American Society of Nephrology Quiz and Questionnaire 2012: Glomerulonephritis

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Summary
Presentation of the Nephrology Quiz and Questionnaire (NQQ) has become an annual tradition at the meetings of the American Society of Nephrology. It is a very popular session, judged by consistently large attendance. Members of the audience test their knowledge and judgment on a series of case-oriented questions prepared and discussed by experts. They can also compare their answers in real time, using audience response devices, to those of program directors of nephrology training programs in the United States, acquired through an Internet-based questionnaire. The topic presented here is GN. Cases representing this category, along with single best answer questions, were prepared by a panel of experts (Drs. Fervenza, Glassock, and Bleyer). The correct and incorrect answers were then briefly discussed after the audience responses and the results of the questionnaire were displayed. This article recapitulates the session and reproduces its educational value for a larger audience—that of the readers of the Clinical Journal of the American Society of Nephrology. Have fun.


GN Case 1
A 20-year-old woman was referred for evaluation of persistent hematuria and proteinuria. She had a history of fever and sore throat lasting 1 week that occurred 6 months earlier and were associated with abdominal pain and dark urine. She did not receive any antimicrobial agents. Significant findings on physical examination at that time included a BP of 140/90 mmHg and 2+ edema. Laboratory evaluation showed a serum creatinine value of 1.4 mg/dl, urinalysis with 2+ protein and 3+ blood, and a 24-hour urinary protein excretion of 680 mg. The C3 level was 46 mg/dl (reference range, 75–175 mg/dl) and the C4 level was 23 mg/dl (reference range, 14–40 mg/dl). A throat culture was negative for streptococci, and an antistreptolysin O titer was 200 IU/ml. The serum albumin concentration was 3.6 g/dl.

A renal biopsy was performed and showed a pattern of membranoproliferative GN on light microscopy. No crescents were observed. Immunofluorescence microscopy showed bright (3+) mesangial and capillary wall C3 staining. Electron microscopy showed mesangial, intramembranous, and subendothelial deposits, as well as a few subepithelial hump-like deposits. Postinfectious GN and type III membranoproliferative GN were diagnosed. The patient was treated symptomatically, but proteinuria and hematuria persisted. Current evaluation shows a hemoglobin level of 11.8 g/dl, a serum creatinine concentration of 1.3 mg/dl, urinalysis with 3+ blood and 3+ protein, quantitative proteinuria of 2.2 g/24 hours, C3 concentration of 44 mg/dl, and C4 concentration of 22 mg/dl.

Question 1 (see Figure 1 for responses of program directors and attendees)
To further evaluate this patient, you would now order:
A. Antinuclear antibodies
B. Hepatitis B and C serology
C. Anti-deoxyribonuclease (DNase) antibody titers
D. Serum C1q level
E. Antibodies to complement-regulating proteins

Discussion of Question 1
Correct answer is E. Antibodies to complement-regulating proteins.

Postinfectious GN is a form of GN that develops after an infection; it is especially common in children and in the elderly (1–4). Often the infection is minor (e.g., pharyngitis) and has usually resolved by the time clinical evidence of an ongoing GN is manifested. The lag time between the start of the infection and the clinical manifestations can vary from a few days to weeks, and disease severity ranges from asymptomatic hematuria to an acute nephritic syndrome, renal failure, and fluid overload. On renal biopsy, postinfectious GN is characterized by proliferative GN on light microscopy, mesangial and/or capillary wall bright C3 staining with or without immunoglobulin on immunofluorescence microscopy, and subepithelial hump-like deposits on electron microscopy (4,5). In some cases, there is no clinical or serologic evidence of a preceding infection, and the diagnosis of postinfectious GN is based solely on these renal biopsy findings. It should be also recognized that over the past 30 years, an important

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shift in epidemiology, bacteriology, and outcome of GN related to infection has occurred (5). A substantial number of cases now occur in adults, particularly the elderly, alcoholic persons, and patients who are immunocompromised. Because the presence of an infection is often ongoing at the time of renal biopsy, the term infection-related GN has been suggested as more appropriately describing this condition (6). In contrast to children, adults are more likely to have infection-related GN secondary to nonstreptococcal infections, particularly staphylococcal infection, and the overall prognosis in terms of renal and patient survival is poor.

The pathogenesis of acute postinfectious GN has been the subject of recent reviews (7,8). It is important to recognize that no animal model can reproduce the classic findings of abundant neutrophil infiltration and subepithelial humps characteristic of human postinfectious GN (8). Nevertheless, it has been proposed that the initial phase of the process is characterized by deposition of bacterial antigens (e.g., streptococcal) in the glomeruli (planted). This phase is followed by production of antibodies that interact in situ with the planted antigens (2,9). Antigens that enter the circulation after the antibody response is fully underway form immune complexes in circulation. Most of these immune complexes are cleared from the circulation by the liver and spleen, but the ones that escape the phagocytic system may deposit in the glomeruli and thus induce immune complex–mediated GN (7). Reduction in serum C3 complement levels is a constant feature during the initial phase of postinfectious GN (10,11). The hypocomplementemia is due to activation of the alternative pathway (AP) of complement while C1q, C2, and C4 complement levels (classic pathway) are usually normal. In some patients with poststreptococcal GN, low C3 levels have been associated with the transient expression of circulating autoantibodies against the C3 convertase complex (i.e., C3 nephritic factors) (12). These antibodies result in stabilization of the enzyme, increase in convertase activity, and enhanced C3 cleavage by the AP of complement (13).

In most cases, GN resolves in a matter of weeks without specific treatment. Similarly, hypocomplementemia usually resolves within 8 weeks. However, in a minority of cases, urinary and complement abnormalities may persist or take longer to resolve, with some patients even progressing to ESRD (1,2,4,14–17). These cases have been labeled as “atypical,” “persistent,” or “chronic” postinfectious GN. Until recently, the cause of these atypical cases was unknown. A recent study postulates that cases of atypical postinfectious GN are due to a defect in the regulating mechanisms of the AP of complement that prevent downregulation of complement activation after resolution of the infection (18). As a consequence, there is excessive deposition of complement proteins and breakdown products in the glomeruli, resulting in persistence of the inflammatory response.

That study included 11 patients (5 women and 6 men; mean age, 35.1 years [range, 2–71 years]) who fulfilled the diagnostic criteria of atypical postinfectious GN, defined as (1) persistent hematuria and proteinuria, with or without history of preceding infection; (2) renal biopsy showing features of postinfectious GN; and (3) abnormalities of the complement AP. Five of the 11 patients had a history of upper respiratory tract infection or impetigo. In the remaining 6, no antecedent illness was documented. Serum creatinine at presentation ranged from 0.5 to 3.1 mg/dl (mean, 1.4 mg/dl), with mean proteinuria of 5139 mg/24 hours (range, 500–15,760 mg/24 hours). C3 levels were low in 7 patients, and C4 levels were normal in all patients. All biopsies showed proliferative GN; the most common pattern was diffuse endocapillary proliferative GN, followed by mesangial proliferative and membranoproliferative GN on light microscopy. On immunofluorescence microscopy, bright (3+) mesangial and capillary wall C3 staining was seen in all but one case, which showed mild (1+) C3 staining. Two cases also showed mild mesangial and capillary wall staining for IgG (1–2+). Electron microscopy showed the hallmark of postinfectious GN in all cases: hump-like subepithelial deposits, which were numerous in 6 patients. Ten of 11 patients also had mesangial and subendothelial deposits. Functional and genetic studies of the AP identified autoantibodies or mutations in complement genes in 10 of 11 patients. Seven patients were positive for C3 nephritic factors, which were associated with other functional abnormalities of the AP in 6 patients. Four patients had mutations of complement genes, including 3 patients with mutations in CFH and 1 patient with a mutation in CFHR5.

The study shows that patients with atypical postinfectious GN have an underlying defect in the complement AP. Results of the study are supported by recent case reports of patients initially diagnosed as having a postinfectious GN who subsequently were found to have a proliferative form of GN called C3GN (19,20). Thus, it can be postulated that under normal circumstances, the activation of the AP by an infection is quickly brought under control once the infection abates. However, in patients with a defect in AP regulation, there is continual AP activation with deposition of complement proteins and their breakdown products in the glomeruli, even after resolution of the infection, leading to the development of atypical proliferative GN. If the defect is mild, AP control eventually occurs with resolution of the GN. If the defect in AP regulation is more severe, hematuria and proteinuria persist, often
exacerbated by recurrent bouts of infection. Recent studies have shown that dysregulation of the AP also results in C3GN. C3GN is characterized by glomerular C3 deposition and the presence of numerous deposits in the mesangium and capillary walls, including subepithelial deposits (21). Thus, there is considerable overlap in the biopsy findings of patients with atypical postinfectious GN and those with C3GN (25). This overlap is not surprising because both types are due to abnormalities of complement AP. Indeed, review of previous reports on prolonged hypocomplementemia in patients with poststreptococcal GN show kidney biopsy findings compatible with the diagnosis of C3GN (15). It is also likely that many cases of familial poststreptococcal GN represent undiagnosed C3 glomerulopathy (26). Therefore, atypical postinfectious GN should be considered a C3 glomerulopathy, and testing for abnormalities in the AP of complement in all patients atypical postinfectious GN is recommended (option E).

The clinical presentation, laboratory evaluation, and renal biopsy findings of this case are not compatible with the diagnosis of systemic lupus erythematosus (option A) or cryoglobulinemic GN (option B). In lupus nephritis, immunofluorescence microscopy usually shows a “full house,” meaning that all or almost all immunoreactants (IgG, IgA, IgM, κ, and λ light chains, C1q, C3) are present. This is unusual in other forms of GN. On electron microscopy evaluation, in addition to immune complex deposits (discrete electron dense immune-type deposits), a very common ultrastructural finding is the presence of tubuloreticular inclusions. On the other hand, in cryoglobulinemic GN, immunofluorescence microscopy typically shows diffuse, pseudo-linear peripheral capillary wall and mesangial staining for IgM, IgG, and C3, with a relatively stronger staining for IgM and κ (compared with λ) light chain, which reflects the typical clonal restriction of type II cryoglobulins. On electron microscopy, cryoglobulin deposits often display an organized substructure: short, curved, thick-walled tubular structures with a diameter of about 30 nm that appear annular on cross-sections.

Anti-DNase antibody testing (option C) detects antigens produced by group A streptococcus and is elevated in most patients with rheumatic fever and poststreptococcal GN. This test is often done concurrently with the ASO titer, and subsequent testing is usually performed to detect differences in the acute and convalescent blood samples. Anti-DNase testing was not performed at presentation and will probably have negative results at this time. The first component of complement (C1) is composed of three subunits, designated as C1q, C1r, and C1s. C1q recognizes and binds to immunoglobulin complexed to antigen and initiates activation of the classic pathway of complement. Serum C1q levels (option D) are usually normal in conditions associated with abnormalities of the AP of complement (e.g., C3GN), although they may be mildly decreased early in the course of postinfectious GN.

**Question 2 (see Figure 2)**

In this patient, you would now recommend:

A. Urinary IgG and β2-microglobulin excretion
B. Serum IgG4 level
C. Repeat anti-PLA2R antibody level
D. Renal ultrasonography with Doppler examination of renal veins
E. Repeat renal biopsy

**Discussion of Question 2**

Correct answer is E. Repeat renal biopsy.

Idiopathic membranous nephropathy (MN) is a common immune-mediated glomerular disease and remains the leading cause of nephrotic syndrome in white adults (27). Although in most patients the disease progresses relatively slowly, approximately 40% of patients eventually develop ESRD (28). Because of its frequency, it remains the

GN Case 2

A 38-year-old man has a history of apparently idiopathic membranous nephropathy diagnosed 3 years ago. Proteinuria at diagnosis was 15 g/24 hours, and the serum creatinine level was 1.4 mg/dl. Anti–phospholipase 2 receptor antibodies (PLA2R) on ELISA were 7350 U/ml (negative < 40 U/ml) measured on a stored serum sample 1 year later. He was treated with angiotensin II blockade for 4 months, but proteinuria persisted at >10 g/24 hours. He was then treated with a combination of methylprednisolone, 1 g intravenously, at the start of months 1, 3, and 5; oral prednisone, 0.5 mg/kg per day, on months 1, 3, and 5; and oral cyclophosphamide, 2.0 mg/kg per day, on months 2, 4, and 6. His proteinuria declined to 9 g/24 hours and 4 g/24 hours at 6 and 12 months, respectively, after the end of the treatment but has increased to 5.5 g/24 hours over the last 3 months. He is now asking you about further therapy. Current medications are lisinopril, 20 mg orally each day, and atorvastatin, 10 mg daily.

Pertinent findings on physical examination include BP of 110/75 mmHg, pulse of 72 beats/min, and trace edema of the ankles. Laboratory tests show a hemoglobin concentration of 12.8 g/dl, serum creatinine concentration of 1.3 mg/dl, serum albumin level of 3.4 g/dl, urinalysis showing heme 1+, and proteinuria of 5.4 g/24 hours. The serum C3 level is 110 mg/dl (reference range, 75–175 mg/dl).

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![Figure 2](image_url)
second or third cause of a primary glomerulopathy leading to ESRD (29). Patients with MN who remain nephrotic are at an increased risk for thromboembolic (30) and cardiovascular (31,32) events. Available immunosuppressive therapies include the use of corticosteroids combined with cytotoxic agents, as well as calcineurin inhibitors. These therapies are at least partially successful in reducing proteinuria, but their use is controversial, is associated with significant adverse effects, and carries a high rate of relapse (33). To date, the best proven long-term therapy for patients with MN consists of the combined use of corticosteroids and cyclophosphamide—the Ponticelli protocol—and it was used in this patient. Proteinuria decreased substantially, but it never decreased to <4 g/24 hours and more recently has increased to 5.5 g/24 hours. The question to answer is: Does proteinuria in this patient reflect active immunologic disease, or is it a consequence of renal damage? This question is clinically relevant and introduces the concept that immunologic remission should be added to clinical remission (based on proteinuria) and that both be used in judging treatment (conservative versus immunosuppressive) in MN.

Some qualitative aspects of proteinuria, such as urinary excretion of α1-microglobulin, β2-microglobulin, IgG, and IgM (option A), have been reported as strong predictors for renal disease progression (34–36). However, little is known regarding factors that may predict response to therapy, and the only studies that have evaluated the use of urinary markers for this purpose concluded that neither absolute levels of urinary IgG, β2-microglobulin, or α1-microglobulin at baseline or 12 months nor the percentage of reduction between baseline and 12 months clearly predicted the occurrence of a remission, a relapse to nephrotic range proteinuria, or longer-term outcomes (37,38). It is unknown whether the use of these markers could predict active immunologic disease.

IgG4-related disease represents a recently recognized group of multiorgan diseases characterized by hypergammaglobulinemia with elevated serum total IgG and/or IgG4 levels, a high level of serum IgG4, and dense infiltration of IgG4-positive cells into multiple organs (39,40). The term IgG4-related sclerosing disease is also used for this entity because it results in a sclerosing lesion of multiple organs, including the kidney, with formation of pseudotumors (41,42). Patients with renal involvement are often elderly men presenting with progressive renal failure. There are several other characteristics. Patients often have elevated serum total IgG and/or IgG4 levels or hypergammaglobulinemia. Kidney biopsy usually shows a tubulointerstitial nephritis (TIN) with moderate to marked increase in IgG4-positive plasma cells, with or without tubular basement membrane deposits. The term IgG4-related TIN has been proposed to describe this entity (39). A paucity of cases of IgG4-related TIN with MN has also been described (option B) (43,44). The absence of systemic organ involvement and TIN in this case make the diagnosis of an IgG4-related MN unlikely.

Renal ultrasonography with Doppler examination of renal veins (option D) is indicated to rule out renal vein thrombosis. Acute renal vein thrombosis is usually characterized by a recent episode of acute flank pain, macroscopic hematuria, flank tenderness at percussion, worsening proteinuria, and deterioration of renal function (45). Hypoalbuminemia, particularly a serum albumin concentration <2.8 g/dl, is the most significant independent predictor of venous thrombotic risk in patients with MN (46). In view of the above, it is unlikely that our patient had developed acute renal vein thrombosis, although asymptomatic chronic renal vein thrombosis cannot be ruled out. However, there is no convincing evidence that chronic renal vein thrombosis is associated with worsening renal function or proteinuria (45).

Anti-PLA2R antibodies (option C) are present in 70%–82% of patients with idiopathic MN but are not present in the serum of healthy controls or patients with other glomerular and autoimmune diseases (47,48). Levels of anti-PLA2R correlate strongly correlated with disease activity and response to therapy: Disappearance of the antibody is associated with remission of proteinuria, and reappearance of the antibody heralds a relapse of nephrotic syndrome (47–50). Taken together, these observations suggest that detection and quantification of circulating anti-PLA2R levels may provide a tool for monitoring disease activity and treatment efficacy in patients with MN (51). On the other hand, low titers of anti-PLA2R have been detected in patients in remission (48), whereas the presence of high titers of anti-PLA2R antibodies did not preclude development of spontaneous remission (52). Similarly, discrepancies between circulating anti-PLA2R antibodies and detectable PLA2R in glomerular deposits have been reported in a study of 42 consecutive patients with primary MN (53). In 21 patients, anti-PLA2R antibodies were present in circulation and PLA2R was seen in glomerular deposits. However, 3 patients with high levels of circulating anti-PLA2R antibodies did not have detectable PLA2R in glomerular deposits, suggesting that antibodies were not nephritogenic or that epitopes were poorly accessible at the time of kidney biopsy; among the 18 patients with no detectable circulating anti-PLA2R antibodies, 10 had positive PLA2R glomerular staining. Debiec and Ronco (53) suggest that these apparently discordant findings might be due to rapid clearance of antibodies from the circulation and deposition in glomeruli or to patients with persistent proteinuria due to glomerular ultrastructural damage but immunologically inactive disease. Therefore, the persistence of proteinuria in some cases may be the consequence of an altered architecture of the filtration barrier due to longstanding disease and remodeling process in the glomerular basement membrane.

In the present case, serial anti-PLA2R testing was not available and although a current negative test result would probably reflect immunologic remission, a single positive anti-PLA2R test result would not necessarily correlate with immunologic activity. As such, we believe the only way to accurately establish disease activity in this patient is to perform renal biopsy (option E).

Further Information for GN Case 2

A repeat renal biopsy is performed and shows thickened capillary loops, positivity for IgG and C3 on immunofluorescence, and markedly disrupted glomerular basement membrane with lucent deposits (stage IV MN) on electron microscopy (Figure 3).
You would now recommend:

A. Continue current therapy
B. Repeat 6-month course of corticosteroids alternating with cyclophosphamide
C. Start tacrolimus
D. Start adrenocorticotropin-releasing hormone gel
E. Start rituximab

Discussion of Question 3

Correct answer is A. Continue current therapy.

The repeat renal biopsy shows “burnt out” (stage IV MN) with grossly disrupted glomerular basement membrane but without new epimembranous deposits on electron microscopy. Therefore, we consider the patient to be in immunologic remission and to have proteinuria due to damage of the glomerular basement membrane. Immunosuppressive therapy has no role in this patient, and the aim should be to maximize conservative therapy (option A).


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