Glomerular Diseases

Review Article

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Immunofluorescence Use and Techniques in Glomerular Diseases: A Review

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Abstract
Background: Immunofluorescence (IF) studies play an essential role in the evaluation of medical renal biopsies. Particularly, in the study of renal glomerular diseases, where it provides fundamental data for the diagnosis, classification, and etiology of the glomerular pathologies. Diverse techniques may be used to optimize the utilization of IF studies, from variations on the test methodologies to expertise on the interpretation of the results and knowledge of potential pitfalls. ***Summary:*** This manuscript presents a brief review on the history of IF and its utilization in kidney pathology, followed by a description of the IF methods, including the use of IF on paraffin-embedded tissue (paraffin IF), and other novel techniques. Guidelines on how to best report IF findings are reviewed, along with a description of antibodies commonly used in glomerular diseases, highlighting their distribution within the normal kidney and potential pitfalls in interpretation. Finally, the use and interpretation of IF are discussed in more detail in individual entities on a range of glomerular diseases. ***Key Messages:*** IF is crucial for interpretation of renal biopsies and diagnosis of glomerular diseases. Knowledge of IF techniques, alternative procedures, its use and proper interpretation is essential for optimal utilization of IF in renal pathology, and this review proposes to serve as a simplified and practical guide on this topic.

Introduction

It is not an understatement to say that renal pathology and the study of glomerular diseases rest heavily on the use of immunofluorescence (IF). IF has its place firmly enshrined, along with light microscopy and electron microscopy (EM), in the morphologic triad that serves as the mainstays of the pathologic evaluation of glomerular diseases. These features, in conjunction with clinical history, generate information leading to diagnosis and classification of kidney diseases, and may shed light on their etiology and prognosis, making the renal biopsy essential on working up patients with glomerular diseases. Knowledge of IF techniques and procedures, along with their uses and interpretation, is fundamental, as optimal utilization of IF is a powerful tool for diagnosis, and conversely, the misinterpretation of IF results may bring disastrous consequences to patient care.

We will concentrate on the impact of IF in the study of glomerular diseases, with a brief review on the history of IF, and a description of the IF methods, including the use of IF on paraffin-embedded tissue (paraffin IF), and other novel

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techniques. A practical approach on how to best report IF findings will be discussed, and the use and interpretation of IF in individual entities on a range of glomerular diseases will be studied in more detail. Hopefully, this work will serve as a practical guide to the use of IF in glomerular diseases.

The Beginnings of IF

"If I have seen further, it is by standing on the shoulders of Giants" is a sentence attributed to Sir Isaac Newton. In science, it is always wise to acknowledge the giants and their contributions, prior to delving into matters in one's own fields. On studying IF, we acknowledge the pivotal role of Albert H.Coons' works. For the first time, in 1942, Dr. Coons described a method for the detection of antigens in tissue cells using an antibody labeled with fluorescein as a specific histochemical stain, as described in "*The demonstration of Pneumococcal antigen in tissues by the use of fluorescent antibody.*" Dr. Coons and colleagues described the development and preparation of a fluorescein isocyanate later conjugated and linked to pneumococcal 3 antibody [1], which was used to identify pneumococcus 3 with fluorescence in ultra-violet light. Conjugates with bright green fluorescence were chosen given the rarity of such coloration in human tissue, and until today green remains as the chosen color in IF, including in renal pathology, whereas fluorescein isothiocyanate (FITC) is the most widely used fluorescent dye. Dr. Coons' experiments were interrupted during the war, but he continued his works, and his methods left an indelible mark in several fields, specifically in pathology and immunology, and their favorite child – renal pathology. An inspiring narrative about "The beginnings of immunofluorescence" is recounted by none less than Dr. Coons himself, addressing the American Association of Immunologists [2].

Robert C. Mellors was the first to apply the novel IF technique to kidney tissue [3]. He induced glomerulonephritis (GN) in animal models and proceeded to concomitant identification of glomerular IF in the nephritic animals. He deduced that: (1) nephrotoxic antibodies to the kidney centered in the glomeruli, more than in tubules; (2) antibodies to foreign antigens do not prefer a specific glomerular localization; and (3) within the glomeruli, antibodies preferred a distribution toward capillary tufts. He speculated that this method should permit the study of the role of antigen-antibody interactions in the pathogenesis of diseases such as GN, lupus erythematosus (SLE), and others.

From then on, experimental models led to a better understanding of the role that antigen-antibody interactions had in the development of GN, and IF was the primordial method to study those interactions. The importance of complement was also recognized. Masters in immunology – Frank Dixon, Fred Germuth, Robert McCluskey, Emil R. Unanue, and others, led the charge in developing the IF techniques and asserting its place in the study of kidney diseases [4, 5].

IF Methods and Techniques

The two main methods of antigen detection by IF are direct and indirect methods. Direct IF is the most used method in renal pathology diagnosis. It consists of applying an antibody directly conjugated with a fluorophobe, such as FITC (the fluorescent "stain") to bind to the target antigen. Direct IF is the routine method for detection of components in the traditional IF renal pathology panel – IgA, IgG, IgM, C3, C1q, albumin, fibrinogen, kappa, and lambda light chains. In indirect IF, there are two steps, in which first a primary antibody is bound to the desired antigen, followed by the detection of the primary antibody by a secondary antibody conjugated with a fluorophobe. The indirect technique is useful to increase sensitivity. In renal pathology, C4d staining is routinely visualized through an indirect method.

Most IF studies performed in renal pathology are based on detection of individual antibodies in a singleplex manner, with no more than one marker present in the same tissue section. Dual-plex IF is seen in rare occasions in the clinical setting, as in dual IF for alpha 2 (Texas red) and 5 (FITC) chains of collagen type IV in evaluating Alport disease. Multiplex IF allows the detection of more targets at once, in the same tissue section, and its current use is limited to research.

IF studies are routinely performed in frozen sections from fresh tissue or tissue preserved in a transport medium. Michel solution is the most commonly used transport medium, containing ammonium sulfate, N-ethylmaleimide, and magnesium sulfate in citrate buffer. It keeps the tissue preserved until ready for processing, when the tissue is washed in buffer and may be snap frozen. Alternatively, IF studies may be performed on formalin-fixed paraffin-embedded tissue following antibody retrieval methods (paraffin IF) and are well recognized as a salvage technique [6]. Paraffin IF has gained increased recognition as a method that may lead to "unmasking" of deposits. In our series, it made a significant contribution to diagnosis in more than one-third of cases when performed [7]. Paraffin IF is particularly helpful in the work-up of the lesions associated with

monoclonal glomerulopathy of renal significance (MGRS). The theory behind unmasking deposits using paraffin IF is that formalin fixation would cause protein cross-linking and an antigen retrieval step would "unmask" those proteins/antigens. Antigen retrieval is routinely used in surgical pathology with immunohistochemistry (IHC) stains, and this application in IF has a similar purpose. Common methods for paraffin IF include antigen retrieval with proteolytic enzymes (trypsin, pronase, proteinase) or through heat, and have been previously described [7, 8]. Indications for paraffin IF use will be discussed individually in the sections to follow.

Classically, visualization of the IF sections happens with a fluorescence microscope, in a dark room. Advances in digital pathology and scanning equipment are allowing the practice of interpreting IF remotely on a computer screen to become feasible and even routinely employed [9]. Artificial intelligence will eventually play a role in IF interpretation, and published data show that convolutional neural networks trained to classify "appearance," "distribution," "location," and "intensity" of glomerular deposits have diagnostic accuracy comparable with experienced pathologists [10].

The Diagnostic IF Toolbox: The Magnificent Seven and Their Companions

The standard IF panel used for diagnosis in renal pathology relies heavily on the interpretation of the presence and distribution of seven main antibodies that give insight into heavy and light chain immunoglobulin deposition – IgA, IgG, IgM, kappa, and lambda light chains – and complement activation – C3, C1q. In addition to that, the optimal panel includes albumin and fibrinogen [11, 12]. C4d is also included in allograft biopsies, primarily to evaluate peritubular capillaries, but its role in glomerular diseases has also been assessed, and it is essential for the diagnosis of the newly described C4 glomerulopathy (C4G) [13, 14]. Additional IF stains are employed as needed. IgG subclasses have been studied and advocated in a range of entities, from immune-complex mediated (IC-MPGN) diseases to paraprotein associated diseases, and in our experience, they are particularly helpful in the latter case [15, 16]. IF markers are routinely used in determining the etiology of membranous nephropathy (MN), particularly targeting phospholipase A2 receptor (PLA2R). IF and/or IHC may be performed to discover other antigens in MN [17, 18].

Nasr et al. [19] developed a heavy chain/light chain (HLC) tissue IF protocol to target conformational epitopes at the junctions of the heavy chain and light chain constant regions of immunoglobulins. The authors advocate HLC IF as a valuable technique for the diagnosis of monoclonal gammopathy-associated diseases, outperforming paraffin and IgG subclasses in confirming or ruling out monoclonal deposits. Table 1 summarizes additional IF ancillary methods and stains used in renal pathology. Finally, tissue submitted for IF may also be used when insufficient material is present in the light microscopy sections, and special stains and IHC may be applied to the tissue, after fixation and paraffin embedding, with a salvage purpose.

Reporting IF Findings

The report of the IF findings should include an assessment of the quality of the biopsy, including the number of glomeruli, and how many are globally sclerotic. The IF stains should be listed, and adequacy of controls should be documented. H&E or Diff-Quick stained sections accompany the IF slides and should be reviewed, with reporting of pertinent findings. Assessment of the morphology of the glomeruli, tubulointerstitium, and vessels in all stains may lead to valuable information. Polarization of the tissue may reveal cholesterol crystals dissolved in formalin-fixed paraffin-embedded tissue.

Interpretation of the IF studies is dependent on assessment of each individual stain. Detailed reports should provide the intensity, pattern, and distribution of staining. Intensity is to be scored, and clearly stated, with scales commonly ranging from "0 to 3+" or "0 to 4+". Descriptors as "trace" or intermediate descriptions (such as "1 to 2+") may be used, particularly when staining varies from one glomerulus to another in the same section. The most recent Mayo Clinic/Renal Pathology Society Consensus guideline [12] recommends an intensity scale of "negative, ±, 1+, 2+, and 3+." Patterns of staining are descriptive and include terms such as punctate, finely granular, coarsely granular, linear, and smudgy, shown in Figure 1. Distribution within the glomeruli should describe involvement of mesangium, capillary walls, and Bowman capsule. Extraglomerular distribution includes cell nuclei (in vivo ANA), tubular basement membranes (TBMs), interstitium, and vessels. In allograft biopsies, peritubular capillary staining for C4d, and the percentage of involvement is documented. Documentation of the findings using photomicrographs is encouraged but does not substitute the description. In photographing, particularly using digital methods, one should avoid over-

Table 1. Ancillary techniques and additional stains in IF in renal pathology

IF technique/ stain	Common indications and potential uses	Limitations
C4d (IF)	PTC evaluation in transplant, diagnosis of C4G, contributory in differential of C3G	Mild nonspecific glomerular stain may be observed in normal glomeruli, particularly in allografts. Intense staining may be seen in transplant glomerulopathy
Paraffin IF	Salvage technique; discrepancy with EM findings, rule out masked deposits – MGRS lesions, crystalline deposits, proliferative/MPGN pattern with negative IF, membranous pattern with negative/weak IgG, monotypic FGN, suspicion of MGRS-associated lesions with negative IF, C3G/C4G	High background (serum) may lead to false positives; not sensitive in anti-GBM cases. Weak staining in complement deposits, IgA and MN; and lower sensitivity in AL amyloidosis, PGNMID, and MIDD
IgG subclasses	MGRS lesions, particularly PGNMID and heavy chain MIDD, and monotypic anti-GBM.	Distinct antibody clones have variable sensitivity; use to distinguish "primary" or "secondary" MN disfavored, replaced by specific markers
HLC IF	Monotypic deposits, MGRS lesions, to confirm or rule out monoclonality	Limited availability and reported cases, reproducibility to be confirmed

PTC, peritubular capillaries; C4G, C4 glomerulopathies; C3G, C3 glomerulopathies; MGRS, monoclonal gammopathy of renal significance; MPGN, membranoproliferative; GBM, glomerular basement membrane; PGNMID, proliferative glomerulonephritis with monoclonal Ig deposits; MIDD, monoclonal immunoglobulin deposition diseases; HLC, heavy chain/light chain.

exposure, and maintain the images representative of what is observed.

Knowledge of distribution of the commonly utilized IF stains in the normal kidney tissue is important to prevent misinterpretation during examination. As some of the IF stains are naturally seen in the normal kidney, these function as positive internal controls. In some instances, staining may be present, but not necessarily carry diagnostic significance or represent immune-complex deposition, these should be documented, but care should be taken on interpretation (see Table 2).

Kappa and lambda light chains should always be compared with each other and demonstrate similar intensities, otherwise the possibility of a monoclonal protein should be entertained. Document any discrepancies in intensity, with scoring and detailed location – casts, basement membranes, protein droplets, glomeruli, vessels. Similarly, identification of an isolated heavy chain, or heavy chain accompanied by a restricted light chain in certain scenarios raises the possibility of monoclonality.

IF Findings in Glomerular Diseases

In this section, we will focus on the role of IF on a selection of glomerular diseases with significant IF findings. Figure 2 illustrates examples of IF use on glomerular diseases.

Podocytopathies

Podocytopathies in their multiple forms, including minimal changes disease (MCD) and focal segmental glomerulosclerosis (FSGS), were classically considered lesions in which IF was negative/noncontributory, or at most would show nonspecific staining with IgM and C3 associated with areas of sclerosis. Although there were clues indicating that an autoimmune etiology was involved in some cases of podocytopathies, this was not corroborated by observations on IF and EM studies. Recently, there has been a paradigm shift on the understanding of these disorders and in the significance of IF in the diagnosis, specifically in MCD and FSGS – Watts et al. [20] reported the discovery of autoantibodies targeting nephrin, which were reported in 29–44% of the patients with MCD, and 9% of cases of "primary" FSGS. The report also identified a delicate punctate staining for IgG in the podocytes of a subset of MCD patients who had positive anti-nephrin antibodies, and IgG colocalized with nephrin on confocal microscopy. The punctate deposits were not positive for albumin [21]. Since then, punctate glomerular IgG colocalizing with nephrin was described in transplant patients with recurrent podocytopathy [22, 23]. Based on these findings, it is advisable that pathologists take note of delicate punctate IgG deposits when examining MCD and FSGS cases, as they may serve as indicators of an autoimmune process and/or surrogates for a more

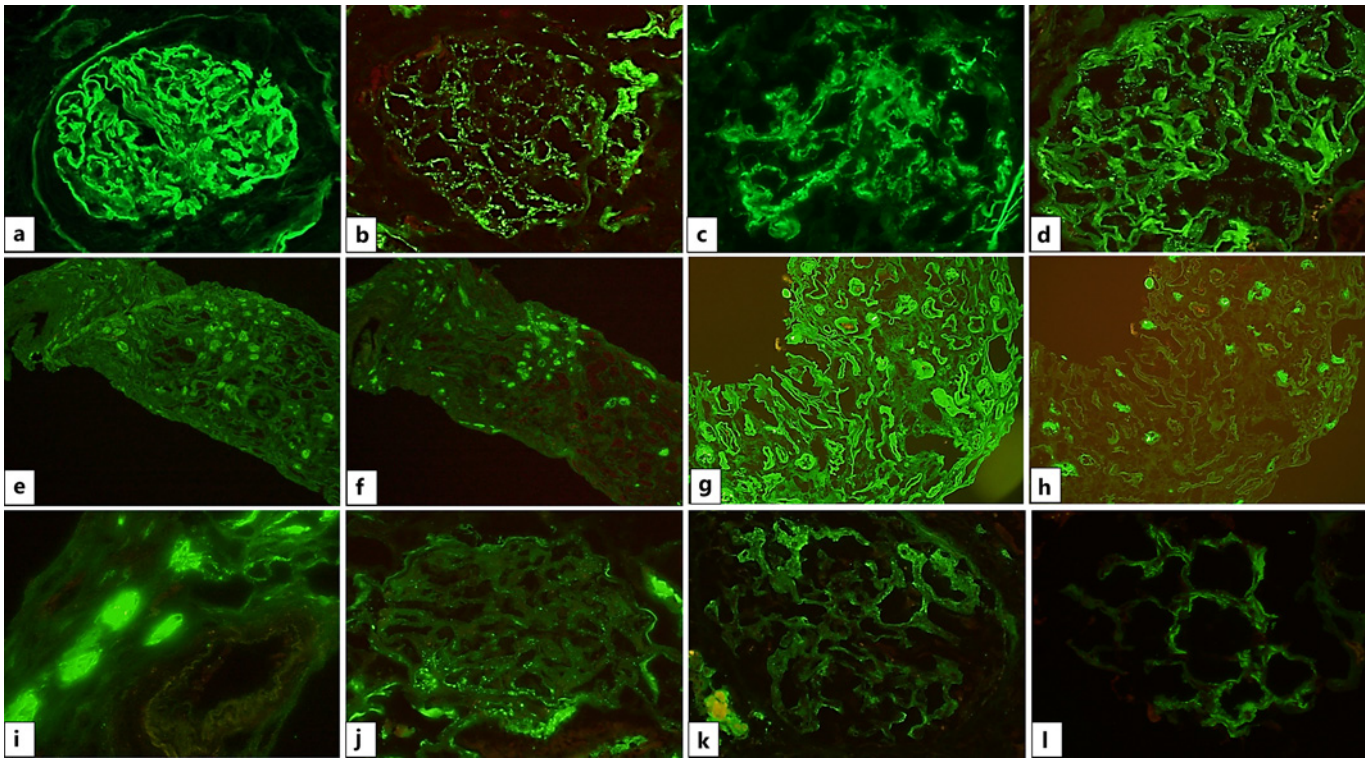

Fig. 1. Common glomerular IF patterns and pearls on IF interpretation. From (**a**–**d**) the common patterns of IF in the glomeruli include linear (**a**), granular (**b**), smudgy (**c**), and punctate (**d**), here seen respectively in anti-GBM disease, postinfectious GN, fibrillary GN, and minimal change disease (MCD). Kappa and lambda light chains should show similar intensity in the tissue as illustrated in (**e**, **f**). Remarkable difference in intensity of staining between the light chains or heavy chains supports MGRS lesions, as illustrated by the difference between kappa (**g**) and lambda (**h**) light chains in this case of kappa light chain deposition disease. Certain stains are observed normally in kidney tissue and may serve as internal control, such as IgA in tubuli (**i**) and C3 in vascular poles (**j**). Other stains may be nonspecific or have no significant or known clinical impact as IgM in FGS lesion (**k**), and C1q in transplant (**l**).

specific anti-nephrin test that is not yet widely available.

In the topic of podocytopathies, we will address the controversial entity of C1q nephropathy. Debate regarding the definition and meaning of C1q nephropathy has taken place, with some studies allowing for a broader presentation of the disease [24], while others argue that C1q nephropathy is within the spectrum of MCD/FSGS [25, 26]. Marked mesangial C1q staining on IF is necessary for the diagnosis, and in their initial cases series Jennette and Hipp adopted "dominant or co-dominant" C1q mesangial staining as diagnostic criteria. This was later revised to mesangial C1q ≥2+ (scale of 0 to 4+), with or without IgM and/or IgG, which may be the dominant stain. SLE should be excluded [27]. It remains to be proved if C1q deposition leads to glomerular injury or if it is a nonspecific marker in the setting of proteinuria. Adding to this debate, C1q-dominant mesangial deposition is often observed in renal allografts with no apparent clinical significance [28], and it should not be overemphasized in these circumstances.

IgA Nephropathy

The hallmark of IgA nephropathy (IgAN) is dominant or codominant mesangial glomerular deposition of IgA, thought to be related to higher levels of circulating galactose-deficient IgA1 which deposit on mesangial areas. IgAN used to be defined by the presence of 2+ or greater IgA (out of 0 to 4+) in the absence of lupus [29], but updated criteria described in the Oxford classification of IgAN focus on dominant staining rather than the intensity of the staining [30]. IgAN is thus defined as dominant or codominant staining, with IgA in glomeruli more than trace in intensity, even if not all glomeruli are positive. SLE-related nephritis should be absent. IgA deposits have mesangium localization, with or without capillary loop staining. IgG and IgM may be present, but

Table 2. Normal or nonspecific distribution of IF stains in the renal biopsy

IF staining	Location and/or scenario	Interpretation notes
IgA	Tubular casts	Normal, internal control
IgG	Areas of fibrinoid necrosis in ANCA	Nonspecific, pitfall
IgG	Linear accentuation of TBMs an GBMs in diabetes	Characteristic, auxiliary on diagnosis, no clinical significance if intensity similar to albumin
IgM	Areas of segmental sclerosis or hyalinosis	Commonly seen, nonspecific
C3	Blood vessels, vascular poles	Normal, internal control
C3	Areas of segmental sclerosis or hyalinosis	Commonly seen, nonspecific
C1q	Areas of segmental sclerosis or hyalinosis	Occasionally seen, nonspecific
C1q	Mesangium, transplant	Commonly seen, no known clinical significance
Fibrinogen	Widespread deposition or along the edges of tissue core	Nonspecific, pitfall
Albumin	Linear accentuation of TBMs an GBMs in diabetes	Characteristic, auxiliary on diagnosis, intensity similar to IgG
Albumin	Tubular reabsorption protein droplets	Characteristic, intense on proteinuric diseases
Kappa and lambda light chains[a]	Tubular reabsorption protein droplets	Characteristic, intense on proteinuric diseases
Kappa and lambda light chains[a]	Tubular casts	Normal, internal control

[a]Kappa and lambda light chains present with similar intensity.

not more intense than IgA, and have been reported in 43% and 54% of cases, respectively. IgM may be prominent in areas of sclerosis or hyalinosis, usually with a focal and/or segmental distribution [31].

Further studies in the Oxford classification patient cohort [32] suggested that capillary wall IgA deposits and presence of IgG were associated with a higher mesangial cellularity score and endocapillary proliferation, but not with loss of renal function. In a series from Shin et al. [33] IgG deposits were independently associated with poor renal outcomes in patients with IgAN and correlated with histological activity and clinical severity. These findings were contested by Turgutalp et al. [34] in a series in which glomerular IgG positivity was not associated with poor renal prognostic risk factors.

IgAN may be secondary to several forms of injury, and some studies have focused on distinguishing a primary form of IgAN from secondary forms, but a definitive answer to this conundrum has not been found yet. IF for galactose-deficient IgA1 showed mesangial deposits in primary IgAN but not in secondary IgA deposition after HCV infection and cirrhosis. The use of this marker for this purpose was currently not advised, and further studies are needed to determine its utility [35]. When comparing primary IgAN to infection-related IgAN, the presence of IgA deposits in sclerotic glomeruli has been reported to strongly favor primary IgAN; conversely, negative staining in the sclerotic glomeruli is seen in about a third of cases with primary IgAN, and only slightly favors infection-related IgA deposits [36]. Mild-to-moderate IgA staining and moderate-to-strong C3 were observed more frequently in patients with staphylococcus infection compared to primary IgAN biopsies where IgA staining is moderate-to-strong (2+ to 3+), with weaker C3 staining; however, there is a spectrum of findings in both situations [37].

Studies on the role of complement in the pathogenesis of IgAN point toward activation of the alternative and lectin pathways. Therefore, C3 is often present in IgAN and may be intense, while C1q staining is rare, and raises the possibility of lupus nephritis. Glomerular C4d and absent C1q can be seen in up to 40% of patients,

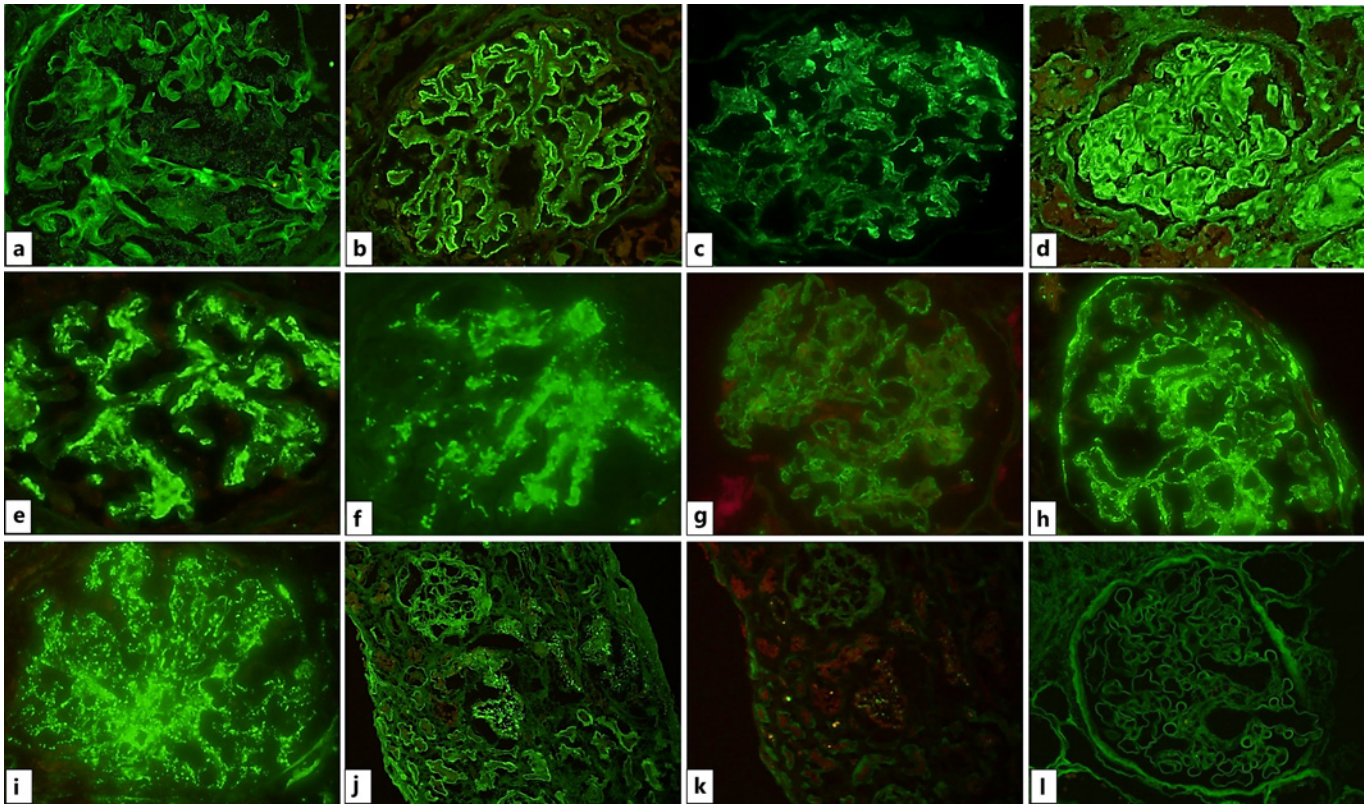

Fig. 2. Examples on IF use in glomerular disease. In podocytopathies, punctate IgG deposits in podocytes (**a**) may suggest anti-nephrin antibodies. PLA2R-positive MN has usually global and diffuse capillary loop staining with IgG (**b**), while in NELL1 positive MN, IgG may be segmental (**c**). Extraglomerular staining is more often seen in MN associated with SLE (**d**). Primary IgAN usually shows mesangial deposits (**e**) which may be accompanied by C3 (**f**), of same or less intensity. In IgA deposits secondary to MRSA, IgA (**g**) may be less intense than C3 (**h**) and extends into capillary loops. However, the IF features alone do not confidently distinguish primary IgA versus secondary causes. In images, a case of C3G associated with MGRS shows intense C3 (**i**), with background kappa predominance in proximal tubules (**j**) when compared with lambda (**l**) and paraffin IF negative for IgG (**l**) or other possible masked Igs, confirming C3G diagnosis.

supporting lectin pathway activation. Investigation of other elements of the complement system showed presence of properdin and C5b-9 on mesangial deposits, and other factors were also studied but currently are not used for diagnostic purposes. New therapies targeting complement activation in IgAN may lead to changes in IF use over time [30, 38]. IgAN may show light chain restriction, most often lambda type, and there is an ongoing debate on the significance of light chain restriction. Close to 10% of IgAN cases appear to display mono-isotypic mesangial deposits, without circulating monoclonal proteins nor other significant clinical or pathological differences from cases with mesangial polyclonal IgA [39]. Other studies have suggested that light chain restriction, particularly kappa light chain type, may lead to a proliferative GN with monoclonal IgA deposits (IgA-PGNMID) which shares common features with proliferative GN with monoclonal IgG deposits (IgG-PGNMID) [40]. Nasr et al. [19] showed recently through use of HLC antibodies testing that half of cases of monotypic IgAN had monoclonal deposits excluded by revealing staining for IgAκ and IgAλ with this technique.

Membranous Nephropathy

IF in MN shows characteristic presence of granular subepithelial deposits of IgG and C3, with or without lesser amounts of IgM or IgA, and uncommonly C1q. Full house staining is more often seen in lupus nephritis or autoimmune etiology. In the majority of cases, the distribution of the deposits is diffuse and global, but segmental MN is also encountered, associated with certain etiologies.

MN was used to be classified as either primary (idiopathic) or secondary, but this current classification

favors an approach geared toward the identification of specific target antigens and antibodies leading to MN. While the discovery of the antigens involves refined techniques combining laser microdissection and mass spectrometry, IF as well as IHC are helpful in identifying the markers in the glomeruli. PLA2R is the most frequently targeted autoantigen in MN, and it can be identified per IF, being performed routinely in many practices, with protocols widely available [18]. Neural epidermal factor growth-like 1 protein (NELL1) and thrombospondin type 1 domain-containing 7A (THSD7A) are the most common antigens, following PLA2R, and are also somewhat routinely available. A gamut of other antigens has been identified, most commonly identified by IHC rather than IF. In interpreting IF, some clues may lead to possible targets – segmental IgG is more frequent in NELL1-positive MN, extraglomerular staining is more often seen in lupus and markers associated with SLE and may also be seen in children. PLA2R-positive MN shows predominance of IgG4 subclass, which is not always the case in other causes of MN; however, if the specific antigen can be tested that would be preferred over IgG subclasses testing. A summary of their pattern of staining, predominant IgG subclass, other pathologic features, and clinical associations can be found on Table 3. Due to the large number of target antigens identified in MN, a targeted approach based on clinical or other morphological features optimizes the selection of markers. Mass spectrometry has been proposed as a substitute for the identification of such antigens, but this approach is not yet disseminated [41, 42]. Light chain restriction in MN is rare, and the largest series evaluating such cases indicates investigation for lymphopoliferative disorders, in cases with combined light chain restriction, negative PLA2R and IgG subclass restriction [43].

Lupus

The epitome of immune-complex deposition in the kidney is found in lupus cases. Immune deposits are widely distributed, and IF positivity is necessary for the diagnosis and contributory to the classification of lupus nephritis. IF in the most active forms is characteristically represented by the "full house" (FH) pattern of staining: three of a kind (immunoglobulins–IgG, IgA, and IgM) plus two of a kind (complement factors–C3 and C1q). Light chains are present, accompanying their heavy chain counterparts, and fibrinogen may be striking in areas of crescents, fibrinoid necrosis, and microangiopathic changes. The pattern of staining in the glomeruli is diffuse and global in most glomeruli, and the localization will depend on the form of lupus nephritis – ranging from mesangial staining only in LN classes I and II, to membranous type granular capillary loop staining in LN class V, to a combination of mesangial and capillary loop with subendothelial type deposits and hyaline intracapillary deposits in classes III and IV [44].

It is important to keep in mind that there is wide variation in the presentation of lupus, both clinically and histologically, and the IF follows this varied pattern. FH staining in lupus is present in the majority of LN, but this varies according to LN class, ranging from 38% in class II to 87% in class IV in a series by Kudose et al. [45] In the same series, up to 10% of all 560 non-lupus biopsies had FH pattern of staining. While we believe that the diagnosis of lupus should precede that of lupus nephritis, the above study showed that only a combination of IF with other morphologic findings would have robust sensitivity and specificity for a diagnosis of lupus based on biopsy alone. Five morphologic features that together favor the diagnosis of lupus include FH staining, intense C1q staining, extraglomerular deposits, tubuloreticular inclusions, and combined subendothelial and subepithelial deposits. Of note, the authors emphasize that some non-lupus cases also presented such features, including cases of HCV-associated immune-complex-mediated GN, IgM-dominant membranoproliferative GN (MPGN) in the setting of low-grade lymphoma, fibrillary GN (FGN), and infection-related GN, and they advise for full clinical, pathologic and laboratorial evaluation for proper diagnosis.

While FH staining is not per se sufficient for a diagnosis of LN, non-full house staining presents a conundrum particularly in cases of MLN. A series from Ye et al. [46] showed 12.6% incidence of non-full house MLN among all MLN biopsies, and those cases happened in older patients and with fewer extrarenal manifestations. Those biopsies had a lower intensity of C3 glomerular deposits, and only 1 of 11 cases were PLA2R positive when testing was performed. Of note, PLA2R positivity is rare in MLN, in just about 5% of cases [47]. Currently only about 1/3 of all MLN cases have known target antigens–exostosin 1/exostosin 2 complex (EXT1/2), neural cell adhesion-molecule 1, and transforming growth factor β receptor 3. Of these, EXT1/EXT2 have been associated with favorable outcomes in patients with MLN [48, 49].

Immune-complex deposition in lupus cases may extend to vessels and/or the tubulointerstitium compartment, with extraglomerular deposition in 40–80% of cases [45, 50]. Cell nuclei may be positive – in vivo *ANA*. TBM deposits may be limited to proximal or non-proximal tubules, or involve both non-proximal and proximal tubules, and IF is more sensitive in detecting

Table 3. Features of antigens and deposits in membranous nephropathy and associated clinical conditions

Antigen	Distribution of IgG deposits	Predominant IgG subclass	Additional deposits on IF	Clinical associations
PLA2R	Global	IgG4	–	None
HTRA1	Global, rare segmental	IgG4	–	None
NTGN1	Global	IgG4	–	None
THSD7A	Global	IgG4	–	Malignancy
NELL1	Segmental or global	IgG1	–	Malignancy
SEMA3B	Global	IgG1	Mesangial, TBM	Children
PCDH7	Global	Variable	–	Older patients
EXT1/ EXT2	Global	IgG1	Mesangial, IgM frequent	SLE, autoimmune
NCAM1	Global	Variable	Mesangial, full house frequent	SLE
TGFBR3	Global	Variable, IgG4 usually absent	Mesangial, TBM	SLE, neuropsychiatric symptoms
CNTN1	Global	IgG4	–	CIDP
FAT1	Global	IgG4	TBM	HSCT, ABMR in kidney tx
NDNF	Global, "lumpy"	Variable, IgG4 usually absent	–	Syphilis
PCSK6	Global	IgG1 and IgG4	–	NSAIDs

HTRA1, serine protease HTRA1; NTNG, netrin G1; SEMA3B, semaphorin 3B; PCDH7, protocadherin 7; EXT1/2, Exostosins 1 and 2; NCAM1, neural cell adhesion-molecule 1; TGFBR3, transforming growth factor beta receptor 3; CNTN1, contactin 1; CIPD, chronic inflammatory demyelinating polyradiculoneuropathy; FAT1, protocadherin FAT1; HSCT, hematopoietic stem cell transplant; NDNF, neuron-derived neurotrophic factor; and PCSK6, proprotein convertase subtilisin/kexin type 6.

these deposits than EM. Curiously, the IgG subclasses on the TBMs and vascular deposits differ from the subclasses in the glomeruli in a majority of LN biopsies, pointing to a different set of antibodies involved in these pathologies [51].

Activation of the complement system in lupus is well known, and complement serum levels are usually low in lupus flares. In the biopsy, C3 and C1q are positive in the majority of the cases and are related with activity. C4d is not usually assessed in LN, but is also positive in the glomeruli, and might be seen in extraglomerular locations as well. C4d deposits in the TBMs have been associated with activity, and vascular C4d deposition with worse renal outcomes [52].

Anti-Glomerular Basement Membrane

Anti-glomerular basement membrane (anti-GBM) disease is classically defined and distinguished from other forms of crescentic GN based on the linear pattern of staining observed along glomerular basement membranes (GBMs) on IF studies. Linear IgG is the most common immunoglobulin, with diffuse and strong staining pattern. IgA or IgM linear staining is much less common. C3 is usually positive, in lesser intensity, and often discontinuous. Due to the very severe and destructive nature of the disease, one caveat on the interpretation of IF stains rests in the identification of relatively intact capillary loops allowing for identification of the linear staining, as glomerular capillary loops may be almost completely destroyed by crescent formation [53].

An "atypical" form of anti-GBM was described in 2016 by Nasr et al. [54] Oddly, it may present with negative serologic anti-GBM studies and histologically it does not show a diffuse crescentic GN. Regarding IF studies, about half of cases show monotypic Ig staining. Although circulating monoclonal proteins matching the deposits on the biopsy are not identified in most of those cases, the monotypic variant of atypical anti-GBM nephritis is considered as a form of MGRS-associated lesions. This

monotypic variant of atypical anti-GBM was shown to recur in transplants, with IF staining pattern similar to the native biopsy [55].

Complement-Mediated Glomerulopathies

C3 glomerulopathies (C3G) encompass the diseases mediated by activation of the alternative pathway of the complement cascade, including C3GN and dense deposit disease (DDD), which are distinguished from each other based on EM findings. IF studies are essential for the diagnosis of C3G, which requires C3 dominant or codominant deposits in the glomeruli, defined as C3 staining stronger than the other reactants, by at least 2+ levels of intensity (0 to 3+ scale) [56]. Immunoglobulins may be present, in smaller amounts. Adoption of stricter criteria requiring isolated C3 deposits would ignore the known presence of Igs described on DDD. Walker et al. [57] reported presence of IgG in about one-third of DDD cases with mesangial proliferative or acute proliferative presentations. Discarding cases with low levels of Igs deposition would also lead to low sensitivity in identifying C3G patients [58]. Localization of the deposits within glomeruli in C3G is variable. Granular deposits may be concentrated in the mesangial areas, with variable extension to the capillary loops. Areas of sclerosis stain even more intensely with C3, and for this reason a diagnosis of C3G should not be based on observing sclerotic or segmentally sclerotic glomeruli only, but include intact glomeruli. C3 deposits in DDD were classically described as railroad tracks along the GBMs and circular rings in the mesangium [59], but less distinct mesangial and capillary loop deposits may be seen. C3 deposition in the Bowman's capsule and TBMs has also long been described in DDD [60].

C1q is usually absent; however, it was previously identified in few cases of DDD, and its presence does not rule out C3G [56, 57]. There are divergent studies regarding the value of C4d staining to distinguish C3G from other forms of GN. Sethi et al. [61] proposed that negative C4d helps confirm the diagnosis of C3G, while a positive C4d staining, equal to or more intense than C3, would favor other IC-MPGNs, but other authors contest those findings, showing C3G cases with significant C4d deposition, although these latter works used IHC rather than IF for C4d staining [62]. C3G may be related to a monoclonal gammopathy–an MGRS lesion. However, most C3G cases with a monoclonal Ig do not show monoclonality in the biopsy, and present isolated C3 deposits with minimal or no other significant staining [63]. Nevertheless, if there is suspicion of MGRS in C3G cases, paraffin IF is suggested to unmask possible deposits.

A rarer form of complement-mediated glomerulopathy, C4G, was described by Sethi et al. [14] and the diagnosis relies on the presence of bright C4d staining, without significant C3 or immunoglobulin deposits. Similarly to C3G, the authors described two forms of the disease–C4 DDD and C4 GN. Mass spectrometry studies showed spectra matching C4, supporting the IF observations. The authors noted that mild C4d positivity can be present in normal glomeruli, particularly in allografts, and bright (3+) staining is necessary to consider a C4G diagnosis. In allografts with transplant glomerulopathy, C4d positivity may be strong; however, there are no electron dense deposits identified on EM, in contrast to C4G. As in C3G, the use of paraffin IF is recommended to rule out "masked" deposits.

Membranoproliferative Glomerular Pattern of Injury

MPGN was a reference to glomerular disorders characterized by capillary wall thickening, with double contours, mesangial expansion, and mesangial, and endocapillary hypercellularity. Historically, biopsies with this morphology were classified as MPGN types I, II, and III, based on ultrastructural findings [64]. Currently, the terminology "Membranoproliferative Glomerular Pattern of Injury" (MPGN pattern) is preferred to describe a group of entities with this morphologic pattern caused by diverse etiologies [65]. IF is crucial in searching for an etiology driven classification of MPGN pattern, as a new classification approach defines three categories: immunoglobulin or IC-MPGN, complement mediated, and MPGN pattern without Ig or complement deposits. The first category includes cases with IF positive for immunoglobulins, with or without complement deposits. The main etiologies in this case are infections, auto-immune diseases, and MGRS lesions. If an etiology is not identified, it may be considered idiopathic IC-MPGN. In biopsies with a complement dominant pattern, C3G or C4G should be considered. Negative IF raises several differential diagnoses, most prominently thrombotic microangiopathy (TMA).

In IC-MPGN, the nature of the Igs deposited depends on the etiology; hence, an MPGN form of lupus nephritis may show "full house" pattern of staining, while MGRS lesions might show Ig restriction. Infection-associated MPGN is one of the common manifestations of hepatitis C, with or without cryoglobulinemia and there is often presence of IgG, IgM, and C3 in glomeruli [66]. One of the main features of IC-MPGNs is the presence of subendothelial deposits, in addition to mesangial

deposits. On IF, the subendothelial deposits are seen along the capillary loops and may be granular or adopt a more rounded contour, as they are bound by the GBMs. Intracapillary deposits, equivalent to hyaline deposits or pseudo-thrombi, are usually positive on IF, and may be seen in cryoglobulinemic GN and others.

Monoclonal Gammopathy of Renal Significance

MGRS refers to any B cell or plasma cell clonal lymphoproliferation with kidney lesion(s) related to a nephrotoxic monoclonal immunoglobulin, without tumor complications or haematological criteria for specific therapy. Renal diseases associated with MGRS are referred to as MGRS-associated lesions and have a broad morphologic spectrum.

MGRS lesions may also be seen in a full-blown malignancy, such as MM. Involvement of glomeruli may be isolated in MGRS or may be seen along with injury to vascular and/or tubulointerstitial compartments. Presentations of MGRS affecting the glomeruli include amyloidosis Ig-related, monoclonal immunoglobulin deposition disease (MIDD), cryoglobulinemic GN (types I and II), proliferative GN with monoclonal immunoglobulin deposits (PGNMID), immunotactoid GN ITG, C3G, TMA, and diverse forms of crystalline nephropathies with glomerular involvement [67].

It is beyond our scope to review in detail each of these lesions, but considerations on IF use in MGRS will be highlighted. The first consideration is that IF plays a major role in determining a diagnosis of MGRS. In general, the renal deposits will present as monotypic/monoclonal, with restriction of light chains, heavy chains, or both heavy and light =chains. "Restriction" is generally accepted as a dominant staining limited to a specific Ig, with more than 2+ difference of staining compared to the remaining panel. In most cases, the deposits in the biopsy mirror the circulating monoclonal immunoglobulin. Based on the restricted Ig, certain MGRS lesions such as amyloidosis and MIDD can be further divided into subtypes – light chain, heavy chain, or heavy and light chain amyloidosis (AL, AH, AHL) and MIDD, respectively (LCDD, HCDD, and HLCDD). However, not all MGRS lesions have monoclonal deposits on IF. Immunotactoid glomerulopathy usually has deposition of IgG and C3, with or without light chain restriction, and is polyclonal in a third of cases, although it is associated with lymphoproliferative disorders in a quarter of those [68]. C3G and TMA associated to MGRS do not necessarily have monoclonal deposition identified in the biopsy and may look morphologically indistinguishable from other etiologies. Conversely, certain MGRS lesions are distinctly well known for the difficulty of encountering a serologic counterpart for the nephrotoxic monoclonal Ig. PGNMID has no detectable monoclonal protein in up to 2/3 of cases, even after long-term follow-up [69]. Nasr et al. [70] reported that PGNMID with deposition of monoclonal immunoglobulin light chain only (PGNMID-light chain) has a much higher frequency of a detectable pathogenic plasma cell clone than cases with a heavy chain present, with a detectable bone marrow plasma cell clone in 88% of the PGNMID-light chain cases in their series. In addition to the traditional IF panel, other IF stains and techniques are often valuable in diagnosing MGRS. IgG subclasses may confirm deposition of an intact monoclonal/monotypic IgG. IgG3 kappa is the most common form seen in PGNIMD, and IgG subclasses are routinely used in that diagnosis. As mentioned, a HLC IF protocol may be used in confirming or ruling out monoclonal deposits [19]. Finally, paraffin IF is of paramount importance in evaluating suspicious MGRS cases, particularly in "unmasking" of deposits. Crystalline nephropathies tend to have "masked" crystal deposits, which are promptly discoverable with paraffin IF. MPGN with masked monotypic Ig deposits requires paraffin IF for diagnosis. IF with isolated C3G deposits should be followed by paraffin IF, in a quest to unmask deposits. Paraffin IF may also "unmask" polyclonal deposits in instances when MGRS is suspected. On a study of DNAJB9-positive FGN cases with monotypic IF findings, Said et al. [71] demonstrated that most cases with lambda restriction had polyclonal deposits on paraffin IF. Combining paraffin IF and IgG subclasses when considering monoclonal FGN shows that DNAJB9-positive monotypic FGN is very rare (0.7% of cases) and not usually associated with monoclonal gammopathy. In our experience, paraffin IF sensitivity is lower in cases of amyloidosis and MIDD, a finding confirmed by others [8].

A caveat in interpreting IF in the setting of MGRS is that the usual IF renal panel in fact reveals mono-isotypic rather than true monoclonal deposits, although the terms are often used interchangeably. Another caveat is that not all cases of MGRS are revealed by IF, despite multiple efforts, including paraffin IF and others. Further ancillary methods should be used when the suspicion of MGRS is in question. Mass spectrometry (MS) has proven to be particularly helpful in cases of amyloidosis, when typing cannot be determined by IF alone.

Membranous-Like Glomerulopathy with Masked IgG Kappa Deposits

While paraffin IF is helpful in several situations in renal pathology, it is absolutely required for the diagnosis of membranous-like glomerulopathy with masked IgG

kappa deposits (MGMID). MGMID is a distinct entity, first described by Larsen et al. [72], which shows conventional IF positive for isolated C3, with no significant Igs staining. Electron microscopy reveals bodacious deposits, often with a "hump" appearance. Once paraffin IF is performed, it "unmasks" IgG and kappa. The pattern of staining was first described as membranous-like (hence the name), but later on, a variation on the number and mesangial and subepithelial location of deposits was appreciated. Critically, most patients do not have a monoclonal gammopathy. They are mostly young, predominantly female, and may present vague auto-immune phenomena. Later it was shown that serum amyloid P is a sensitive and specific marker of the disorder. Using serum amyloid P as a marker for MGMID, an additional group of patients with similar features was discovered, and in those cases, the deposits were unmasked and consisted of IgG1 kappa deposits [73]. There are potential pitfalls in diagnosing this entity: the initial IF aspect with isolated C3-only deposits may lead to an incorrect diagnosis of C3G, or infection-associated GN due to the hump deposits on EM. One should be familiar with the entity and use of paraffin IF to avoid interpreting it as a diagnostic MGRS lesion.

Conclusion

The use of IF in glomerular diseases has a remarkable history and is evolving toward a bright future. Today as ever, knowledge of classic IF uses, including pitfalls of staining and nonspecific staining patterns, and the acknowledgment of masked antigens, will allow proper interpretation of the results. The adoption of novel technologies and techniques available for further work-up will guarantee that IF keeps its distinguished diagnostic value and provides ever more information for the better understanding of glomerular diseases and benefits for our patients.

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Conflict of Interest Statement

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Author Contributions

Nidia Messias conceptualized the review article, reviewed the literature, acquired images, and wrote the manuscript.

References

1 Coons AH, Creech HJ, Jones RN, Berliner E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. J Immunol. 1942;45(3):159–70. https://doi.org/10.4049/jimmunol.45.3.159

2 Coons AH. The beginnings of immunofluorescence. J Immunol. 1961;87(5):499–503. https://doi.org/10.4049/jimmunol.87.5.499

3 Mellors RC. Histochemical demonstration of the in vivo localization of antibodies: antigenic components of the kidney and the pathogenesis of glomerulonephritis. J Histochem Cytochem. 1955;3(4):284–9. https://doi.org/10.1177/3.4.284

4 Weening JJ, Jennette JC. Historical milestones in renal pathology. Virchows Arch. 2012;461(1):3–11. https://doi.org/10.1007/s00428-012-1254-7

5 Unanue ER. Starting in immunology by way of immunopathology. Annu Rev Pathol. 2011;6:1–18. https://doi.org/10.1146/annurev-pathol-011110-130300

6 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

7 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

8 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

9 L'Imperio V, Brambilla V, Cazzaniga G, Ferrario F, Nebuloni M, Pagni F. Digital pathology for the routine diagnosis of renal diseases: a standard model. J Nephrol. 2021;34(3):681–8. https://doi.org/10.1007/s40620-020-00805-1

10 Ligabue G, Pollastri F, Fontana F, Leonelli M, Furci L, Giovanella S, et al. Evaluation of the classification accuracy of the kidney biopsy direct immunofluorescence through convolutional neural networks. Clin J Am Soc Nephrol. 2020;15(10):1445–54. https://doi.org/10.2215/CJN.03210320

11 Chang A, Gibson IW, Cohen AH, Weening JW, Jennette JC, Fogo AB, et al. A position paper on standardizing the nonneoplastic kidney biopsy report. Hum Pathol. 2012;43(8):1192–6. https://doi.org/10.1016/j.humpath.2012.04.009

12 Sethi S, Haas M, Markowitz GS, D'Agati VD, Rennke HG, Jennette JC, et al. Mayo clinic/renal pathology society consensus report on pathologic classification, diagnosis, and reporting of GN. J Am Soc Nephrol. 2016;27(5):1278–87. https://doi.org/10.1681/ASN.2015060612

13 Drachenberg CB, Papadimitriou JC, Chandra P, Haririan A, Mendley S, Weir MR, et al. Epidemiology and pathophysiology of glomerular C4d staining in native kidney biopsies. Kidney Int Rep. 2019;4(11):1555–67. https://doi.org/10.1016/j.ekir.2019.07.015

14 Sethi S, Quint PS, O'Seaghdha CM, Fervenza FC, Bijol V, Dorman A, et al. C4 glomerulopathy: a disease entity associated with C4d deposition. Am J Kidney Dis. 2016;67(6):949–53. https://doi.org/10.1053/j.ajkd.2016.01.012
15 Bannister KM, Howarth GS, Clarkson AR, Woodroffe AJ. Glomerular IgG subclass distribution in human glomerulonephritis. Clin Nephrol. 1983;19(4):161–5.
16 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
17 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
18 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
19 Nasr SH, Fidler ME, Said SM, Koepplin JW, Altamirano-Alonso JM, Leung N. Immunofluorescence staining for immunoglobulin heavy chain/light chain on kidney biopsies is a valuable ancillary technique for the diagnosis of monoclonal gammopathy-associated kidney diseases. Kidney Int. 2021;100(1):155–70. https://doi.org/10.1016/j.kint.2021.02.038
20 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
21 Hengel FE, Dehde S, Lassé M, Zahner G, Seifert L, Schnarre A, et al. Autoantibodies targeting nephrin in podocytopathies. N Engl J Med. 2024;391(5):422–33. https://doi.org/10.1056/NEJMoa2314471
22 Shirai Y, Miura K, Ishizuka K, Ando T, Kanda S, Hashimoto J, et al. A multi-institutional study found a possible role of anti-nephrin antibodies in post-transplant focal segmental glomerulosclerosis recurrence. Kidney Int. 2024;105(3):608–17. https://doi.org/10.1016/j.kint.2023.11.022
23 Batal I, Watts AJB, Gibier J-B, Hamroun A, Top I, Provot F, et al. Pre-transplant anti-nephrin antibodies are specific predictors of recurrent diffuse podocytopathy in the kidney allograft. Kidney Int. 2024;106(4):749–52. https://doi.org/10.1016/j.kint.2024.07.022
24 Jennette JC, Hipp CG. C1q nephropathy: a distinct pathologic entity usually causing nephrotic syndrome. Am J Kidney Dis. 1985;6(2):103–10. https://doi.org/10.1016/s0272-6386(85)80150-5
25 Iskandar SS, Browning MC, Lorentz WB. C1q nephropathy: a pediatric clinicopathologic study. Am J Kidney Dis. 1991;18(4):459–65. https://doi.org/10.1016/s0272-6386(12)80114-4
26 Markowitz GS, Schwimmer JA, Stokes MB, Nasr S, Seigle RL, Valeri AM, et al. C1q nephropathy: a variant of focal segmental glomerulosclerosis. Kidney Int. 2003;64(4):1232–40. https://doi.org/10.1046/j.1523-1755.2003.00218.x
27 Vizjak A, Ferluga D, Rozic M, Hvala A, Lindic J, Levart TK, et al. Pathology, clinical presentations, and outcomes of C1q nephropathy. J Am Soc Nephrol. 2008;19(11):2237–44. https://doi.org/10.1681/ASN.2007080929
28 Said SM, Cornell LD, Valeri AM, Sethi S, Fidler ME, Cosio FG, et al. C1q deposition in the renal allograft: a report of 24 cases. Mod Pathol. 2010;23(8):1080–8. https://doi.org/10.1038/modpathol.2010.92
29 Jennette JC. The immunohistology of IgA nephropathy. Am J Kidney Dis. 1988;12(5):348–52. https://doi.org/10.1016/s0272-6386(88)80022-2
30 Working Group of the International IgA Nephropathy Network and the Renal Pathology Society; Roberts ISD, Cook HT, Troyanov S, Alpers CE, Amore A, et al. The Oxford classification of IgA nephropathy: pathology definitions, correlations, and reproducibility. Kidney Int. 2009;76(5):546–56. https://doi.org/10.1038/ki.2009.168
31 Magistroni R, D'Agati VD, Appel GB, Kiryluk K. New developments in the genetics, pathogenesis, and therapy of IgA nephropathy. Kidney Int. 2015;88(5):974–89. https://doi.org/10.1038/ki.2015.252
32 Bellur SS, Troyanov S, Cook HT, Roberts ISD; Working Group of International IgA Nephropathy Network and Renal Pathology Society. Immunostaining findings in IgA nephropathy: correlation with histology and clinical outcome in the Oxford classification patient cohort. Nephrol Dial Transpl. 2011;26(8):2533–6. https://doi.org/10.1093/ndt/gfq812
33 Shin DH, Lim BJ, Han IM, Han SG, Kwon YE, Park KS, et al. Glomerular IgG deposition predicts renal outcome in patients with IgA nephropathy. Mod Pathol. 2016;29(7):743–52. https://doi.org/10.1038/modpathol.2016.77
34 Turgutalp K, Cebeci E, Turkmen A, Derici U, Seyahi N, Eren N, et al. The relationship between glomerular IgG staining and poor prognostic findings in patients with IgA nephropathy: the data from TSN-GOLD working group. BMC Nephrol. 2021;22(1):352. https://doi.org/10.1186/s12882-021-02560-2
35 Suzuki H, Yasutake J, Makita Y, Tanbo Y, Yamasaki K, Sofue T, et al. IgA nephropathy and IgA vasculitis with nephritis have a shared feature involving galactose-deficient IgA1-oriented pathogenesis. Kidney Int. 2018;93(3):700–5. https://doi.org/10.1016/j.kint.2017.10.019
36 Brodsky SV, Nadasdy T, Cassol C, Satoskar A. IgA staining patterns differentiate between IgA nephropathy and IgA-dominant infection-associated glomerulonephritis. Kidney Int Rep. 2020;5(6):909–11. https://doi.org/10.1016/j.ekir.2020.03.029
37 Satoskar AA, Suleiman S, Ayoub I, Hemminger J, Parikh S, Brodsky SV, et al. Staphylococcus infection-associated GN - spectrum of IgA staining and prevalence of ANCA in a single-center cohort. Clin J Am Soc Nephrol. 2017;12(1):39–49. https://doi.org/10.2215/CJN.05070516
38 Medjeral-Thomas NR, Cook HT, Pickering MC. Complement activation in IgA nephropathy. Semin Immunopathol. 2021;43(5):679–90. https://doi.org/10.1007/s00281-021-00882-9
39 Nagae H, Tsuchimoto A, Tsuruya K, Kawahara S, Shimomura Y, Noguchi H, et al. Clinicopathological significance of monoclonal IgA deposition in patients with IgA nephropathy. Clin Exp Nephrol. 2017;21(2):266–74. https://doi.org/10.1007/s10157-016-1275-7
40 Vignon M, Cohen C, Faguer S, Noel L-H, Guilbeau C, Rabant M, et al. The clinicopathologic characteristics of kidney diseases related to monotypic IgA deposits. Kidney Int. 2017;91(3):720–8. https://doi.org/10.1016/j.kint.2016.10.026
41 Sethi S, Fervenza FC. Membranous nephropathy-diagnosis and identification of target antigens. Nephrol Dial Transpl. 2024;39(4):600–6. https://doi.org/10.1093/ndt/gfad227
42 Vrana JA, Theis JD, Wegwerth PJ, Dasari S, Madden B, Nasr SH, et al. A reliable clinical test for detection of membranous nephropathy antigens using laser microdissection and mass spectrometry. Kidney Int. 2024;106(5):907–12. https://doi.org/10.1016/j.kint.2024.07.031
43 Best Rocha A, Larsen CP. Membranous glomerulopathy with light chain-restricted deposits: a clinicopathological analysis of 28 cases. Kidney Int Rep. 2017;2(6):1141–8. https://doi.org/10.1016/j.ekir.2017.07.008
44 Bajema IM, Wilhelmus S, Alpers CE, Bruijn JA, Colvin RB, Cook HT, et al. Revision of the International Society of Nephrology/Renal Pathology Society classification for lupus nephritis: clarification of definitions, and modified National Institutes of Health activity and chronicity indices. Kidney Int. 2018;93(4):789–96. https://doi.org/10.1016/j.kint.2017.11.023
45 Kudose S, Santoriello D, Bomback AS, Stokes MB, D'Agati VD, Markowitz GS. Sensitivity and specificity of pathologic findings to diagnose lupus nephritis. Clin J Am Soc Nephrol. 2019;14(11):1605–15. https://doi.org/10.2215/CJN.01570219

46 Ye J, Croom N, Troxell ML, Kambham N, Zuckerman JE, Andeen N, et al. Non-full house membranous lupus nephritis represents a clinically distinct subset. Kidney360. 2023;4(7):935–42. https://doi.org/10.34067/KID.0000000000000161
47 Garcia-Vives E, Solé C, Moliné T, Alvarez-Rios AM, Vidal M, Agraz I, et al. Antibodies to M-type phospholipase A2 receptor (PLA2R) in membranous lupus nephritis. Lupus. 2019;28(3):396–405. https://doi.org/10.1177/0961203319828521
48 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
49 Ravindran A, Casal Moura M, Fervenza FC, Nasr SH, Alexander MP, Fidler ME, et al. In patients with membranous lupus nephritis, Exostosin-positivity and Exostosin-negativity represent two different phenotypes. J Am Soc Nephrol. 2021;32(3):695–706. https://doi.org/10.1681/ASN.2020081181
50 Papa V, Brainer J, Henriksen KJ, Cenacchi G, Chang A. Extraglomerular immune complex deposition in lupus nephritis. Lupus. 2022;31(1):19–27. https://doi.org/10.1177/09612033211062535
51 Satoskar AA, Brodsky SV, Nadasdy G, Bott C, Rovin B, Hebert L, et al. Discrepancies in glomerular and tubulointerstitial/vascular immune complex IgG subclasses in lupus nephritis. Lupus. 2011;20(13):1396–403. https://doi.org/10.1177/0961203311416533
52 Ding Y, Yu X, Wu L, Tan Y, Qu Z, Yu F. The spectrum of C4d deposition in renal biopsies of lupus nephritis patients. Front Immunol. 2021;12:654652. https://doi.org/10.3389/fimmu.2021.654652
53 McAdoo SP, Pusey CD. Anti-glomerular basement membrane disease. Clin J Am Soc Nephrol. 2017;12(7):1162–72. https://doi.org/10.2215/CJN.01380217
54 Nasr SH, Collins AB, Alexander MP, Schraith DF, Herrera Hernandez L, Fidler ME, et al. The clinicopathologic characteristics and outcome of atypical anti-glomerular basement membrane nephritis. Kidney Int. 2016;89(4):897–908. https://doi.org/10.1016/j.kint.2016.02.001
55 Mignano SE, Nasr SH, Fidler ME, Herrera Hernandez LP, Alexander MP, Sethi S, et al. Recurrent atypical antiglomerular basement membrane nephritis in the kidney transplant. Am J Transpl. 2024;24(1):123–33. https://doi.org/10.1016/j.ajt.2023.09.007
56 Pickering MC, D'Agati VD, Nester CM, Smith RJ, Haas M, Appel GB, et al. C3 glomerulopathy: consensus report. Kidney Int. 2013;84(6):1079–89. https://doi.org/10.1038/ki.2013.377
57 Walker PD, Ferrario F, Joh K, Bonsib SM. Dense deposit disease is not a membranoproliferative glomerulonephritis. Mod Pathol. 2007;20(6):605–16. https://doi.org/10.1038/modpathol.3800773
58 Hou J, Markowitz GS, Bomback AS, Appel GB, Herlitz LC, Barry Stokes M, et al. Toward a working definition of C3 glomerulopathy by immunofluorescence. Kidney Int. 2014;85(2):450–6. https://doi.org/10.1038/ki.2013.340
59 Kim Y, Vernier RL, Fish AJ, Michael AF. Immunofluorescence studies of dense deposit disease. The presence of railroad tracks and mesangial rings. Lab Invest. 1979;40(4):474–80.
60 Andres GA, McCluskey RT. Tubular and interstitial renal disease due to immunologic mechanisms. Kidney Int. 1975;7(4):271–89. https://doi.org/10.1038/ki.1975.38
61 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
62 Singh G, Singh SK, Nalwa A, Singh L, Pradeep I, Barwad A, et al. Glomerular C4d staining does not exclude a C3 glomerulopathy. Kidney Int Rep. 2019;4(5):698–709. https://doi.org/10.1016/j.ekir.2019.02.006
63 Ravindran A, Fervenza FC, Smith RJH, Sethi S. C3 glomerulopathy associated with monoclonal Ig is a distinct subtype. Kidney Int. 2018;94(1):178–86. https://doi.org/10.1016/j.kint.2018.01.037
64 Cook HT, Pickering MC. Histopathology of MPGN and C3 glomerulopathies. Nat Rev Nephrol. 2015;11(1):14–22. https://doi.org/10.1038/nrneph.2014.217
65 Sethi S, Fervenza FC. Membranoproliferative glomerulonephritis: pathogenetic heterogeneity and proposal for a new classification. Semin Nephrol. 2011;31(4):341–8. https://doi.org/10.1016/j.semnephrol.2011.06.005
66 Johnson RJ, Gretch DR, Yamabe H, Hart J, Bacchi CE, Hartwell P, et al. Membranoproliferative glomerulonephritis associated with hepatitis C virus infection. N Engl J Med. 1993;328(7):465–70. https://doi.org/10.1056/NEJM199302183280703
67 Leung N, Bridoux F, Batuman V, Chaidos A, Cockwell P, D'Agati VD, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. Nat Rev Nephrol. 2019;15(1):45–59. https://doi.org/10.1038/s41581-018-0077-4
68 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
69 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
70 Nasr SH, Larsen CP, Sirac C, Theis JD, Domenger C, Chauvet S, et al. Light chain only variant of proliferative glomerulonephritis with monoclonal immunoglobulin deposits is associated with a high detection rate of the pathogenic plasma cell clone. Kidney Int. 2020;97(3):589–601. https://doi.org/10.1016/j.kint.2019.10.025
71 Said SM, Leung N, Alexander MP, Cornell LD, Fidler ME, Grande JP, et al. DNAJB9-positive monotypic fibrillary glomerulonephritis is not associated with monoclonal gammopathy in the vast majority of patients. Kidney Int. 2020;98(2):498–504. https://doi.org/10.1016/j.kint.2020.02.025
72 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
73 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