg/dL, with serum albumin 2.5 g/dL and repeat PLA2R 1:2560.
References
Case 3 from Megan L. Troxell, MD, PhD—Stanford University Medical Center

A 60-year-old woman with CKD and new onset renal failure is admitted for dialysis.

Her medical history includes diabetes mellitus with retinopathy and neuropathy, along with a nonhealing wound on the right heel. She has recently experienced head trauma, fatigue, and shortness of breath. Other history includes hypertension, lymphedema, gout, migraines, depression, hypothyroidism, and osteoarthritis.

A baseline serum creatinine concentration of 2.9 mg/dL increased to 6.1 mg/dL at presentation. Laboratory studies also yielded the following values:

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<tr>
<td>Sodium</td>
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<td>Potassium</td>
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<td>Glucose</td>
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<td>Urine protein-creatinine ratio</td>
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</tbody>
</table>

In 2015, urine protein electrophoresis demonstrated an IgG kappa monotypic band; more recently, both serum and urine protein electrophoresis results showed IgA κ monotypia. Current medications include carvedilol, isosorbide, insulin glargine, levothyroxine, pregabalin, and vitamin D.

On examination, the patient's vital signs included a BP of 179/77 mmHg, pulse 72/min, temperature 96.8°F, and oxygen saturation 94% on room air. Physical examination results were significant for a new scar over the left eye, obese abdomen, and 2+ lower extremity edema with dressing on her right foot.

A kidney biopsy was performed.
Immunofluorescence: (not shown) 3+ positivity for amyloid P in the single glomerulus and in the interstitium. Staining for λ was slightly stronger than that for κ, with weak smudgy staining for C3, IgG, amyloid A, and transthyretin. IgM, IgA, C1q, and fibrinogen results were negative.

Electron Microscopy
1. Based on the clinical history, the most likely diagnosis is:
   A. Amyloidosis
   B. **Diabetic nephropathy**
   C. Infection-associated glomerulonephritis
   D. Light chain deposition disease
   E. Myeloma cast nephropathy

2. Based on the light microscopic findings, the most likely diagnosis is:
   A. Amyloidosis
   B. Diabetic nephropathy
   C. **Diabetic nephropathy and amyloidosis**
   D. Diabetic nephropathy and light chain deposition disease
   E. Light chain deposition disease

3. Which is seen on electron microscopy?
   A. Curvilinear tubular deposits of 40–60 nm
   B. **Fibrillary deposits of 8–12 nm**
   C. Fine deposits along the subendothelial aspect of basement membranes
   D. Mesangial basement membrane nodules without deposits
   E. Mesangial granular deposits without substructure

4. The next best step in diagnosis or management is:
   A. Amyloid typing (mass spectroscopy or proteomic)
   B. Bone marrow biopsy
   C. Genetic testing
   D. Repeat serum and urine studies for paraprotein
   E. Treatment for κ paraprotein

**Diagnosis**
- Amyloidosis, typed as ALECT2 by mass spectroscopy
- Diabetic nephropathy

**Discussion**
This renal biopsy demonstrates nodular mesangial sclerosis, but interestingly the nodules have a biphasic appearance by periodic acid-Schiff (PAS) and silver stain. The argyrophilic component (silver positive) represents basement membrane and is typical of diabetic sclerosis, whereas the silver-negative, PAS-pale component is amyloid. In addition, amyloid is quite prominent in the interstitium as well as in the arterioles and arteries. Congo red is strongly positive in each of these areas. Results of immunofluorescence characterization of amyloid were unrevealing; λ light chain staining was only modestly stronger than that for κ, whereas serum and urine studies might have suggested excess κ. The biopsy specimen was sent for amyloid typing by mass spectroscopy after laser capture microdissection and returned leukocyte cell-derived chemotaxin 2 (LECT2) amyloid.

LECT2, alternatively termed leukocyte chemotactic factor 2, is secreted primarily by the liver and has been identified as a human neutrophil chemotactic protein with a molecular mass of approximately 16 kDa. LECT2 was also formerly known as chondromodulin 2, for its cartilage-derived role in bone repair and may have other, as yet uncharacterized functions. LECT2 shares structural similarity to a metalloprotease family (M23); it is unclear whether the human homolog has protease function. The predicted structure of LECT2 includes several β-sheets but no α-helix regions.

LECT2 amyloid was first characterized in 2008 from a nephrectomy specimen of a patient with longstanding proteinuria; since then, more than 160 cases have been reported. The majority of US patients with LECT2...
amyloid have been older and Hispanic, like the patient here.(2-4,7) It is also becoming recognized in Native American peoples and in those from the Indian subcontinent as well as the Middle East.(8-10) About 30% of patients with LECT2 amyloid on biopsy have other concomitant renal pathology, especially diabetes but also IgA or membranous nephropathy.[2,8,11] The clinical presentation of patients with LECT2 renal amyloid has been widely variable, many presenting with markedly elevated creatinine levels and proteinuria ranging from 0.1–7.8 g/d.(2,7,8)

In a recent kidney biopsy series, LECT2 was the second or third most common amyloid fibril protein, behind light chain amyloidosis, likely dependent on the referral population.(4,6–8,11) Sequencing of the LECT2 gene in patients with LECT2 amyloid reveals homozygosity for the codon 172 G polymorphism, resulting in valine at amino acid 40 in the mature protein instead of the slightly more common isoleucine.(2,4,7,8) However, about 60% of people carry at least one copy of this polymorphism, and other factors are hypothesized to contribute to amyloid deposition.(8)

Although first characterized in kidney, LECT2 is now recognized as an important cause of hepatic amyloid, often asymptomatic.(8) Serum LECT2 level is increased in the setting of liver injury, including obesity and fatty liver.(8) LECT2 deposits in a peri-portal and peri-central distribution, in contrast to the peri-sinusoidal distribution of other amyloid types.(8) Bone marrow, spleen, adrenal, and pulmonary involvement by LECT2 amyloid has been documented, but cardiac, brain, and fat involvement is distinctly rare.(8,10,12) Typically, LECT2 amyloid is abundant in the renal cortical interstitium but is often seen in all renal compartments: interstitial, vascular, and glomerular (mesangial).(8) In the case presented here, LECT2 amyloid involved each of these compartments, alongside diabetic mesangial nodular sclerosis.(2–4) Specific therapy for LECT2 amyloid is lacking at present; although patient survival is better than that of patients with light chain (AL) or serum amyloid A (AA) amyloidosis, renal survival remains poor, with ≥20–30% of patients reaching ESRD during follow-up in 2 series.(8,10,11) In patients such as this one with positive serum protein electrophoresis and/or differential light chain staining by immunofluorescence, it is crucial to type the tissue amyloid itself so as to not overdiagnose light chain amyloid, given the different therapeutic implications.(8)

The vast majority of patients with diabetes mellitus and CKD do not undergo tissue sampling. The prototypical progression of diabetic kidney disease begins with hyperfiltration hypertrophy, morphologic lesions without clinical symptoms, then microalbuminuria and macroalbuminuria followed by progressive loss of renal function, and eventually ESRD.(13,14) However, the clinical course and structural-functional correlation is more heterogeneous in patients with type 2 diabetes mellitus as compared with the classic studies in type 1 diabetes.(13,14)

Patients with an atypical clinical course, or clinical suspicion of nondiabetic renal disease, such as microscopic hematuria, uncharacteristic change in kidney function, absence of diabetic retinopathy, or presence of other systemic diseases, are targeted for renal biopsy.(13,15,16) In patients with clinical diabetes selected for biopsy, the spectrum of renal biopsy findings is broad and varied. Renal biopsy findings among patients with diabetes have been tabulated in many single-center retrospective studies, and two recent reviews enumerate findings from across the globe.(13,16) The results are quite variable between studies, likely due to local biopsy practices, demographics, and differences in reporting.(13,16,17) In the larger studies, 60% of biopsy specimens show evidence of nondiabetic pathology, either alone or in conjunction with typical diabetes-associated pathology.(13,15–17) In general, patients with only nondiabetic renal disease on biopsy have a shorter duration of diabetes than those with diabetic renal disease, either alone or in combination with other pathology.(13,15,16) FSGS, membranous nephropathy, and IgA nephropathy are the most commonly reported glomerular lesions, as in the general population (Table 1).(13,15,17) Amyloid has been seen in about 5% of patients with diabetes.(15) Tubular lesions such as acute tubular necrosis and acute interstitial nephritis are also quite common.
### Table 1. Largest study of renal biopsy pathology from patients with a history of type 2 diabetes (15)

<table>
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<th>Findings</th>
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<th>Diabetic Nephropathy and Nondiabetic Renal Disease, No. (%)</th>
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<td>Total diabetes mellitus biopsies</td>
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<td>Hypertensive</td>
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<td>Acute tubular necrosis</td>
<td>38 (17)</td>
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<td>Acute interstitial nephritis</td>
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</tr>
<tr>
<td>FSGS</td>
<td>48 (21)</td>
<td>21 (13)</td>
</tr>
<tr>
<td>IgA nephropathy or Henoch-Schonlein purpura</td>
<td>23 (11)</td>
<td>12 (7)</td>
</tr>
<tr>
<td>Membranous</td>
<td>18 (8)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Crescentic GN</td>
<td>15 (7)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Infection-associated GN</td>
<td>3 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Amyloidosis</td>
<td>10 (5)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Myeloma cast</td>
<td>8 (4)</td>
<td>2 (1)</td>
</tr>
<tr>
<td>Atheroembolic</td>
<td>2 (1)</td>
<td>3 (2)</td>
</tr>
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</table>

### References


Case 4 from Ian Roberts, MBChB—Oxford University Hospitals

A 78-year-old man presented to a nephrology outpatient service after investigations by his primary care physician for nocturia. Laboratory tests revealed progressive AKI (creatinine 208 µmol/L; 2.4 mg/dL), proteinuria (spot protein-creatinine ratio 100 mg/mmol; 1.1 g/g), and nonvisible hematuria. He had no symptoms of systemic disease and no recent infections. Immunological tests identified a 3g IgG λ paraprotein with a normal free light chain ratio. Bence-Jones protein was not present. His serum complement levels were normal, and C3 nephritic factor was not detected. Skeletal survey and serum calcium values were normal, and his bone marrow had 6% plasma cells.

His kidney function deteriorated further (creatinine 284 µmol/L; 3.2 mg/dL), and a kidney biopsy was performed 48 hours after presentation.

Follow-up: After the biopsy diagnosis, he was treated with bortezomib and dexamethasone, and his creatinine value at 1 year after presentation is 209 µmol/L (2.4 mg/dL).

Discussion Questions
1. Light microscopy (more than one correct answer): Images of three glomeruli (hematoxylin and eosin stain) are provided. These show:
   A. Membranoproliferative glomerulonephritis
   B. **Focal endocapillary hypercellularity**
   C. FSGS
   D. Nodular glomerulosclerosis
   E. **Focal segmental necrosis**

2. Immunofluorescence (more than one correct answer): An image of C3 is provided. There is trace positivity for IgM. IgG, IgA, κ, λ, and C1q findings are negative. The immunofluorescence findings are in keeping with:
   A. Postinfectious glomerulonephritis
   B. **C3 glomerulonephritis**
   C. Monoclonal immunoglobulin deposition disease
   D. Dense deposit disease
   E. **Proliferative immune complex-mediated glomerulonephritis with masked deposits**
3. **Electron microscopy (one correct answer):** Four images are provided. The features are most in keeping with:
   - A. Membranous glomerulonephritis
   - **B. Dense deposit disease**
   - C. C3 glomerulonephritis
   - D. Light chain deposition disease
   - E. Postinfectious glomerulonephritis

**Biopsy Description**

**Diagnosis**
- C3 glomerulopathy (dense deposit disease) associated with monoclonal gammopathy

**Light microscopy:** Glomeruli show focal segmental endocapillary hypercellularity with focal segmental fibrinoid necrosis and one glomerulus containing a small cellular crescent. There is focal segmental sclerosis with capsular adhesions and foam cells. There is focal mild mesangial hypercellularity. Basement membranes appear normal on silver stain. There is moderate chronic tubulointerstitial damage with 30% of the cortex showing interstitial fibrosis. There is relatively less established tubular atrophy. There is a focal tubulointerstitial nephritis with a neutrophilic and lymphocytic tubulitis. There are granular protein casts with lysed red cell debris in some tubules. Arteries present show focal mild fibroelastosis. Arterioles appear normal.

**Immunofluorescence:** There is diffuse coarse granular mesangial and capillary wall positivity for C3 (3+) with trace positivity for IgM. IgG, IgA, κ, λ, and C1q findings are negative.

**Electron microscopy:** There is moderate effacement of podocyte foot processes. Basement membranes show focal replacement of the lamina densa by electron dense material, particularly as it crosses the mesangium. The intramembranous deposits are associated with focal cellular interposition and membrane duplication.

**Comment:** There is a focal segmental necrotizing and endocapillary proliferative glomerulonephritis. The immunofluorescence is indicative of a C3 glomerulopathy, and the electron microscopy shows features of dense deposit disease. There is moderate chronic tubulointerstitial damage.

**Discussion**
C3 glomerulopathy (C3G) is a disease process that results from dysregulation of the activation of the alternative pathway of complement, resulting in the deposition of C3 within glomeruli. It is characterized histologically by glomerular C3 deposits with little or no accompanying antibodies or early complement components (C3 intensity at least two orders of magnitude greater than any other immunoreactant on immunofluorescence). (1) C3G cannot be defined purely by histology because dysregulation of the alternative pathway of complement can be demonstrated in
some patients whose biopsies show glomerular immune complex deposits. Furthermore, C3 dominant glomerular deposits are a feature of most cases of self-limiting postinfectious glomerulonephritis.

C3G is subclassified according to the appearances on electron microscopy. It encompasses dense deposit disease, characterized by replacement of the normal lamina densa by intramembranous electron dense deposits, and C3 glomerulonephritis, in which there are discrete electron dense deposits, typically subendothelial with or without subepithelial deposits. The morphology at light microscopy of dense deposit disease is highly variable, with 25%–62% showing a membranoproliferative pattern.(2,3)

C3G is the consequence of uncontrolled C3b amplification. Underlying mechanisms include genetic defects affecting complement factor H (CFH, the main regulator of C3 activation) and CFH-related proteins(4) and autoantibodies to the alternative pathway C3 convertase (C3 nephritic factor)(5) and less commonly to CFH.(6) The clinical presentation of C3G often follows infections, suggesting that activation of the alternative pathway by the infection is a trigger for deposition of C3 in glomeruli.(7)

Low plasma C3 levels are typically seen in complete CFH deficiency and suggest uncontrolled C3 activation in the plasma. However, plasma levels may be near normal if the C3 activation occurs only along the glomerular basement membrane, as in most patients who lack a genetic basis or C3 nephritic factor and those with abnormalities of CFH-related proteins.(8)

There are a number of reports of monoclonal gammopathies in patients with C3G, the frequency being highest in older patients. Monoclonal immunoglobulins were detected in 31% of 32 patients with C3G, mean age 55 years,(9) and in 10 of 14 patients with dense deposit disease, aged ≥50 years.(10) In a series of 12 patients diagnosed with C3G aged ≥50 years (3 with dense deposit disease ; 9 with C3G), a paraprotein was detected in 10, 8 of whom had a plasma cell dyscrasia evident on bone marrow biopsy.(11) The monoclonal immunoglobulin is most frequently IgG k. If the diagnosis of monoclonal gammopathy-associated C3G is based on immunofluorescence with frozen sections, then it is important to carry out immunofluorescence or immunohistochemistry on proteinase-digested paraffin sections in order to exclude the possibility of masked monoclonal immunoglobulin deposits. Light microscopy in monoclonal gammopathy-associated C3G usually shows a membranoproliferative pattern and less frequently mesangial and endocapillary hypercellularity.(11)

Most patients in the reported series progressed to ESRD. Of four patients with C3G and multiple myeloma reported by Lloyd et al, three required renal replacement therapy and one showed improvement in renal function after chemotherapy and stem cell transplant.(11) These series emphasize the importance of screening older patients diagnosed with C3G for an underlying monoclonal gammopathy because treatment of the underlying hematological disorder might alleviate the renal disease.

The frequency of the association of monoclonal gammopathies with C3G in older adults indicates a pathogenetic link. Studies in some patients have demonstrated that monoclonal λ light chains can activate the alternative pathway of complement.(12,13) The mechanism appears to involve binding of the monoclonal light chains to CFH, thus blocking its complement inhibitory activity.
References


Case 5 from Vanesa Bijol, MD—Northwell Health Hofstra University

A 62-year-old man presented to his primary care physician with shortness of breath and lower extremity edema. His medical history includes coronary artery disease status post bypass graft surgery, diastolic heart failure, diabetes mellitus, and hypertension.

At the time of presentation, his vital signs showed a BP of 187/95 mmHg, heart rate 90/min, temperature 98.3°F, and oxygen saturation of 95% on 2L O₂. On physical examination of the chest and neck, he had bilateral basal crackles; no murmurs, gallops, or rubs; and no jugular vein distension. He had bilateral soft pitting edema of lower extremities, up to his knees. The remainder of the physical examination was unremarkable.

Laboratory data included the following:

<table>
<thead>
<tr>
<th>White blood cell count</th>
<th>7800/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>12.9 g/dL</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>37%</td>
</tr>
<tr>
<td>Platelets</td>
<td>120,000/µL</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>68%</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>18%</td>
</tr>
<tr>
<td>Monocytes</td>
<td>11%</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2%</td>
</tr>
<tr>
<td>Basophils</td>
<td>1%</td>
</tr>
<tr>
<td>Glucose</td>
<td>132 mg/dL</td>
</tr>
<tr>
<td>Blood urea nitrogen</td>
<td>36 mg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>1.74 mg/dL (baseline 1.1)</td>
</tr>
<tr>
<td>Sodium</td>
<td>134 mEq/L</td>
</tr>
<tr>
<td>Potassium</td>
<td>4 mEq/L</td>
</tr>
<tr>
<td>Chloride</td>
<td>103 mEq/L</td>
</tr>
<tr>
<td>CO₂</td>
<td>24 mEq/L</td>
</tr>
<tr>
<td>Calcium</td>
<td>8.6 mg/dL</td>
</tr>
</tbody>
</table>

It was felt that the main patient’s issues were coming from his chronic heart failure; however, on further testing, his urinalysis showed 3+ protein by dipstick, his serum albumin level was 2.5 g/dL, and his urine protein-creatinine ratio was 9.7. Renal ultrasound showed a right kidney of 14.9 cm and a left kidney of 11.7 cm.

A planned kidney biopsy was cancelled, and he was treated empirically with methylprednisolone, 48 mg/d, for idiopathic nephrotic syndrome. He had an initial response to this treatment with a decrease in urinary protein to 1.8 g/d and an improvement of his edema. At 2 months, while the patient was taking methylprednisolone, his nephrotic syndrome relapsed with a significant increase in proteinuria (urine protein-creatinine ratio 23) and a marked increase in his edema. He was switched to cyclosporine, but his serum creatinine concentration increased to 6.7 mg/dL, and the cyclosporine was discontinued. His renal function worsened, and hemodialysis was started. A kidney biopsy was performed.
1. Based on the biopsy findings shown here, what is the most likely cause of nephrotic syndrome in this patient?
   A. Diabetic nephropathy
   B. Collapsing glomerulopathy
   C. Minimal change disease
   D. Membranous nephropathy

2. What is the best description of the immunofluorescence (IF) and electron microscopy (EM) findings?
   A. IF is negative, EM shows diffuse effacement of podocyte foot processes
   B. IF is negative, EM shows no significant abnormalities
   C. IF shows granular staining for IgG, with corresponding subepithelial deposits on EM
   D. IF shows granular staining for IgM and κ, with small mesangial deposits on EM

3. Based on these findings, the next step should be to:
   A. Send serum sample for phospholipase A2 receptor antibody testing
   B. Perform work up for lymphoproliferative disease
   C. Send serum sample for APOL1 genetic testing
   D. Continue or modify the treatment regimen, no other tests are needed

Pathologic Diagnosis
- Minimal change disease
- Diffuse diabetic mesangial sclerosis
- Sparse IgM/kappa mesangial deposition, most likely representing an early paraprotein deposition disease

Discussion
The most likely cause of nephrotic syndrome in this patient is minimal change disease (answer C, question 1), based on this patient’s clinical presentation and the biopsy findings. There is no evidence of overt immune complex deposition or subepithelial dense deposits; therefore, membranous nephropathy can be ruled out. Features of collapsing glomerulopathy (collapse of capillaries, proliferative epithelial cell changes, tubular intracytoplasmic coarse protein reabsorption granules, etc.) are not present in this biopsy. Massive proteinuria in this patient is out of proportion to the early stage of diabetic changes in this biopsy.

The ultrastructural finding of widespread podocyte foot process effacement is quite characteristic for minimal change disease or minimal change-like lesion, in the absence of global or segmental glomerulosclerosis on light microscopy.
In addition, as presented by the immunofluorescence microscopy studies in this biopsy, finely granular deposition of IgM and κ light chain is noted in some glomerular segments; the finding is subtle, but as shown on immunofluorescence images, the IgG and λ light chain stains are negative. This particularly becomes relevant when looking at the electron micrographs, where small mesangial dense deposits are visible. Therefore, the correct answer for question 2 is answer D: IF shows granular staining for IgM and κ, with small mesangial deposits on EM. The presence of monoclonal IgM-κ mesangial deposits in this patient eventually led to a workup for lymphoproliferative disease (answer B, question 3), and he was diagnosed with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL). The treatment with rituximab led to much better control of proteinuria, which decreased to <1 g/24 h.

Minimal change disease is often idiopathic but can be associated with lymphoproliferative disorders and hypersensitivity reactions to drugs. The most frequent lymphoproliferative disorders associated with minimal change disease include Hodgkin lymphoma, Waldenström macroglobulinemia, marginal zone B-cell lymphoma, and CLL; other associations include multiple myeloma, mantle cell lymphoma, and peripheral T-cell lymphoma.(1–4) The most common form of kidney involvement in patients with CLL/SLL is the presence of direct infiltration of the kidney parenchyma by leukemic cells; glomerular disease in patients with CLL/SLL is less common. Glomerular diseases frequently seen in patients with CLL/SLL include membranoproliferative glomerulonephritis (immune complex mediated), monoclonal immunoglobulin deposition disease (amyloid and nonamyloid), and minimal change disease.(5) The pathophysiologic mechanism of diffuse podocytopathy in the setting of lymphoproliferative disease is unclear; however, the correlation between the development of lymphoproliferative disease and nephrotic syndrome in these patients, before and after treatment, strongly suggests an immune background involving the clonal proliferation. Both a T-cell dysfunction and the B-cell role in cytokine production have been cited as important pathophysiologic factors.(6,7)

Most commonly, nephrotic syndrome and lymphoproliferative disease occur simultaneously; however, reports of nephrotic syndrome preceding or following the diagnosis of lymphoma are also common, and the occurrence of the two can be separated by as long as 120 months.(8,9) When compared with classic idiopathic minimal change disease, the nephrotic syndrome associated with hematologic malignancies has a higher incidence of relapse and frequently shows resistance to usual treatment modalities while showing better response to lymphoma-specific chemotherapy. This further supports the paraneoplastic nature of minimal change disease in this setting and argues that the occurrence of two diseases is not independent, even if occurring long before or after each other. Furthermore, the presence of steroid-resistant minimal change disease in adults should urge investigation of secondary causes, including lymphoproliferative disease.(9)

No particular risk factors for the development of nephrotic syndrome among patients with lymphoma were identified (sex, age, stage of disease), although some studies showed higher prevalence of systemic symptoms (B symptoms) in patients who do develop this complication.(10)

References


