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Diagnostic Immunostaining of Renal Biopsies: An Overview of Markers for Glomerular Diseases

Vinita Agrawal[a] Alok Sharma[b]

[a]Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India; [b]Renal Pathology and Transmission Electron Microscopy, DrLal PathLabs Ltd., New Delhi, India

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Abstract
Background: The analysis of a renal biopsy is made complex by multifactorial etiologies involving different renal compartments. Recent proteomic data, pattern-based classification, and a better understanding of various glomerular renal diseases have underscored the importance of immunohistology as an integral part of the diagnostic evaluation of renal biopsies. These include immunofluorescence on formalin-fixed paraffin-embedded renal tissue (IF-P), IgG subclass staining, typing of amyloid, and other organized deposits, classification of membranous nephropathy, etc. ***Summary:*** We describe the recent immunohistological markers on immunofluorescence (IF) and immunohistochemistry (IHC) on fresh and formalin-fixed paraffin-embedded renal native biopsies for proper evaluation and classification of glomerular diseases. The article also provides information on the diagnostic utility, interpretation, and established antibody clones described in the literature for various glomerular diseases. The indications of IF-P in renal biopsies are also outlined. ***Key Messages:*** Immunohistology has become integral to diagnosing and classifying various glomerular renal diseases. A specific protein or antigen-based classification has prognostic and therapeutic implications. Additionally, it provides clue for screening the patient for an underlying etiology.

Introduction

Renal biopsy interpretation is complex, requiring recognition of patterns of injury involving the different compartments, namely, glomerular, tubular, interstitial, and vascular. Understanding injury patterns may vary with age and type of biopsies-native or transplant. The analysis is made complex by multifactorial etiologies simultaneously involving different renal compartments. Further, it necessitates a multidisciplinary approach, integrating features of light microscopy, immunofluorescence (IF), immunohistochemistry (IHC), and electron microscopy. Light microscopy of renal biopsy requires evaluation by histochemical stains, namely, hematoxylin and eosin, periodic acid-Schiff (PAS), trichrome, and silver methanamine. Other stains, e.g., Congo red for amyloid and elastin, fibrin, and collagen, are often required for diagnostic evaluation.

IHC and IF workup of renal biopsies requires a panel of immunological tissue markers. Recently, there has

karger@karger.com
www.karger.com/gdz

Correspondence to:
Vinita Agrawal, vinita@sgpgi.ac.in; vinita.agrawal15@gmail.com

been a paradigm shift in understanding diagnostic categories in renal pathology with the importance of pattern recognition. Various new markers are described for proper evaluation and algorithmic diagnosis of renal biopsies. This is a review of the recent markers for diagnostic evaluation and classification of glomerular diseases in renal native biopsies, emphasizing their interpretation and diagnostic utility.

Immunochemical Techniques

Tissue immunostaining uses monoclonal antibodies (MAbs) or polyclonal antibodies (PAbs) targeted against an antigen of interest. MAbs are derived from a single-cell clone, thus exhibiting remarkable specificity by binding to a specific epitope on the target antigen. In contrast, PAbs arise from multiple B-cell clones and, therefore, can recognize multiple epitopes, resulting in lower specificity but higher sensitivity for detecting the target antigen. The decision to choose a monoclonal or polyclonal antibody depends on various factors such as the breadth of epitope recognition of the target antigen, cross-reactivity, cost, and availability, etc.

Immunofluorescence on frozen sections (IF-F) or immunohistochemistry on formalin-fixed paraffin-embedded (FFPE) kidney biopsies for immunoglobulins (IgG, IgM, IgA), immunoglobulin light chains (kappa, lambda), complements (C3, C1q), and fibrin/fibrinogen is routinely performed in most nephropathology laboratories for evaluation of all renal biopsies. Some laboratories do albumin staining as a routine or as and when required. Tubular casts are an internal control for IgA, kappa, and lambda. The blood vessel wall is an internal control for C3. External controls for all markers should be included in every batch of staining.

The renal biopsy for IF-F is received in normal saline, transport medium (Michel/Zeus), or saline wet gauze, depending on the transport time. It is embedded in a cryomedium (OCT-optimum cutting temperature compound), and 3–5 μm sections are cut at minus (–) 15–20°C on a cryo-microtome. Direct IF using fluorescent-tagged primary antibodies is performed. Indirect IF uses an additional step of fluorescent-tagged secondary antibody.

Immune deposits can also be identified using IHC or immunofluorescence on FFPE renal tissue (IF-P). In IF-P techniques, enzyme digestion of paraffin tissue sections by proteinase K, trypsin, or pronase enzymes is required for antigen retrieval.

Masked deposits that are not detected on IF-F can be identified by IF-P (shown in Fig. 1) [1–3]. High concordance has been reported for IF-F and IF-P, especially for immunoglobulins [1]. Some centers perform IF-P routinely, thus avoiding the need of an additional biopsy for IF-F and an additional advantage of good correlation with light microscopy. Messias et al. [4] reported their experience with IF-P in native kidney biopsies over 9 months. Of the 4,969 renal biopsies, IF-P was performed as a salvage technique or to evaluate masked deposits in 207 (4%) and 97 (2%) biopsies, respectively. As a salvage technique, it was necessary to diagnose 24 (11.6%) and significantly contributed to the diagnosis in 63 (30.4%) renal native biopsies. Out of 97 cases evaluated for unmasking of immunoglobulins or light chains, IF-P was useful or had a significant contribution in identifying masked deposits in 22 cases (23%).

However, IF-P has a low sensitivity for detecting immunoglobulins in anti-GBM disease, idiopathic/primary MN, and IgA nephropathy [5]. Table 1 mentions the indications and limitations of IF-P in routine nephropathology practice.

IF staining on FFPE renal tissue sections using dual microwave retrieval (IF-DMP) is another technique that has shown comparable specificity and sensitivity with IF-F in detecting immunoglobulins, complements, kappa, and lambda light chains [12]. The overall inter-observer agreement is numerically higher for IF-F than for IF-P or IHC for the routinely used immunoglobulins and complement components in renal biopsies.

However, IHC is a method of choice for the newer markers available for various glomerular diseases. IHC is done on FFPE tissues after enzyme digestion/heat-induced antigen retrieval, usually using DAB (di-amino benzidine) as a chromogen [13].

Complements and Complement Split Products

Direct IF usually performed by IF-F or IHC on FFPE for complements C3 and C1q is routine for all renal biopsies. C3 is detected usually using an antibody directed against its proteolytic fragment C3c. Blood vessels act as an internal control for C3. C3 deposits are due to the activation of any of the three pathways of complement activation – classical, alternate, or lectin. C1q deposits are usually associated with the activation of the classical pathway. Analysis of the pattern, location, and intensity of staining is vital for diagnosis.

Complement activation by one or more complement activation pathways may cause or be a secondary driver of the progression of primary glomerular diseases [22]. Immune complex-mediated glomerulonephritis (GN)

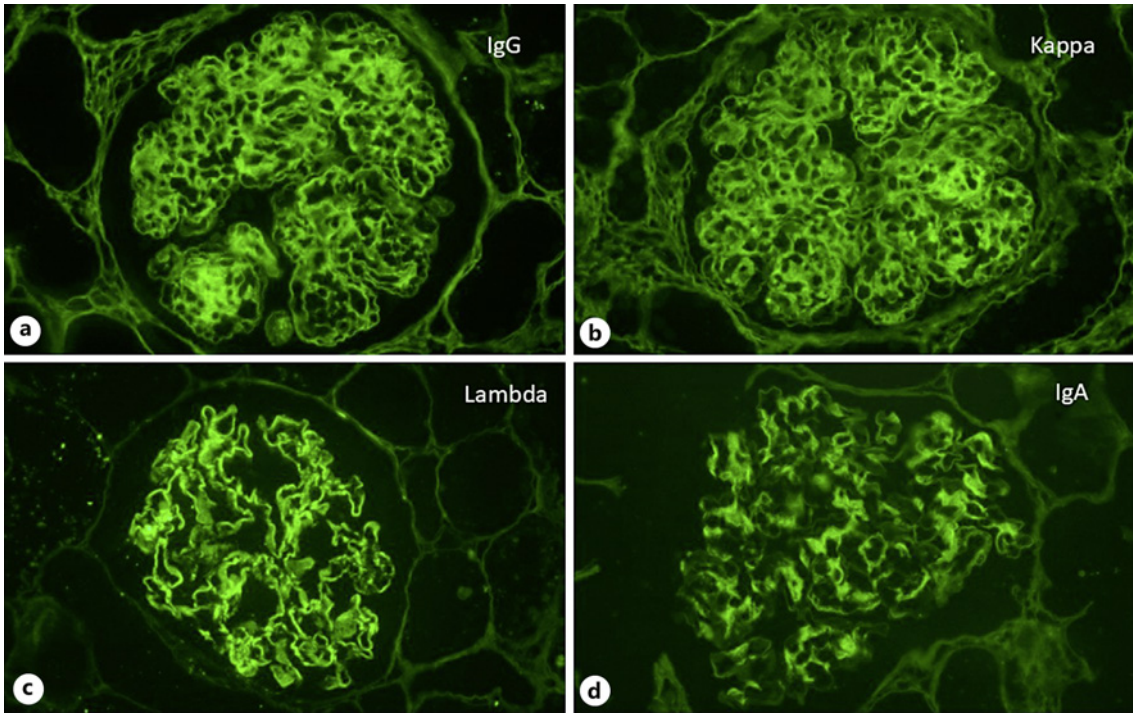

Fig. 1. Paraffin immunofluorescence (IF): 3+ granular capillary wall and mesangial IgG (**a**) and kappa (**b**) deposits in a renal biopsy of membranoproliferative glomerulonephritis (MPGN) with masked kappa deposits, lambda was negative (not shown). **c** Positive control of lambda. **d** IgA mesangial granular deposits (3+) in a formalin-fixed paraffin-embedded renal biopsy with no glomeruli in the fresh tissue sent for IF.

Table 1. Indications of immunofluorescence on formalin-fixed paraffin-embedded renal tissue (IF-P)

Indications	Masked deposits	Reference
Salvage technique		
Monoclonal deposits		
Crystallized deposits		
Light chain proximal tubulopathy	Usually kappa (noncrystalline are usually lambda)	[1]
Light chain crystalline podocytopathy	Kappa	[6]
Crystalglobulin-induced nephropathy	IgG, kappa	[7]
Non-organized deposits		
Membranous-like glomerulopathy with masked IgG kappa deposits (MGMID)	IgG, kappa	[8, 9]
MPGN with masked monotypic Ig deposits	IgG>IgM, kappa>lambda	[2]
MN with masked polyclonal IgG deposits	IgG	[10]
Microtubular deposits (monoclonal/polyclonal)		
Immunotactoid glomerulopathy (in ~15%)	IgG, kappa>lambda	[11]

shows immunoglobulins on IF, along with variable C3. In complement-mediated GN, e.g., C3 glomerulopathy, there is dominant, intense staining for C3, at least two grades higher than immunoglobulins or other immune reactants. It primarily results from alternative complement pathway activation.

Glomerular pathology associated with complement activation by the classical or lectin pathway leads to the

Table 2. Predominant IgG subclass staining on renal biopsies in glomerular diseases[a]

IgG subclass	Glomerular pathology	Reference
IgG1	Ext1/2-associated MLN (also IgG2 and IgG3), NELL-1-associated MN, SEMA3B-associated MN, Immunotactoid GP, IgAN	[11, 14–18]
IgG2	With IgG1 (mentioned above) or in monoclonal deposits (mentioned below)	
IgG3	PGNMID (kappa more common) (IgG1 or IgG2 in some)	[19]
IgG4	PLA2R-associated MN, THSD7A-associated MN, [b]Protocadherin FAT1-associated MN, HTRA1-associated MN	[15, 20, 21]

GP, glomerulopathy; MLN, membranous lupus nephritis; MN, membranous nephropathy; PGNMID, proliferative glomerulonephritis with monoclonal immunoglobulin deposits. [a]There may be weak/moderate/co-dominant expression of more than one IgG subclass in polyclonal deposits. [b]Usually lacks complement deposits.

deposition of a split product of C4 activation, C4d, in renal biopsies. The presence of both C3 and C4d and the absence of C1q is usually an indicator of lectin pathway involvement.

C4d is a degradation product of complement C4 due to activation of the classic and/or the lectin complement pathway. C4d along with C1q and immunoglobulin deposition suggests activation of the classical pathway [23]. C4d staining can be performed on native renal biopsies by indirect IF-F using monoclonal antibody or by IHC using polyclonal antibody on FFPE tissue. Sethi et al. studied the glomerular C4d staining in proliferative GN [24]. They found that it is a marker for immune complex-mediated proliferative GN but is absent or minimally detected in C3 glomerulopathy [24]. However, Bouatou et al. [13] reported that C4d is limited in discriminating immune complex GN from C3 glomerulopathy. Though C4d has limited diagnostic utility, it may help identify patients with a worse renal prognosis in some renal diseases and may guide treatment decisions [23].

C3d, a final degradation product of C3, is a more stable product of both the classic and the alternative complement pathway. Glomerular C3d deposition may be useful for diagnostic and prognostic evaluation of certain glomerular diseases [25, 26].

IgG Subclass Analysis

IgG has four subclasses, named in order of decreasing abundance in serum: IgG1, IgG2, IgG3, and IgG4. These subclasses share more than 90% of the amino acid sequence, with significant variations in the hinge region and N-terminal CH2 domain responsible for differences with respect to half-life, antigen binding, immune complex formation, complement activation, and triggering of effector cells [27]. IgG subclass analysis has diagnostic importance in various glomerular diseases.

Most idiopathic/primary MN patients have IgG4 antibodies to the phospholipase A2 receptor (PLA2R), which co-localize with PLA2R. Most primary MN shows IgG1/IgG4-codominant or IgG4-dominant staining (Fig. 2). Huang et al. [28] reported that in the early stage (stage 1) of primary MN, IgG1 is the dominant IgG subclass, while in later stages, IgG4 dominates. IgG4-dominant/-codominant subclass staining, but absent PLA2R staining may denote primary MN due to autoantibodies targeting an alternate podocyte antigen.

IgG1, IgG2, and IgG3 are expressed in secondary MN, with IgG1 dominance in most renal biopsies [28]. Both mesangial and proliferative lupus nephritis show IgG1, IgG2, and IgG3 staining with much less frequent and weak IgG4 staining (shown in Fig. 2). Similarly, in proliferative GN with polyclonal IgG deposition, IgG1 dominance/codominance with concomitant IgG3 and IgG2 but weak or absent IgG4 staining favors an underlying autoimmune disease [14]. Hemminger et al. [14] reported IgG1-predominant IF staining and frequent IgG3 in infection-associated GN. IgG2 staining was occasionally present with infrequent IgG4.

IgG subclass staining may help distinguish IgA nephropathy with concurrent IgG staining from lupus nephritis. Lupus nephritis shows IgG1, IgG2, and IgG3 staining, particularly with C1q staining, whereas IgA nephropathy usually shows only IgG1 staining without C1q staining [14].

Proliferative GN with monoclonal IgG deposits shows monotypic IgG3 with less frequent IgG1 and rarely IgG2 [19]. IgG subclass and kappa/lambda light chain staining are required to differentiate PGNMID, which shows

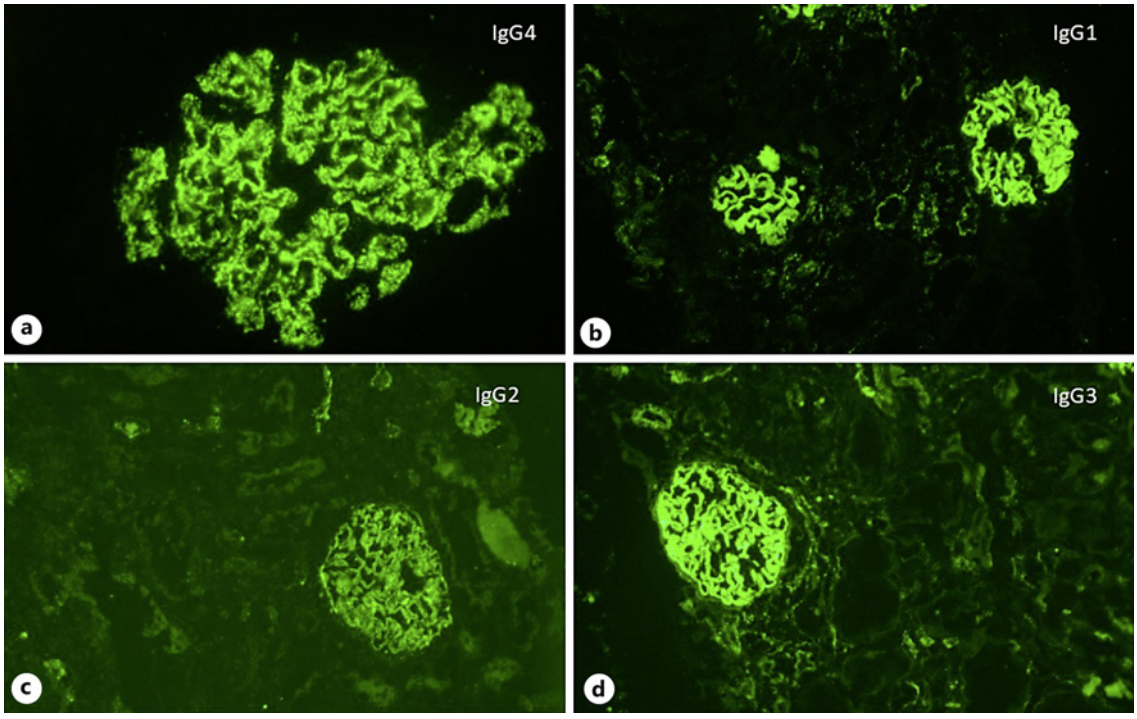

Fig. 2. IgG subclass staining: granular capillary wall (3+) IgG4 (**a**) in renal biopsy of PLA2R-associated membranous nephropathy; granular capillary wall and mesangial (2–3+) IgG1 (**b**), IgG2 (**c**), and IgG3 (**d**) in renal biopsy of lupus nephritis.

monoclonal monotypic IgG staining (usually kappa), from other proliferative GN, which shows polyclonal IgG deposits. Heavy chain and heavy and light chain deposition diseases also show monotypic IgG subclass staining. However, unlike PGNMID, heavy chain deposition disease will lack light chain staining, and both heavy chain and heavy and light chain deposition are associated with tubular basement membrane and vascular deposits [14]. Table 2 shows the predominant IgG subclasses in various glomerular conditions. Table 3 shows the immunostains useful for the diagnostic categorization and classification of glomerular diseases and the common sources of antibodies from the published literature.

MN-Associated Antigens

The antibodies, staining methods, staining patterns, and cited clones that help characterize MN in renal biopsies are enumerated in Table 3. If available, mass spectrometry and an algorithmic approach are recommended for immunostaining for multiple antigens of MN on a renal biopsy [15, 29]. Some of the commonly demonstrated antigens are described below.

Phospholipase A2 Receptor (PLA2R)

Phospholipase A2 receptor (PLA2R) is a transmembrane receptor that belongs to the mannose-receptor family. It is expressed in the glomerular podocyte's cytoplasm and plasma membrane [65].

Historically, MN was classified as primary (idiopathic) or secondary. Secondary MN has an underlying causal disease process or condition. In 2009, Beck et al. [52] discovered that PLA2R1 is the major antigen involved in the pathogenesis of most adult primary MN (70%) with circulating autoantibodies (mainly of the IgG4 subclass) to PLA2R.

Though PLA2R localizes to the cell body and processes of the normal podocyte, the antigen is detected in the subepithelial deposits in MN, where it co-localizes with IgG (shown in Fig. 3a). Studies show that demonstrating PLA2R antigen in the immune deposits on tissue staining is more sensitive than PLA2R antibody in the serum [66]. Therefore, the absence of circulating PLA2R autoantibody during kidney biopsy does not rule out a diagnosis of PLA2R-related MN. Combining biopsy antigen testing with serum antibody testing raises the assay's negative predictive value [67, 68].

PLA2R antigen can be detected on a renal biopsy using anti-PLA2R1 antibody by any of the two methods:

Table 3. Immunohistological markers for diagnostic categorization of glomerular diseases, their staining methods, controls, and interpretation in native renal biopsies

S.No.	Indication	Marker	Common staining method	Staining pattern	Interpretation	Sources	Positive controls	Reference
						primary antibody		
1	Amyloid	SAP (serum amyloid protein)	IHC	Extracellular deposits	Amyloid protein	Multiple	Known positive	[8]
		SAA (serum amyloid A)	IHC	Extracellular deposits	AA amyloid	Multiple	Hepatocyte (cytoplasm)	
		Lambda/kappa light chains	IHC	Extracellular deposits	AL amyloid	Multiple	Renal tubular casts	
		LECT2 (leukocyte chemotactic factor 2)	IHC	Extracellular deposits (commonly interstitial)	ALECT amyloid	R&D systems (MAb); Vector Labs (MAb)	Hepatocyte, Leydig cells (cytoplasm)	[30–33]
		Gelsolin (Familial Amyloidosis of Finnish type [FAF])	IHC	Extracellular deposits (commonly glomerular)	AGel amyloid	Abcam (Clone EPR1942); Sigma-Aldrich (Merck) (Clone 3G5)	Known positive	[34, 35]
		Fibrinogen alpha chain		Extracellular deposits (commonly glomerular)	AFib-amyloid	DakoCytomation (PAb)	Known positive	[35–37]
		Prealbumin	IHC	Extracellular deposits	ATTR amyloid	DakoCytomation (PAb); Abcam (Mab)	Hepatocyte (cytoplasm)	[36, 38, 39]
		Lysozyme	IHC	Extracellular deposits (commonly glomeruli and ± medulla)	ALys amyloid	DakoCytomation (PAb)	Known positive	[36, 40]
		Apolipoprotein AI, AII, AIV, CII, CIII	IHC	Extracellular deposits (AI and AIV-medullary interstitium; AII and CII-glomeruli±arteries)	AApoAI, AApoAII, AApoAIII, AApoCII, AApoCIV	Calbiochem (ApoAI); DakoCytomation (ApoAI); Atlas Antibodies (Apo-AIV)	Known positive	[35, 39, 41]
2	Nodular GS/ other patterns with deposits	DNAJB9	IHC	Extracellular deposits	Fibrillary GN	Sigma-Aldrich (Merck) (HPA040967 and HPA041553) Thermo Fisher Scientific (PA5-59621)	Many cells; renal tubular epithelial cells, podocytes (weak cytoplasmic)	[42–45]
		Collagen III	IHC	Extracellular deposits	Collagenofibrotic/ collagen type III GP	BioGenex, (Clone HWD1.1) Cosmo Bio USA, Carlsbad, CA, USA	Renal interstitium, skin dermis, blood vessel wall	[46, 47]
		Fibronectin	IHC	Extracellular deposits	Fibronectin GP	Sigma-Aldrich (Merck) (clone IST-4); Serotec (Mab)	–	[48–51]
3	MN-associated antigens	PLA2R (phospholipase A2 receptor)	IHC; IF-P	Granular, glomerular capillary walls	PLA2R-associated MN	Sigma-Aldrich (Merck) (PA012657); Novus Biologicals (Alexa Fluor® 488-conjugated mouse anti-PLA2R1-clone 12-6-5)	–	[21, 52–54]
		THSD7A (Thrombospondin type-1 domain-containing 7A)	IHC, IF-P	Granular, glomerular capillary walls	THSD7A-associated MN	Atlas Antibodies (AMAB91234) Sigma-Aldrich (Merck) (clone CL3778)	–	[21, 54–56]
		NCAM-1 (CD56)	IHC; IF-P	Granular, along GBM	NCAM-1-associated MN	Many sources Invitrogen, Sigma-Aldrich (PAbs)	–	[56]
		EXT1/2 (exostosin 1 and 2)	IHC; IF-P	Granular, along GBM	EXT1/2-associated MN	EXT1: Invitrogen/ ThermoFisher Scientific (PAb) (PA5-60699); Abcam (ab126305) EXT2: PAb Abcam; Arigo, (ARG58606)	Not known	[16, 21, 57]

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Table 3 (continued)

S.No.	Indication	Marker	Common staining method	Staining pattern	Interpretation	Sources	Positive controls	Reference
						primary antibody		
		NELL-1 (neural epidermal growth factor-like 1 protein)	IHC; IF-P	Granular, along GBM	NELL-1-associated MN	Invitrogen (PA5-27958); Sigma-Aldrich (Merck) (HPA051535)	TEC (cytoplasm)	[58, 59]
		Semaphorin 3B	IHC; IF-P	Granular, along GBM	Sema3B-associated MN	Abcam (ab48197)	Not known	[18]
		PCDH7 (Protocadherin 7)	IHC; IF-P	Granular, along GBM	PCDH7-associated MN	Abcam (clone OT12G6)	TBM, TEC (cytoplasm)(moderate)	[60]
		HTRA1 (high temperature recombinant protein A1)	IHC; IF-P	Granular, along GBM	HTRA1-associated MN	R&D Systems (clone 275,603) Thermo Fisher Scientific (PA511412)	-	[21]
		TGFBR3 (transforming growth factor beta receptor 3)	IHC	Granular, along GBM	TGFBR3-associated MN	Thermo Fisher Scientific (PAb)	–	[61]
		Contactin 1	IF-P, IHC	Granular, along GBM	MN associated with chronic inflammatory demyelinating polyneuropathy (CIDP)	R&D Systems (AF904)		[54, 62]
4	GBM disorders	Collagen IV alpha-3, alpha-4, and alpha-5	IF-P; IF	Absent staining in GBM, TBM (also in EBM)	Alport Syndrome (AS)	[a]	Normal Glom.-GBM, TBM	[63]
				Reduced/discontinuous/ segmental staining in GBM, TBM (also in EBM)	(X-linked dominant, males) (X-linked dominant AS, females)		Normal skin-EBM	[64]
				Absent staining in GBM only	AR AD			
		Collagen IV alpha-6		Absent staining Reduced/discontinuous/ segmental staining Retained staining	Alport syndrome (X-linked dominant; males) (X-linked dominant; females) AR and AD	[a]	Normal glomerulus-Bowman capsule, collecting duct, DT TBM	
		Collagen IV alpha-1 and -2		Retained staining	Alport syndrome (X-linked dominant, AR, AD)	[a]	Mesangium, subendothelial aspect of GBM	

IHC, immunohistochemistry; Mab, monoclonal antibody; PAb, polyclonal antibody; GS, glomerulosclerosis; GBM, glomerular basement membrane; TBM, tubular basement membrane; EBM, epidermal basement membrane; AR, autosomal recessive; AD, autosomal dominant. [a]Double staining by a collagen IV alpha-5 and alpha-2 chain antibody cocktail can be performed. Anti-collagen antibodies available for direct immunofluorescence for frozen sections are from Shigei Medical Research Institute in Japan (https://www.shigei.or.jp/smri/alport_eg.html). It has monoclonal antibodies against alpha-2 and alpha-5 subunits of type IV collagen (alpha-5 and -2 conjugated with fluorochrome FITC and Texas red, respectively).

indirect IF using the IF-P method or IHC. Larsen et al. [69] described sensitive and specific staining by the IF-P method using rabbit polyclonal anti-PLA2R1 antibody. Gudipati et al. [70] described tissue staining by the IHC method with a sensitivity of 91.8% and specificity of 95.1% for diagnosing PLA2R-related MN.

Glomerular PLA2R antigen expression can be seen in HBV infection-associated MN and rarely in MN secondary to HCV infection, lupus nephritis, sarcoidosis, malignancy, and Sjogren's syndrome [66, 71]. Clinical presentation and demonstration of the target antigen and glomerular IgG subclass staining with dominant/codominant IgG4 staining can help classify MN and aid in proper management.

Thrombospondin Type-1 Domain-Containing 7A (THSD7A)

In 2014, Tomas et al. [55] identified Thrombospondin type-1 domain-containing 7A (THSD7A) as a second autoantigen involved in 2.5–5% of the patients of adult idiopathic MN. Similar to PLA2R, THSD7A is also a large transmembrane glycoprotein expressed by the podocyte. It induces an IgG4-predominant humoral immune response that produces circulating autoantibodies that can be used clinically for diagnostic and monitoring purposes [53].

Larsen et al. [72] described the procedure for detecting glomerular THSD7A antigen by IHC/immunoperoxidase on FFPE tissue using rabbit polyclonal anti-THSD7A antibody. THSD7A-associated MN shows intense diffuse global granular staining of THSD7A along the capillary loops (Fig. 3b) [72].

Staining of renal biopsies for THSD7A antigen may be more sensitive for the identification of THSD7A-associated MN than serum autoantibody measurement, similar to that for PLA2R1-associated MN [73].

THSD7A-associated MN has increased rates of malignancy as compared with PLA2R-associated MN. Hoxha et al. [74] found that 7 of the 25 patients with circulating THSD7A antibodies had a malignant tumor. Rarely, patients with MN may show dual positive antibodies against THSD7A and PLA2R, with tissue staining for both antigens [72].

Exostosin1/2

Sethi et al. [16] described two proteins, exostosin 1 (EXT1) and exostosin 2 (EXT2), in the GBM of PLA2R-negative MN by proteomics and IHC, using rabbit polyclonal antibodies. Most EXT1/EXT2-associated MN were secondary, present in patients with autoimmune manifestations (shown in Fig. 3c). Eighty percent were patients of membranous lupus nephritis. Circulating anti-EXT1/EXT2 antibodies are not detected [16].

NELL-1

Neural epidermal growth factor-like 1 protein (NELL-1)-associated MN was described as a distinct subtype of PLA2R-negative MN, characterized by accumulation and co-localization of NELL-1 protein with IgG along the glomerular basement membrane. Often, the immunostaining is segmental (involving <75% of the glomerular tuft) (shown in Fig. 3d, e) [17]. The patients have anti-NELL-1 antibodies in the serum [58]. It may be associated with an underlying malignancy [75]. Reports suggest that the use of skin fairness creams with high mercury content and indigenous medicines may be responsible for a high percentage of NELL-1-associated MN in certain regions [59].

Figure 3 and Table 3 detail the various antigens described for evaluating MN. The etiological assessment of renal biopsies with MN requires immunostaining for the various antigens using an algorithmic approach [15].

Larsen et al. [8] have shown that immunostaining for serum amyloid P (SAP) can identify Membranous-like glomerulopathy with masked IgG kappa deposits (MGMID). SAP protein was identified by mass spectrometry of laser capture micro-dissected glomeruli and on confocal microscopy of IF-P sections where it co-localized with glomerular IgG deposits.

Markers for Organized Deposition Diseases

Amyloid Protein

The tissue diagnosis of amyloidosis is defined by hyaline acellular congophilic deposits on a Congo red stain showing apple-green birefringence under polarized light microscopy. The deposits can involve one or more compartments of the kidney. The typical clinical presentation is nephrotic proteinuria due to glomerular involvement. Interstitial involvement by deposits manifests as concentrating defects. All pathogenic amyloid fibrils can be stained by antibody to the plasma glycoprotein, SAP component, also known as pentraxin-2 (Table 2). Certain proteins, like SAP, heparin sulfate proteoglycans, apolipoprotein AIV and E, are grouped as "amyloid signatures," forming part of all mature amyloid fibrils, irrespective of the type [76]. Till date, 42 amyloid fibril proteins are identified and are named as per the Nomenclature Committee of the International Society of Amyloidosis [77]. The kidney is a site of amyloid deposition in immunoglobulin (Ig) light chain (AL), Ig heavy chain (AH), amyloid A (AA), leukocyte chemotactic factor 2 (ALECT-2), fibrinogen, gelsolin, lysozyme, apolipoprotein AII (apoAII), and, rarely, transthyretin-variant, apoAI, AIV, CII, and ApoCIII disease [77]. The more common AL and AA amyloids frequently involve the glomeruli and the blood

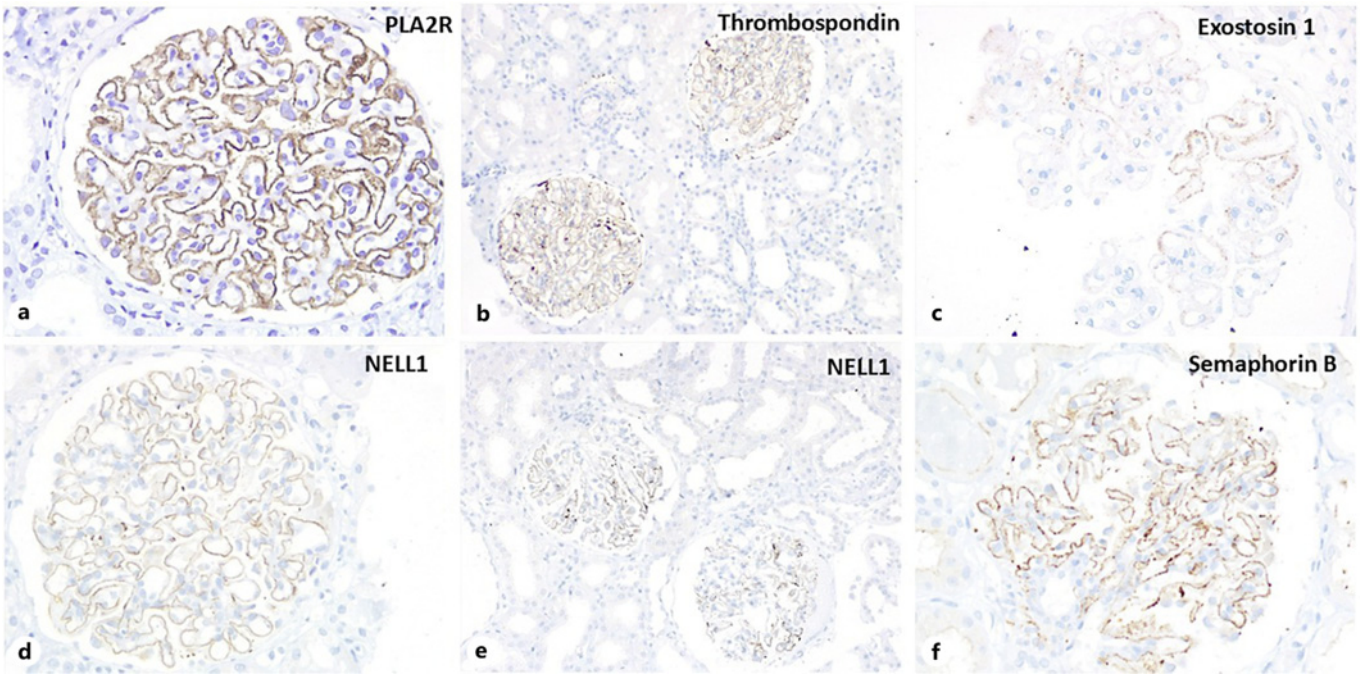

Fig. 3. IHC for classification of membranous nephropathy (MN): glomerular capillary wall granular staining in PLA2R-associated MN (**a**), Thrombospondin-associated MN (**b**), EXT1 in lupus nephritis (**c**), NELL-1-associated MN (**d**), often with segmental deposits (**e**), and semaphorin-associated MN (**f**).

vessels but can involve all compartments. Serum amyloid A is an acute-phase reactant produced by hepatocytes in response to inflammatory or neoplastic conditions. LECT2 and ApoA amyloid often show prominent interstitial involvement, while AFib predominantly involves glomeruli.

Proteomics is considered the gold standard for amyloid typing. Laser dissection and tandem mass spectrometry is an accurate tool for identifying and typing amyloid [78]. It can be performed on formalin-fixed paraffin-embedded tissue. However, it needs standard approaches and is only available at some centers.

Fibril protein type can be typed by IHC using fibril protein-specific antibodies or IF for kappa/lambda light chains. A panel of antibodies for more common types can subtype most amyloids (shown in Fig. 4). This is a valuable and easily accessible tool for amyloid subtyping available in most institutions. In a study on 117 systemic amyloidosis, IHC for amyloid typing was highly specific and sensitive, permitting a definite classification in 110 of 117 (94%) specimens tested [36].

However, IHC for typing amyloid fibril can have limitations of nonspecific staining due to epitope cross-reactivity and trapping of plasma proteins. False-positive staining is reported for ALECT-2 and amyloid light chain IHC [30, 79]. In our experience, immunostaining using standardized protocols, an algorithmic approach using a panel of antibodies performed on an automated platform, and integration of clinical features is helpful for interpretation and amyloid typing (Fig. 5). Table 3 lists the IHC markers available for different amyloid types and their common location in the renal compartments.

In a study comparing IHC with LDMS for amyloid typing, Rezk et al. [80] reported that MS is superior to IHC for amyloid fibril typing. MS could determine the amyloid type in 85% of Congo red-positive systemic amyloidosis and in 80% of the cases where IHC could not type. LDMS is a powerful tool for diagnosing and typing amyloid when used with clinical and histological assessment. It is essential for rare amyloid types, as antibodies to all amyloid are unavailable globally. Genetic testing for gene mutations for hereditary transthyretin-associated amyloidosis (ATTR) and some non-ATTR amyloids is also an important tool for diagnosing amyloid types [81].

DNAJB9

DNAJ heat-shock protein family member B9 (DNAJB9) has been identified as a significant component of fibrillary GN fibrils. IHC using an anti-DNAJB9 antibody can reliably diagnose fibrillary GN and differentiate it from other organized deposition diseases with high

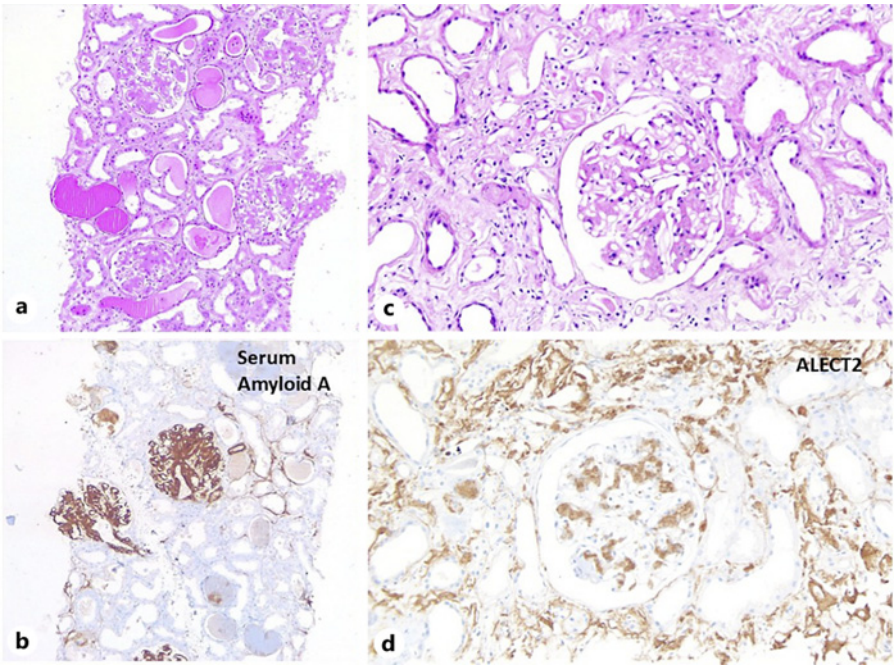

Fig. 4. Immunohistochemistry (IHC) for amyloid typing: renal biopsy showing deposits of hyaline acellular weak PAS-positive extracellular material (**a**, **c**), which was congophilic with apple-green birefringence on polarized light, in glomeruli and also interstitial (in **c**). **a** Periodic acid-Schiff. **b** IHC for serum amyloid A. **c** Periodic acid-Schiff. **d** IHC for LECT2.

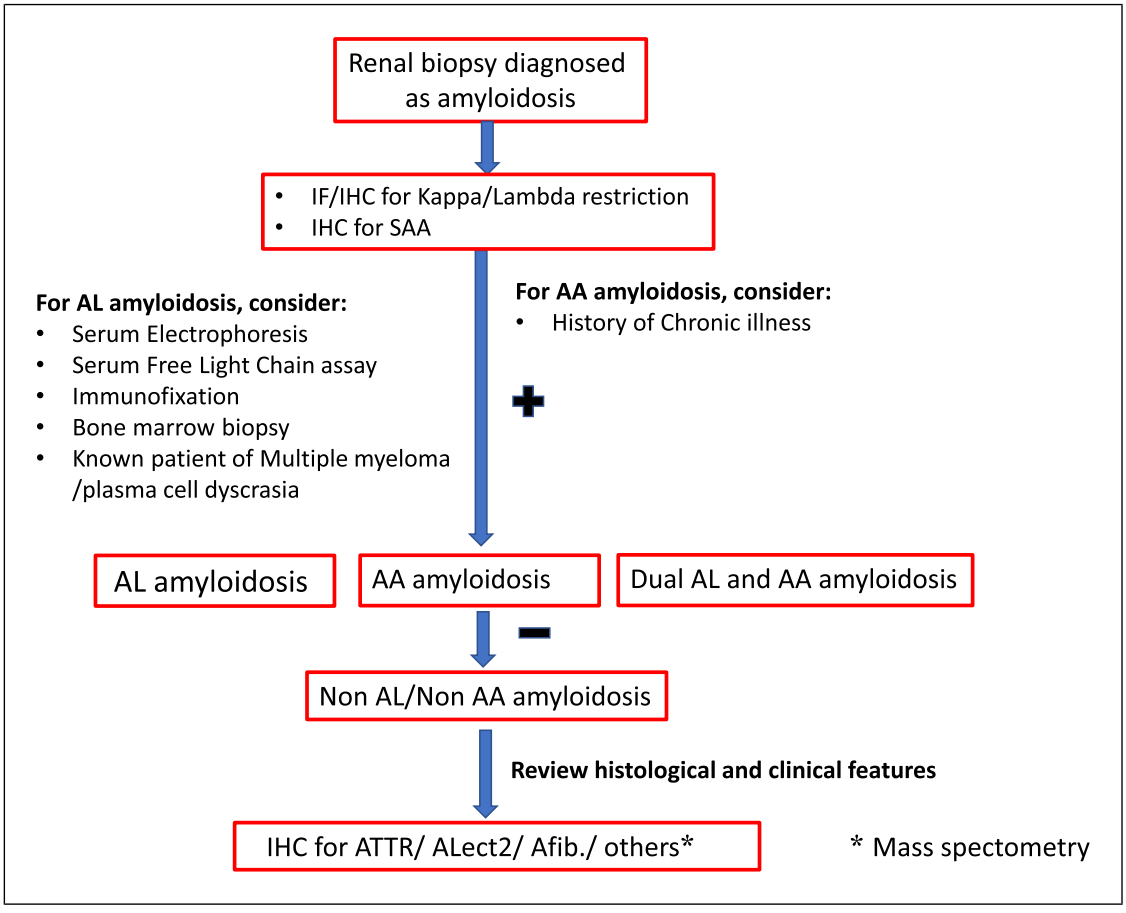

Fig. 5. Algorithmic approach for typing of renal amyloidosis by IHC.

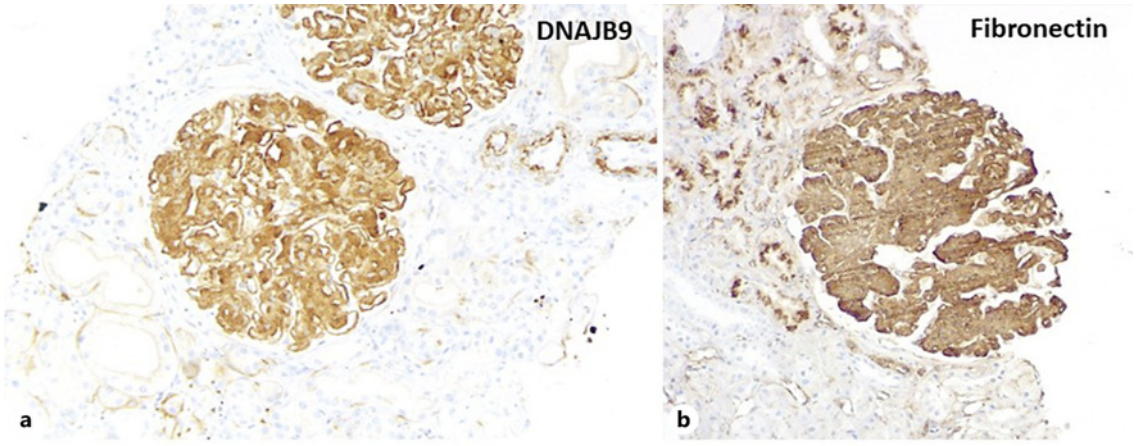

Fig. 6. IHC for organized deposits, other than amyloid: renal biopsy showing smudgy glomerular deposits of DNAJB9 in fibrillary GN (**a**) and fibronectin in fibronectin glomerulopathy (**b**).

sensitivity and specificity (shown in Fig. 6a) [82]. However, a false-positive and weak staining was observed in renal serum amyloid A (AA) amyloidosis [83].

Fibrillary GN is an organized deposition disease characterized on electron microscopy by randomly oriented non-branching fibrils measuring 10–30 nm in diameter in the mesangium and/or the glomerular basement membrane. On IF, the deposits stain intensely for IgG, usually accompanied by C3, kappa, and lambda, and sometimes are associated with staining for C1q, IgM, or IgA. Approximately 5% of fibrillary GN show kappa or lambda light chain restriction. IF staining for IgG subtypes shows predominantly IgG4 and IgG1 staining in the absence of IgG2 and IgG3. Tubular basement positivity may be seen in a minority of cases [84]. Nasr et al. [85] have developed an immunoprecipitation-based multiple reaction monitoring method to measure serum levels of DNAJB9 as a biomarker of fibrillary GN.

Collagen III

Collagenofibrotic glomerulopathy, or collagen type III glomerulopathy, is a rare glomerular disease characterized by abnormal mesangial and subendothelial collagen type III fibril deposits. Most cases were initially reported from Japan [86]. The glomeruli show lobular accentuation with mesangial expansion and often a nodular or membranoproliferative pattern of glomerulopathy. Indirect IF or IHC using anti-collagen III antibody shows staining for collagen type III in the expanded mesangium and thickened glomerular capillary walls (Table 3). Electron microscopy helps demonstrate haphazard, irregular bundles of curved and frayed banded curvilinear collagen fibers.

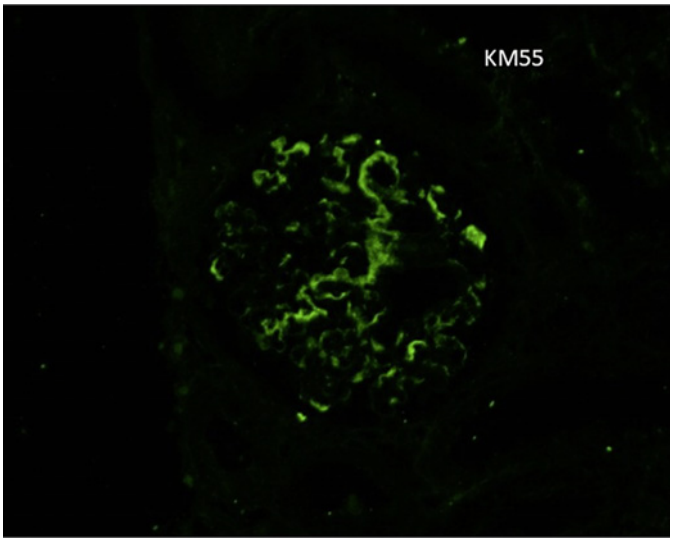

Fig. 7. Granular mesangial 2+ galactose-deficient IgA1 (Gd-IgA1) demonstrated on IF by the monoclonal antibody for Gd-IgA1 (KM55) in IgA nephropathy.

Fibronectin

Fibronectin glomerulopathy, an autosomal-dominant condition characterized on electron microscopy by deposition of granular and fibrillary (12–16 nm) organized deposits in glomeruli, usually manifests as mild to nephrotic proteinuria in the middle-aged. It may gradually progress to end-stage renal disease. The glomeruli show lobular accentuation with mesangial expansion and glomerular basement membrane thickening, producing a membranoproliferative pattern with minimal cellularity. The extracellular material deposited is fuschinophilic (periodic acid-Schiff stain-

positive), non-argyrophilic (Silver stain-negative), and non-congophilic (Congo red stain-negative). It stains intensely on IHC using fibronectin antibodies against the plasma-derived isoforms [48–51] (shown in Fig. 6b and Table 3). Variable fibronectin expression can be seen in the normal mesangial matrix and conditions associated with mesangial sclerosis [87]. Therefore, immunostaining should be strong and interpreted in correlation with histological and IF features for diagnosing fibronectin glomerulopathy.

Strom et al. [48] demonstrated that the glomerular deposits stain with antibody to fibronectin isoform IST-4, which detects both cellular and plasma fibronectins, while they are negative with antibody to IST-9, which stains only cellular fibronectin. This suggests that most of the fibronectin deposited in fibronectin glomerulopathy is plasma-derived [88].

GBM Disorders: Alport Spectrum Disorders

Renal biopsies with suspected Alport syndrome disorders can be stained for alpha chains of collagen IV for diagnostic evaluation (Table 3). Antibodies with a cocktail of alpha-5 and alpha-2 chains of collagen IV are available; however, genetic testing is preferred for a specific diagnosis.

KM55 in IgA Nephropathy

Mucosal-derived galactose-deficient IgA1 (Gd-IgA1) is a critical molecule affecting IgA nephropathy's pathogenesis and progression. Gd-IgA1 circulates in the bloodstream of patients with IgA nephropathy, and its deposition in the glomeruli is considered to be involved in its pathogenesis. KM55 is a monoclonal rat antibody that detects Gd-IgA1 (shown in Fig. 7). The utility of KM55 staining as a diagnostic tool in IgA-containing glomerular diseases is uncertain; however, it can provide some insight into the origin of IgA. The positivity of KM55 in IgA-dominant glomerular disease suggests a mucosal-derived IgA (e.g. primary IgA, HSP, and secondary IgA). On the other hand, negative staining suggests systemic derivation of IgA (e.g. SLE and IgA-dominant MPGN.) [89].

In certain situations, staining for other markers, such as podocyte proteins, podocyte proliferation, maturity markers, CD68, and infectious agents, may help evaluate renal biopsies [89–94].

Conclusion

Renal biopsy interpretation requires a multidisciplinary approach incorporating light microscopy using hematoxylin-eosin and histochemical stains, IF, IHC, and electron microscopy. IHC has become integral to the diagnosis and management of glomerular renal diseases. Understanding the importance of immunohistology using various markers is required to classify the various glomerular diseases for proper patient management.

Conflict of Interest Statement

There is nothing to disclose. Prof. Vinita Agrawal was a member of the journal's Editorial Board at the time of submission.

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Author Contributions

V.A. performed the literature search and drafted the manuscript and tables. A.S. contributed to the content. V.A. and A.S. approved the final manuscript.

References

1 Nasr SH, Fidler ME, Said SM. Paraffin immunofluorescence: a valuable ancillary technique in renal pathology. Kidney Int Rep. 2018;3(6):1260–6. https://doi.org/10.1016/j.ekir.2018.07.008

2 Larsen CP, Messias NC, Walker PD, Fidler ME, Cornell LD, Hernandez LH, et al. Membranoproliferative glomerulonephritis with masked monotypic immunoglobulin deposits. Kidney Int. 2015;88(4):867–73. https://doi.org/10.1038/ki.2015.195

3 Larsen CP, Boils CL, Cossey LN, Sharma SG, Walker PD. Clinicopathologic features of membranous-like glomerulopathy with masked IgG kappa deposits. Kidney Int Rep. 2016;1(4):299–305. https://doi.org/10.1016/j.ekir.2016.08.012

4 Messias NC, Walker PD, Larsen CP. Paraffin immunofluorescence in the renal pathology laboratory: more than a salvage technique. Mod Pathol. 2015;28(6):854–60. https://doi.org/10.1038/modpathol.2015.1

5 Nasr SH, Galgano SJ, Markowitz GS, Stokes MB, D'Agati VD. Immunofluorescence on pronase-digested paraffin sections: a valuable salvage technique for renal biopsies. Kidney Int. 2006;70(12):2148–51. https://doi.org/10.1038/sj.ki.5001990

6 Nasr SH, Preddie DC, Markowitz GS, Appel GB, D'Agati VD. Multiple myeloma, nephrotic syndrome and crystalloid inclusions in podocytes. Kidney Int. 2006;69(3):616–20. https://doi.org/10.1038/sj.ki.5000144

7 Gupta V, El Ters M, Kashani K, Leung N, Nasr SH. Crystalglobulin-induced nephropathy. J Am Soc Nephrol. 2015;26(3):525–9. https://doi.org/10.1681/ASN.2014050509
8 Larsen CP, Sharma SG, Caza TN, Kenan DJ, Storey AJ, Edmondson RD, et al. Serum amyloid P deposition is a sensitive and specific feature of membranous-like glomerulopathy with masked IgG kappa deposits. Kidney Int. 2020;97(3):602–8. https://doi.org/10.1016/j.kint.2019.10.026
9 Larsen CP, Ambuzs JM, Bonsib SM, Boils CL, Cossey LN, Messias NC, et al. Membranous-like glomerulopathy with masked IgG kappa deposits. Kidney Int. 2014;86(1):154–61. https://doi.org/10.1038/ki.2013.548
10 Nagahama K, Isomura A, Shimoyamada H, Masuko S, Shimoda S, Karube M, et al. Membranous nephropathy with masked polyclonal IgG deposits associated with primary Sjögren's syndrome. CEN Case Rep. 2021;10(1):53–8. https://doi.org/10.1007/s13730-020-00516-3
11 Nasr SH, Kudose SS, Said SM, Santoriello D, Fidler ME, Williamson SR, et al. Immunotactoid glomerulopathy is a rare entity with monoclonal and polyclonal variants. Kidney Int. 2021;99(2):410–20. https://doi.org/10.1016/j.kint.2020.07.037
12 Shi S, Cheng Q, Zhang P, Wang N, Zheng Y, Bai XY, et al. Immunofluorescence with dual microwave retrieval of paraffin-embedded sections in the assessment of human renal biopsy specimens. Am J Clin Pathol. 2013;139(1):71–8. https://doi.org/10.1309/AJCPRZG8EXN7BAID
13 Bouatou Y, Kers J, Chevalier-Florquin MSN, Claessen N, Nguyen TQ, Damman J, et al. Diagnostic accuracy of immunofluorescence versus immunoperoxidase staining to distinguish immune complex-mediated glomerulonephritis and C3 dominant glomerulopathy. Histopathology. 2018;72(4):601–8. https://doi.org/10.1111/his.13385
14 Hemminger J, Nadasdy G, Satoskar A, Brodsky SV, Nadasdy T. IgG subclass staining in routine renal biopsy material. Am J Surg Pathol. 2016;40(5):617–26. https://doi.org/10.1097/PAS.0000000000000605
15 Sethi S, Beck LH Jr, Glassock RJ, Haas M, De Vriese AS, Caza TN, et al. Mayo Clinic consensus report on membranous nephropathy: proposal for a novel classification. Kidney Int. 2023;104(6):1092–102. https://doi.org/10.1016/j.kint.2023.06.032
16 Sethi S, Madden BJ, Debiec H, Charlesworth MC, Gross L, Ravindran A, et al. Exostosin 1/exostosin 2-associated membranous nephropathy. J Am Soc Nephrol. 2019;30(6):1123–36. https://doi.org/10.1681/ASN.2018080852
17 Kudose S, Santoriello D, Debiec H, Canetta PA, Bomback AS, Stokes MB, et al. The clinicopathologic spectrum of segmental membranous glomerulopathy. Kidney Int. 2021;99(1):247–55. https://doi.org/10.1016/j.kint.2020.06.014
18 Sethi S, Debiec H, Madden B, Vivarelli M, Charlesworth MC, Ravindran A, et al. Semaphorin 3B-associated membranous nephropathy is a distinct type of disease predominantly present in pediatric patients. Kidney Int. 2020;98(5):1253–64. https://doi.org/10.1016/j.kint.2020.05.030
19 Nasr SH, Satoskar A, Markowitz GS, Valeri AM, Appel GB, Stokes MB, et al. Proliferative glomerulonephritis with monoclonal IgG deposits. J Am Soc Nephrol. 2009;20(9):2055–64. https://doi.org/10.1681/ASN.2009010110
20 Sethi S, Madden B, Casal Moura M, Nasr SH, Klomjit N, Gross L, et al. Hematopoietic stem cell transplant-membranous nephropathy is associated with protocadherin FAT1. J Am Soc Nephrol. 2022;33(5):1033–44. https://doi.org/10.1681/ASN.2021111488
21 Al-Rabadi LF, Caza T, Trivin-Avillach C, Rodan AR, Andeen N, Hayashi N, et al. Serine protease HTRA1 as a novel target antigen in primary membranous nephropathy. J Am Soc Nephrol. 2021;32(7):1666–81. https://doi.org/10.1681/ASN.2020101395
22 Vivarelli M, Barratt J, Beck LH Jr, Fakhouri F, Gale DP, Goicoechea de Jorge E, et al. The role of complement in kidney disease: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) controversies conference. Kidney Int. 2024;106(3):369–91. https://doi.org/10.1016/j.kint.2024.05.015
23 Chandra P. C4d in native glomerular diseases. Am J Nephrol. 2019;49(1):81–92. https://doi.org/10.1159/000496059
24 Sethi S, Nasr SH, De Vriese AS, Fervenza FC. C4d as a diagnostic tool in proliferative GN. J Am Soc Nephrol. 2015;26(11):2852–9. https://doi.org/10.1681/ASN.2014040406
25 Villacorta J, Diaz-Crespo F, Acevedo M, Guerrero C, Campos-Martin Y, García-Díaz E, et al. Glomerular C3d as a novel prognostic marker for renal vasculitis. Hum Pathol. 2016;56:31–9. https://doi.org/10.1016/j.humpath.2016.05.015
26 Snijders MLH, van de Wall-Neecke BJ, Hesselink DA, Becker JU, Clahsen-van Groningen MC. Utility of immunohistochemistry with C3d in C3 glomerulopathy. Mod Pathol. 2020;33(3):431–9. https://doi.org/10.1038/s41379-019-0348-z
27 Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. Front Immunol. 2014;5(520):520. https://doi.org/10.3389/fimmu.2014.00520
28 Huang CC, Lehman A, Albawardi A, Satoskar A, Brodsky S, Nadasdy G, et al. IgG subclass staining in renal biopsies with membranous glomerulonephritis indicates subclass switch during disease progression. Mod Pathol. 2013;26(6):799–805. https://doi.org/10.1038/modpathol.2012.237
29 Caza TN, Al-Rabadi LF, Beck LH Jr. How times have changed! A cornucopia of antigens for membranous nephropathy. Front Immunol. 2021;12:800242. https://doi.org/10.3389/fimmu.2021.800242
30 Paueksakon P, Fogo AB, Sethi S. Leukocyte chemotactic factor 2 amyloidosis cannot be reliably diagnosed by immunohistochemical staining. Hum Pathol. 2014;45(7):1445–50. https://doi.org/10.1016/j.humpath.2014.02.020
31 Larsen CP, Kossmann RJ, Beggs ML, Solomon A, Walker PD. Clinical, morphologic, and genetic features of renal leukocyte chemotactic factor 2 amyloidosis. Kidney Int. 2014;86(2):378–82. https://doi.org/10.1038/ki.2014.11
32 Larsen CP, Walker PD, Weiss DT, Solomon A. Prevalence and morphology of leukocyte chemotactic factor 2-associated amyloid in renal biopsies. Kidney Int. 2010;77(9):816–9. https://doi.org/10.1038/ki.2010.9
33 Murphy CL, Wang S, Kestler D, Larsen C, Benson D, Weiss DT, et al. Leukocyte chemotactic factor 2 (LECT2)-associated renal amyloidosis: a case series. Am J Kidney Dis. 2010;56(6):1100–7. https://doi.org/10.1053/j.ajkd.2010.08.013
34 Sethi S, Dasari S, Amin MS, Vrana JA, Theis JD, Alexander MP, et al. Clinical, biopsy, and mass spectrometry findings of renal gelsolin amyloidosis. Kidney Int. 2017;91(4):964–71. https://doi.org/10.1016/j.kint.2016.11.017
35 Li Z-Y, Wang S, Li D-Y, Liu D, Wang S-X, Yu X-J, et al. Fibrinogen A alpha-chain amyloidosis in two Chinese patients. Front Med. 2022;9:869409. https://doi.org/10.3389/fmed.2022.869409
36 Schönland SO, Hegenbart U, Bochtler T, Mangatter A, Hansberg M, Ho AD, et al. Immunohistochemistry in the classification of systemic forms of amyloidosis: a systematic investigation of 117 patients. Blood. 2012;119(2):488–93. https://doi.org/10.1182/blood-2011-06-358507
37 Stangou AJ, Banner NR, Hendry BM, Rela M, Portmann B, Wendon J, et al. Hereditary fibrinogen A α-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood. 2010;115(15):2998–3007. https://doi.org/10.1182/blood-2009-06-223792
38 Sousa MM, Cardoso I, Fernandes R, Guimarães A, Saraiva MJ. Deposition of transthyretin in early stages of familial amyloidotic polyneuropathy: evidence for toxicity of nonfibrillar aggregates. Am J Pathol. 2001;159(6):1993–2000. https://doi.org/10.1016/s0002-9440(10)63050-7
39 Murakami T, Takasawa A, Moriki A, Igaki Y, Ikeda H, Murase K, et al. A systemic apolipoprotein A-IV-associated amyloidosis confirmed by proteome analysis. Virchows Arch. 2021;479(5):1041–6. https://doi.org/10.1007/s00428-021-03073-x
40 Nasr SH, Dasari S, Mills JR, Theis JD, Zimmermann MT, Fonseca R, et al. Hereditary lysozyme amyloidosis variant p.Leu102Ser associates with unique phenotype. J Am Soc Nephrol. 2017;28(2):431–8. https://doi.org/10.1681/ASN.2016090951

41 Lu C, Zuo K, Lu Y, Liang S, Huang X, Zeng C, et al. Apolipoprotein A-1-related amyloidosis 2 case reports and review of the literature. Medicine. 2017;96(39):e8148. https://doi.org/10.1097/MD.0000000000008148
42 Nasr SH, Vrana JA, Dasari S, Bridoux F, Fidler ME, Kaaki S, et al. DNAJB9 is a specific immunohistochemical marker for fibrillary glomerulonephritis. Kidney Int Rep. 2018;3(1):56–64. https://doi.org/10.1016/j.ekir.2017.07.017
43 Said SM, Rocha AB, Valeri AM, Sandid M, Ray AS, Fidler ME, et al. Characteristics of patients with coexisting DNAJB9-associated fibrillary glomerulonephritis and IgA nephropathy. Clin Kidney J. 2021;14(6):1681–90. https://doi.org/10.1093/ckj/sfaa205
44 El Ters M, Bobart SA, Cornell LD, Leung N, Bentall A, Sethi S, et al. Recurrence of DNAJB9-positive fibrillary glomerulonephritis after kidney transplantation: a case series. Am J Kidney Dis. 2020;76(4):500–10. https://doi.org/10.1053/j.ajkd.2020.01.018
45 Avasare RS, Robinson BA, Nelson J, Woltjer R, Krajbich V, Nguyen V, et al. DNAJB9 is not transcriptionally upregulated in the glomerulus in fibrillary glomerulonephritis. Kidney Int Rep. 2020;5(3):368–72. https://doi.org/10.1016/j.ekir.2019.12.004
46 Wilson AV, Costigliolo F, Farris AB, Rengen R, Arend LJ. Collagen type III glomerulopathy. Kidney Int Rep. 2021;6(6):1738–42. https://doi.org/10.1016/j.ekir.2021.03.887
47 Kurien AA, Larsen CP, Cossey LN. Collagenofibrotic glomerulopathy. Clin Kidney J. 2015;8(5):543–7. https://doi.org/10.1093/ckj/sfv061
48 Strøm EH, Banfi G, Krapf R, Abt AB, Mazzucco G, Monga G, et al. Glomerulopathy associated with predominant fibronectin deposits: a newly recognized hereditary disease. Kidney Int. 1995;48(1):163–70. https://doi.org/10.1038/ki.1995.280
49 Hata M, Mori T, Hirose Y, Nishida Y, Mandai S, Ando F, et al. A case of unexpected diagnosis of fibronectin glomerulopathy with histological features of membranoproliferative glomerulonephritis. BMC Nephrol. 2024;25(1):25. https://doi.org/10.1186/s12882-024-03456-7
50 Hara M, Kusaba T, Ono K, Masuzawa N, Nakamura I, Urata N, et al. Extraglomerular vascular involvement of glomerulopathy with fibronectin deposits. Intern Med. 2021;60(13):2103–7. https://doi.org/10.2169/internalmedicine.6558-20
51 Takii M, Suehiro T, Shima A, Yotsueda H, Hisano S, Katafuchi R. Fibronectin glomerulopathy complicated with persistent cloaca and congenital esophageal atresia: a case report and literature review. BMC Nephrol. 2017;18(1):288. https://doi.org/10.1186/s12882-017-0704-5
52 Beck LH Jr, Bonegio RGB, Lambeau G, Beck DM, Powell DW, Cummins TD, et al. M-type phospholipase A2 receptor as target antigen in idiopathic membranous nephropathy. N Engl J Med. 2009;361(1):11–21. https://doi.org/10.1056/NEJMoa0810457
53 Beck LH. PLA2R and THSD7A: disparate paths to the same disease? J Am Soc Nephrol. 2017;28(9):2579–89. https://doi.org/10.1681/ASN.2017020178
54 Le Quintrec M, Teisseyre M, Bec N, Delmont E, Szwarc I, Perrochia H, et al. Contactin-1 is a novel target antigen in membranous nephropathy associated with chronic inflammatory demyelinating polyneuropathy. Kidney Int. 2021;100(6):1240–9. https://doi.org/10.1016/j.kint.2021.08.014
55 Tomas NM, Beck LH, Meyer-Schwesinger C, Seitz-Polski B, Ma H, Zahner G, et al. Thrombospondin type-1 domain-containing 7A in idiopathic membranous nephropathy. N Engl J Med. 2014;371(24):2277–87. https://doi.org/10.1056/NEJMoa1409354
56 Caza TN, Hassen SI, Kuperman M, Sharma SG, Dvanajscak Z, Arthur J, et al. Neural cell adhesion molecule 1 is a novel autoantigen in membranous lupus nephritis. Kidney Int. 2021;100(1):171–81. https://doi.org/10.1016/j.kint.2020.09.016
57 Xia X, Li S, Wang Z, Ye S, Fan Y, Peng W, et al. Glomerular exostosin-positivity is associated with disease activity and outcomes in patients with membranous lupus nephritis. Kidney Int Rep. 2024;9(4):1040–6. https://doi.org/10.1016/j.ekir.2024.01.008
58 Sethi S, Debiec H, Madden B, Charlesworth MC, Morelle J, Gross L, et al. Neural epidermal growth factor-like 1 protein (NELL-1) associated membranous nephropathy. Kidney Int. 2020;97(1):163–74. https://doi.org/10.1016/j.kint.2019.09.014
59 Narayanan R, Sivadas S, Kurien AA. NELL-1-associated membranous nephropathy linked to skin fairness cream use: insights from an Indian case series. Kidney Int. 2024;105(6):1316–9. https://doi.org/10.1016/j.kint.2024.03.025
60 Sethi S, Madden B, Debiec H, Morelle J, Charlesworth MC, Gross L, et al. Protocadherin 7-associated membranous nephropathy. J Am Soc Nephrol. 2021;32(5):1249–61. https://doi.org/10.1681/ASN.2020081165
61 Caza TN, Hassen SI, Kenan DJ, Storey A, Arthur JM, Herzog C, et al. Transforming Growth Factor Beta Receptor 3 (TGFBR3)-associated membranous nephropathy. Kidney360. 2021;2(8):1275–86. https://doi.org/10.34067/KID.0001492021
62 Shida R, Iwakura T, Ohashi N, Ema C, Aoki T, Tashiro T, et al. Anti-contactin 1 antibody-associated membranous nephropathy in chronic inflammatory demyelinating polyneuropathy with several autoantibodies. Intern Med. 2024;63(5):699–705. https://doi.org/10.2169/internalmedicine.2126-23
63 Akihisa T, Sato M, Wakayama Y, Taneda S, Horita S, Hirose O, et al. Glomerular basement membrane protein expression and the diagnosis and prognosis of autosomal dominant Alport syndrome. Kidney Med. 2019;1(6):391–6. https://doi.org/10.1016/j.xkme.2019.06.007
64 Kagawa M, Kishiro Y, Naito I, Nemoto T, Nakanishi H, Ninomiya Y, et al. Epitope-defined monoclonal antibodies against type-IV collagen for diagnosis of Alport's syndrome. Nephrol Dial Transplant. 1997;12(6):1238–41. https://doi.org/10.1093/ndt/12.6.1238
65 Beck LH Jr, Salant DJ. Membranous nephropathy: from models to man. J Clin Investig. 2014;124(6):2307–14. https://doi.org/10.1172/JCI72270
66 Pourcine F, Dahan K, Mihout F, Cachanado M, Brocheriou I, Debiec H, et al. Prognostic value of PLA2R autoimmunity detected by measurement of anti-PLA2R antibodies combined with detection of PLA2R antigen in membranous nephropathy: a single-centre study over 14 years. PLoS One. 2017;12(3):e0173201. https://doi.org/10.1371/journal.pone.0173201
67 Debiec H, Ronco P. PLA2R autoantibodies and PLA2R glomerular deposits in membranous nephropathy. N Engl J Med. 2011;364(7):689–90. https://doi.org/10.1056/NEJMc1011678
68 Bomback AS. Management of membranous nephropathy in the PLA_2R era. Clin J Am Soc Nephrol. 2018;13(5):784–6. https://doi.org/10.2215/CJN.12461117
69 Larsen CP, Messias NC, Silva FG, Messias E, Walker PD. Determination of primary versus secondary membranous glomerulopathy utilizing phospholipase A2 receptor staining in renal biopsies. Mod Pathol. 2013;26(5):709–15. https://doi.org/10.1038/modpathol.2012.207
70 Gudipati A, Uppin MS, Kalidindi RK, Swarnalatha G, Das U, Taduri G, et al. Immunohistochemical analysis of anti-phospholipase A2 receptor antibody on renal biopsies: a single tertiary care center study. Indian J Nephrol. 2017;27(5):353–8. https://doi.org/10.4103/ijn.IJN_79_17
71 Xie Q, Li Y, Xue J, Xiong Z, Wang L, Sun Z, et al. Renal phospholipase A2 receptor in hepatitis B virus-associated membranous nephropathy. Am J Nephrol. 2015;41(4–5):345–53. https://doi.org/10.1159/000431331
72 Larsen CP, Cossey LN, Beck LH. THSD7A staining of membranous glomerulopathy in clinical practice reveals cases with dual autoantibody positivity. Mod Pathol. 2016;29(4):421–6. https://doi.org/10.1038/modpathol.2016.32
73 Hoxha E, Beck LH, Wiech T, Tomas NM, Probst C, Mindorf S, et al. An indirect immunofluorescence method facilitates detection of Thrombospondin type 1 domain–containing 7A–specific antibodies in membranous nephropathy. J Am Soc Nephrol. 2017;28(2):520–31. https://doi.org/10.1681/ASN.2016010050
74 Hoxha E, Wiech T, Stahl PR, Zahner G, Tomas NM, Meyer-Schwesinger C, et al. A mechanism for cancer-associated membranous nephropathy. N Engl J Med. 2016;374(20):1995–6. https://doi.org/10.1056/NEJMc1511702

75 Caza TN, Hassen SI, Dvanajscak Z, Kuperman M, Edmondson R, Herzog C, et al. NELL1 is a target antigen in malignancy-associated membranous nephropathy. Kidney Int. 2021;99(4):967–76. https://doi.org/10.1016/j.kint.2020.07.039
76 Dasari S, Theis JD, Vrana JA, Rech KL, Dao LN, Howard MT, et al. Amyloid typing by mass spectrometry in clinical practice: a comprehensive review of 16,175 samples. Mayo Clin Proc. 2020;95(9):1852–64. https://doi.org/10.1016/j.mayocp.2020.06.029
77 Buxbaum JN, Eisenberg DS, Fändrich M, McPhail ED, Merlini G, Saraiva MJM, et al. Amyloid nomenclature 2024: update, novel proteins, and recommendations by the International Society of Amyloidosis (ISA) nomenclature committee. Amyloid. 2024;31(4):249–56. https://doi.org/10.1080/13506129.2024.2405948
78 Leung N, Nasr SH. 2024 update on classification, etiology, and typing of renal amyloidosis: a review. Am J Kidney Dis. 2024;84(3):361–73. https://doi.org/10.1053/j.ajkd.2024.01.530
79 Chen J, Chen H, Zhou L, Liu D, Du F, Xiang H. Strong positive light chain immunostaining in a patient with transthyretin amyloidosis. Hematology. 2023;28(1):2244315. https://doi.org/10.1080/16078454.2023.2244315
80 Rezk T, Gilbertson JA, Mangione PP, Rowczenio D, Rendell NB, Canetti D, et al. The complementary role of histology and proteomics for diagnosis and typing of systemic amyloidosis. J Pathol Clin Res. 2019;5(3):145–53. https://doi.org/10.1002/cjp2.126
81 Solignac J, Delmont E, Fortanier E, Attarian S, Mancini J, Daniel L, et al. Kidney involvement in hereditary transthyretin amyloidosis: a cohort study of 103 patients. Clin Kidney J. 2022;15(9):1747–54. https://doi.org/10.1093/ckj/sfac118
82 Nasr SH, Vrana JA, Dasari S, Bridoux F, Fidler ME, Kaaki S, et al. DNAJB9 is a specific immunohistochemical marker for fibrillary glomerulonephritis. Kidney Int Rep. 2018;3(1):56–64. https://doi.org/10.1016/j.ekir.2017.07.017
83 Andeen NK, Kung VL, Robertson J, Gurley SB, Avasare RS, Sitaraman S. Fibrillary glomerulonephritis, DNAJB9, and the unfolded protein response. Glomerular Dis. 2022;2(4):164–75. https://doi.org/10.1159/000525542
84 Rosenstock JL, Markowitz GS. Fibrillary glomerulonephritis: an update. Kidney Int Rep. 2019;4(7):917–22. https://doi.org/10.1016/j.ekir.2019.04.013
85 Nasr SH, Dasari S, Lieske JC, Benson LM, Vanderboom PM, Holtz-Heppelmann CJ, et al. Serum levels of DNAJB9 are elevated in fibrillary glomerulonephritis patients. Kidney Int. 2019;95(5):1269–72. https://doi.org/10.1016/j.kint.2019.01.024
86 Alchi B, Nishi S, Narita I, Gejyo F. Collagenofibrotic glomerulopathy: clinicopathologic overview of a rare glomerular disease. Am J Kidney Dis. 2007;49(4):499–506. https://doi.org/10.1053/j.ajkd.2007.01.020
87 Nadamuni M, Piras R, Mazbar S, Higgins JP, Kambham N. Fibronectin glomerulopathy: an unusual cause of adult-onset nephrotic syndrome. Am J Kidney Dis. 2012;60(5):839–42. https://doi.org/10.1053/j.ajkd.2012.04.029
88 Borsi L, Carnemolla B, Castellani P, Rosellini C, Vecchio D, Allemanni G, et al. Monoclonal antibodies in the analysis of fibronectin isoforms generated by alternative splicing of mRNA precursors in normal and transformed human cells. J Cell Biol. 1987;104(3):595–600. https://doi.org/10.1083/jcb.104.3.595
89 Raj R, Sharma A, Barwad A, Bagchi S, Agarwal SK, Bagga A, et al. KM55 in the evaluation of IgA-containing glomerular diseases. Glomerular Dis. 2022;2(2):59–74. https://doi.org/10.1159/000520640
90 Watts AJB, Keller KH, Lerner G, Rosales I, Collins AB, Sekulic M, et al. Discovery of autoantibodies targeting nephrin in minimal change disease supports a novel autoimmune etiology. J Am Soc Nephrol. 2022;33(1):238–52. https://doi.org/10.1681/ASN.2021060794
91 Agrawal V, Prasad N, Jain M, Pandey R. Reduced podocin expression in minimal change disease and focal segmental glomerulosclerosis is related to the level of proteinuria. Clin Exp Nephrol. 2013;17(6):811–8. https://doi.org/10.1007/s10157-013-0775-y
92 Bu L, Ye B, Kouri AM, Kim Y. Diagnostic utility of galactose-deficient immunoglobulin A1 immunostaining in the differentiation of lupus nephritis and immunoglobulin A nephropathy. Glomerular Dis. 2021;1(1):34–9. https://doi.org/10.1159/000511056
93 Soares MF, Genitsch V, Chakera A, Smith A, MacEwen C, Bellur SS, et al. Relationship between renal CD68(+) infiltrates and the oxford classification of IgA nephropathy. Histopathology. 2019;74(4):629–37. https://doi.org/10.1111/his.13768
94 Oumarou Hama H, Aboudharam G, Barbieri R, Lepidi H, Drancourt M. Immunohistochemical diagnosis of human infectious diseases: a review. Diagn Pathol. 2022;17(1):17. https://doi.org/10.1186/s13000-022-01197-5