Can a Single Glomerulus Morphology Implicate Successful Therapy?

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Abstract

Recurrent gross hematuria of glomerular origin is frequently encountered in clinical practice, and in absence of specific serological marker, renal biopsy is mandatory to address the definitive diagnosis, and set out an appropriate therapeutic protocol. Technical deficiencies associated with practice of renal biopsy are frequently encountered, as inadequate number of glomeruli or poor immunofluorescence staining of kidney biopsy specimen; however, these deficiencies can be offset by detailed electron microscopy analysis of a single abnormal glom.

We present a single middle-aged Libyan woman, with a rare glomerular disease, related to abnormal activation of alternative complement pathway, where renal biopsy report was initially not adequate and lacking immunohistochemistry workup. However, electron microscopy reports a characteristic abnormal glomerular deposit, coupled with clinical and biochemical data that guided our therapeutic protocol.

In a middle-aged female who presented with recurrent gross hematuria and nephrotic range proteinuria, we should suspect a glomerular pathology. Further to immunoglobulin A nephropathy or lupus nephritis, particularly in presence of complement abnormalities and negative serology for glomerulopathy-related autoantibodies, dense deposit disease and C3 glomerulonephritis that are rare complement mediated glomerulopathy should be considered as a seronegative lupus nephritis-equivalent, in terms of their membranoproliferative features on light microscopy, and when setting out appropriate therapeutic protocol. Patient and family counseling for C3 glomerulopathy is essential because this type of glomerulopathy has a recurrence rate after kidney transplant.

Keywords

► recurrent gross hematuria
► C3 glomerulopathy
► dense deposit disease
► complement system
► renal biopsy

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**Introduction**

Clinical manifestations of glomerulonephritis (GN) range from the asymptomatic person who is discovered to have hypertension or microscopic hematuria during a routine medical examination to patients who may present with proteinuria, gross recurrent hematuria, or even rapidly progressing GN. Awareness of such spectrum of clinical presentation is mandatory to practicing physicians, for early detection and management of these nephritides to avoid progression to end-stage renal disease.

Renal biopsy is considered a valuable diagnostic tool in glomerular diseases, particularly in the setting of overlapping clinical presentation, and paucity of definitive serological marker: moreover, it provides direct exploration of the type and extent of renal pathology, providing a robust guide for the therapy. Nonetheless, many renal biopsy reports are considered inadequate based on number of glomeruli obtained in the biopsy core; ideally, they should not be less than 10 to 12 glomeruli, for proper processing for light microscopy or immunohistochemistry staining (immunofluorescence and immunoperoxidase sections). Nevertheless, even with a single glom, use of detailed electron microscopy (EM) examination can provide valuable information. Furthermore, abnormalities of complement system (primary or secondary defects) play central role in pathogenesis of many glomerulopathies, and we should consider them in differential diagnosis of patients presenting with recurrent hematuria.

We present a case with rare glomerular disease that her renal biopsy report was initially not adequate and lacking immunohistochemistry workup. However, E/M report eventually addressed abnormal glomerular deposits, coupled with clinical and biochemical data that guided our therapy protocol with remarkable patient outcome.

**Case Presentation**

In October 2021, a single Libyan woman, in her mid-thirties (35 years) with no previous medical problems, presented to our nephrology clinic, complaining of several episodes of red discoloration of her urine over the last 3 months, lasting few days, and subside spontaneously. No history of dietary or drug was found that may cause urine discoloration. These episodes of gross hematuria were not associated with pain or other significant associated urinary tract symptoms. There was no history of lower limb swelling or facial puffiness. She had reported two episodes of hematuria that were linked to concurrent upper respiratory infection. She denied bleeding from any site, skin rash, or oral ulceration. She had no
significant family history of similar illness. She denied any history of coronavirus disease 2019 (COVID-19) infection. Her physical examination revealed average body-built woman, her blood pressure was 110/70 mm Hg, pulse 88 beats per minute, pale conjunctivae, normal throat, and mild pitting lower limb edema. No skin rash, photosensitivity, or alopecia was found. Precordial, chest, and breast examination were normal.

Her initial blood testing showed that hemoglobin was 10.9 g/dL (12–15 g/dL), mean corpuscular volume 76 fl (80–100 fl), white blood cell (WBC) was 6.1 × 10^3/µL (4–11 × 10^3/µL), platelet count was 254 × 10^3/µL (150–450 × 10^3/µL). Urine sediment examination showed protein +++, WBC was 1–4/HPF (high-power filed [0–5]), red blood cell (RBC) was 25–50/HPF (0–3) with irregular shape, no cast, no crystals. Blood chemistry showed urea was 41 mg/dL (15–50 mg/dL), creatinine was 0.5 mg/dL (0.5–0.9 mg/dL), K⁺ was 3.64 mmol/L (3.5–5.5 mmol/L), Na was 137 mmol/L (135–148 mmol/L), serum albumin was 3.1 g/dL (4–6 g/dL), calcium was 7.6 mg/dL (8.4–10.2 g/dL), was uric acid 4.3 mg/dL (3.5–5.7 g/dL), low-density lipoprotein was 134 mg/dL (50–200 g/dL), hemoglobin A1c was 5.4% (5.5–6.4%), transferrin saturation was 14% (20–50), C3 was 0.2 g/L (0.9–1.8 g/L), and C4 was 0.3 g/L (0.2–0.5 g/L). Chest imaging showed no signs of pulmonary or pleural disease. Serological workup for autoantibodies showed antistreptolysin-O titer was normal, positive for antinuclear antibodies with titer of 1:64, and was negative for anti-ds-DNA level 0.1 and 0.3 μL/mL, and large globular mesangial densities, going with the picture of membranoproliferative C3 GP or lupus nephritis. Presence of significant proteinuria mandates renoprotective measures, using low dose enalapril 2.5–5mg per day, statins for hyperlipidemia, atorvastatin 40 mg once daily, and starting steroid therapy, prednisolone 1mg/kg, for 6–8 weeks, to achieve clinical and renal remission, and to proceed with gradual tapering of steroid therapy thereafter. Patient treatment is further supplemented with iron tablet, to treat her iron deficiency anemia.

A month later, she came with cushingoid facies, blood pressure of 120/70 mm Hg, biochemical data—fasting blood glucose, 87 mg/dL (70–110 mg/dL); urea, 34 mg/dL; creatinine, 0.7 mg/dL—and with improvement of her lipid profile. Urine analysis showed ongoing hematuria of 12–20/HPF and significant proteinuria (UPCR 3 mg/g) and for this reason azathioprine tablet (50 mg twice daily) was added.

Over a period of 2 months, the patient was monitored. We observe improvement in her clinical picture (no gross hematuria no more pitting edema) but on laboratory level she had persistent microscopic hematuria and nephrotic range proteinuria (urine RBC 60–70/HPF, 24 urine collection for protein 4.8 g). Low C3 and normal C4 indicate that complement-mediated glomerulopathy and immunoglobulin A (IgA) nephropathy have been excluded on this basis. Renal biopsy was advised for the patient (3 months after her first presentation) and because of technical issue the biopsy report (January 2022) was deficient as under light microscopic examination of serial sections only one glomerulus was seen and showed segmentally thickened capillary basement membrane and mesangial expansion, EM ultrastructural examination (Fig. 1) revealed GBM thickening with extensive subendothelial electron dense deposits (ribbon like), and large globular mesangial densities, going with the picture of membranoproliferative C3 GP or lupus nephritis. At this stage, mycophenolate mofetil (MMF) tablet 1 g twice per day was prescribed for 2 months, replacing azathioprine, with prednisolone 0.5 mg/kg mg per day, as we adopted lupus nephritis-equivalent treatment regimen. In close follow-up over next 6 months, in August 2022, patient showed remarkable clinical and biochemical improvement, with complete remission, as no microscopic hematuria was found; urine RBC was 0–2 HPF, her UPCR was 0.14, and C3 and C4 returned to normal level of 0.64 and 0.28, respectively, with normal renal function. She is currently on MMF tablet 500 mg twice per day, and prednisolone tablet 10 mg daily.

Based on renal biopsy report, together with persistently low C3 level, normal C4 level, and no serological markers of lupus nephritis, we conveniently diagnosed C3 glomerulopathy; possibly dense deposit disease (DDD pattern).

**Discussion**

Recurrent hematuria is significant clinical problem; screening programs show a prevalence of 0.18 to 16.1% among apparently healthy individuals, and glomerular hematuria contributes to a significant proportion of these cases in adults. Presence of glomerular hematuria alerts physicians' attention to broad differential diagnosis (Table 1).
<table>
<thead>
<tr>
<th>Disease characters</th>
<th>IgA nephropathy</th>
<th>Lupus nephritis</th>
<th>C3 glomerulopathy</th>
<th>Atypical hemolytic uremic syndrome</th>
<th>Thin membrane disease</th>
<th>Alport syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence; per 100,000 of population (related reference)</td>
<td>3.1–4.5 cases, according to geographic area⁴</td>
<td>20–150 cases, may be higher in woman and black race⁷</td>
<td>0.1–0.2 cases, a rare glomerulopathy</td>
<td>&lt;0.1 cases, a rare glomerulopathy</td>
<td>1000, a common benign condition</td>
<td>1–2cases, relatively rare hereditary disease</td>
</tr>
<tr>
<td>Precipitating factor</td>
<td>Synpharyngitic hematuria</td>
<td>Provoked by systemic flares of lupus</td>
<td>No specific precipitating factor</td>
<td>- Pregnancy, kidney transplantation or its therapy, non-enteric bacterial infections</td>
<td>No specific precipitating factor</td>
<td>No specific precipitating factor</td>
</tr>
<tr>
<td>Family history /genetic mutations</td>
<td>Majority are sporadic cases. But familial clustering possible</td>
<td>Association with HLA-DR2/DR3 alleles</td>
<td>- Mostly acquired form</td>
<td>- Mostly acquired form</td>
<td>Autosomal dominant from parents to their children</td>
<td>Autosomal dominant X-linked, AR, both males and females are affected</td>
</tr>
<tr>
<td>Systemic manifestations</td>
<td>- No systemic manifestations - Clinical associations; celiac disease, and dermatitis herpetiformis</td>
<td>- Involving several organs; skin, joints, pulmonary, cardiac, renal, nervous system</td>
<td>Few cases demonstrate partial lipodystrophy, and macular degeneration</td>
<td>- Symptomatic anemia - Petechial skin rash</td>
<td>- No systemic manifestations</td>
<td>- Sensorineural deafness - Ocular abnormalities</td>
</tr>
<tr>
<td>Specific serological markers</td>
<td>- No specific autoantibody - Measurement of serum galactosylated-IgA level and IgA/C3 ratio</td>
<td>- Several autoantibodies; - Anti-ds DNA - Anti-histone antiphospholipid</td>
<td>- Low C3, normal C4 level - Circulating C3- nef autoantibodies - Complement factor- H mutations analysis</td>
<td>- Thrombocytopenia - Peripheral blood schistocytes - Complement factor- H mutations analysis</td>
<td>- No specific autoantibody</td>
<td>- No specific autoantibody</td>
</tr>
<tr>
<td>Renal biopsy</td>
<td>- Characteristic IgA mesangial deposition - Few C3 or other Ig deposits - No C1q deposits</td>
<td>- Several histological classes, wire-loop lesions - Full house immune complex, C3, C4, and C1q deposits</td>
<td>- Membranoproliferative pattern on light microscopy - C3 deposits on immunofluorescence - Characteristic dense deposits according to DDD or C3 GN pattern (see discussion paragraph)</td>
<td>- Thrombotic microangiopathy pattern with glomerular and arteriolar involve-ment, and double contouring of GBM</td>
<td>- Uniformly thinning of lamina densa of GBM - Occasional IgM or IgG deposits, - No complement deposits</td>
<td>- Thick GBM - Loss of normal staining for alpha 3 and alpha 4 proteins - No complement deposits</td>
</tr>
</tbody>
</table>

Abbreviations: AR, autosomal recessive; CT, computed tomography; GBM, glomerular basement membrane; HLA-DR2/DR3, human leukocyte antigen; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M.
The case presented with recurrent episodes of painless hematuria, no history of coagulopathy medications, no evidence of renal stones, and no evidence of urological malignancies; urine sediment examination repeatedly showed hematuria with nephrotic range proteinuria (UPCR 4.2) associated with hyperlipidemia, makes hematuria of glomerular origin is the most likely pathology. These episodes of hematuria are not associated with history of Pharyngitis. No features of systemic lupus (photosensitivity, malar rash, arthritis, or oral ulcers), no features of systemic vasculitis, acrocyanosis, or cutaneous lipodystrophy. Family history was negative for similar episodes or any renal disease. No evidence of previous streptococcal infection or COVID-19 infection was found. This clinical presentation was further supplemented by laboratory data, which showed persistently low level of C3 and normal C4 level (excluding IgA nephropathy) and absence of thrombocytopenia (excluding hemolytic uremic syndrome). Patient’s screening was negative for glomerulopathy-related autoantibodies. Due to persistence of microscopic hematuria with no serological diagnosis, request for renal biopsy was made. Renal biopsy report showed, light microscopic examination of serial sections, only one glomerulus was seen and segmentally thickened capillary basement membrane and mesangial expansion. E/M ultrastructural examination (Fig. 1) revealed GBM thickening with extensive subendothelial electron dense deposits (ribbon-like). Segmental GBM duplication was detected, mesangial expansion by electron dense deposits (ribbon-like appearance). There had been a segmental GBM duplication with mesangial expansion due to electron dense deposits. Therefore, the diagnosis is in favor of C3 GP of DDD type, rather than IgA nephropathy or lupus nephritis. Many case reports addressed the nonbenign nature of this emerging glomerulopathy. We do treat our patient as lupus nephritis-equivalent disease, with combination therapy (MMFþ prednisolone) used in 12 cases, resulted in complete remission in 17% of cases, partial remission (stable disease) in 58% of cases, while, ESRD occurred in 50% of untreated cases, compared to 25% in treated cases. Recurrence after kidney transplantation is high; up to 50% of recipients eventually lose their graft within 5 years of transplantation, a fact that requires patient and family counselling, and need for specific complement 5 (C5) blocking therapy as eculizumab to save kidney graft from progressive damage and loss.

Conclusions

IgA nephropathy and lupus nephritis are both characterized by recurrent gross hematuria with nephrotic range proteinuria. Others, such as C3-GN or DDD that are considered seronegative lupus nephritis-equivalents, must be ruled out in the context of complement abnormalities and negative serology results for glomerulopathy-related autoantibodies. Validated C3-GN diagnosis with EM report is essential for appropriate treatment protocol, planning for kidney transplantation, and prognosis.

Conflict of Interest

None declared.

References

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