Recent advances in glomerular biology have expanded our understanding of glomerular diseases, leading to more precise therapeutic options. Since the discovery of the autoantigen phospholipase A2 receptor in primary membranous nephropathy 10 years ago, the serologic evaluation of glomerular diseases has become more detailed and nuanced for nephrologists. In addition to phospholipase A2 receptor antibodies, circulating autoantibodies now include thrombospondin type 1 domain—containing 7A and most recently, neural epidermal growth factor–like 1 protein for membranous nephropathy. Additionally, discoveries in C3 glomerulopathy and fibillary glomerulonephritis are poised to improve the diagnostic approach to these disorders by using novel biomarkers to complement traditional histologic patterns on kidney biopsy. Although kidney biopsies are considered the gold standard in profiling glomerular diseases, validated novel glomerular biomarkers contribute substantially to the diagnostic and therapeutic approaches through their ability to improve sensitivity, permit dynamic longitudinal monitoring of disease activity, and capture genetic heterogeneity. We describe the value of specific biomarkers in selected glomerular diseases, with the major focus on their clinical applicability.

Introduction

Guidance on the management of patients with glomerular diseases necessitates an accurate diagnosis together with the ability to predict the clinical course and responsiveness to therapy. For glomerular diseases, kidney biopsy remains the gold standard that for decades has provided diagnostic and prognostic information that forms the basis of current therapies. There are certain limitations to biopsies. Biopsies are processed for light (LM), immunofluorescence (IF), and electron microscopy (EM) and provide a “snapshot” in time of the disease. They do not necessarily reflect on the dynamic nature of disease activity and course governed by a complex pathogenesis. Biopsies do not always differentiate between primary or secondary disease and do not provide an association between appearance and prognosis or responsiveness to treatment. Moreover, biopsies are invasive.

Traditional biomarkers, including serum creatinine, estimated glomerular filtration rate (eGFR), albuminuria, and proteinuria, lack sensitivity and specificity. Thus, the search for specific and sensitive serologic and or tissue biomarkers is an active area of investigation in glomerular diseases. There are a large number of glomerular biomarkers under investigation. We have chosen to review a select few, for which considerable research advances have been made that offer clinical applicability and or lend insight to pathogenesis.

Membranous Nephropathy

Overview

Membranous nephropathy (MN), the most common nondiabetic cause of nephrotic syndrome in adults, is caused by antibodies targeting autoantigens at the podocyte cell membrane–basement membrane interface, resulting in immune complex formation (Table 1).\(^1\) Traditionally the diagnosis was based on the histologic pattern resulting from immune complex formation as seen on kidney biopsy. LM demonstrates thickened glomerular basement membrane with “spikes” and “holes” on silver stain, granular capillary wall staining of polyclonal immunoglobulin G (IgG) with variable C3 staining on IF, and podocyte effacement with subepithelial deposits on EM.\(^2\) Various biopsy findings differentiate primary from secondary MN (mesangial deposits, full house staining [ie, all 5 major immunofluorescent stains on a kidney biopsy—IgM, IgG, IgA, C3, and C1q—are all positive]) in the presence of associated conditions such as systemic lupus erythematosus, Sjögren syndrome, malignancy, and nonsteroidal anti-inflammatory drug use.

However, the discovery of circulating antibodies to the phospholipase A2 receptor (PLA2R) autoantigen as a specific marker for primary MN has created a new schema of categorizing and treating primary MN. Most autoantigens in primary MN have been discovered; PLA2R and thrombospondin type 1 domain—containing 7 (THSD7A) are found in 70% and 1% to 5% of patients with primary MN, respectively. Neural epidermal growth factor–like 1 protein (NELL-1) identifies 16% of PLA2R-negative MN cases, making it the second most common autoantigen in primary MN.\(^3\)\(^-\)\(^5\) Exostosin 1 and 2 (EXT1/EXT2)-related MN in PLA2R-negative THSD7A-negative cases has expanded the diagnostic spectrum in secondary MN.\(^6\) These serologic-based approaches to identify subtypes of MN are transforming our molecular understanding of MN.\(^7\)
The diagnosis of PLA2R MN. In a recent meta-analysis, the pooled sensitivity of serum anti-PLA2R antibody was 65% (range, 63%-67%), depending on the assay (IF or enzyme-linked immunosorbent assay [ELISA]), stage, and ethnicity. A widely used commercially available autoantibody test for PLA2R is highly sensitive (96.5%) and specific (~100%) and correlates with proteinuria, relapse, chronic kidney disease outcomes, and response to therapy, with faster and more sensitive assays on the horizon. These studies prove that the serum anti-PLA2R antibody is a true biomarker and is a primary target of disease control. Monitoring of antibodies to PLA2R is the current standard of care for PLA2R MN.

**Table 1. Biomarkers of Membranous Nephropathy in Adults**

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Disease</th>
<th>Method of Detection</th>
<th>Malignancy Screening and Rate</th>
<th>Incidence</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phospholipase A2 receptor 1 (PLA2R)</td>
<td>Primary MN</td>
<td>Serum: ELISA, IIF, WB; Tissue: IHC, IF</td>
<td>Age-appropriate screening; rate of malignancy: ~9%</td>
<td>~70%-80% of idiopathic MN</td>
<td>• Most common antigen in primary MN • Biopsy not necessary if eGFR &gt; 60 without evidence of secondary/superimposed cause • IgG4 dominant</td>
</tr>
<tr>
<td>Neural epidermal growth factor-like 1 protein (NELL-1)</td>
<td>Primary MN</td>
<td>Serum: WB; Tissue: IF, IHC</td>
<td>Search for malignancy; rate of malignancy: 11.7-33%</td>
<td>~3.8%-16% of PLA2R, THD7A-negative idiopathic MN</td>
<td>• 2nd most common antigen in MN • IgG1 dominant</td>
</tr>
<tr>
<td>Thrombospondin type 1 domain containing 7A (THSd7A)</td>
<td>Primary MN</td>
<td>Serum: ELISA, IIF, WB; Tissue: IHC, IF</td>
<td>Aggressive screening including urogenital and gastrointestinal/colorectal; rate of malignancy: 6%-20%</td>
<td>1%-5% of idiopathic MN (~10% of PLA2R negative)</td>
<td>• 3rd most common antigen in MN • ELISA not commercially available • IgG4 dominant</td>
</tr>
<tr>
<td>Exostosin 1/exostosin 2 (EXT1/EXT2)</td>
<td>Secondary MN</td>
<td>Tissue: IHC, IF</td>
<td>Limited data to recommend screening; rate of malignancy: 7.6%</td>
<td>11.6% of PLA2R-negative MN</td>
<td>• Tissue marker of class V lupus ~1/3 of cases &amp; autoimmune disease, typically young, female • IgG1 dominant</td>
</tr>
</tbody>
</table>

**Abbreviations:** eGFR, estimated glomerular filtration rate (in mL/min/1.73 m²); ELISA, enzyme-linked immunosorbent assay; IF, immunofluorescence; IgG4, immunoglobulin G4; IHC, immunohistochemical; IIF, indirect immunofluorescence; MN, membranous nephropathy; PLA2R, phospholipase A2 receptor; WB, Western blot.

*Commercially available.*

**Figure 1. Intramolecular epitope spreading of the anti-PLA2R antibody (anti-PLA2R) versus baseline multido-main recognition.** The reactivity of anti-PLA2R antibodies to a ubiquitous epitope in the cysteine-rich (CysR) domain (left) “spreads” to include subdominant epitopes of the first (center, C-type lectin domain [CTLD1]) and seventh and eighth CTLDs (right, CTLD7 and CTLD8) distinct from the CysR epitope. Disease progression is positively correlated with greater urinary protein excretion and patient age and inversely correlated with the likelihood of remission. An alternative hypothesis is that antibodies to multiple domains are present at the time of diagnosis, and progression of disease is correlated with total anti-PLA2R antibody levels. Abbreviation: FNII, fibronectin type II domain.
The variability of assays drives this difference because both in-house and commercially available ELISAs are in use. Therefore, it is important to interpret an ELISA titer value with respect to the assay and the studies using the assay. It is not advised to use assays interchangeably and it is not clear whether one assay is superior to another.

“Epitope spreading” in PLA2R MN is thought to represent resistant disease. PLA2R is a transmembrane protein of 180 kDa that has an extensive extracellular region consisting of 10 globular domains of 7 to 17 kDa; these comprise a cysteine-rich domain, a fibronectin type II domain, and 8 C-type lectin domains (CTLD1-8). The cysteine-rich domain is the dominant primary epitope; CTLD1 and CTLD7 are the 2 other independent epitopes targeted by antibodies in PLA2R MN. Theoretically, epitope spreading beyond the original cysteine-rich domain allows for diversification of the immunologic response to the antigen. A high titer (>369 RU/mL) has been found to be consistently associated with epitope spreading, and “spreaders” have more resistant disease, more proteinuria, and lower eGFR. High titer values are considered a poor prognostic indicator (Fig 1).

In a large prospective cohort, CTLD8 was identified as a fourth epitope, all patients had baseline epitope spreading, recognizing at least 2 epitopes (cysteine-rich domain–fibronectin type II domain–CTLD1 and CTLD7 to CTLD8). Furthermore, when 150 patients were followed up for a median of 54 months, anti-PLA2R antibody titer and not epitope-specific titers predicted outcomes. The discrepancy between the 2 findings may be attributed to variabilities in assay detection (Western blot [WB] or ELISA), serum dilution, cross-reactivity, construct design, and threshold for positivity. The same challenges in defining clinically relevant titer thresholds also apply to defining epitope-specific subtypes in PLA2R MN. Thus, further studies are needed to clarify the importance of epitope spreading.

**PLA2R MN with GFR > 60 mL/min/1.73 m²**

In patients in whom primary MN is suspected, accurate diagnosis by ELISA can be obtained while simultaneously screening for secondary causes, including viral hepatitis, antinuclear antibodies, IgG4, sarcoidosis, age-appropriate malignancy screening, and medication and nonsteroidal anti-inflammatory drug use. This noninvasive serologic-based approach is reasonable, especially if risk of biopsy is high or if the patient refuses. This serologic-based approach to the diagnosis of PLA2R MN is supported by a recent retrospective study in which 838 patients were tested for anti-PLA2R antibody; 97 were positive by ELISA (>2 RU/mL) confirmed with indirect IF (IIF), a kidney biopsy, and a negative workup for secondary causes. They were then analyzed in groups according to eGFR (>60 vs <60 mL/min/1.73 m²). The rate of superimposed disease was higher in those with eGFR <60 mL/min/1.73 m² (13.5% [5/37]), which included diabetic nephropathy, acute tubular necrosis, and 2 patients with focal segmental glomerulosclerosis. Notably, 2 patients with superimposed disease (crescentic and diabetic nephropathy) had eGFR ≤12 mL/min/1.73 m² and high total renal chronicity score on biopsy, limiting the benefit of immunosuppressive therapy. Of 97 patients, only 2 would have required more extensive workup or change in immunosuppression (allergic interstitial nephritis and crescentic disease). Thus, for patients with suspected MN and eGFR >60 mL/min/1.73 m² with no evidence of secondary causes, a biopsy to prove serologic-positive PLA2R MN is not necessary (Fig 2).

As ELISA becomes more widely used, the utility of a kidney biopsy in this situation is likely to be diminished; however, more false-positives are likely to be found. In one study, although investigators confirmed anti-PLA2R antibody with both ELISA and IIF, there was only 1 patient with an ELISA result >20 RU/mL and negative IF results. Additionally, there was only 1 patient with negative ELISA (<2 RU/mL) and positive IIF results. In both cases, MN was diagnosed on biopsy. Therefore, although it is common practice to confirm the diagnosis of PLA2R MN serologically with both ELISA and the more sensitive IIF, the use of combined testing may not significantly improve sensitivity and specificity substantially for routine cases, however this will require further study.

**PLA2R MN With GFR < 60 mL/min/1.73 m²**

The severity of tubulointerstitial scarring is a prognostic indicator in glomerular disease. However, it is not clear if this is superior to clinical data in MN. In a prospective observational study, 243 patients who had MN, circulating antibody to PLA2R, and biopsy-proven PLA2R tissue antigen positivity were followed up for a median of 48 months. Patients were stratified according to ELISA PLA2R antibody tertiles, and 36 patients reached the study end point of doubling of serum creatinine level or kidney failure. Baseline independent predictors of the primary outcome were anti-PLA2R antibody level by ELISA (hazard ratio [HR], 1.36; 95% CI, 1.11–1.66; \( P = 0.01 \)) and interstitial fibrosis and tubular atrophy (HR, 1.32; 95% CI, 1.03–1.68; \( P = 0.03 \)). This study did not control for immunosuppressive therapy, which was most often cyclosporine (81/243). Therefore, conclusions about optimal therapy as it relates to baseline characteristics of glomerulosclerosis, fibrosis, or anti-PLA2R antibody level cannot be made. Patients with higher anti-PLA2R antibody levels tended to be older, with lower eGFR and higher proteinuria. These data suggest that little prognostic information is gained by obtaining a biopsy when clinical data including anti-PLA2R antibody level are available and a secondary cause clinically is excluded. However, advanced chronic kidney disease was not well represented in this study, and the point at which immunosuppression
becomes futile and progression to kidney failure becomes inevitable is not well defined.

Although detection of circulating antibody and tissue staining for PLA2R antigen is highly specific, its presence does not exclude the possibility of superimposed disease. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients. Hypertensive damage has been seen in cases of higher anti-PLA2R antibody titer and is associated with progressive decline in kidney function in MN and poor response to immunosuppression in diabetic patients.

Few clinical scenarios reduce the utility of PLA2R serologic testing and necessitate a kidney biopsy for diagnosis. As mentioned, crescentic disease in MN is a rare (<1% of MN) but well-described entity. It is often associated with antineutrophil cytoplasmic antibody, anti–glomerular basement membrane positivity, or very rarely monoclonal gammopathy of unknown significance. However, some are serologically negative. Often patients present with heavy proteinuria, hematuria, and acute kidney injury. In a case series of MN with crescents, 38% (6/16) stained for PLA2R on biopsy. In a recent case series of MN with necrotizing and crescent glomerulonephritis, 2 of 15 were positive for both serum anti-PLA2R antibody and tissue antigen while being negative for antineutrophil cytoplasmic antibody and anti–glomerular basement membrane. Both had MN diagnosed initially and transformed into crescentic disease; 40% with dual crescentic and MN reached kidney failure in a 72-month median follow-up. PLA2R MN has also been reported post–hematopoietic stem cell transplantation with crescent formation and as a manifestation of graft-versus-host disease that responded to corticosteroid therapy. Therefore, isolated positive PLA2R serologic results do not obviate the search for secondary causes or always permit eliminating kidney biopsies (and potentially missing a crescentic lesion) and should always be interpreted with clinical context. Although MN with crescents is extremely rare, a rapid decline in kidney function with hematuria should alert the nephrologist to the possibility of crescentic disease, even in the presence of PLA2R positivity.

**THSD7A MN**

THSD7A is a glycosylated 250-kDa type 1 transmembrane protein highly expressed on podocytes and is a specific biomarker for primary MN. In a recent meta-analysis of more than 4,000 patients with MN, 3% (95% CI, 3%-4%) had autoantibodies directed against THSD7A, and prevalence was 10% (95% CI, 6%-15%) in PLA2R-negative patients. Similarly, in a study of 1,276 patients with MN in German and North American cohorts, the combined prevalence was 3.1%. One of 130 patients with MN enrolled in the MENTOR trial had THSD7A MN. Taken together, THSD7A prevalence is low, and providing detailed clinicopathologic characteristics is challenging. THSD7A MN may be associated with malignancy. In a cohort of 1,276 patients with MN, 40 had THSD7A MN by

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**Figure 2.** Proposed approach to serologic diagnosis of phospholipase A2 receptor (PLA2R) membranous nephropathy (MN). Sensitivity of the serologic assays is not uniform and depends on ethnicity. This algorithm is based on the study by Bobart et al (predominantly White North American cohort). In general it should include a search for autoimmune disease (lupus), medications (nonsteroidal anti-inflammatory drugs), malignancy, and infections (viral hepatitis). Abbreviations: eGFR, estimated glomerular filtration rate; ELISA, enzyme-linked immunosorbent assay; IFT, immunofluorescence testing.
IIF: 8 (20%) developed malignancy within 3 months of diagnosis.56 THSD7A is expressed in human tumors, including gall bladder, colorectal, endometrial, and breast.58-60 Reduction in THSD7A antibodies and/or proteinuria has been seen following treatment of the tumor, thus potentially linking malignancy and MN.58,60,61 However, malignancy in MN was found to be less common in other studies (6%-16%).56,59,60 These discrepancies may be due to differences in study protocol, local practice of cancer screening, and selection bias. Given the association between THSD7A and MN, an in-depth search for malignancy is warranted when THSD7A positivity is encountered.

THSD7A antibodies are thought to be pathogenic. A THSD7A MN–positive patient with kidney failure with a titer of 1:1,000 at the time of transplantation was reported to have recurrence in less than 1 year, with the allograft staining positive for THSD7A.54 Moreover, sera from 2 patients with THSD7A MN bound to mouse THSD7A antigen on podocytes, colocalizing with nephrin at the slit diaphragm 2 hours after intravenous injection, and induced histologic features of MN with proteinuria.54 THSD7A localizes to the slit diaphragm of podocyte foot processes and is associated with enhanced stabilization of the slit diaphragm in cultured podocytes, supporting a pathogenic role.62 The positivity rate of antibody in serum and tissue is highly correlated.53 Serum IIF has 92% diagnostic sensitivity and 100% specificity.56 In a small study, 24 patients with serologic positivity to THSD7A also had positivity on tissue by IIF.50 ELISA detected anti-THSD7A autoantibodies with sensitivity comparable to IIF and WB in 45 of 49 patients with THSD7A MN.63 Only 3 were negative for circulating antibody (WB, ELISA, and IIF) but positive on biopsy. At baseline, patients in the lowest anti-THSD7A antibody level tertile (23-122 RU/mL) saw the highest rate of remission (92%), whereas patients in combined middle (134-566 RU/mL) and high (606-13,920 RU/mL) tertiles saw statistically significant less remission (P = 0.006).65 Although ELISA titers for anti-THSD7A antibody appear to correlate with disease activity similar to PLA2R, ELISA is not yet commercially available. IIF testing is commercially available to clinicians and can be acquired from other specialized laboratories, thus allowing for differentiating primary from secondary MN in PLA2R-negative cases. However, IIF is not suited for longitudinal monitoring.

Summary
The discovery of circulating antibodies to autoantigens in MN has led to an exponential growth in our understanding of MN. Testing for these antibodies is likely to continue to expand in clinical practice. However, as with any test, there are important clinical caveats, such as superimposed glomerular disease, progressed chronic kidney disease, and the association of malignancy, that need to be taken into account when interpreting these tests. As testing for autoantibodies increases and potentially fewer biopsies are performed, we must remain informed and judicious in their clinical application.

C3 Glomerulopathy
Overview
C3 glomerulopathy (C3G) is a spectrum of diseases with the common mechanism of dysregulation of alternative complement in the fluid phase. C3G is a remarkably heterogeneous and an ultrarare disease (about 2–3 cases per million) and often results from trigger events such as infection, autoimmunity, or monoclonal gammopathy.64 Underlying abnormalities in the alternative complement pathway are manifest as acquired drivers (the autoantibodies C3 nephritic factor [C3Nef], C4Nef, C5Nef, monoclonal gammapathy, and anti-factor H) or genetic drivers (mutations of C3 or the complement factor genes CFB, CFH, CFI, and CFHR1-CHRS).65 Although mutations in complement genes are common (13%-37%), a fraction of these are known to be or likely be pathogenic (12.9% in a large Mayo Clinic case series).64,66,67 Our understanding of the genetics and genotype-phenotype correlation of C3G is incomplete. A KDIGO (Kidney Disease: Improving Global Outcomes) controversies report of 2017 concluded that there is no clear benefit of performing genetic analysis in every case of C3G. However, genetic results may assist in treatment decisions.68 Additionally, genetic testing should be undertaken when familial causes are suspected (ie, CFHR5 nephropathy).

Outcomes of C3G are varied, some with rapid progression of kidney disease compared with other reports showing kidney failure in 36.5% of patients at 10 years.66 The decision to treat with immunosuppression is largely dependent on non–disease-specific factors, including severity of proteinuria, kidney function, and degree of tubular atrophy/interstitial fibrosis.69 Although no specific therapy for C3G is currently available, retrospective studies suggest that immunosuppressive treatment with mycophenolate mofetil and corticosteroids is beneficial compared with conservative treatment.64,67,70 Given the remarkable heterogeneity of presentation, associated disease, and pathogenesis, with poor outcomes, the discovery of a viable biomarker is critical.

Tissue Biomarkers in C3G
C3G causes significant glomerular deposition of complement C3, C5, C6, C7, C8, and C9, with C3 being most abundant.71 A study by Sethi et al71-73 using laser microdissection and mass spectrometry to analyze the composition of complement deposits in 6 cases of C3G and dense deposit disease found that C3dg, which is cleaved from surface bound C3b, was the most abundant C3 protein detected (Fig 3). Importantly, the degradation products of C3 (iC3c and C3dg) do not form new convertases, but are opsonins and participate in adaptive immune stimulation. This is important because routine evaluation for C3 by IIF detects C3c, which does not include C3dg. Therefore, routine IF studies cannot accurately reflect complement activation as a disease biomarker.71-73
A recent small retrospective study of patients with C3G further explored the characterization of complement proteins by analyzing an array of proteins (including factor H-related protein 5 [CFHR5], FHR1, FH, C3b/iC3b/C3c, C3dg, C5b-9, properdin, C4d, and C1q) through targeted antibodies on formalin-fixed paraffin-embedded kidney biopsy samples. They found similar results to those found by mass spectrometry by Sethi et al, with a notable addition that FH5 was the most prevalent protein, was more prevalent than FH1, and was detected in 96% of native samples and 100% of transplant samples with at least 1+ intensity. The staining intensity of FH5 correlated with the intensities of C3b/iC3b/C3c, C3dg, and C5b9, yet only the staining intensity of FH5 and C5b9 negatively correlated with eGFR. Serial biopsies obtained in recurrent crescentic C3G showed that FH5 correlated with disease activity but was unaltered by C5 inhibition with eculizumab. This suggests that clinical improvement with eculizumab may result from reduced production of C5a, a potent anaphylatoxin, and inflammatory cell recruitment and not inhibition of terminal complement. Further analysis of FH5 as a biomarker will require larger cohorts to capture a difference in disease courses, non-complement-mediated comparators, and correlations with serial biopsies to understand the evolution of FH5 staining with respect to treatment, progression, proteinuria, and changes in morphology.

The diagnosis of C3G remains challenging and often relies on both a structural and functional assessment, which poses significant challenges in interpretation for many nephrologists given that they do not routinely use these assays. These studies represent intriguing concepts in the pathogenesis of C3G and potential emerging biomarkers, which are desperately needed not only to guide diagnosis but also treatment.

**Fibrillary Glomerulonephritis**

Fibrillary glomerulonephritis (FGN) is a rare proliferative glomerulopathy defined ultrastructurally by haphazardly arranged fibrils that are 10 to 30 nm in diameter and a lack of Congo red staining for amyloid. Determining the fibril size is both challenging and crucial due to overlaps in size with amyloid fibrils (8-12 nm) and immunotactoid glomerulopathy fibrils (>35 nm). FGN occurs mainly in adults around 50 years of age, with hematuria (82%), proteinuria (average protein excretion of 5.7 g/d), or full nephrotic syndrome (~33%). It has been associated with dysproteinemia in 4% to 42% of cases depending on the population, with 5% to 11% and 7% to 27% also having an autoimmune disease or hepatitis C virus infection, respectively. Prognosis is abysmal, with 44% of patients reaching kidney failure at a mean follow-up of 52.3 months according to the large case series from the Mayo Clinic, and there remains no established treatment.

Clinicians are dependent on biopsy for diagnosis, and histologic features can vary significantly. The patterns by LM are diverse, including membranoproliferative glomerulonephritis, mesangial proliferative, diffuse proliferative glomerulonephritis, and membranous and diffuse sclerosing. Patients with diffuse sclerosing and diffuse proliferative glomerulonephritis experience the worst outcomes, reaching kidney failure in 7 and 20 months, respectively. The presence of fibrils, described in diabetic nephropathy, obesity-related glomerulopathy, focal segmental glomerulosclerosis, malignant hypertension, and hemolytic uremic syndrome, is relatively nonspecific. Cryoglobulinemia can have a similar appearance and must be excluded.

Despite its diagnostic advantages, EM is not widely available and takes much longer than LM and IF. A specific biomarker to properly classify the disease is needed. Recently, DNAJ homolog subfamily B member 9 (DNAJB9) was identified as an abundant protein in FGN by laser-capture microdissection and mass spectrometry. DNAJB9 is a molecular chaperone and functions by binding to immunoglobulins that assist in folding and degrading misfolded proteins to protect cells from stress apoptosis. DNAJB9 is present in podocytes as well as mesangial and endothelial cells in the glomerulus. DNAJB9 was detected in serum using an immunoprecipitation-linked multiple reaction monitoring assay with sensitivity of 67% and specificity of 98% for FGN when compared with amyloidosis, myeloma, non-FGN glomerular disease, and healthy individuals and was negatively associated with eGFR. These results suggest that serum detection could prove useful as a biomarker for diagnosis and potentially longitudinal monitoring, pending an effective therapy. Of course, additional studies will need to replicate these findings. The sensitivity (98%) and specificity (99%) of DNAJB9 for FGN by immunohistochemistry is impressive when FGN is compared with amyloidosis, other glomerular diseases (n = 98), and...
healthy individuals. DNAJB9 localizes to FGN fibrils and colocalizes with IgG, giving credence to FGN autoimmune association.

However, the mechanism by which DNAJB9 contributes to pathogenicity is far from being fully understood. Congo red staining, although routinely performed and widely available, is imperfect in differentiating amyloid and nonamyloid diseases. In 18 patients with rare congoophilic FGN, DNAJB9 was found to be specific for FGN and excluded amyloid that was confirmed by laser microdissection and mass spectrometry, currently the gold standard in the diagnosis of amyloid. In the largest retrospective study from multiple North American sites (1997-2017) analyzing 296 biopsies, clinicopathologic features, and outcomes of FGN, DNAJB9 staining confirmed the diagnosis in 100% of patients with FGN. DNAJB9 further established the diagnosis in 79% (23/29) of cases labeled as possible FGN due to atypical features that would raise the possibility of paraprotein-mediated disease (including Congo red positivity, IgG monotypic light chain staining on IF, and fibril size outside the norm). Taken together, DNAJB9 is highly specific and testing should be performed when FGN is suspected.

It is truly remarkable that for a disease as rare as FGN, a highly sensitive and specific biomarker has been discovered in the form of DNAJB9. Although much remains to be understood concerning DNAJB9 and its role in the pathogenesis of FGN, we agree with Nasr and Fogo that the diagnostic strength of DNAJB9 should result in altering the disease name to “DNAJB9-associated FGN” from FGN. It will be exciting to see how DNAJB9 will lend to our understanding of pathogenesis and treatment of this unique disease.

Conclusions

Novel glomerular biomarkers have provided clinicians with insight into glomerular disease pathogenesis and have advanced care by enabling tailored therapy. The clinical applicability of the various biomarkers presented here varies greatly. Although some are highly relevant clinically, widely accessible, and scalable, others are currently more exploratory and should remain complementary to the kidney biopsy. Despite inherent limitations, kidney biopsy still remains the gold standard for the diagnosis of glomerular disease when used in its current form to understand pathogenesis. Whereas exciting progress has been made in certain kidney diseases, the field is only beginning to appreciate the power of identifying biomarkers aided by molecular profiling and large-scale data generation and integration, along with prospectively collected clinical data. With new molecular techniques such as proteomics and transcriptomics currently being studied and applied to kidney tissue, a much deeper level of understanding of glomerular disease will soon be realized. As new glomerular biomarkers of glomerular disease continue to be discovered and validated, it is imperative to have a clear understanding of their use and limitations in relation to the role of kidney biopsies. Overcoming these challenges in the future, the nephrologist has the opportunity to affect the care of patients through personalized treatment regimens leading to improved outcomes.

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Support: None.

Financial Disclosure: Dr Okusa reports being a prior holder of equity in Adenosine Therapeutics, LLC; receipt of royalty payments from UpToDate; travel and/or honoraria not related to the subject of this review (from Yale O’Brien External Advisory Committee, Yale University, UAB O’Brien Center, Mount Sinai, Northwestern University, CRRT Taipei and India, Hong Kong Society of Nephrology, Japanese Society of Nephrology, Johns Hopkins University, University of Florida, Tokyo University, University of Maryland, FASEB, and Keystone Symposia); and receipt of research grants not related to this review (from Am Pharma, Pfizer, John Bower Foundation).

Peer Review: Received March 2, 2020, in response to an invitation from the journal. Evaluated by 3 external peer reviewers, with direct editorial input from an Associate Editor and a Deputy Editor. Accepted in revised form June 6, 2020.

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