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Review

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Primary Glomerular Diseases and Novel Biomarkers

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ABSTRACT

Glomerulonephritis is the third most common cause of end-stage kidney disease. The presentation, clinical course, and outcome of glomerular diseases are highly variable. A kidney biopsy is always needed to clarify the diagnosis; however, performing a kidney biopsy is limited by several factors such as bleeding disorders, obesity, and other comorbid conditions. There is a need for less invasive, simple, and reproducible tests, especially by using blood and urine samples, which could replace kidney biopsy. Our review focuses on the novel clinical, histopathological, blood, and urine biomarkers to make an accurate diagnosis and predict the prognosis of primary glomerular diseases including immunoglobulin A nephropathy, membranous nephropathy, focal segmental glomerulosclerosis, and membranoproliferative glomerulonephritis. Overall, although there are promising biomarkers for glomerulonephritis, long-term evaluation of these biomarkers is still needed.
Keywords: Focal segmental glomerulosclerosis, glomerular disease, immunoglobulin A nephropathy, membranoproliferative glomerulonephritis, membranous nephropathy

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INTRODUCTION

Glomerulonephritis (GN) is the third most common cause of end-stage kidney disease (ESKD).[1] As the treatment is disease-specific, making a well-defined diagnosis is important.[2] The presentation, clinical course, and outcome of glomerular diseases are highly variable.[3] A kidney biopsy is always needed to clarify the diagnosis; however, performing a kidney biopsy is limited by several factors such as bleeding disorders, obesity, and other comorbid conditions.[2] There is a need for less invasive, simple, and reproducible tests especially by using blood and urine samples, which could replace kidney biopsy. Here, we review the novel clinical, histopathological, blood, and urine biomarkers to make an accurate diagnosis and predict the prognosis of primary glomerular diseases including immunoglobulin A (IgA) nephropathy (IgAN), membranous nephropathy (MN), focal segmental glomerulosclerosis (FSGS), and membranoproliferative glomerulonephritis (MPGN) (Table 1).

IMMUNOGLOBULIN A NEPHROPATHY

Immunoglobulin A nephropathy is the most common primary GN worldwide[4,5] and the prevalence varies with the geographic region, being the highest in Southeast Asia.[4,5] Although different histologic appearances can be seen, the major diagnostic feature is mesangial hypercellularity and mesangial IgA deposition.[4] The disease pathophysiology has not been completely understood yet. The suggested mechanism is a "four hit" model, starting with the improper galactosylation of IgA1 (first hit).[4,5] The second hit is the formation of IgA or IgG autoantibodies against this galactose-deficient IgA1 (Gd-IgA1) resulting in immunocomplex (third hit) deposits in the mesangium of the glomerulus (fourth hit).[4-7] At the end of this scenario, complement pathway activation[4] and cytokine release could change podocyte gene expression and permeability of glomeruli[8] which may result in kidney failure.[4]

Recent studies have shown associations between specific complement proteins and IgAN severity[9] which lead investigators to study alternative and lectin complement pathways in IgAN. As the disease may result in spontaneous remission in some patients,[4] 40% of IgAN patients progress to ESKD within 20 years.[10] The variability of the clinical course anticipates different treatment options and response rates are highly variable. There is an absolute need for validated biomarkers to predict the risk of progression and indication for treatment at early stages when lesions can be reversible.

Clinical Markers

Patients with IgAN have a wide range of clinical presentations, including episodes of macroscopic hematuria with concurrent pharyngitis, microscopic hematuria as a part of nephritic, nephrotic syndrome, or rapidly progressive GN.[4] Baseline clinical factors of patients with IgAN including decreased kidney function at the time of diagnosis measured by glomerular filtration rate (GFR),[11-14] histologic grading,[11-15] and proteinuria[15-17] were most consistently found to be independently associated with progressive kidney disease.

Blood pressure at diagnosis is also a risk factor for IgAN progression and takes place in international prediction tool.[5,18] In a recent study, the study outcome, dialysis, or death at 20 years was reached in 5%, 19%, and 42% of normotensive, controlled hypertension, and uncontrolled hypertension groups, respectively.[15] The presence of proteinuria and particularly higher levels are associated with a decline in kidney function in glomerular diseases.[19,20] Time-averaged proteinuria (TAP) is the strongest independent prognostic factor in IgAN.[5] When the TAP is less than 1 g/day, the 10-year risk of ESKD is 5%; when TAP is more than 3 g/day, this risk increases to 60%.[17] An early reduction in proteinuria is associated with improved survival.[17] Proteinuria is an activator of tubular chemokine and complement pathways resulting in kidney inflammation, as well as a biomarker of glomerular injury.[21] Thus, changes in proteinuria level are important for follow-up of the patients on treatment.[10] Hematuria has been the most controversial risk factor for IgAN. In an Asian cohort, hematuria with mild proteinuria was found as a risk factor for progression to ESKD.[22] On the contrary, in a Japan cohort, it was not found as a risk factor for progression.[23] Another Japan study showed that while mild hematuria (urinary red blood cell (RBC) <30 per high-power field) was an independent risk factor for progression, severe hematuria was not found as a risk factor.[14] So, the importance of hematuria is still under debate.

The estimated GFR (eGFR) at diagnosis is a predictor of future kidney function in patients with IgAN.[5,19] The patients with a lower eGFR at presentation are assumed to have less immunological activity and more fibrotic remodeling in the kidneys which means most likely they will be unresponsive to the immunosuppressive treatment.[5] In a Japanese cohort study, serum creatinine level at presentation was found to be the most important risk factor for IgAN progression.[24] In recent studies, being male[24] and older,[23] higher uric acid,[23] lower serum albumin[25] or total protein levels,[19] dyslipidemia,[26] and obesity[27] were found as risk factors for IgAN progression.

In 2019, the International IgAN Prediction Tool using eGFR and proteinuria at the time of biopsy, systolic blood pressure, diastolic blood pressure, age, usage of angiotensin-converting enzyme inhibitor or angiotensin receptor blocker, and immunosuppressive treatment before biopsy to predict progression was developed. It encompasses the MEST score that we mention below, as well.[5]

Histopathologic Markers

Immunoglobulin A nephropathy was first described histologically in 1968 by Berger[28] as intercapillary deposits of IgA-IgG. Since then, IgAN can be diagnosed by immunohistological examination of a kidney biopsy and is usually characterized by mesangioproliferative changes in glomeruli with deposition of IgA in the mesangium, although the light microscopical pattern may vary widely.[4] Many other features may be seen as well and 4 of them are used in the MEST scoring system identified by the 2009 Oxford Classification working group.[4] MEST score includes mesangial hypercellularity (M), endocapillary hypercellularity (E), segmental sclerosis (S), and tubular atrophy/interstitial fibrosis (T) and was defined to predict the prognosis of IgAN.[5,29] MEST score determines the risk and time to progression to ESKD.[4] In 2016, the Oxford classification was revised to include cellular and fibrocellular crescents[30] and named the MEST-C score.[4] Risk stratification according to MEST-C score helps clinicians to determine the therapy.[4] Endocapillary hypercellularity, a measure of glomerular macrophage accumulation, leads to improved outcomes due to responding well to immunosuppressive treatment, although there is also contrary evidence.[5,31] The presence of crescents leads clinicians to treat disease with cyclophosphamide which improves the outcome[4] because non-treated crescent-containing glomerulus were found associated with lower survival.[5]

MAIN POINTS

- There is a need for less invasive, simple, and reproducible tests especially by using blood and urine samples, which could replace kidney biopsy for glomerular diseases.
- Autoantibodies targeting galactose-deficient IgA1, microRNA-148b, C3 and C4d deposition are promising biomarkers for IgAN.
- Anti-PLA2R1 is a well-defined biomarker for MN. However, NELL-1, serine protease HTRA1, Contactin, THSD7A and kidney tissue EXT1/EXT2 staining are promising novel biomarkers for MN.
- Anti-CD40 antibodies, podocyte gene expression-based assay, hih molecular weight forms of ApoA-1 and CD44 staining in parietal cells are promising novel biomarkers for FSGS.

It is not well known if the presence and intensity of IgA, IgG co-deposition, and complement C3 deposition in glomeruli is able to contribute to the prognostic value of traditional histological features yet.[32,33] However, Kim et al[34] claimed that glomerular C3 deposition and the presence of terminal complement complex (C5b-9) were correlated with the severity of histological damage and independent risk factors for progression. This finding shows that complement activation plays a pathogenic role in IgAN. Supporting this, Caliskan et al[33] found that high levels of mesangial C3 immune staining had a better predictive value for renal outcome compared to proteinuria.

Previous work showed that IgA activates alternative pathway of complement and complement system can be triggered by lectin pathway in IgAN as well.[35] Espinosa et al[36] found that C4d staining was associated with the severity of histological damage and an independent risk factor for IgAN progression. C4d deposition without C1q staining shows lectin pathway activation[5] and this finding showed that complement activation may be involved in the pathogenesis of IgAN.[36]

Blood Markers

Circulating immune complex development due to IgG and/or IgA antiglycan autoantibodies against Gd-IgA1 plays a key role in the pathogenesis of IgAN.[8] Serum levels of Gd-IgA1 have been reported to be elevated in patients with IgAN rather than healthy individuals.[6,37,38] Thus, serum levels of Gd-IgA1 have been studied in previous studies to learn whether it is associated with clinical and histological parameters or not.[39] In a recent study, the serum Gd-IgA1 level was found to be correlated with eGFR at diagnosis, urine protein creatinine ratio, and histological parameters including MEST-C score.[39] On the contrary, Shimozato et al[40] reported that serum Gd-IgA1 levels were not associated with the severity of the disease.

The second hit of IgAN pathogenesis is the production of IgA or IgG autoantibodies against Gd-IgA1[5,6]; thus, the levels of these autoantibodies were studied to understand whether they might be used for the diagnosis of IgAN.[37,41] In recent studies on Japanese and French cohorts, Gd-IgA1-specific IgA and IgG autoantibodies were found to be elevated in patients with IgAN rather than healthy control populations.[37,41] Supporting these studies, Suzuki et al[42] were able to differentiate the patients with IgAN from healthy individuals and the other kidney disease controls by measuring anti-Gd-IgA1 levels. Berthoux et al[41] suggested that there was a correlation between levels of autoantibodies and the progression of IgAN as well. We assume that anti-Gd-IgA1 level is a promising biomarker that will be used for monitoring disease progression and response to therapy.[29]

Genetic Markers

Immunoglobulin A nephropathy is most common in Asians, moderately prevalent in Europeans, and rare in Africans.[43] It was reported that associated genetic variants vary between different ethnicities as well.[43] Genome-wide association studies (GWAS) showed that single-nucleotide polymorphisms (SNP)s in alternative complement pathway genes have importance in IgAN pathogenesis and prognosis.[44] Kiryluk et al[43] performed a GWAS that found 5 loci that are important contributors to IgAN and used them for the calculation of genetic risk score for IgAN. Among the 5 loci, Chr.1q32 (*CFHR3/R1* locus) encodes complement factor H (CFH), CFH-related protein 1 (CFHR1), CFHR2, and CFHR3.[43] The combined deletion of *CFHR1* and *CFHR3* genes reduced the risk of IgAN.[44] Another GWAS performed by Kiryluk et al[45] also showed 6 new genome-wide significant signals including known SNPs in the *HLA-DQB1* and alfa defensins.

MicroRNAs (miRs) are non-coding oligonucleotides that suppress gene expression and have a pathophysiological role in IgAN.[46] MicroRNAs were found to be associated with Gd-IgA1 levels,[47] glomerular inflammation, fibrosis,[48] and endocapillary hypercellularity.[49] In a recent study, upregulated microRNAs were shown by microarray analysis in peripheral blood mononuclear cells of IgAN patients. MicroRNA-148b was found to be associated with the glycosylation process of IgA1 by regulating the enzyme core 1 beta-1,3-galactosyltransferase 1. Its expression levels changed significantly between Caucasians and East Asians irrespective of the disease status.[50] These results suggest that miR-148b could be considered as a specific biomarker.

MEMBRANOUS NEPHROPATHY

Membranous nephropathy is the most common cause of adult-onset non-diabetic nephrotic syndrome.[51-54] Traditionally, diagnosis is based on the histologic pattern of kidney biopsy[52] including thickening of the glomerular basement membrane with spikes and holes, and podocyte effacement with subepithelial immunocomplex deposits consisting of autoantibodies, especially IgG4 targeting podocyte autoantigens.[51,52,55,56] The prognosis of MN is variable.[55] While one-third of patients with MN are followed with spontaneous complete remission, the other one-third of patients develop ESKD.[55] This dilemma should be solved with early and accurate prediction of prognosis to allow early treatment of high-risk patients and to avoid unnecessary exposure of therapy in low-risk patients. A delay in treatment may result in complications of the nephrotic syndrome such as thrombosis and infections as well.[57] Hence, we aimed to highlight the importance of the newer biomarkers used in the management of MN.

Clinical Markers

Nephrotic range proteinuria is seen in 70%-80% of patients with MN.[53] Patients with nephrotic syndrome at diagnosis of MN are related to a lack of complete remission on follow up.[51] Ghiggeri et al[51] showed that patients with the nephrotic syndrome had more worsening kidney function after antiproteinuric treatment rather than the patients with MN without nephrotic syndrome at diagnosis. Although patients with nephrotic syndrome have a poor prognosis, the level

of proteinuria did not help to discriminate between those patients with a poor prognosis or with a better outcome.[57] Proteinuria does not always reflect MN activity because a change in proteinuria may be seen after several months of a change in immunologic activity.[58] Proteinuria may reflect irreversible damage on podocytes without disease activity as well.[58] According to all these factors, the clinical course of idiopathic MN is predicted by using initial proteinuria levels,[59] and it is well known that patients with limited proteinuria have favorable prognosis.[57]

Toronto risk score, a prediction tool created by Cattran et al,[57] is calculated with 24-hour creatinine clearance at diagnosis, the slope of creatinine clearance over 6 months, and the level of proteinuria during 6 months period of maximum proteinuria and predicts MN prognosis with 90% accuracy.[53]

Histopathologic Markers

Traditional histopathologic staging of MN is not well correlated with the prognosis of MN.[60] Even though glomerular C4d staining may reflect disease activity,[61] there is a need for prognostic histological markers. Membranous nephropathy could be primary or secondary to malignancies, autoimmune diseases, drugs, and chronic infections in 20% of the patients with MN.[55,56] In primary MN, most patients have autoantibodies directed against podocyte antigens detected in plasma or kidney tissue.[55] Phospholipase A2 receptor 1 (PLA2R1), neural epidermal growth factor-like protein, and thrombospondin type 1 domain-containing 7A (THSD7A) are tissue markers that could be detected in the immune deposits on kidney biopsy specimens of MN patients by immunohistochemical and immunofluorescence staining.[52]

Although PLA2R1 staining is seen with primary MN more commonly, some cases such as sarcoidosis, hepatitis C virus infection, malignancies may also show PLA2R1 antibody staining on biopsy.[52] In some MN cases, serum anti PLA2R1 antibody may be falsely negative which is explained by the "kidney as a sink" phenomenon when antibodies bind to the antigen on podocytes. When antibody production exceeds the buffering capacity of the kidney, anti-PLA2R1 antibodies can be detectable in serum.[56] The inverse scenario is uncommon and suggests a technical artifact.[56] Recently, 2 new autoantigens were identified, exostosins 1 and 2 (EXT1-EXT2).[51,52] The main difference is that exostosins are more present in secondary MN such as class 5 lupus nephritis.[51,52] EXT1 and 2 are tissue markers that could be detected by immunohistochemical and immunofluorescence staining on kidney biopsy. There is limited data to recommend screening malignancies when biopsies show EXT1-2 staining.[52] Autoimmune diseases are common in this group of patients.[62] In a recent study, EXT 1 and 2 were detected in 21 of 224 patients with PLA2R1-negative MN but not in all 47 PLA2R1-associated MN. Tests in 7 patients with EXT1/EXT2-associated MN did not show any circulating antibody against exostosin.[62]

Blood Markers

Glomerular biomarkers contribute to the diagnostic and therapeutic approach of GN and permit clinicians to monitor disease activity and responsiveness to therapy although kidney biopsy is the gold standard for the diagnosis of GN.[52,56]

In MN, some biopsy findings can help to differentiate primary from secondary causes. These findings include histologic patterns such as intramembranous, mesangial deposits, and full house pattern, particularly in systemic lupus erythematosus, suggesting secondary causes.[52,56] Validated biomarkers are needed to be used because kidney biopsy is invasive, can not always differentiate between primary or secondary GNs, and has limitations to reflect disease activity.[52] Biomarker discovery in MN has started with the identification of circulating autoantibodies against PLA2R1 in 2009 that was found in 70% of patients with primary MN.[53] More recently, THSD7A was found in 1%-5% of patients with primary MN.[52] Autoantibodies against membrane-bound podocyte antigens aldose reductase, superoxide dismutase 2, and alfa enolase were also characterized.[51]

Recent studies showed anti-PLA2R1 antibodies are highly specific (%100) and sensitive (96.5%) to MN.[52,53,56] Anti-PLA2R1 autoantibody titers are correlated with proteinuria, chronic kidney disease outcomes, response to therapy, and relapses.[52,56,63] The absence of anti-PLA2R1 at the time of biopsy increases the risk of malignancy-related MN.[64] The anti-PLA2R1 antibody may be detected in patients with sarcoidosis and hepatitis B virus (HBV)-related MN with a high prevalence.[53] If eGFR is higher than 60% of baseline level and secondary causes of MN are excluded, a biopsy may not be necessary for PLA2R1-positive patients with MN.[52] Monitoring anti-PLA2R1 antibodies while therapy is the current recommended approach for evaluating the role of PLA2R1 during MN treatment course.[52] In addition, in a study, more immunosuppressive medication-related adverse effects were seen in patients with positive anti-PLA2R1 antibody MN rather than negative of those.[65] High titer of anti-PLA2R1 antibodies is found to be associated with epitope spreading that is thought to be related to the resistant disease.[52] Ghiggeri et al[51] showed that epitope spreaders had higher anti-PLA2R1 titers and were also more positive for intracellular autoantigens. Among anti-PLA2R1 antibody-positive patients, epitope spreading was found to be associated with a risk of lower GFR at diagnosis and 12 months after diagnosis as well.[51]

Neural epidermal growth factor-like 1 protein (NELL 1), autoantigen more present in primary MN, could be found in 16% of anti-PLA2R1-negative MN patients. When NELL 1 is found in serum or tissue, malignancies should be screened because the frequency of malignancy in NELL 1-positive cases is 33%.[66] In a recent study, NELL 1-positive cases of MN had unique histopathology with segmental to incomplete IgG capillary loop staining (93%) and dominant or co-dominant IgG1 subclass staining

(95.5%).[66] It is the second most common antigen in primary MN, but it is not commercially available in practice.[52]

Thrombospondin type 1 domain-containing 7A is the third most common antigen with a rate of 1%-5% of primary MN and 10% of PLA2R1-negative MN.[52] Serum THSD7A is highly specific and sensitive for MN,[67] as 100% and 92%, respectively.[52] In positive THSD7A cases, the rate of malignancies is as high as 6%-20%; hence, aggressive screening for malignancies should be done specifically for the urogenital and gastrointestinal system because THSD7A is expressed in human tumors as well.[52] After treatment for malignancies, THSD7A and proteinuria levels can reduce.[52] In contrast, some studies claim that there is no correlation between THSD7A and proteinuria levels.[53] Higher titers of anti-THSD7A antibodies correlate with a lower remission rate and higher disease activity.[52] Anti-THSD7A antibody is associated with recurrence after transplantation as well.[53] Serine protease HTRA1 is a novel antigen in idiopathic MN. Anti-HTRA1 antibodies can be detected in primary MN. In a recent study, high titers of anti-HTRA1 antibodies were found to be correlated with disease activity.[68] Recently, several antigens have been identified including contactin-1 (CNTN1). Anti-CNTN1 antibodies precipitate in both autoimmune neuropathy and MN.[69] The presence of CNTN1 protein and antibodies in both peripheral nerve and diseased glomeruli, as well as the temporal correlation of these disorders, is an evidence for a common antibody-mediated pathological process.

FOCAL SEGMENTAL GLOMERULOSCLEROSIS

Focal and segmental glomerulosclerosis (FSGS) is a podocytopathy[70] which is the cause of 40% nephrotic syndrome cases in adults and 20% in children.[71] Focal and segmental glomerulosclerosis is classified into 2 forms: primary or idiopathic FSGS and secondary FSGS which includes genetic, adaptive, medication-associated, infection, and inflammation-associated FSGS.[71] Primary FSGS is thought to be caused by a permeability factor that damages the podocytes.[71] A patient with primary FSGS usually presents with edema, severe hypoalbuminemia, and nephrotic-range proteinuria. Kidney biopsy specimens by electron microscopy show foot process effacement of podocytes.[72] It is not easy to differentiate primary FSGS from secondary forms and also from other glomerulopathies such as minimal change disease (MCD).[73] The biopsy obtains only a small portion of the kidney, and if the affected portion of the kidney is not sampled,[2] it is not always possible to diagnose FSGS because initially lesions are confined to a limited number of glomeruli and they are segmental.[72] Therefore, several studies have been conducted on biomarkers to distinguish between primary FSGS and all these entities that we discuss in this review.

Clinical Markers

Medical history and a careful examination are needed to differentiate primary (idiopathic) FSGS from secondary forms. The distinction is important because while the treatment of secondary forms of FSGS (except genetic forms) is the cure of underlying disease, primary FSGS requires an immunosuppressive regimen.[74] Therefore, many studies have been focused on the clinical biomarkers for FSGS.

Patients with FSGS always present with proteinuria. While patients with primary FSGS usually have nephrotic range proteinuria (>3.5 g/day/1.73 m^2) with severe hypoalbuminemia, the patients with secondary forms of FSGS present with either subnephrotic or nephrotic range proteinuria and do not develop complete nephrotic syndrome despite the presence of nephrotic range proteinuria. Kidney failure develops more in patients with primary FSGS than others.[75] Genetic forms of secondary FSGS are an exception that may have a very aggressive course resulting in ESKD and are associated with extrarenal findings.[76]

Histopathologic Markers

Focal and segmental glomerulosclerosis is a histological term that means segmental (in parts) and focal (of some) sclerosis of glomeruli. Thus, it is important to obtain representative biopsy specimen.[74] Focal and segmental glomerulosclerosis histological findings are classified into 5 subtypes according to the Columbia classification[77]: perihilar, tip, collapsing, cellular, and not otherwise specified.[78] Collapsing variant is associated with poor prognosis, whereas the patients with tip variant have the best outcome.[79] These biopsy findings detected by light microscopy[77] may not be seen in the early phase of FSGS, so electron microscopy should be done to diagnose. Diffuse foot process effacement (FPE) >80% of the analyzed podocitary surface may let us think that the disease is primary FSGS or MCD, whereas <80% FPE is most likely associated with secondary FSGS.[80]

Podocyte loss is the main feature of glomerulosclerosis. It has been suggested that activated parietal epithelial cells can transition to podocytes and thus can have a role in the pathogenesis of glomerulosclerosis. Increased expression of CD44 which is a marker for activated parietal epithelial cells[81] in biopsy specimen is correlated with sclerosis,[82] thus CD44-positive staining in the glomerular parietal cells is associated with FSGS.[83] Although, it still remains unclear whether parietal epithelial cell activation contributes to the pathogenesis of sclerosis in idiopathic FSGS or is a regenerative response to podocytes' injury.[81]

Blood Markers

It has been thought that a circulating permeability factor causes primary FSGS, and many studies have been focused on that.[74,84] One of the most promising permeability factor candidates is the soluble urokinase-type plasminogen activator receptor (suPAR).[85,86] In a study, suPAR levels were lower in healthy individuals than patients with glomerular diseases, whereas suPAR levels were not significantly different between the patients with FSGS and other glomerular disease controls.[84] As a high suPAR level has been suggested as a risk factor for the progression of chronic kidney disease, it is also related to poor outcomes

in patients with FSGS.[87] The role and importance of intact and cleaved forms of suPAR remain controversial.[84]

An additional circulating factor, anti-CD40 autoantibody, has been implicated in the pathogenesis of FSGS which is involved in immunity and inflammation.[88] Pretransplant serum anti-CD40 levels were associated with post-transplant FSGS recurrence with 78% accuracy.[89] Further studies are needed to confirm this result. As FSGS involves ongoing podocyte injury and death, mRNA profiling of cultured podocytes with plasma from patients with recurrent FSGS showed upregulated genes involved in podocyte injury. Three upregulated genes (*IL1β*, *BMF*, and *IGFBP3*) were selected and transfected into podocytes. When these podocytes were exposed to plasma from patients with recurrent FSGS, the increased specific response was seen contrary to those of non-recurrent FSGS. The assay diagnoses FSGS patients with high sensitivity and specificity.[90]

Urine Markers

Diagnostic protein biomarkers available in urine provide a non-invasive way for the diagnosis of FSGS.[91] The synthesis of ApoA-1, the main protein in high-density lipoprotein,[92] is increased with the severity of the nephrotic syndrome.[93] In a recent study, many patients with the diseases including tubulopathies, renal dysplasia/congenital anomalies of the kidney and urogenital tract, GN, and nephrotic syndrome in relapse, especially with FSGS, had increased urinary ApoA-1 isoforms.[92] In addition, urinary ApoA-1b, a high molecular weight form of ApoA-1, was found to be associated with the recurrence of FSGS with high specificity and sensitivity[94] although the role of ApoA-1b has not been well understood yet.

MEMBRANOPROLIFERATIVE GLOMERULONEPHRITIS

Membranoproliferative GN has been classified into 3 subgroups: type I, II, and III which are characterized by immune deposition in the subendothelial space and mesangium, C3 deposition within the mesangium and in the basement membrane, and a variant of type I, respectively.[95] In an expert meeting in 2012, MPGN was re-classified into 2 subgroups: immune complex-mediated MPGN (IC-MPGN) and complement-mediated GN.[96] In other words, type I and III MPGN were renamed as IC-MPGN and type II MPGN as complement-mediated GN, C3 glomerulopathy (C3G).[95] C3G was classified into 3 subgroups: dense deposit disease (DDD), C3 GN, and complement factor H-related protein 5 (CFHR5) nephropathy[97] which are related to abnormal activation of alternative complement pathway and abnormal C3 deposition in the glomeruli.[95,98] Assessment with a kidney biopsy, complement system components, and genetic tests have been focused on making an accurate diagnosis.[95]

C3 GLOMERULOPATHY

Histopathologic Markers

Diagnosis of the primary IC-MPGN and C3G depends on immunofluorescence staining on the kidney biopsy specimen.[99] C3G is divided into 3 major subgroups, (i) DDD is characterized by intramembranous dense osmophilic deposits, (ii) C3 GN could be differentiated from others with the C3 deposits in mesangial, subendothelial, and subepithelial areas of glomeruli, and (iii) CFHR5 glomerulopathies is caused by a genetic variant of *CFHR5* gene.[97]

C3 staining with the absence or low presence of immunoglobulins and classical complement pathway components is detected by the immunofluorescence on a kidney biopsy of the patients with C3G.[100] C3 has a major role in complement activity with its proteolytic cleavage first generating C3a and C3b, followed by inactivation of C3b generating iC3b (which includes C3α and C3β), which undergoes further breakdown yielding C3c and terminal breakdown fragment C3dg.[101] In a recent study, C3dg was detected as a major fragment of C3 in glomeruli.[101] However, there was no difference found on C3dg staining between DDD and C3 GN cases.[101] It is also noteworthy that routine immunofluorescence studies can miss the deposition of C3dg. Thus, the detection of C3 is limited.[101] It would be unclear whether C3dg is correlated with disease activity or not.

The factor H protein family includes 5 factor H-related proteins (FHR1-5), which are thought to act as positive regulators that promote complement activity and are named because of their structural similarity to factor H. A recent finding was a high prevalence of FHR5 in glomeruli with C3G.[102] Glomerular FHR5 staining intensity was positively correlated with disease severity[103] and worse kidney function.[99] Where FHR5 staining intensity was high, the presence of a membranoproliferative pattern and staining intensity of C3 activation products were also found high there.[102]

Genetic Markers

Abnormal control of complement activation as a result of acquired and genetic complement abnormalities[104] causes predominant C3 fragment deposition within the glomerulus and causes glomerular injury.[105] One of these mutations, *CFHR5* mutation, is associated with a familial form of C3G, known as the CFHR5 glomerulopathy[103] which is caused by an internal duplication of exons 2 and 3 of the *CFHR5* gene. In recent years, the effect of the mutations on the patients with the atypical hemolytic uremic syndrome, IgAN, and MPGN have been explored, but the pathophysiological role is still under debate.[106] The patients with *CFHR5* mutations present with macroscopic hematuria and kidney failure.[106] Therefore, the clinical course is occasionally confused with IgAN.[107] In a previous study, it was demonstrated that *CFHR5* mutations led to C3G by disrupting the homeostatic regulation of complement within the kidney.[108] Interestingly, in a study, low FHR5 levels and the presence of *CFHR5* variations were found in the patients with good renal outcome. Specific forms of FHR5 protein can be disease-modifying. However, the role of missense variations and frameshift mutations has still not been well known.[106] This pathway needs further evaluation to find out FHR5 targeting treatment on complement-mediated kidney injury.[108]

Table 1. Biomarkers in Primary Glomerular Diseases

Biomarker	Disease	Detected Sample
Autoantibodies targeting galactose-deficient IgA1	IgAN	Serum[41]
MiR-148b	IgAN	Genetic[50]
SNPs	IgAN	Genetic[45]
C3 deposition	IgAN	Kidney tissue[33]
C4d deposition	IgAN	Kidney tissue[36]
Phospholipase A2 receptor 1 (PLA2R)	MN	Serum[64]
Neural epidermal growth factor-like 1 protein (NELL-1)	MN	Serum[66]
Serine protease HTRA1	MN	Serum[68]
Contactin-1	MN	Serum[69]
Thrombospondin type 1 domain containing 7A (THSD7A)	MN	Serum[67]
Exostosin 1/exostosin 2 (EXT1/EXT2) staining	MN	Kidney tissue[62]
Antibodies against CD40	FSGS	Serum[89]
Podocyte gene expression-based assay	FSGS	Serum[90]
High molecular weight forms of ApoA-1	FSGS	Urine[92]
CD44 in parietal cells	FSGS	Kidney tissue[81]
FHR5 staining	C3G	Kidney tissue[103]
C3dg staining	C3G	Kidney tissue[101]
CFHR5 mutation	C3G	Genetic[103]

C3G, C3 glomerulopathy; CFHR5, complement factor 5-related peptide; IgAN, IgA nephropathy; FSGS, focal segmental glomerulosclerosis; MN, membranous nephropathy; SNP, single-nucleotide polymorphism.

CONCLUSION

This review aimed to identify current biomarkers associated with primary GN. Although kidney biopsy is still the gold standard for diagnosis, it is an invasive and potentially risky procedure that can not be routinely performed to guide the therapy closely. Therefore, the diagnoses and management of GN are based on blood, urine, and genetic markers as well as clinical and histological features. Reproducibility to get information on the severity of the disease, the likelihood of benefit of a given therapy, and to identify treatment responders provide insight into the underlying pathophysiology and guide the treatment. Overall, although there are promising biomarkers for GN, long-term evaluation of these biomarkers is still needed.

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REFERENCES

1. Ali AA, Sharif DA, Almukhtar SE, Abd KH, Saleem ZSM, Hughson MD. Incidence of glomerulonephritis and non-diabetic end-stage renal disease in a developing middle-east region near armed conflict. *BMC Nephrol*. 2018;19(1):257. [CrossRef]
2. Varghese SA, Powell TB, Budisavljevic MN, et al. Urine biomarkers predict the cause of glomerular disease. *J Am Soc Nephrol*. 2007;18(3):913-922. [CrossRef]
3. Caliskan Y, Kiryluk K. Novel biomarkers in glomerular disease. *Adv Chronic Kidney Dis*. 2014;21(2):205-216. [CrossRef]
4. Hassler JR. IgA nephropathy: a brief review. *Semin Diagn Pathol*. 2020;37(3):143-147. [CrossRef]
5. Selvaskandan H, Shi S, Twaij S, Cheung CK, Barratt J. Monitoring immune responses in IgA nephropathy: biomarkers to guide management. *Front Immunol*. 2020;11:572754. [CrossRef]
6. Kim JS, Hwang HS, Lee SH, et al. Clinical relevance of serum galactose deficient IgA1 in patients with IgA nephropathy. *J Clin Med*. 2020;9(11). [CrossRef]
7. Tomana M, Novak J, Julian BA, Matousovic K, Konecny K, Mestecky J. Circulating immune complexes in IgA nephropathy consist of IgA1 with galactose-deficient hinge region and antiglycan antibodies. *J Clin Invest*. 1999;104(1):73-81. [CrossRef]
8. Maixnerova D, Reily C, Bian Q, Neprasova M, Novak J, Tesar V. Markers for the progression of IgA nephropathy. *J Nephrol*. 2016;29(4):535-541. [CrossRef]

9. Tortajada A, Gutierrez E, Pickering MC, Praga Terente M, Medjeral-Thomas N. The role of complement in IgA nephropathy. *Mol Immunol*. 2019;114:123-132. [CrossRef]
10. Moriyama T. Clinical and histological features and therapeutic strategies for IgA nephropathy. *Clin Exp Nephrol*. 2019;23(9):1089-1099. [CrossRef]
11. Radford MG, Jr, Donadio JV, Jr, Bergstralh EJ, Grande JP. Predicting renal outcome in IgA nephropathy. *J Am Soc Nephrol*. 1997;8(2):199-207. [CrossRef]
12. Li PK, Ho KK, Szeto CC, Yu L, Lai FM. Prognostic indicators of IgA nephropathy in the Chinese--clinical and pathological perspectives. *Nephrol Dial Transplant*. 2002;17(1):64-69. [CrossRef]
13. Lv J, Zhang H, Zhou Y, Li G, Zou W, Wang H. Natural history of immunoglobulin A nephropathy and predictive factors of prognosis: a long-term follow up of 204 cases in China. *Nephrology*. 2008;13(3):242-246. [CrossRef]
14. Goto M, Wakai K, Kawamura T, Ando M, Endoh M, Tomino Y. A scoring system to predict renal outcome in IgA nephropathy: a nationwide 10-year prospective cohort study. *Nephrol Dial Transplant*. 2009;24(10):3068-3074. [CrossRef]
15. Berthoux F, Mohey H, Laurent B, Mariat C, Afiani A, Thibaudin L. Predicting the risk for dialysis or death in IgA nephropathy. *J Am Soc Nephrol*. 2011;22(4):752-761. [CrossRef]
16. Donadio JV, Bergstralh EJ, Grande JP, Rademcher DM. Proteinuria patterns and their association with subsequent end-stage renal disease in IgA nephropathy. *Nephrol Dial Transplant*. 2002;17(7):1197-1203. [CrossRef]
17. Reich HN, Troyanov S, Scholey JW, Cattran DC, Toronto Glomerulonephritis Registry. Remission of proteinuria improves prognosis in IgA nephropathy. *J Am Soc Nephrol*. 2007;18(12):3177-3183. [CrossRef]
18. Okabayashi Y, Tsuboi N, Haruhara K, et al. Reduction of proteinuria by therapeutic intervention improves the renal outcome of elderly patients with IgA nephropathy. *Clin Exp Nephrol*. 2016;20(6):910-917. [CrossRef]
19. Alamartine E, Sabatier JC, Guerin C, Berliet JM, Berthoux F. Prognostic factors in mesangial IgA glomerulonephritis: an extensive study with univariate and multivariate analyses. *Am J Kidney Dis*. 1991;18(1):12-19. [CrossRef]
20. Gorriz JL, Martinez-Castelao A. Proteinuria: detection and role in native renal disease progression. *Transplant Rev*. 2012;26(1):3-13. [CrossRef]
21. Cravedi P, Remuzzi G. Pathophysiology of proteinuria and its value as an outcome measure in chronic kidney disease. *Br J Clin Pharmacol*. 2013;76(4):516-523. [CrossRef]
22. Szeto CC, Lai FM, To KF, et al. The natural history of immunoglobulin A nephropathy among patients with hematuria and minimal proteinuria. *Am J Med*. 2001;110(6):434-437. [CrossRef]
23. Moriyama T, Tanaka K, Iwasaki C, et al. Prognosis in IgA nephropathy: 30-year analysis of 1,012 patients at a single center in Japan. *PLoS ONE*. 2014;9(3):e91756. [CrossRef]
24. Wakai K, Kawamura T, Endoh M, et al. A scoring system to predict renal outcome in IgA nephropathy: from a nationwide prospective study. *Nephrol Dial Transplant*. 2006;21(10):2800-2808. [CrossRef]
25. Lee H, Kim DK, Oh KH, et al. Mortality of IgA nephropathy patients: a single center experience over 30 years. *PLoS ONE*. 2012;7(12):e51225. [CrossRef]
26. Syrjänen J, Mustonen J, Pasternack A. Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant*. 2000;15(1):34-42. [CrossRef]
27. Shimamoto M, Ohsawa I, Suzuki H, et al. Impact of body mass index on progression of IgA nephropathy among Japanese patients. *J Clin Lab Anal*. 2015;29(5):353-360. [CrossRef]
28. Berger J. IgA glomerular deposits in renal disease. *Transplant Proc*. 1969;1(4):939-944.
29. Hastings MC, Moldoveanu Z, Suzuki H, et al. Biomarkers in IgA nephropathy: relationship to pathogenetic hits. *Expert Opin Med Diagn*. 2013;7(6):615-627. [CrossRef]
30. Trimarchi H, Barratt J, Cattran DC, et al. Oxford classification of IgA nephropathy 2016: an update from the IgA Nephropathy Classification Working Group. *Kidney Int*. 2017;91(5):1014-1021. [CrossRef]
31. Caliskan Y, Demir E, Karatay E, et al. Oxidative stress and macrophage infiltration in IgA nephropathy. *J Nephrol*. 2021. [CrossRef]
32. Becker JU, Mayerich D, Padmanabhan M, et al. Artificial intelligence and machine learning in nephropathology. *Kidney Int*. 2020;98(1):65-75. [CrossRef]
33. Caliskan Y, Ozluk Y, Celik D, et al. The clinical significance of uric acid and complement activation in the progression of IgA nephropathy. *Kidney Blood Press Res*. 2016;41(2):148-157. [CrossRef]
34. Kim SJ, Koo HM, Lim BJ, et al. Decreased circulating C3 levels and mesangial C3 deposition predict renal outcome in patients with IgA nephropathy. *PLoS ONE*. 2012;7(7):e40495. [CrossRef]
35. Roos A, Rastaldi MP, Calvaresi N, et al. Glomerular activation of the lectin pathway of complement in IgA nephropathy is associated with more severe renal disease. *J Am Soc Nephrol*. 2006;17(6):1724-1734. [CrossRef]
36. Espinosa M, Ortega R, Sánchez M, et al. Association of C4d deposition with clinical outcomes in IgA nephropathy. *Clin J Am Soc Nephrol*. 2014;9(5):897-904. [CrossRef]
37. Yanagawa H, Suzuki H, Suzuki Y, et al. A panel of serum biomarkers differentiates IgA nephropathy from other renal diseases. *PLoS ONE*. 2014;9(5):e98081. [CrossRef]
38. Zhang L, Wang Y, Shi X, Zou H, Jiang Y. A higher frequency of CD4(+) CXCR5(+) T follicular helper cells in patients with newly diagnosed IgA nephropathy. *Immunol Lett*. 2014;158(1-2):101-108. [CrossRef]
39. Bagchi S, Lingaiah R, Mani K, et al. Significance of serum galactose deficient IgA1 as a potential biomarker for IgA nephropathy: a case control study. *PLoS ONE*. 2019;14(3):e0214256. [CrossRef]
40. Shimozato S, Hiki Y, Odani H, Takahashi K, Yamamoto K, Sugiyama S. Serum under-galactosylated IgA1 is increased in Japanese patients with IgA nephropathy. *Nephrol Dial Transplant*. 2008;23(6):1931-1939. [CrossRef]
41. Berthoux F, Suzuki H, Thibaudin L, et al. Autoantibodies targeting galactose-deficient IgA1 associate with progression of IgA nephropathy. *J Am Soc Nephrol*. 2012;23(9):1579-1587. [CrossRef]
42. Suzuki H, Fan R, Zhang Z, et al. Aberrantly glycosylated IgA1 in IgA nephropathy patients is recognized by IgG antibodies with restricted heterogeneity. *J Clin Invest*. 2009;119(6):1668-1677. [CrossRef]
43. Kiryluk K, Li Y, Sanna-Cherchi S, et al. Geographic differences in genetic susceptibility to IgA nephropathy: GWAS replication study and geospatial risk analysis. *PLoS Genet*. 2012;8(6):e1002765. [CrossRef]
44. Gharavi AG, Kiryluk K, Choi M, et al. Genome-wide association study identifies susceptibility loci for IgA nephropathy. *Nat Genet*. 2011;43(4):321-327. [CrossRef]
45. Kiryluk K, Li Y, Scolari F, et al. Discovery of new risk loci for IgA nephropathy implicates genes involved in immunity against intestinal pathogens. *Nat Genet*. 2014;46(11):1187-1196. [CrossRef]

46. Selvaskandan H, Pawluczyk I, Barratt J. MicroRNAs: a new avenue to understand, investigate and treat immunoglobulin A nephropathy? *Clin Kidney J*. 2018;11(1):29-37. [CrossRef]
47. Serino G, Sallustio F, Cox SN, Pesce F, Schena FP. Abnormal miR-148b expression promotes aberrant glycosylation of IgA1 in IgA nephropathy. *J Am Soc Nephrol*. 2012;23(5):814-824. [CrossRef]
48. Szeto CC, Ching-Ha KB, Ka-Bik L, et al. Micro-RNA expression in the urinary sediment of patients with chronic kidney diseases. *Dis Markers*. 2012;33(3):137-144. [CrossRef]
49. Bao H, Chen H, Zhu X, et al. MiR-223 downregulation promotes glomerular endothelial cell activation by upregulating importin alpha4 and alpha5 in IgA nephropathy. *Kidney Int*. 2014;85(3):624-635. [CrossRef]
50. Serino G, Pesce F, Sallustio F, et al. In a retrospective international study, circulating miR-148b and let-7b were found to be serum markers for detecting primary IgA nephropathy. *Kidney Int*. 2016;89(3):683-692. [CrossRef]
51. Ghiggeri GM, Seitz-Polski B, Justino J, et al. Multi-autoantibody signature and clinical outcome in membranous nephropathy. *Clin J Am Soc Nephrol*. 2020;15(12):1762-1776. [CrossRef]
52. Cavanaugh C, Okusa MD. The evolving role of novel biomarkers in glomerular disease: a review. *Am J Kidney Dis*. 2021;77(1):122-131. [CrossRef]
53. Maifata SM, Hod R, Zakaria F, Abd Ghani F. Primary membranous glomerulonephritis: the role of serum and urine biomarkers in patient management. *Biomedicines*. 2019;7(4). [CrossRef]
54. Salant DJ. Genetic variants in membranous nephropathy: perhaps a perfect storm rather than a straightforward conformeropathy? *J Am Soc Nephrol*. 2013;24(4):525-528. [CrossRef]
55. Rodriguez EF, Nasr SH, Larsen CP, Sethi S, Fidler ME, Cornell LD. Membranous nephropathy with crescents: a series of 19 cases. *Am J Kidney Dis*. 2014;64(1):66-73. [CrossRef]
56. De Vriese AS, Glassock RJ, Nath KA, Sethi S, Fervenza FC. A proposal for a serology-based approach to membranous nephropathy. *J Am Soc Nephrol*. 2017;28(2):421-430. [CrossRef]
57. van den Brand JA, Hofstra JM, Wetzels JF. Prognostic value of risk score and urinary markers in idiopathic membranous nephropathy. *Clin J Am Soc Nephrol*. 2012;7(8):1242-1248. [CrossRef]
58. Parker SE. Use and abuse of volatile substances in industry. *Hum Toxicol*. 1989;8(4):271-275. [CrossRef]
59. An C, Akankwasa G, Liu J, et al. Urine markers of renal tubular injury in idiopathic membranous nephropathy: a cross sectional study. *Clin Chim Acta*. 2019;492:7-11. [CrossRef]
60. Sprangers B, Bomback AS, Cohen SD, et al. Idiopathic membranous nephropathy: clinical and histologic prognostic features and treatment patterns over time at a tertiary referral center. *Am J Nephrol*. 2012;36(1):78-89. [CrossRef]
61. Val-Bernal JF, Garijo MF, Val D, Rodrigo E, Arias M. C4d as a diagnostic tool in membranous nephropathy. *Nefrologia*. 2012;32(4):536; author reply 537. [CrossRef]
62. Sethi S, Madden BJ, Debiec H, et al. Exostosin 1/exostosin 2-associated membranous nephropathy. *J Am Soc Nephrol*. 2019;30(6):1123-1136. [CrossRef]
63. Mahmud M, Pinnschmidt HO, Reinhard L, et al. Role of phospholipase A2 receptor 1 antibody level at diagnosis for long-term renal outcome in membranous nephropathy. *PLoS ONE*. 2019;14(9):e0221293. [CrossRef]
64. Timmermans SA, Ayalon R, van Paassen P, et al. Anti-phospholipase A2 receptor antibodies and malignancy in membranous nephropathy. *Am J Kidney Dis*. 2013;62(6):1223-1225. [CrossRef]
65. Hoxha E, Harendza S, Pinnschmidt H, Panzer U, Stahl RA. PLA2R antibody levels and clinical outcome in patients with membranous nephropathy and non-nephrotic range proteinuria under treatment with inhibitors of the renin-angiotensin system. *PLoS ONE*. 2014;9(10):e110681. [CrossRef]
66. Caza TN, Hassen SI, Dvanajscak Z, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. *Kidney Int*. 2021;99(4):967-976. [CrossRef]
67. Sharma SG, Larsen CP. Tissue staining for THSD7A in glomeruli correlates with serum antibodies in primary membranous nephropathy: a clinicopathological study. *Mod Pathol*. 2018;31(4):616-622. [CrossRef]
68. Al-Rabadi LF, Caza T, Trivin-Avillach C, et al. Serine protease HTRA1 as a novel target antigen in primary membranous nephropathy. *J Am Soc Nephrol*. 2021;32(7):1666-1681. [CrossRef]
69. Le Quintrec M, Teisseyre M, Bec N, et al. Contactin-1 is a novel target antigen in membranous nephropathy associated with chronic inflammatory demyelinating polyneuropathy. *Kidney Int*. 2021;100(6):1240-1249. [CrossRef]
70. D'Agati VD, Kaskel FJ, Falk RJ. Focal segmental glomerulosclerosis. *N Engl J Med*. 2011;365(25):2398-2411. [CrossRef]
71. Rosenberg AZ, Kopp JB. Focal segmental glomerulosclerosis. *Clin J Am Soc Nephrol*. 2017;12(3):502-517. [CrossRef]
72. Sprangers B, Meijers B, Appel G. FSGS: diagnosis and diagnostic work-up. *BioMed Res Int*. 2016;2016:4632768. [CrossRef]
73. Maas RJ, Deegens JK, Smeets B, Moeller MJ, Wetzels JF. Minimal change disease and idiopathic FSGS: manifestations of the same disease. *Nat Rev Nephrol*. 2016;12(12):768-776. [CrossRef]
74. Jacobs-Cachá C, Vergara A, García-Carro C, et al. Challenges in primary focal segmental glomerulosclerosis diagnosis: from the diagnostic algorithm to novel biomarkers. *Clin Kidney J*. 2021;14(2):482-491. [CrossRef]
75. Praga M, Morales E, Herrero JC, et al. Absence of hypoalbuminemia despite massive proteinuria in focal segmental glomerulosclerosis secondary to hyperfiltration. *Am J Kidney Dis*. 1999;33(1):52-58. [CrossRef]
76. Trautmann A, Schnaidt S, Lipska-Ziętkiewicz BS, et al. Long-term outcome of steroid-resistant nephrotic syndrome in children. *J Am Soc Nephrol*. 2017;28(10):3055-3065. [CrossRef]
77. D'Agati VD, Fogo AB, Bruijn JA, Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am J Kidney Dis*. 2004;43(2):368-382. [CrossRef]
78. Gipson DS, Trachtman H, Kaskel FJ, et al. Clinical trial of focal segmental glomerulosclerosis in children and young adults. *Kidney Int*. 2011;80(8):868-878. [CrossRef]
79. Thomas DB, Franceschini N, Hogan SL, et al. Clinical and pathologic characteristics of focal segmental glomerulosclerosis pathologic variants. *Kidney Int*. 2006;69(5):920-926. [CrossRef]
80. Sethi S, Zand L, Nasr SH, Glassock RJ, Fervenza FC. Focal and segmental glomerulosclerosis: clinical and kidney biopsy correlations. *Clin Kidney J*. 2014;7(6):531-537. [CrossRef]
81. Fatima H, Moeller MJ, Smeets B, et al. Parietal epithelial cell activation marker in early recurrence of FSGS in the transplant. *Clin J Am Soc Nephrol*. 2012;7(11):1852-1858. [CrossRef]
82. Zhang J, Pippin JW, Krofft RD, Naito S, Liu ZH, Shankland SJ. Podocyte repopulation by renal progenitor cells following glucocorticoids treatment in experimental FSGS. *Am J Physiol Ren Physiol*. 2013;304(11):F1375-F1389. [CrossRef]
83. Froes BP, de Almeida Araújo S, Bambirra EA, Oliveira EA, Simões E Silva AC, Pinheiro SVB. Is CD44 in glomerular parietal epithelial

cells a pathological marker of renal function deterioration in primary focal segmental glomerulosclerosis? *Pediatr Nephrol*. 2017;32(11):2165-2169. [CrossRef]
84. Harel E, Shoji J, Abraham V, et al. Identifying a potential biomarker for primary focal segmental glomerulosclerosis and its association with recurrence after transplantation. *Clin Transplant*. 2019;33(3):e13487. [CrossRef]
85. Wei C, Trachtman H, Li J, et al. Circulating suPAR in two cohorts of primary FSGS. *J Am Soc Nephrol*. 2012;23(12):2051-2059. [CrossRef]
86. Wei C, El Hindi S, Li J, et al. Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis. *Nat Med*. 2011;17(8):952-960. [CrossRef]
87. Hayek SS, Sever S, Ko YA, et al. Soluble urokinase receptor and chronic kidney disease. *N Engl J Med*. 2015;373(20):1916-1925. [CrossRef]
88. Peev V, Hahm E, Reiser J. Unwinding focal segmental glomerulosclerosis. *F1000Res*. 2017;6:466. [CrossRef]
89. Delville M, Sigdel TK, Wei C, et al. A circulating antibody panel for pretransplant prediction of FSGS recurrence after kidney transplantation. *Sci Transl Med*. 2014;6(256):256ra136. [CrossRef]
90. Srivastava P, Solanki AK, Arif E, et al. Development of a novel cell-based assay to diagnose recurrent focal segmental glomerulosclerosis patients. *Kidney Int*. 2019;95(3):708-716. [CrossRef]
91. Nafar M, Kalantari S, Samavat S, Rezaei-Tavirani M, Rutishuser D, Zubarev RA. The novel diagnostic biomarkers for focal segmental glomerulosclerosis. *Int J Nephrol*. 2014;2014:574261. [CrossRef]
92. Clark AJ, Jabs K, Hunley TE, et al. Urinary apolipoprotein AI in children with kidney disease. *Pediatr Nephrol*. 2019;34(11):2351-2360. [CrossRef]
93. Kaysen GA, de Sain-van der Velden MG. New insights into lipid metabolism in the nephrotic syndrome. *Kidney Int Suppl*. 1999;71:S18-S21. [CrossRef]
94. Lopez-Hellin J, Cantarell C, Jimeno L, et al. A form of apolipoprotein a-I is found specifically in relapses of focal segmental glomerulosclerosis following transplantation. *Am J Transplant*. 2013;13(2):493-500. [CrossRef]
95. Schena FP, Esposito P, Rossini M. A narrative review on C3 glomerulopathy: a rare renal disease. *Int J Mol Sci*. 2020;21(2). [CrossRef]
96. Pickering MC, D'Agati VD, Nester CM, et al. C3 glomerulopathy: consensus report. *Kidney Int*. 2013;84(6):1079-1089. [CrossRef]
97. Cook HT, Pickering MC. Clusters not classifications: making sense of complement-mediated kidney injury. *J Am Soc Nephrol*. 2018;29(1):9-12. [CrossRef]
98. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement system part II: role in immunity. *Front Immunol*. 2015;6:257. [CrossRef]
99. Medjeral-Thomas NR, Pickering MC, Cook HT. Complement and kidney disease, new insights. *Curr Opin Nephrol Hypertens*. 2021;30(3):310-316. [CrossRef]
100. Fakhouri F, Frémeaux-Bacchi V, Noël LH, Cook HT, Pickering MC. C3 glomerulopathy: a new classification. *Nat Rev Nephrol*. 2010;6(8):494-499. [CrossRef]
101. Sethi S, Vrana JA, Fervenza FC, et al. Characterization of C3 in C3 glomerulopathy. *Nephrol Dial Transplant*. 2017;32(3):459-465. [CrossRef]
102. D'Agati VD, Bomback AS. In search of C3G tissue biomarkers. *Kidney Int Rep*. 2019;4(10):1359-1361. [CrossRef]
103. Medjeral-Thomas NR, Moffitt H, Lomax-Browne HJ, et al. Glomerular complement factor H-related protein 5 (FHR5) is highly prevalent in C3 glomerulopathy and associated with renal impairment. *Kidney Int Rep*. 2019;4(10):1387-1400. [CrossRef]
104. Servais A, Noël LH, Roumenina LT, et al. Acquired and genetic complement abnormalities play a critical role in dense deposit disease and other C3 glomerulopathies. *Kidney Int*. 2012;82(4):454-464. [CrossRef]
105. Dalili N, Behnam B, Vali F, et al. C3 glomerulonephritis with multiple mutations in complement factor H. *Iran J Kidney Dis*. 2018;12(6):376-381.
106. Garam N, Cserhalmi M, Prohászka Z, et al. FHR-5 serum levels and CFHR5 genetic variations in patients with immune complex-mediated membranoproliferative glomerulonephritis and C3-glomerulopathy. *Front Immunol*. 2021;12:720183. [CrossRef]
107. Athanasiou Y, Voskarides K, Gale DP, et al. Familial C3 glomerulopathy associated with CFHR5 mutations: clinical characteristics of 91 patients in 16 pedigrees. *Clin J Am Soc Nephrol*. 2011;6(6):1436-1446. [CrossRef]
108. Malik TH, Gitterman DP, Lavin DP, et al. Gain-of-function factor H-related 5 protein impairs glomerular complement regulation resulting in kidney damage. *Proc Natl Acad Sci USA*. 2021;118(13). [CrossRef]