

Recurrent Glomerular Diseases in Renal Transplantation with Focus on Role of Electron Microscopy

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Keywords

Renal transplantation · Recurrent diseases · Electron microscopy · Focal segmental glomerulosclerosis · Membranous glomerulonephritis · IgA nephropathy · C3 glomerulopathies · Thrombotic microangiopathy

Abstract

Background: The common causes of renal transplant complications include active or chronic rejection process, infections, and toxicity but also recurrent or de novo diseases, which play an important role in affecting long-term graft function or graft loss. **Summary:** Recurrent disease in renal transplantation is defined as recurrence of the original kidney disease leading to end-stage kidney disease. They comprise a heterogeneous group of predominantly glomerular and some tubulointerstitial and vascular lesions, which include primary kidney diseases (e.g., focal segmental glomerulosclerosis, membranous glomerulonephritis, and IgA nephropathy) or those secondary to systemic autoimmune, metabolic, and infectious processes that can range from subclinical to clinically overt acute, subacute, or chronic clinical presentations. In addition to the knowledge of prior renal disease and routine/periodic serum and urine testing for kidney function, a complete transplant renal biopsy examination is essential in the identification and differentiation of

these diseases. The time of onset and severity of these diseases depend on the underlying etiopathogenetic mechanisms and the varied rates of recurrence in the early or late posttransplant period, often being modified by the current immunosuppressive protocols and other donor and recipient predisposing characteristics. **Key Messages:** Transplant kidney biopsy findings provide diagnostic accuracy and prognostic information regarding the potential for reversibility along with detection of unsuspected or clinically symptomatic recurrent diseases, with any concomitant rejection process or toxicity, for appropriate therapeutic decision-making. Routine electron microscopy in transplant kidney biopsies is a valuable tool in recognizing fully developed or early/subtle features of evolving recurrent diseases, often during the subclinical phases, in for cause or surveillance allograft biopsies.

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Introduction

The renal transplantation is by far the best form of renal replacement therapy for patients with chronic renal failure. Progress in donor selection including HLA matching, refined surgical techniques, and immunosuppressive

Table 1. Renal parenchymal lesions that recur in renal transplants (modified from Seshan [5])

Immune complex glomerular lesions
MGN
IgA nephropathy
MPGN, type 1
C3GN
DDD (primarily C3 deposits)
LN
Glomerular podocytopathy
Minimal change disease with mesangial hypercellularity, rare
FSGS
FSGS, not otherwise specified
FSGS, collapsing variant
Glomerular disease with organized deposits
Amyloidosis, secondary form (AA protein type), rare, related to familial Mediterranean fever or other chronic inflammatory conditions
FGN
Immunotactoid glomerulopathy
Paraproteinemia-associated renal diseases with or without organized deposits
MIDD (light and heavy chain types)
Amyloidosis, monoclonal light chain (AL type), common
Proliferative glomerulonephritis with monoclonal IgG deposits
Other paraproteinemia-related diseases – less common, for example, light chain tubulopathy
Lesions related to vascular diseases
ANCA-associated crescentic GN, pauci-immune type
SVV
Antiphospholipid antibody-mediated renal lesions
TMA-aHUS
Inherited or metabolic diseases
DKD
Fabry disease/other lipidoses
Primary hyperoxaluria 1/renal oxalosis
Cystinosis
2,8 hydroxyadenine deposits/adenine phosphoribosyltransferase deficiency

MGN, membranous glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; C3GN, C3 glomerulonephritis; DDD, dense deposit disease; LN, lupus nephritis; FSGS, focal segmental glomerulosclerosis; MIDD, monoclonal immunoglobulin deposition disease; ANCA, antineutrophil cytoplasmic antibody; GN, glomerulonephritis; SVV, small vessel vasculitis; TMA-aHUS, thrombotic microangiopathy-atypical hemolytic uremic syndrome; FGN, fibrillary glomerulonephritis; DKD, diabetic kidney disease.

protocols have contributed toward prolonged survival of the kidney allograft, minimizing the chances of graft losses to rejection processes. This paper deals with the important role that electron microscopy (EM) plays in identification and confirmation of recurrent diseases within the renal allograft, with histologic, immunofluorescence, and clinical correlations. This has improved the prognostic and therapeutic significance of the diseases diagnosed. Although information related to the exact proportion of end-stage kidney disease (ESKD) patients lacking a specific diagnosis with or without a native kidney biopsy is not readily available, it could vary in different parts of the world. This may have an impact on proper differentiation of recurrent diseases from preexisting donor-related dis-

eases (in the early posttransplant period) as well as de novo diseases (usually late posttransplant period), particularly when common primary glomerular diseases such as IgA nephropathy, membranous glomerulonephritis (MGN), and diabetic kidney disease (DKD) are considered.

Recurrent Renal Diseases

Definition

Recurrent disease in a renal transplant is defined as recurrence of the original cause of renal disease (primary or secondary) leading to ESKD. This group of diseases

forms the third leading cause (following rejection and infection) of allograft dysfunction and failure, particularly glomerular diseases, which constitute the primary cause of ESKD around the world. Based on retrospective studies of large cohorts of patients from transplant registries, they comprise a significant proportion of cases (35–50%), although the actual estimated range of recurrent disease is from 18 to 22% of renal allografts [1–4]. A wide range of glomerular and some tubulointerstitial and vascular lesions tend to recur at various times. Despite proper donor selection and effective immunosuppressive therapies, the risk for recurrence causing allograft dysfunction has not been diminished significantly (Table 1). This paper deals with the pathologic features and the significant role of EM, mainly in the diagnosis of recurrent glomerular diseases.

Clinical Manifestations and Spectrum of Diseases

A minimal or modest degree of protein in urine in the posttransplant period is not unusual under normal circumstances or an acute rejection process or chronic allograft failure. But, higher levels of proteinuria that are close to or over the nephrotic range (2.5 to >5 g/24 h) indicate the presence of a glomerular disease regardless of etiology, often associated with recurrent/de novo disease processes [6]. Additionally, gross or microscopic hematuria with varying degrees of renal insufficiency, acute kidney injury, or chronic renal failure, along with varying degrees of proteinuria, are largely dependent on the type and severity of glomerular, tubulointerstitial, or vascular lesions encountered. Results of various routine laboratory tests, urinalysis, and common serological tests for infections, autoimmune diseases, vasculitis, and paraproteinemia aid in the diagnosis of specific type of suspected recurrent glomerular or other diseases. Recurrent renal disease may have a subclinical or clinically symptomatic presentation in the posttransplant period. It is preferable to obtain any pertinent information of the primary disease in the native kidney including kidney biopsy results and clinical activity of disease at end stage where feasible. This will not only confirm recurrence but also helps to establish the reproducibility of specific patterns/disease variants. The age, gender, donor status (living related vs. unrelated and deceased), and dialysis of <5 years before transplant were predictors of higher recurrent disease rates. The posttransplant period preceding the recurrence of disease varies in frequency among specific disease entities, which can span from within the first week to 5–10 years (Table 2).

Pathologic Features

The common variables with regard to accounting for the frequency or the rate of detection of recurrent diseases are dependent on a number of factors. They are the variability in follow-up times, diagnostic criteria used and threshold to determine a recurrent lesion, the common biopsy practices with different institutions and nephrologists, and availability of comprehensive processing of renal biopsies. Some institutions have adopted routine protocol biopsies at regular intervals for early detection of rejection process or recurrent lesions. A systematic examination of the renal biopsy with routine special stains for light microscopy (LM), using a complete panel of immunofluorescence (IF) staining, and conducting EM is recommended in suspected cases [5]. LM, IF, and EM are useful to identify early/mild or established features of the original disease.

Critical Role of EM and When to Use It in Diagnosing Recurrent Glomerular Diseases

Routine EM in the setting of transplant kidney biopsies plays a crucial role in the diagnosis and confirmation of both subclinical and overt clinical recurrent diseases as well as some early and late features of transplant rejection process. While most of the recurrent tubulointerstitial and vascular lesions are recognized by LM, almost all the glomerular lesions require EM, providing further ultrastructural details for a specific diagnosis. EM enables detection of early immune deposits and subtle findings of extra- and intraglomerular cellular changes, basement membrane alterations, intracellular and extracellular inclusion bodies, and nature and location of immune complex (IC) deposits [1–5]. Most immune complex-mediated diseases may show some degree of modification such as delay in recurrence in the renal allograft, milder lesions, lack of abundant or “full-house” deposits, slower progression, or evidence of resolving changes. Ongoing transplant-associated immunosuppression could alter the typical pathology and may uncover an early or an atypical form of glomerular lesion, when compared to the native kidney disease. Specifically, many entities described in this paper require EM for an accurate and complete diagnosis including recurrent focal segmental glomerulosclerosis (FSGS) to detect extent of foot process effacement in its initial phase, IC diseases to confirm deposits and location of deposits (MGN, IgA nephropathy, lupus nephritis [LN], membranoproliferative glomerulonephritis [MPGN], and C3 glomerulonephritis [C3GN]/dense deposit disease [DDD]) and early basement membrane and mesangial alterations (DKD and thrombotic

Table 2. Recurrent and de novo disease occurrence at specific posttransplant time interval

1. Early posttransplant period
1–4 weeks
Detection of preexisting disease, mostly from deceased donor
DKD
IgA nephropathy
MGN
Podocytopathy
Amyloidosis
1–6 months
Early diagnosis of lesions in the subclinical or overt clinically manifest diseases
Minimal change nephrotic phase of recurrent FSGS
Early stages of MGN
Glomerular IgA deposits, often asymptomatic
C3GN
Proliferative glomerulonephritis with monoclonal IgG deposits
Early glomerular endothelial injury TMA secondary to recurrent HUS, complement-mediated HUS
De novo disease (rejection process, toxicity, infection)
2. Late posttransplant period (>6 mo to several years)
Developed or established FSGS, all variants
IgA nephropathy
MGN
MPGN
DDD
Diseases of organized deposits: fibrillary and immunotactoid GN
Diseases associated with monoclonal immunoglobulin-associated diseases
Amyloidosis
C1q nephropathy
Lipidoses, for example, Fabry disease, lipoprotein glomerulopathy
LN
De novo infection related – postinfectious GN
3. Beyond 5 years (generally de novo diseases, slowly progressive)
Recurrent fibrillary GN
Recurrent amyloidosis
De novo MGN
De novo Diabetic kidney disease
Collapsing glomerulopathy, a form of ischemic podocytopathy
Proliferative glomerulonephritis with monoclonal IgG deposits

DKD, diabetic kidney disease; MGN, membranous glomerulonephritis; GN, glomerulonephritis; FSGS, focal segmental glomerulosclerosis; C3GN, C3 glomerulonephritis; TMA, thrombotic microangiopathies; HUS, hemolytic uremic syndrome; MPGN, membranoproliferative glomerulonephritis; DDD, dense deposit disease; LN, lupus nephritis.

microangiopathy [TMA]), or to confirm the absence of deposits (crescentic glomerulonephritis [GN] and TMA). For example, in patients with LN, majority of the recurrent lesions are a mild form of mesangial LN, membranous LN, or rarely focal LN, sometimes without the “full-house” pattern of deposits by IF.

There are certain clinical or pathologic scenarios when EM is more useful or essential to make the diagnosis of a recurrence. Clinically, in patients with proteinuria, hematuria, or who have an unknown cause of renal disease,

especially without a suggestion of a rejection or an infectious process, EM may be indicated. Based on LM and IF findings, one may wish to do EM to confirm or describe in greater detail deposits or in cases in which there are equivocal IF findings. The pathologic findings should also be distinguished from concurrent rejection-related findings or nephrotoxic lesions (e.g., glomerulitis, transplant glomerulopathy [devoid of immune deposits], tubular injury and toxic epithelial changes, TMA, and small vessel vasculitis [SVV]). Although, de novo glomerular

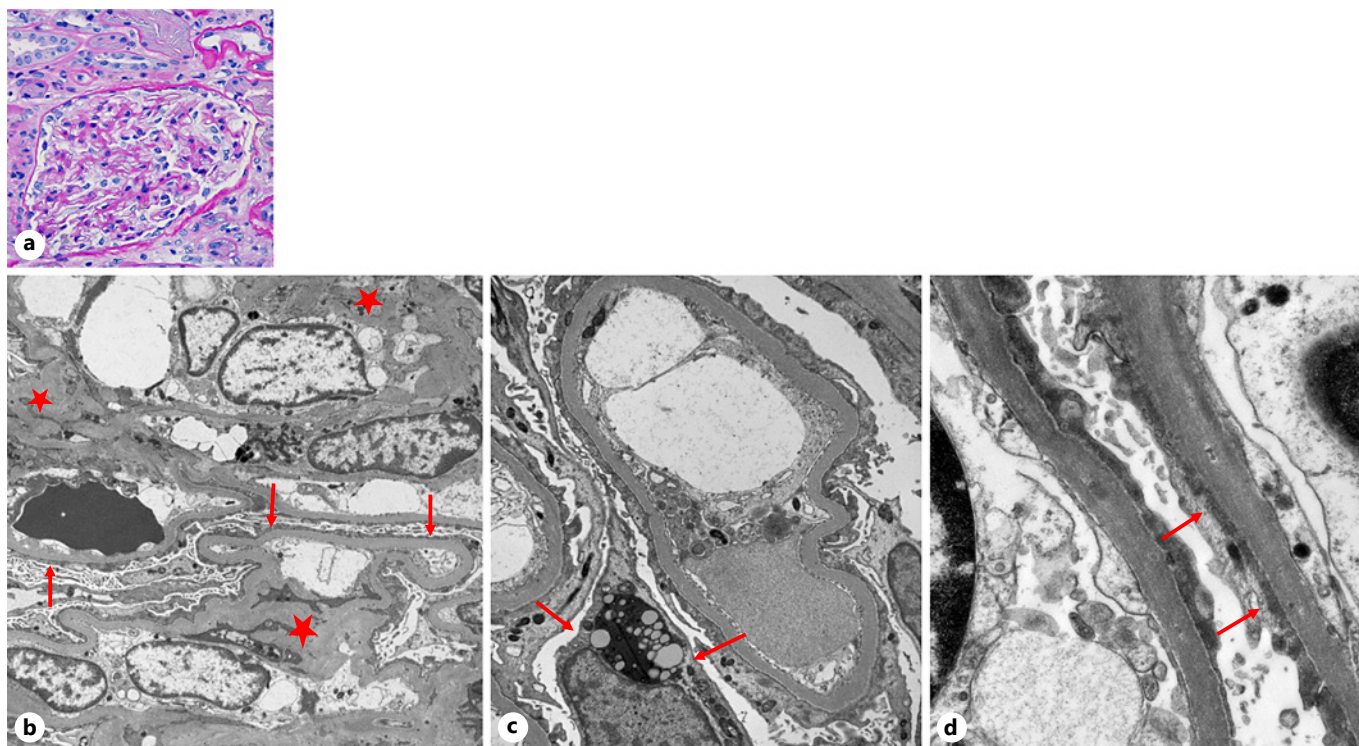


Fig. 1. Recurrent minimal change-like FSGS. **a** Normal glomerular capillary tuft architecture without segmental sclerosis (PAS, $\times 400$). **b** On EM, the salient feature is almost total foot process effacement (arrows) covering normal thickness of GBMs with microvillous transformation in the urinary space and normal mesangial areas (asterisks) $\times 6,000$. **c** High magnification of a glomerular capillary loop with foot process effacement, an epithelial cell containing an

aggregate of lysosomal protein and lipid droplets (arrows), and intact endothelial fenestrations ($\times 10,000$). **d** Higher magnification of glomerular capillary basement membranes with foot process effacement and condensation of the actin and elements adjacent to the lamina densa (arrows) ($\times 20,000$). FSGS, focal segmental glomerulosclerosis; PAS, periodic acid-Schiff; EM, electron microscopy; GBM, glomerular basement membrane.

diseases, preexisting donor-related disease, and rejection-associated pathology may also benefit in certain instances from EM, these entities will not be addressed in this paper.

Morphology of Common Recurrent Lesions in Renal Transplantation

Focal Segmental Glomerulosclerosis in Children and Adults (All Variants)

Definition

Recurrence of FSGS, an established primary form of podocytopathy, is defined by the rapid onset of proteinuria, often nephrotic range, usually in the early posttransplant period within a few weeks to a year. This is characterized by mainly extensive effacement of foot processes by EM with eventual development of segmental collapse and sclerosing lesions by LM in a few weeks to months.

Clinical Features

The hallmark of recurrent FSGS is onset of proteinuria or nephrotic syndrome, with a rate of recurrence that ranges from 25 to 55% from different studies, accounting for an average of one-third of the cases [7, 8]. The risk factors for this condition include young age of onset, progression to end stage within 3 years of onset, living related donors, previous recurrence in an allograft, and mesangial hypercellularity in the original disease [1–3]. In adults, older age groups, white race, and higher BMI are risk factors [8]. Although graft failure can occur in 13–20% of cases in children and may be as high as 39% in adults, a substantial proportion of them exhibit partial or complete remission following plasmapheresis and anti-CD20 therapy (rituximab) therapy. Idiopathic collapsing glomerulopathy as a variant of a severe form of primary FSGS also manifests marked proteinuria frequently associated with rising renal insufficiency. Inherited or familial forms of FSGS rarely recur, but certain polymorphisms in

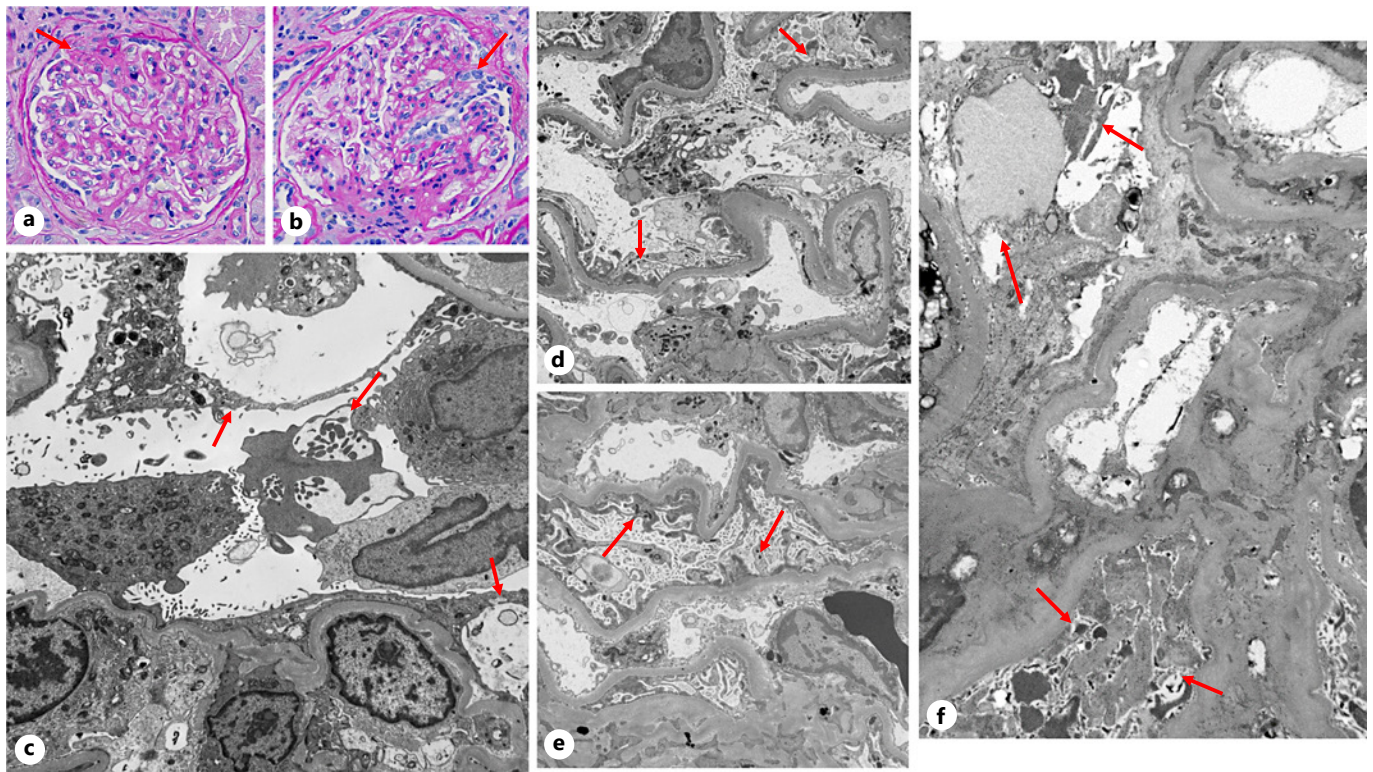


Fig. 2. Recurrent FSGS, not otherwise specified (NOS type). **a, b** Glomeruli showing mild to moderate enlargement and peripheral segmental sclerosing change with capsular adhesion and focal hyperplastic epithelial cells (arrows) (PAS $\times 400$). **c** Diffuse foot process effacement, in some cases is also accompanied by visceral epithelial injury such as prominent and increased active cytoplasmic organelles with small or confluent vacuolization (arrows) ($\times 6,000$). **d, e** Although some of the glomerular epithelial

foot processes appear preserved, others may display variable flattening or distortion (arrows) ($\times 6,000$; **d, e**). **f** This is a case of recurrent FSGS with severe proteinuria within a month of transplantation with sustained nephrotic syndrome. Here, the glomerular epithelial cells show foot process effacement as well as severe injury with fragmentation and degenerative changes ($\times 6,000$). FSGS, focal segmental glomerulosclerosis; PAS, periodic acid-Schiff.

podocin or nephrin encountered in some of these patients are regarded as potential risk factors (some heterozygous NPHS2 variants), although still with a low frequency. Serum permeability factors may be mediators of FSGS; the biochemical nature of a few are identified as soluble urokinase receptor (SuPAR), cardiotrophin-like 1, and an autoantibody to CD40. Secondary forms of FSGS generally do not recur. Sometimes, the challenge will be to differentiate from de novo form of FSGS, since the distinction may be somewhat difficult based on the posttransplant interval, degree of proteinuria, and morphologic findings including the extent of foot process effacement. A thorough clinical evaluation and consideration of underlying pathophysiologic mechanisms may be useful.

Light Microscopy

The glomeruli in the early stage of recurrence, particularly within a few hours to several weeks posttransplantation, disclose relatively normal glomerular architecture (Fig. 1a), despite significant proteinuria, often termed the “minimal change phase of recurrent FSGS.” The glomerular epithelial cells or podocytes may show variable swelling, vacuolization, and focal detachment. The later lesions are more typical of the original disease in native kidneys, with segmental collapse of glomerular capillaries and sclerosis, visceral epithelial hyperplasia, luminal foam cells, and focal capsular adhesions (Fig. 2a, b). The following variants of FSGS can occur as recurrent disease: (1) glomerular tip, (2) cellular, (3) segmental/global collapsing, and (4) peripheral or not otherwise specified as seen in LM. One study has shown the relative high degree of fidelity in the recurrence of the various subtypes of FSGS, re-

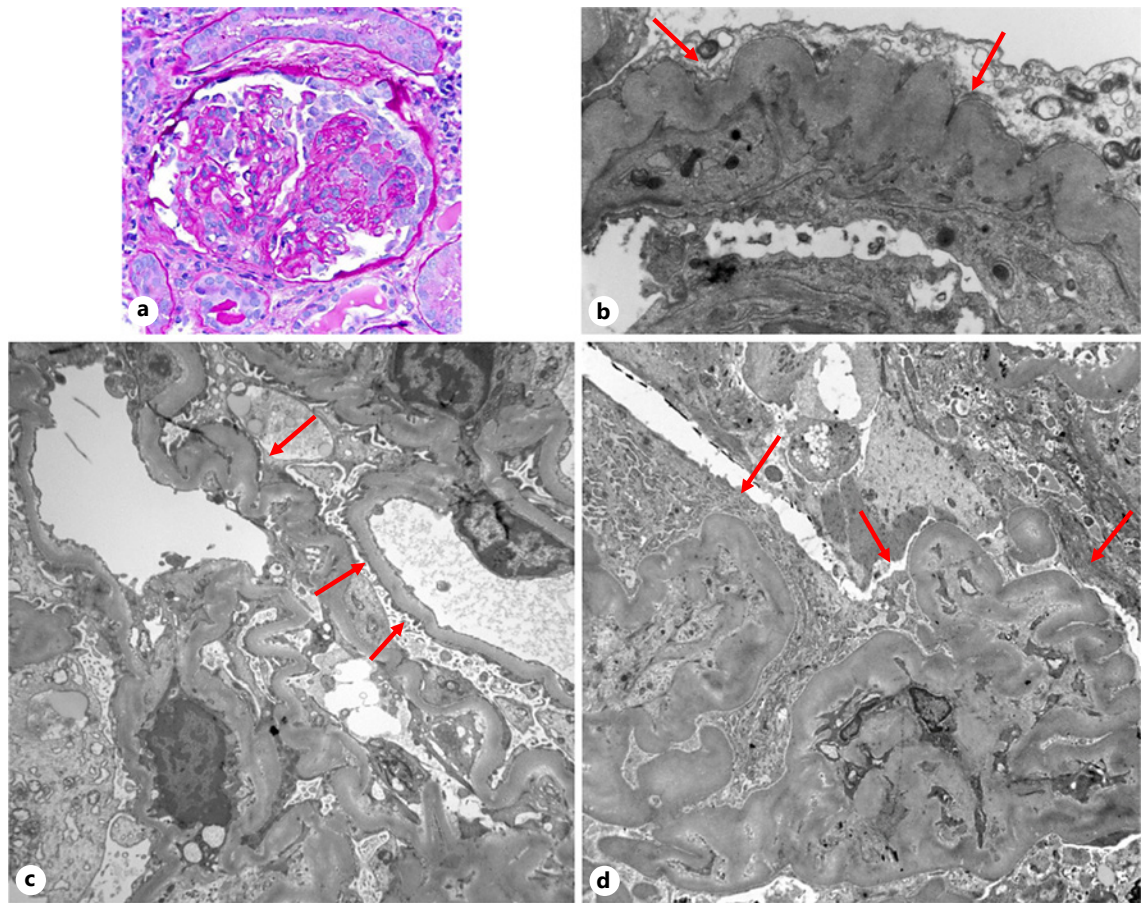


Fig. 3. Recurrent FSGS, collapsing glomerulopathy variant. This is a patient who progressed to ESKD secondary to FSGS (information of a specific variant was not available) developed progressive nephrotic syndrome 7 months posttransplantation and renal failure. **a** The glomerulus shows global wrinkling and collapse covered partially by hyperplastic epithelial cells in the Bowman space, some containing PAS+ protein resorption droplets. It is surrounded by a few tubules containing protein casts and minimal interstitial in-

flammatory infiltrate (PAS, $\times 400$). **b-d** The electron microscopic findings are relatively nonspecific and recapitulate the thickening, wrinkling, and collapsing features of the glomerular capillary basement membranes with extensive foot process effacement (arrows) and prominent active epithelial cells in the Bowman space, containing occasional protein and lipid droplets ($\times 6,000$ (**b-d**)). FSGS, focal segmental glomerulosclerosis; ESKD, end-stage kidney disease; PAS, periodic acid-Schiff.

capitulating the original lesion of the native biopsy [9]. The presence of a parietal epithelial marker CD44 is shown to be upregulated in recurrent FSGS [1]. This may be useful in a patient with new-onset proteinuria and no known prior diagnosis of FSGS in the native kidney, when an actual FSGS lesion is lacking in the biopsy tissue. The perihilar variant, a secondary form of FSGS, does not recur. The tubulointerstitial compartment is generally preserved but may display increased lysosomal protein resorption and lipid droplets in the tubular epithelial cells as well as scattered protein casts within the tubular lumina, as a consequence of substantial proteinuria.

The collapsing variant of FSGS also shows a high tendency to recur, almost always recapitulating the morpho-

logical changes of the original disease. The pathologic findings are segmental and/or global wrinkling and collapse of the glomerular capillary tufts with occlusion of the lumina, covered by hyperplastic and vacuolated epithelial cells containing prominent protein droplets (Fig. 3a). This lesion needs to be differentiated from the de novo form of collapsing glomerulopathy, which generally occurs later in the posttransplant period. The latter appears to be secondary to certain infections and microvascular obliterative changes in the setting of vascular rejection, arteriolar hyalinosis in DKD, and calcineurin inhibitor therapies, to name a few instances (see De novo diseases).

Electron Microscopy

The earliest ultrastructural change observed in recurrent FSGS, also known as “minimal change phase,” is widespread glomerular epithelial foot process effacement with condensation of actin filaments adjacent to the basement membranes and varying amounts of microvillous transformation within the urinary space (Fig. 1b–d). In light of ongoing immunosuppression, the disposition of the foot processes may sometimes be modified, showing patchy or focal effacement with irregular flattening or distortion. Other changes of podocyte injury include varying degrees of cytoplasmic swelling, discrete or confluent vacuolization, frequent protein and lipid droplets within the lysosomal bodies, and focal cytoplasmic degenerative changes (Fig. 2c–f). The endothelial cells may also display varying degrees of swelling and loss of fenestrations, lacking endothelial honeycombing pattern. While no IC deposits are identified, occasionally fine proteinaceous hyaline-like material may be seen within the capillaries or in the area of sclerosing change. The EM findings of collapsing glomerulopathy disclose extreme wrinkling and collapse of the glomerular capillaries (Fig. 3b–d), which express evidence of severe podocyte injury and focal detachment with no hyaline deposits.

Membranous Glomerulonephritis

Definition

Recurrence of primary MGN is defined by the reappearance of subepithelial deposits in the glomerular capillary basement membranes composed of polyclonal IgG and C3 deposits by IF, confirmed by the presence of M-type phospholipase A2 receptor (PLA2R) staining or other less common antigens that have been recently identified and EM localization of deposits [1–4, 10–13]. This may be accompanied by severe nephrotic syndrome early in the posttransplant period or more often begin as subnephrotic proteinuria in cases of later onset.

Clinical Features

On an average, 15–40% of cases of primary MGN recur in transplants during the life of the allograft. While most recur within 2 years, a small proportion of them are seen in a few weeks manifesting a rapidly progressive course to end stage with reappearance of high titers of PLA2R antibodies. Both primary and secondary MGN may not recur in renal transplants, if the underlying cause is effectively and adequately treated (e.g., infections [HBV and HCV], sometimes paraproteinemic diseases and autoimmune diseases) [14]. However, some allograft biopsies from patients who may be considered completely treated

with remission prior to transplantation, present with recurrent secondary disease, probably due to residual/persistent subclinical systemic disease, recent relapse, or activation of the disease posttransplantation.

Since antibodies to PLA2R are documented in only about 70% of primary MGN, additional putative autoantigens have emerged as targets, such as N-terminal region of thrombospondin 7A, exostosin 1/exostosin 2, neural epidermal growth factor-like 1 protein [10–13]. As serologic testing and immunohistochemical stains become available, these could be identified definitively and differentiate them from the PLA2R-negative cases, now deemed to fall under the category of *de novo* disease. The risk factors identified for a potential recurrence include shorter clinical course to chronic renal failure in the native kidney, older recipient age, previous recurrence in allograft, steroid-free immunosuppressive protocols, and living related donor with more compatible donor-recipient HLA molecular haplotypes and risk alleles (HLA-A3, HLA-DRB1/DQA1 loci) [1, 15]. The presenting clinical feature is nephrotic-range proteinuria, with generally normal renal function unless complicated by other transplant-related processes such as rejection or an infection. Graft failure ranges from 20 to 50% in over 5–10 years.

Light Microscopy

At initial presentation, LM can be noncontributory with only minimal glomerular changes or no obvious capillary wall alterations in recurrent MGN [16] (Fig. 4a). With longer posttransplant duration, there is progressive irregular thickening of the capillary walls, forming basement membrane spikes, best seen by PAS and silver stains (in the later stages) (Fig. 5a). Silver staining can also uncover small subepithelial/intramembranous lucencies or “pinholes” early in the disease. The tubulointerstitial compartment is unaffected, except increased epithelial cell protein resorption droplets, as a consequence of the proteinuric state. Any evidence of chronic tubulointerstitial scarring and vascular sclerosis have to be assessed in the context of the original status of the allograft, the duration of the disease, and other concomitant transplant-related changes.

IF Microscopy

IF is the best diagnostic modality that can detect the earliest recurrence of MGN [16], even before LM or EM features of deposits become apparent and is termed stage 0 MGN. A faint-to-low intensity of finely granular staining is seen for polyclonal IgG and C3 (Fig. 4b) (as seen in primary MGN in the native kidney) and C4d (routinely

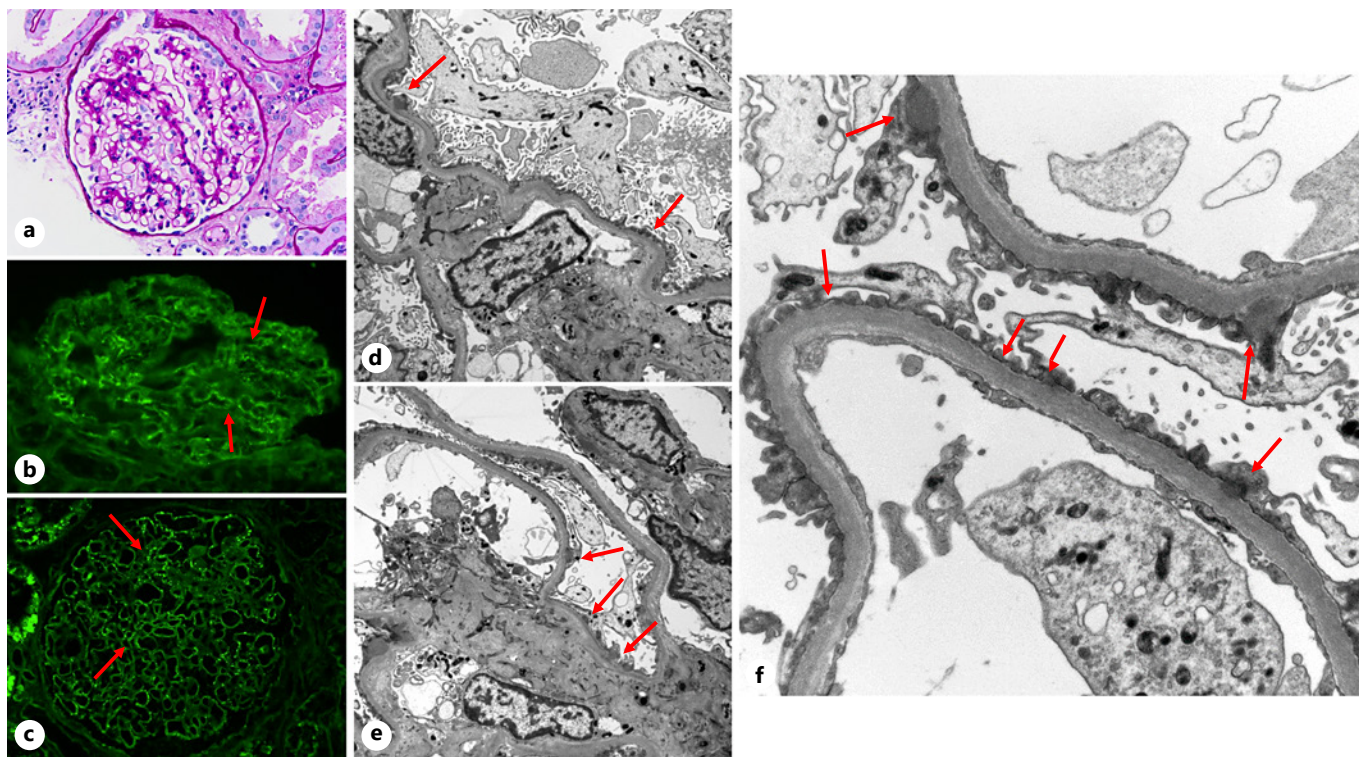


Fig. 4. Recurrent early MGN. **a** The LM features of the glomeruli in early cases may be minimal or reflect the underlying status of the allograft, showing no significant thickening of the capillary walls or glomerular hypercellularity (PAS, $\times 200$). **b** Focal segmental relatively low-intensity IgG localization within the glomerular capillary walls is noted by IF. As in idiopathic/primary form of MGN, C3, kappa, and lambda light chains, as well as C4d are also localized in a similar distribution (IgG, $\times 400$). **c** Immunofluorescence staining on protease-digested paraffin-embedded tissue using antibodies to PLA2R shows focal stretches of small, low-intensity positive staining within the glomerular capillary walls (PLA2R, $\times 400$). **d, e** Scattered, small subepithelial deposits are detected in

some capillary basement membranes (arrows), which may be imperceptible in some glomerular capillary loops and can be easily missed in low magnification or if routine IF with immunoglobulins and complement components are not available. An important clue is the extent of foot process effacement and microvilli, even if the deposits do not become apparent ($\times 8,000$). **f** High magnification of 2 glomerular capillary loops showing small subepithelial electron-dense deposits without basement membrane spikes and intervening preserved foot processes. The endothelial cells show intact fenestrations ($\times 12,000$). MGN, membranous glomerulonephritis; LM, light microscopy; PAS, periodic acid-Schiff; IF, immunofluorescence; PLA2R, phospholipase A2 receptor.

used for transplant biopsies), which increases in strength with progression of the lesion (Fig. 5b). Although these IF findings are similar to those of de novo MGN, the presence of mainly IgG4 subtype and localization of PLA2R [17] within the deposits along with detectable serum PLA2R antibodies are suggestive of recurrent primary MGN (Fig. 4c, 5c). The later lesions of recurrent MGN may also contain codominant IgG1 or IgG3 with IgG4 subtype [1]. It is also suggested that about 20% of primary MGN may be negative for IgG4, demonstrating the relative heterogeneity of IgG subset staining in this setting. Varying degrees of IgG1 and IgG2 may be present along with IgG4 in a proportion of cases associated with the newly described antigens in MGN [10–13].

Electron Microscopy

EM is helpful in recognizing extensive early foot process effacement, epithelial swelling, and variable microvillous transformation, even if only scant deposits without spikes or no visible deposits may be apparent and is termed “stage 0” [16] (Fig. 4d–f). The lamina densa is intact and exhibits mild attenuation or thickening with increasing subepithelial deposits. The endothelial cells are largely unremarkable with preserved fenestrations. As the disease progresses, small or well-developed spikes appear (Fig. 5d–f), enclosing resolving deposits of varying densities and rarefaction in long-standing lesions, as seen in various stages of evolution of MGN proposed by Ehrenreich and Churg [18]. In advanced lesions, fragments of epithelial cells may be trapped in these spaces, giving the

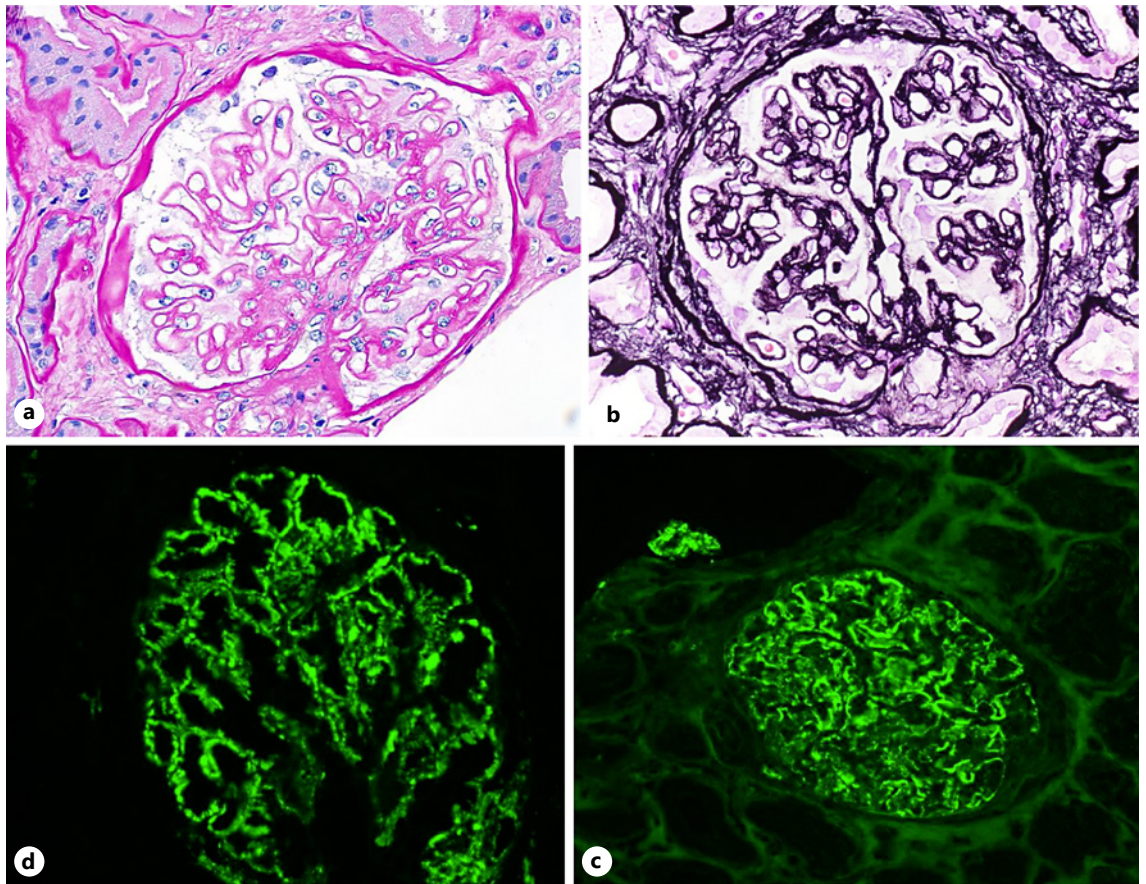


Fig. 5. Recurrent advanced MGN. **a** Glomerulus showing global, conspicuous thickening of the capillary walls with some suggestion of irregularity on the outer aspect, visualized more clearly in a silver-stained section (PAS, $\times 400$). **b** Glomerulus showing global, irregular thickening of the capillary walls with frequent spikes on the outer aspect of the capillary walls (PAMS, $\times 400$). **c** Global, coarse to finely granular, strong IgG staining by IF mainly along the glomerular capillary walls (IgG, $\times 400$). **d** IF staining on paraffin-embedded tissue for PLA2R shows strong coarsely granular positive staining along the glomerular capillary wall deposits (PLA2R, $\times 400$). **e-g** EM of the glomerular capillary basement membranes

frequent finely granular subepithelial electron dense deposits (arrows) along with early to well-developed spikes interspersed between them and total foot process effacement. The lamina densa is largely intact ($\times 6,000$ (**e-g**)). **h** In long-standing or resolving membranous glomerulonephritis, varying stages of resorption or resolution of the deposits occur along with rarefied spaces and trapping of fragments of cellular material within the spaces, even in the absence of deposits, sometimes known as “podocyte infolding glomerulopathy” ($\times 6,000$). MGN, membranous glomerulonephritis; PAS, periodic acid-Schiff; IF, immunofluorescence; PLA2R, phospholipase A2 receptor; EM, electron microscopy.

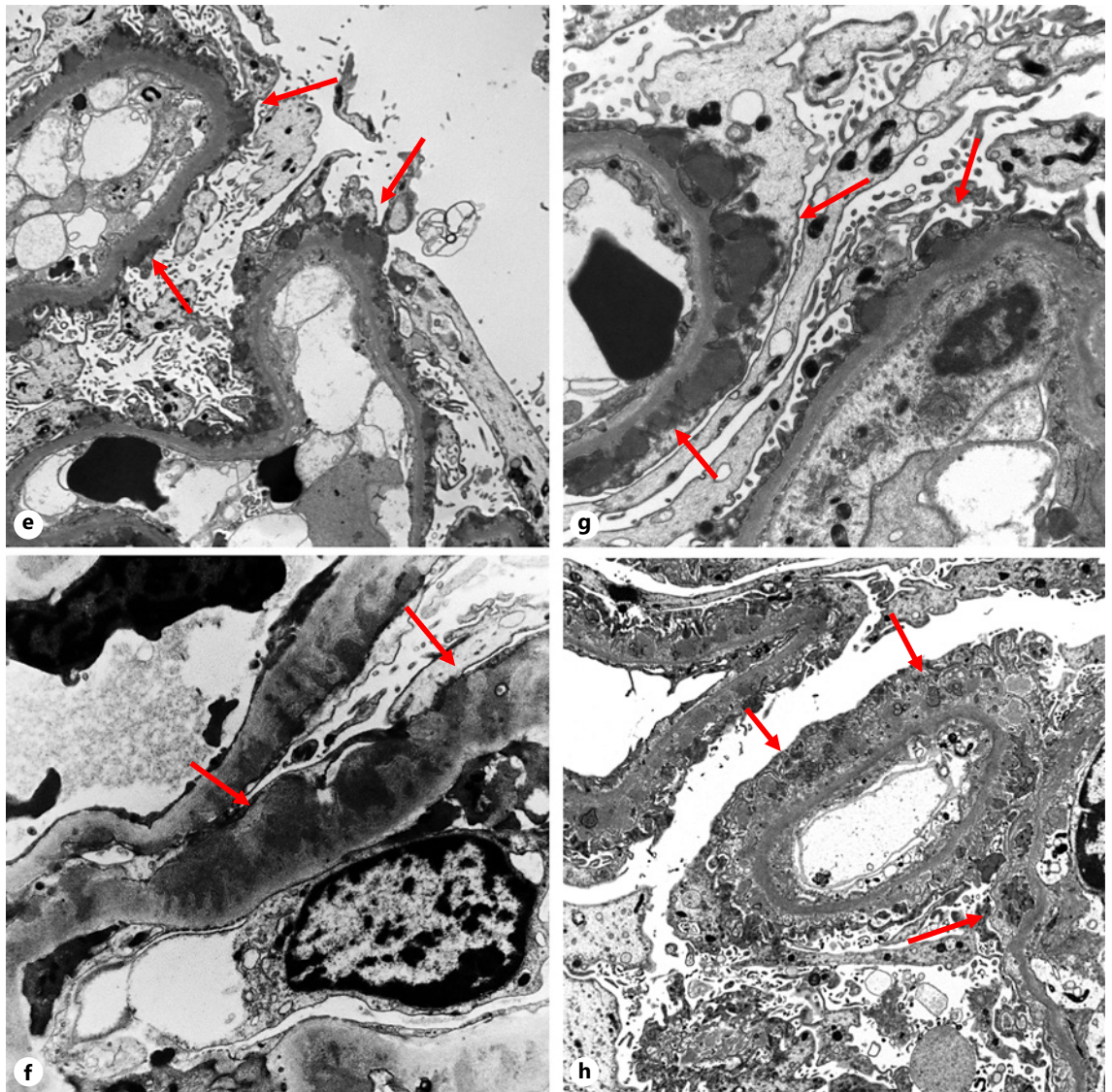
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appearance of “podocyte infolding” (Fig. 5g). Such findings in an evolving disease are not uncommon, because of the modifying effects of the ongoing immunosuppressive therapies. As in the native kidney, no mesangial or subendothelial deposits are evident in this setting. However, when they are present, the possibility of a secondary form of MGN should be entertained and investigated further clinically.

IgA Nephropathy

Definition

Recurrent IgA nephropathy is the most common primary glomerular disease in the renal transplant, where glomerular mesangial deposits of IgA are found. This may be initially asymptomatic for an indefinite period or present with isolated microhematuria and/or mild proteinuria on routine urinalysis, often having an indolent course, but remains an important cause of graft dysfunction and failure in the long term [19].



Clinical Features

The clinical presentation of recurrent IgA nephropathy is fairly heterogeneous, and although it is a hematuric disease, this feature is not a reliable marker of recurrent disease. Transplant protocol biopsies have shown that over 60% of cases did not have this urinary finding, despite the presence of mesangial IgA deposits by IF. In addition to isolated hematuria or proteinuria, mild to moderate elevation of creatinine may be observed with or without abnormal urinary findings. The incidence of recurrence ranges from 8 to 53% in different series reported, and on average, around 30%, the variability being partly influenced by racial and geographical background, institutional biopsy practices, and the duration of clinical

follow-up. The availability and extent of transplant renal biopsy workup to include IF and EM workup also contributes to the rate of diagnosis [19]. The usual risk factors for recurrence and of prognostic significance for the course of disease in the graft and outcome are young age of the patient at transplantation, IL-10 genotype, living related donors with close to 0 HLA mismatch, and more aggressive proliferative glomerular disease in the native kidney with increased crescents with rapid progression. However, the data on 0 HLA mismatch as a recurrence risk factor have been inconsistent, and there is no current recommendation to avoid living-related transplantation in these patients. The ongoing immunosuppression or induction protocols in these patients have not shown to be

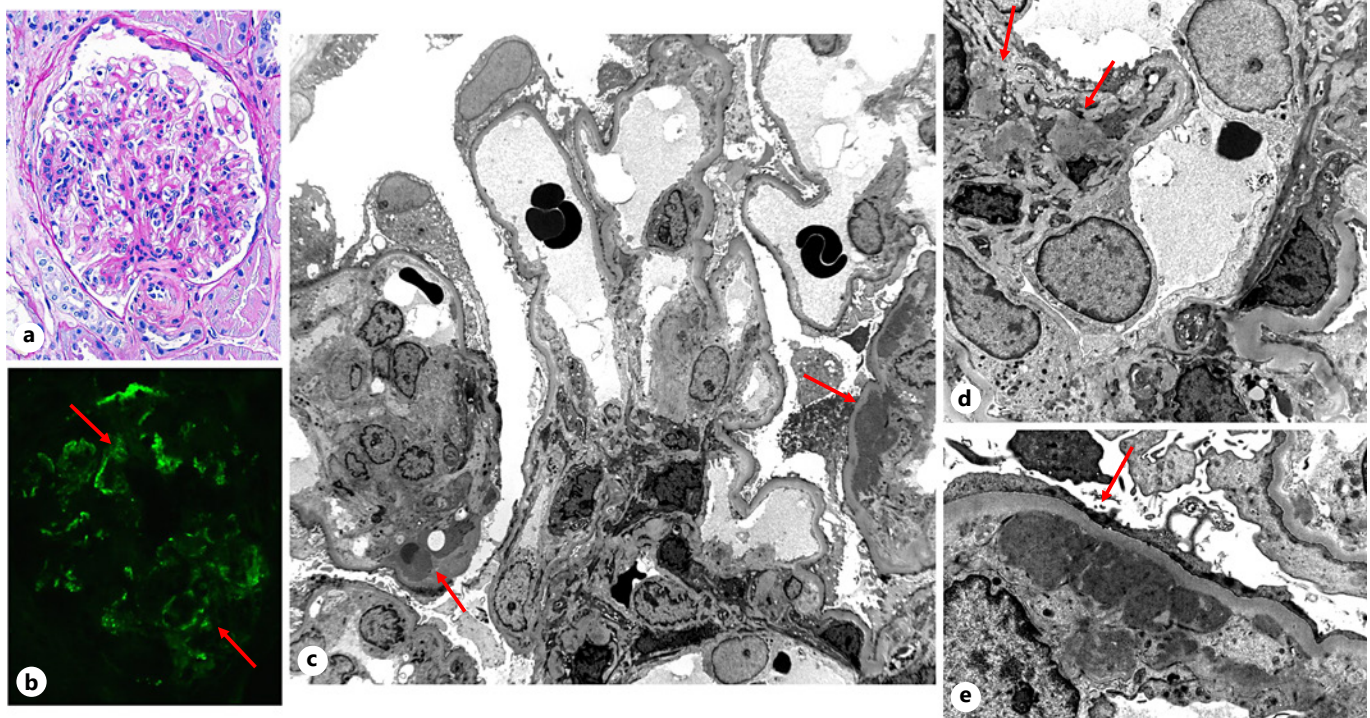


Fig. 6. Recurrent IgA nephropathy. **a** Glomerulus with mild to moderate mesangial hypercellularity and increased matrix, partly encroaching on the peripheral capillary lumina. Occasional infiltrating mononuclear cells are seen marginating within capillaries in the transplant setting of mild active cellular rejection in this case (not shown here) (PAS, $\times 400$). **b** Granular, dominant, and varying amounts of IgA staining predominantly in the mesangial areas are seen, in a global distribution (IgA, $\times 400$). **c** Electron micrograph showing prominent mesangial areas with hypercellularity, some

matrix, and paramesangial electron-dense deposits beneath the basement membranes reflected on the mesangial areas (arrows). The capillary basement membranes are within the normal range of thickness with relatively preserved foot processes and intact endothelial fenestrations ($\times 1,800$). **d, e** EM of the glomerular mesangial areas displaying finely granular mesangial matrix (**d**) and paramesangial (**e**) deposits (arrows) with focal overlying foot process effacement ($\times 6,000$ (**d**), $\times 10,000$ (**e**)). PAS, periodic acid-Schiff; EM, electron microscopy.

effective in prevention of recurrence or slow the progression of disease to graft loss. Nevertheless, the overall 10-years graft survival is over 60%. While the recurrence rate for IgA vasculitis (Henoch-Schonlein purpura) nephritis is lower than that for IgA nephropathy and the posttransplant period to recurrence is relatively shorter, the pathological findings are similar.

Light Microscopy

Although serum biomarkers are being developed to monitor the recurrence of IgA nephropathy in known cases, both native and transplant renal biopsy evaluation remains the mainstay of definitive diagnosis of IgA nephropathy. The recurrence in asymptomatic cases can be purely a “histological,” found in nearly one-third of protocol biopsies in the first 2 years posttransplant or an incidental finding in “for cause” biopsies. Regardless of the

clinical scenario, when renal function is normal, the glomeruli may appear relatively normal or express mild mesangial hypercellularity and increased matrix (Fig. 6a). A more typical morphologic finding is variable mesangial proliferation, and in more active cases, focal segmental glomerulonephritis characterized by endocapillary hypercellularity (Fig. 6b), with or without cellular or fibrocellular crescents and with associated patchy active interstitial inflammation. The chronic lesions may progress to segmental sclerosis with capsular adhesions. Some of the tubules may contain RBCs or RBC casts suggesting active disease and when present in large numbers obstructing the lumina and can cause acute kidney injury. Chronic tubulointerstitial scarring and vascular sclerosis are late features or depend on the status of the donor kidney at transplantation.

IF Microscopy

The localization of dominant polyclonal IgA deposits (Fig. 6c) with a lesser intensity of IgM and C3, restricted to the mesangial areas is a standard feature. This may be performed by IF or immunohistochemistry to confirm the diagnosis. In a few cases with follow-up or protocol biopsies of mild cases, partial or complete resolution of the mesangial staining for IgA has been observed.

Electron Microscopy

Those cases subjected to EM studies disclose variable finely granular electron-dense deposits in the paramesangial areas in the earlier stages, involving the mesangial areas (Fig. 6d–f). In the absence of considerable proteinuria, the foot processes and the visceral epithelial cells are largely preserved. No capillary basement membrane deposits are noted. On occasion, rarified mesangial areas are seen, indicating evidence of resolving deposits.

Membranoproliferative Glomerulonephritis

In contrast to a previous classification of MPGN as types 1, 2, and 3 [20], in the last 10–15 years, this pattern of glomerular injury has shown that the pathophysiologic categorization of MPGN is appropriately done based on the immunopathologic findings [21]. Thus, it is dependent on the composition of the glomerular deposits by IF or immunohistochemistry, where the 2 main categories are IC-mediated MPGN (primary and secondary forms to infections, autoimmune diseases, and paraproteinemias) and MPGN with predominantly C3 deposits (also known as C3 glomerulopathies) [21, 22].

IC-Mediated MPGN (Formerly MPGN Type 1)

Definition

Recurrence of IC-mediated MPGN (formerly known as MPGN type 1) is fairly common with characteristic glomerular histologic pattern in renal transplants [7, 21]. The glomerular features include global proliferative GN with a lobular architecture, thickening of the peripheral capillary walls by cellular interposition giving rise to double contours, and subendothelial and mesangial, polyclonal immunoglobulin deposits [7]. By virtue of significant glomerular capillary remodeling, requiring longer period of time, the diagnosis of recurrence is delayed as compared to other forms of glomerular diseases, up to nearly 2 years posttransplantation. Both primary and secondary types of IC-mediated MPGN (hepatitis B virus, hepatitis C virus, and monoclonal protein) have the potential to recur; however, it should be noted that in adequately treated patients with quiescent disease activity

and remission of serologic evidence prior to renal transplantation, recurrence is generally unlikely to develop. As some secondary forms have no specific treatment, but instead are treated with generic immunosuppressive agents, complete treatment may not always be possible.

Clinical Features

The clinical renal presentation is relatively nonspecific, with nephritic features of hematuria, subnephrotic to nephrotic range proteinuria, and slowly rising creatinine depending on the activity and chronicity of the lesions and parenchymal scarring [20]. Serologic work-up in this setting shows mainly persistent hypocomplementemia with low C3, CH50, and C4, with appropriate positive parameters in the secondary forms related to infections and autoimmune diseases. A smaller proportion of patients with IC-mediated MPGN have also had complement protein abnormalities and underlying mutations. Though the disease itself is relatively rare constituting about 2% of all children with ESKD, a high rate of recurrence spanning 30–70% has been recorded from various studies and registries with 25–30% graft loss [2]. Some of the known risk factors are younger age at diagnosis, living related donors, persistent hypocomplementemia, and severe glomerular lesions with crescents and an aggressive course in the native kidney.

Light Microscopy

The earlier lesions in the allograft may not express the full morphologic picture of the glomerular disease (especially when subjected to protocol biopsies), but may show only minimal or mild mesangial proliferation or inflammatory cell infiltration (Fig. 7a). Occasionally, more active segmental/global endocapillary hypercellularity with occasional crescents and minimal to moderate interstitial inflammation are evident. With increasing duration of the disease, mesangial cellularity may increase, leading to lobular accentuation, and advancing subendothelial cellular interposition giving rise to “double contours,” resulting in more irreversible pattern of glomerular injury termed MPGN (Fig. 7b). These are best visualized by PAS and silver stains. While initially the tubulointerstitial and vascular compartments may be relatively preserved, the disease progression with segmental and global glomerulosclerosis can bring about cortical scarring, contributing to CKD.

IF Microscopy

In the broad scheme of MPGN patterns of glomerular lesions, IF plays a major role in delineating the more spe-

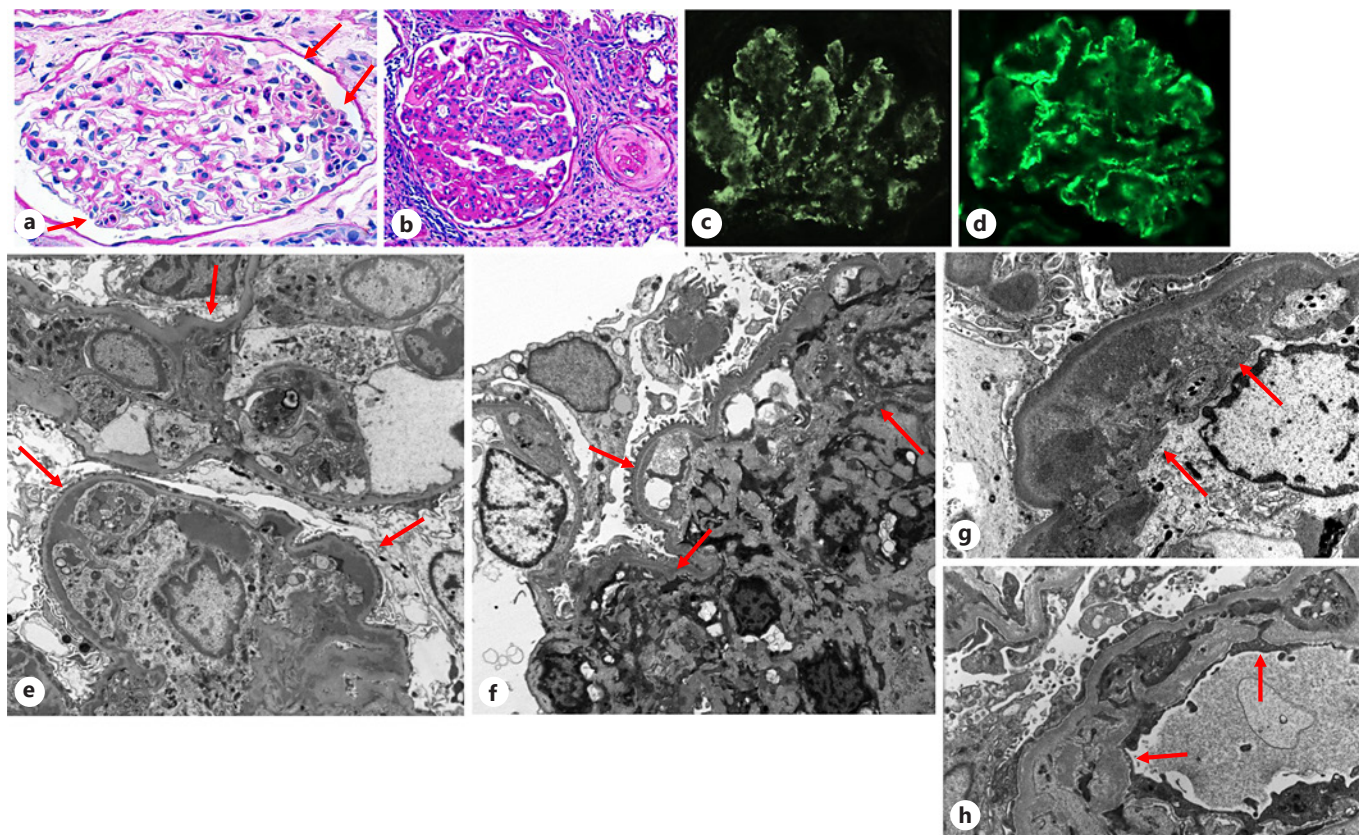


Fig. 7. MPGN, type 1. **a** In the early stages of recurrence with a clinical presentation of proteinuria and microhematuria, only mild glomerular alterations are seen as in this glomerulus showing small focal segmental endocapillary hypercellularity due to influx of inflammatory cells (arrows) (PAS, $\times 400$). **b** As the disease progresses with increased glomerular hypercellularity, an early lobular configuration and irregular peripheral capillary wall thickening with suggestion of double contours, diagnostic of membranoproliferative pattern of glomerular injury becomes visible. Since a significant duration of disease is required for the development of such a lesion, other transplant-related processes of rejection or chronic parenchymal changes have also developed in the background with a globally sclerosed glomerulus in this image (PAS, $\times 200$). **c, d** Mild and heavy staining of IgG by IF may be found along the glomerular capillary walls and mesangial areas in the early and well-developed lesions, respectively. In this entity, polyclonal IgG with

C3 are seen predominantly with localization of C4d, while other parameters may indicate different etiologic diagnoses (IgG, $\times 200$ (**c**); IgG, $\times 400$ (**d**)). **e** Focal endocapillary hypercellularity and small subendothelial and mesangial deposits (arrows) mark the early phase of MPGN type 1, as seen in this image ($\times 6,000$). **f** Small to confluent subendothelial deposits involving capillary subendothelial areas along with expanding nodular mesangial areas with increased cells, matrix, and focal deposits (arrows) ($\times 6,000$). **g, h** Hilar magnification of a later stage of MPGN type 1 showing abundant or lesser subendothelial deposits with intact lamina densa and foot processes along with focal cellular interposition and varying thickness of new basement membrane formation (arrows) lined by displaced endothelial cells ($\times 10,000$ (**g**), $\times 12,000$ (**h**)). MPGN, membranoproliferative glomerulonephritis; PAS, periodic acid-Schiff; IF, immunofluorescence.

cific entities of primary and secondary forms of disease, namely primary IC-mediated MPGN with predominantly polyclonal IgG and C3 staining (Fig. 7c, d), including positive C4d within the deposits because of classical pathway of complement involvement. A different panel of immune reactants are positive for hepatitis C-related MPGN with strong IgM kappa, C1q with weaker IgG and lambda, or appropriate monoclonal proteins in association with hematological malignancies (see below).

Electron Microscopy

The unique glomerular pattern of IC-mediated MPGN is best visualized by EM, where the relationship of the glomerular matrix alterations, the epithelial, mesangial, and endothelial cells, as well as the immune deposits in the various locations is clear. Since the immune deposits of fine to coarsely granular in texture are the first to arrive accumulating in the subendothelial and mesangial areas, local cellular activation with proliferation of intraglomer-

Table 3. Differential diagnosis of membranoproliferative (MPGN) pattern of glomerular injury in renal transplantation

	MPGN	TG	TMA
Etiologies	Primary, secondary (infections, autoimmune diseases, paraproteinemias, C3 glomerulopathies)	Antibody-mediated rejection, some cases of cell-mediated rejection	Atypical HUS (recurrent, de novo), known genetic mutations, drug induced, infections, APS
Clinical presentation	Subacute, chronic onset Hematuria, proteinuria (subnephrotic or nephrotic range) and/or rise in creatinine	Chronic onset Mainly subnephrotic or nephrotic proteinuria, variable renal insufficiency	Acute, subacute, or chronic onset Acute renal failure, hematuria, proteinuria Rise in creatinine
Serologic findings	Low complement levels, serology associated with viral, autoimmune, or paraprotein diseases	HLA, non-HLA alloantibodies, anti-endothelial antibodies	Low or normal complement levels, antiphospholipid antibodies, infections
LM	Mild to severe mesangial to endocapillary hypercellularity in active lesions, lobular accentuation, subendothelial/mesangial deposits, segmental to global capillary wall double contours, no specific vascular lesions	Variable intracapillary hypercellularity (transplant glomerulitis), mesangial thickening and sclerosis, segmental to global capillary wall double contours. Evidence of active/chronic vascular rejection in some cases	Fresh or organized capillary microthrombi, no intraglomerular cellularity, mesangiolytic or mesangial sclerosis, segmental to global capillary wall double contours, active or chronic microvascular thrombosis, intimal endothelial injury
IF	Presence of IC/paraprotein deposits dependent on the specific entity C4d positive in immune deposits	Nonspecific IgM and/C3 staining Strong C4d capillary wall staining	Nonspecific IgM and/C3 staining Strong variable C4d capillary wall staining
EM	Variable hypercellularity, infiltrating macrophages, subendothelial and mesangial deposits cellular interposition, new layer of basement membrane material, endothelial swelling	Mild hypercellularity, infiltrating macrophages, subendothelial lucent space, cellular interposition, multiple layers of new basement membranes, no immune deposits	Subendothelial lucent space with fibrin tactoids, plasma proteins, cellular interposition, multiple layers of new basement membranes in chronic lesions, no immune deposits

MPGN, membranoproliferative glomerulonephritis; TG, transplant glomerulopathy; TMA, thrombotic microangiopathy; HUS, hemolytic uremic syndrome; APS, antiphospholipid antibody syndrome; LM, light microscopy; IF, immunofluorescence; IC, immune complex; EM, electron microscopy.

ular cells and influx of inflammatory cells constitute the hypercellularity (Fig. 7e). Mesangial expansion into the loose zone of lamina rara interna with cells, matrix, and deposits is observed in well-developed lesions, also known as mesangial interposition (Fig. 7f–h). There is conspicuous endothelial separation with new layers of basement membrane formation corresponding to “double contours” by LM and irreversible basement membrane remodeling, following considerable period of time. Although the clinical renal findings may be relatively nonspecific, the MPGN pattern of glomerular injury should be differentiated from transplant glomerulopathy and healed forms of TMA (all causes) (Table 3).

C3 Glomerulopathies

Definition

In this group of diseases as the term suggests, the glomerular lesions are mediated by almost exclusively C3 deposits, and the 2 common entities that are familiar to renal pathologists are C3GN and DDD. While they have some clinical similarities, they are morphologically dif-

ferent, requiring EM for a definite diagnosis [22]. Both result from hyperactivation of the alternate pathway of complement due to genetic mutations or development of autoantibodies to complement-related proteins or regulatory factors. The former are constitutive abnormalities unaffected by immunosuppressive therapies, which persist in the posttransplant period, setting up a relatively early and high rate of recurrence [22].

C3GN and DDD

Clinical Features

The salient clinical renal manifestation at the time of recurrence is shared by both C3GN and DDD, such as relatively asymptomatic microhematuria or proteinuria initially, or an acute nephritic or nephrotic syndrome. While persistent hypocomplementemia is a common feature in most all of the cases pretransplantation, nearly 35% of them may have normal C3 levels posttransplantation. C3GN has been seen to recur in 30–50% of the cases within the first 2 years, having a higher rate of graft loss [16], while DDD is known to recur in 80–100% of cases,

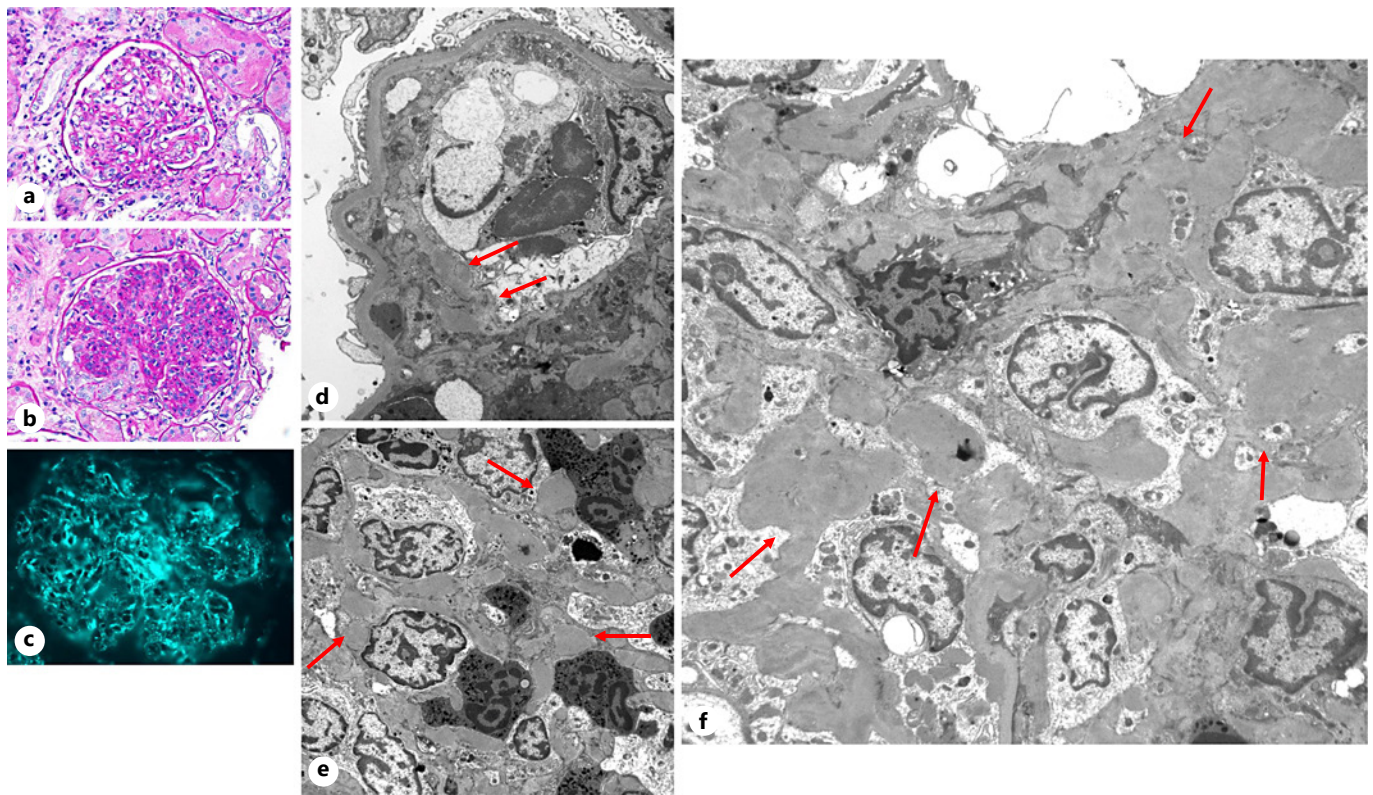


Fig. 8. C3GN. **a, b** The glomeruli show the early and fully developed active lesions with minimal to severe mesangial and endocapillary hypercellularity with exudative features and lobular accentuation in the later stages, mostly occluding the capillary lumina (PAS, $\times 400$ (**a, b**)). **c** Coarsely granular and global distribution of exclusively C3 deposits are noted along the glomerular capillary walls and mesangial areas (C3, $\times 400$). **d, e** Mild to abundant, fine

to coarsely granular subendothelial and mesangial electron-dense deposits are seen (arrows), having a relatively pale staining homogeneous texture, often observed in mainly C3 glomerulonephritis ($\times 6,000$ (**d, e**)). **f** An expanded mesangial area with increased cellularity and abundant deposits of similar less dense texture (arrows) and replacing most of the matrix ($\times 6,000$). C3GN, C3 glomerulonephritis; PAS, periodic acid-Schiff.

as early as 2 weeks posttransplant [1, 7, 23, 24] and progresses in an indolent manner with about 15–25% graft loss in 5–10 years.

Light Microscopy

The glomerular lesions by LM in both diseases are diverse and can broadly range from mild to severe, active or chronic glomerular changes, with heterogeneous involvement of the glomeruli on the same biopsy. Glomerular patterns of injury include mesangial proliferative, endocapillary proliferative with or without exudative features, MPGN, and crescentic GN with segmental or global sclerosing lesions. Recurrent C3GN can manifest mesangial or endocapillary proliferative lesions (Fig. 8a, b) fairly early in the posttransplant period, whereas early recurrent DDD may not manifest any or variable mesangial hypercellularity (Fig. 9a, b)

correlating with the asymptomatic clinical presentation requiring IF and EM to identify the complement deposits.

IF Microscopy

The dominant or the only parameter that is localized in C3GN and DDD is C3, varying in intensity depending on the quantity of deposits. There is granular pattern of staining along the glomerular capillary walls and mesangial areas in C3GN (Fig. 8c), while mesangial and capillary wall stretches of granular or ribbon-like pseudolinear staining is noted in DDD (Fig. 9c). No significant amounts of immunoglobulins are noted in the glomerular deposits. Staining for C4d is negative within the deposits as the alternate pathway of complement pathway is involved.

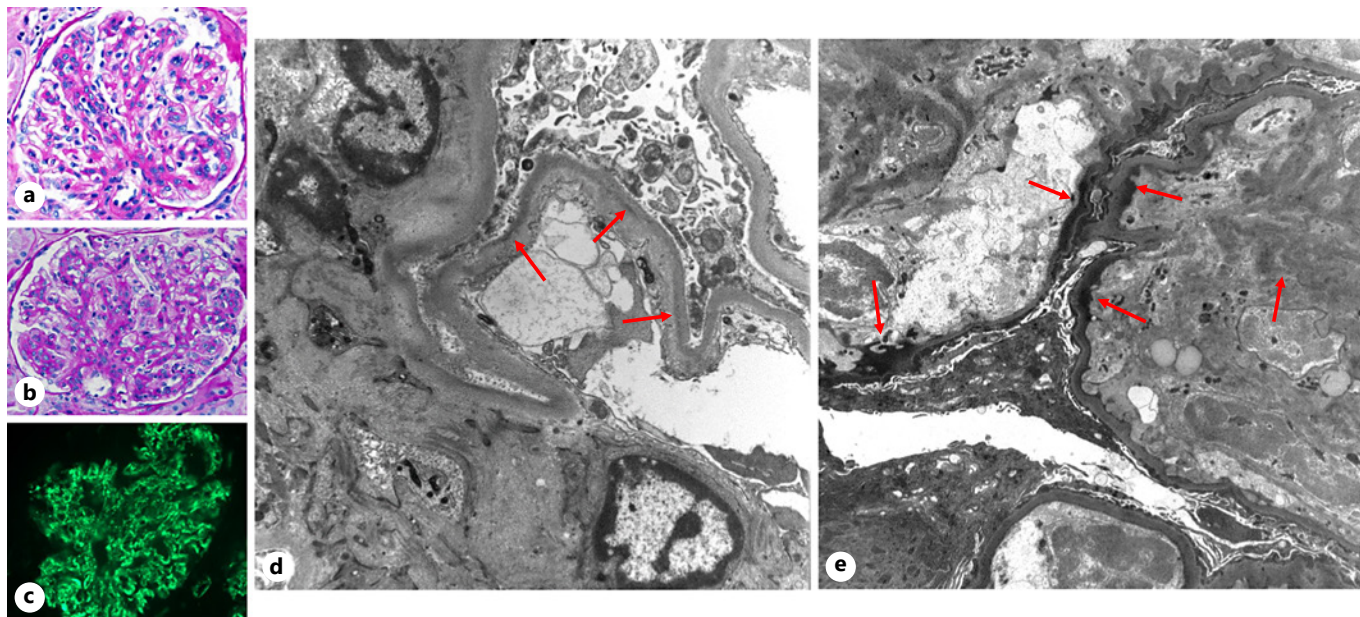


Fig. 9. Recurrent DDD. **a, b** Focal or global, mesangial hypercellularity and increased matrix and mild glomerulitis are noted in these glomeruli, partly encroaching on the capillary lumina (PAS, $\times 400$). **c** Strong granular C3 deposits are localized along the glomerular capillary walls and mesangial areas, suggesting abundant glomerular deposits (C3, $\times 400$). **d** EM in a mild case who was on treatment with anti-C5 antibody therapy, now presenting with sub-nephrotic proteinuria, demonstrates focal, short, interrupted stretches of intramembranous dense deposits of homogeneous

texture (arrows) ($\times 10,000$). **e** A repeat biopsy from this same patient 2 years later after discontinuation of therapy for over a year shows extremely dense partly intramembranous and subendothelial deposits involving interrupted or short stretches of capillary basement membranes and focally within the mesangial matrix (arrows). The foot processes appear preserved, while endothelial fenestrations are lost ($\times 6,000$). DDD, dense deposit disease; PAS, periodic acid-Schiff; EM, electron microscopy.

Electron Microscopy

EM is critical for distinguishing C3GN from DDD and therefore is required for the diagnosis of recurrence of either entity in the allograft [21]. Even with EM, there are instances that show significant overlap having features of both entities, particularly in the early phases of recurrences and a strong C3 staining by IF.

In C3GN, the deposits may be more widespread involving the mesangium, subendothelial, focal intramembranous (Fig. 8d, e), and occasional subepithelial areas, a few resembling “hump-like” deposits. The texture of the deposits appears of lower density closer to the matrix, finely granular to waxy, and amorphous in character (Fig. 8f).

In DDD, there may be minimal suggestion of early deposits (Fig. 9d), but longer duration of lesions show stretches or interrupted pattern of extremely dense homogeneous osmiophilic deposits within the glomerular basement membranes (GBMs) and smaller aggregates replacing the mesangial matrix (Fig. 9e). In lesions of longer duration of subsequent biopsies, the dense deposits of

similar characteristics may extend into the subendothelial zones. In some cases, tubular basement membrane and vascular dense deposits are also recognized by IF and EM.

The foot processes may be focally or partially effaced depending on the extent of the deposits. There is considerable endothelial swelling with loss of fenestrae in the proliferative glomerular lesions.

Lupus Nephritis

Definition

Recurrence of LN in the renal allograft generally represents milder forms of the glomerular lesions with IC deposits composed of mostly “full-house” IgG, IgM, IgA, C3, and C1q. The lesions represented are classes 1 and 2 mesangial LN, class 3 focal proliferative LN, and class 5 membranous LN. Diffuse proliferative LN seldom develops in the transplant setting, partly related to the ongoing immunosuppressive protocols that could modify the severity of the lesions [25].

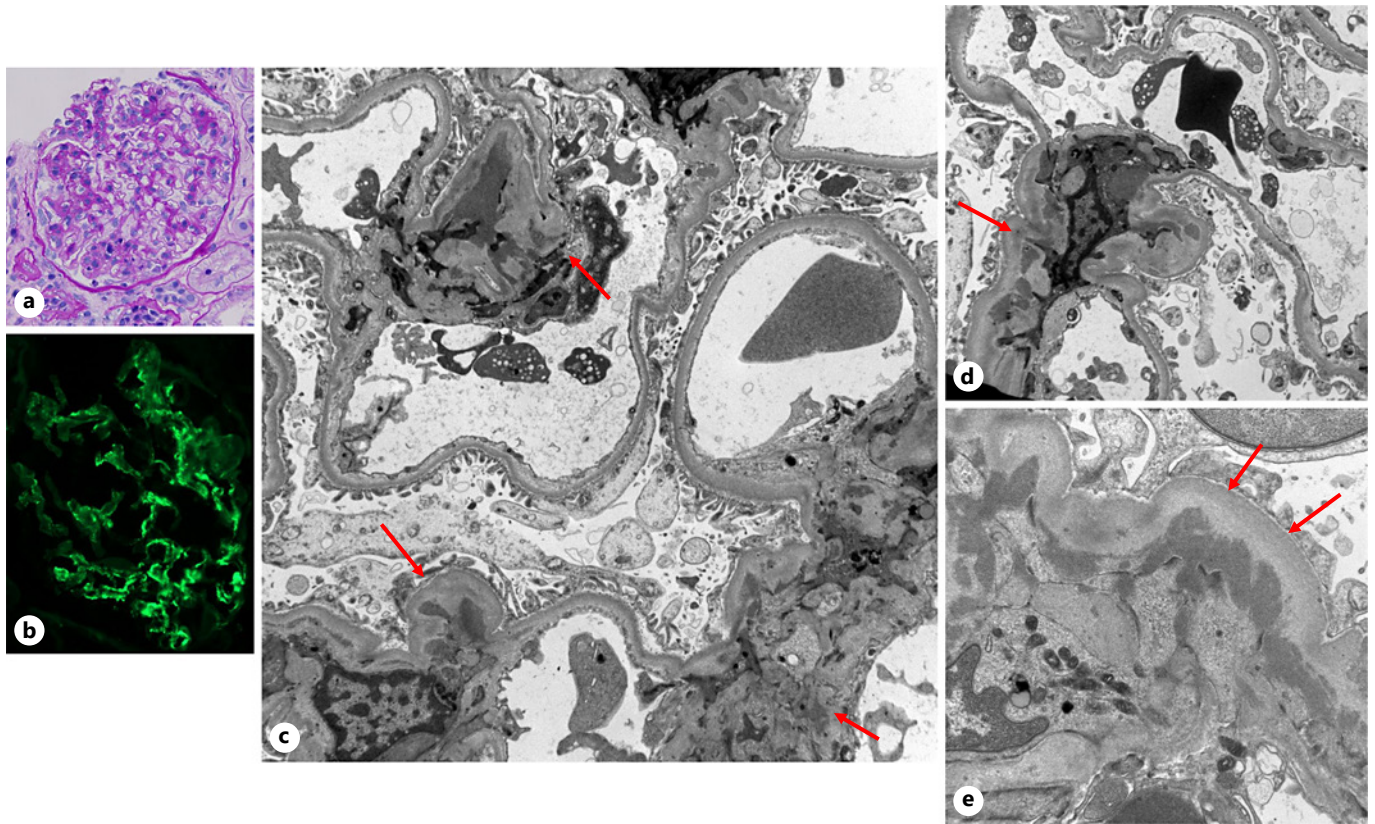


Fig. 10. Recurrent mesangial lupus GN (ISN/RPS class 2). **a** Glomerulus showing mild to moderate mesangial expansion and hypercellularity and normal-appearing peripheral capillaries with patent lumina (PAS, $\times 400$). **b** Granular IgG deposits are found restricted within the glomerular mesangial areas (IgG, $\times 400$). **c–e** Recurrent mesangial lupus glomerulonephritis showing vari-

able amounts of granular dense deposits mainly in the mesangial areas (arrows), while the peripheral capillary basement membranes are of normal thickness and texture with preserved foot processes ($\times 6,000$ (**c**), $\times 6,000$ (**d**), $\times 20,000$ (**e**)). GN, glomerulonephritis; PAS, periodic acid-Schiff.

Clinical Features

Based on the nature of the recurrent LN lesions, most appear to be subclinical or indolent with new-onset asymptomatic hematuria or proteinuria, without significant impact on renal function. This may often not be associated with clinical lupus symptoms or have full serologic evidence of systemic lupus erythematosus. In these cases, biopsy is required to make the diagnosis. The overall recurrence rate reported in some series is as high as 30–50% based on histologic diagnoses from protocol biopsies, though only around 10% show symptomatic clinical renal disease with low complement levels, contributing to relatively low graft loss. The risk factors for recurrence are younger age; living related donors, especially those having closer to 0 antigen mismatch; and patients of African descent, with a graft loss of 5–15% in 10 years.

Renal Pathology

The most common pattern that is noted in recurrent disease is mesangial LN (Fig. 10a), with a few focal LN and membranous LN cases (Fig. 11a) and very rarely diffuse LN. Unless a complete transplant renal biopsy examination including IF and EM is conducted, the diagnosis may be missed in the milder forms of glomerular lesions, particularly in asymptomatic patients. IF in most cases reveals positive staining for polyclonal IgG, IgM, C3, and C1q (Fig. 10b, 11b) in the glomerular mesangial and/or capillary wall locations in various stages of resolution, depending on the type and class of LN. EM exhibits the following: mesangial deposits observed in all classes (Fig. 10c–e); subendothelial and/or subepithelial deposits, depending on the class of LN; and endothelial tubuloreticular inclusions may or may not be readily visible. In cases of membranous LN, the subepithelial deposits may be small, frequently intramembranous, and in various

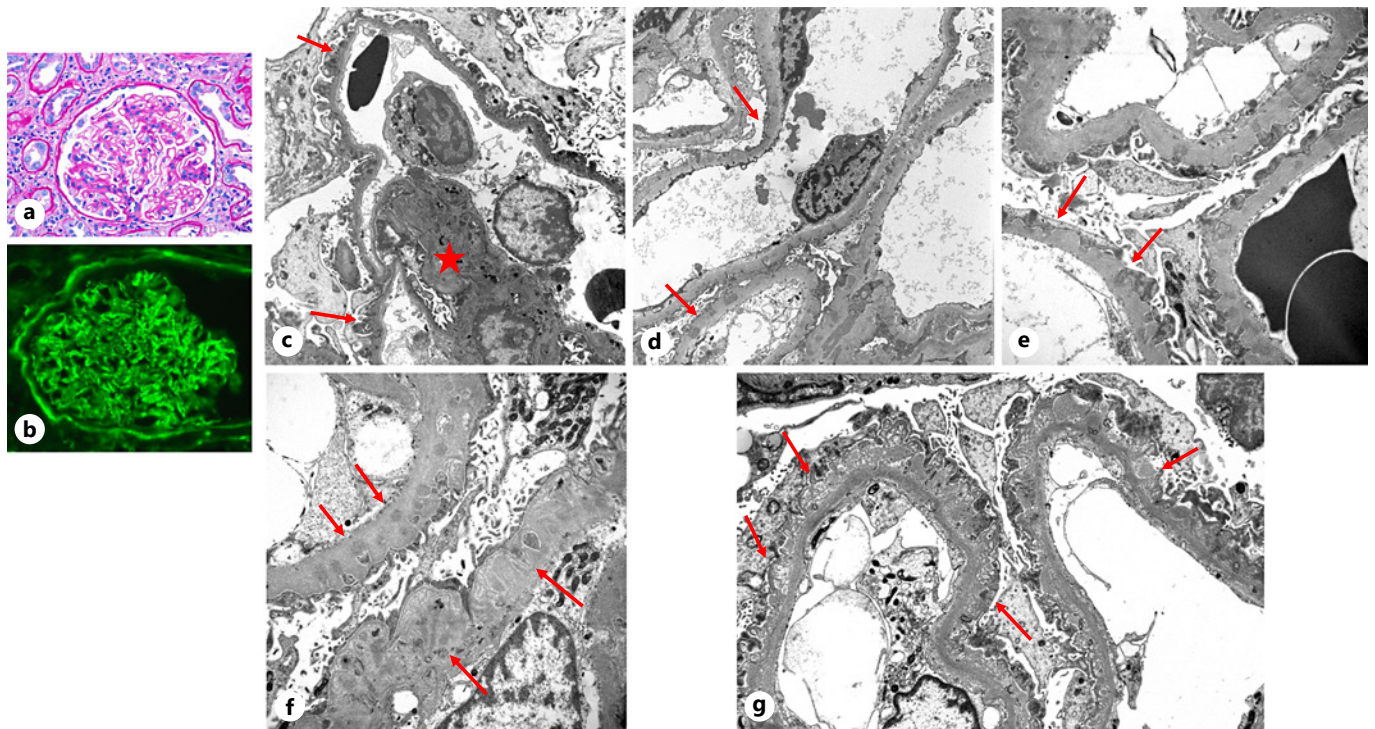


Fig. 11. Recurrent membranous lupus GN (ISN/RPS class 5). **a** Glomerulus displaying focal, mild to moderate mesangial hypercellularity and moderate thickening of the capillary walls with patent lumina, surrounded by mild chronic interstitial inflammation and tubular atrophy (PAS, $\times 400$). **b** The global, granular strong IgG staining along the glomerular capillary walls and mesangial areas (lesser intensity of IgM, IgA, C3, and C1q was also present) (IgG, $\times 400$). **c** A single glomerular capillary loop from an early membranous LN lesion shows small subepithelial electron-dense deposits with rare basement membrane spikes (arrows), localized foot process effacement, and small mesangial deposits (asterisk). No subendothelial deposits are visible ($\times 6,000$). **d–f** With disease

progression, the glomerular deposits are more numerous with frequent spike formation, partly intramembranous, with occasional evidence of resorption, leaving lucent spaces and accompanied by extensive foot process effacement. The endothelial cells can show segmental or extensive loss of fenestrations and mild swelling ($\times 6,000$ (**d**), $\times 12,000$ (**e**), $\times 12,000$ (**f**)). **g** More advanced cases show resolving intramembranous deposits with rarefaction or loose spaces containing small fragments of trapped epithelial cells and persistent foot process distortion or effacement. The lamina densa appears thickened and intact with no evidence of subendothelial deposits ($\times 6,000$). GN, glomerulonephritis; PAS, periodic acid-Schiff; LN, lupus nephritis.

stages of resolution with variable foot process effacement (Fig. 11c–g).

Fibrillary Glomerulonephritis

Definition and Clinical Background

FGN is a primary form of glomerular disease, accounting for about 1% or less of all the renal biopsies performed, with predominantly organized fibrillar polyclonal IC deposits in the capillary walls and the mesangial areas causing asymptomatic proteinuria, nephrotic or a nephritic syndrome, hypertension, and varying renal insufficiency depending on the pathological lesions. FGN has been encountered mainly in adult patients with a slight predilection for females. Although no specific serological marker is known, a proportion of these patients have underlying

malignancies or autoimmune diseases and most (>85%) reach end-stage disease in 4 years. Transplant recurrence of FGN is not uncommon, and over one-third of cases may recur within 1–10 years presenting with subnephrotic or nephrotic range proteinuria, causing graft loss in <50% in a study [26–28]. Another larger outcome study reported that the renal allograft survival was comparable to other causes of ESKD [26].

Light Microscopy

The glomerular lesions of FGN in the native kidney range from a mild form of mesangial GN, MGN with variable mesangial involvement, and proliferative/exudative GN with or without crescents to an MPGN pattern. This is usually accompanied by mild to moderate tubular atro-

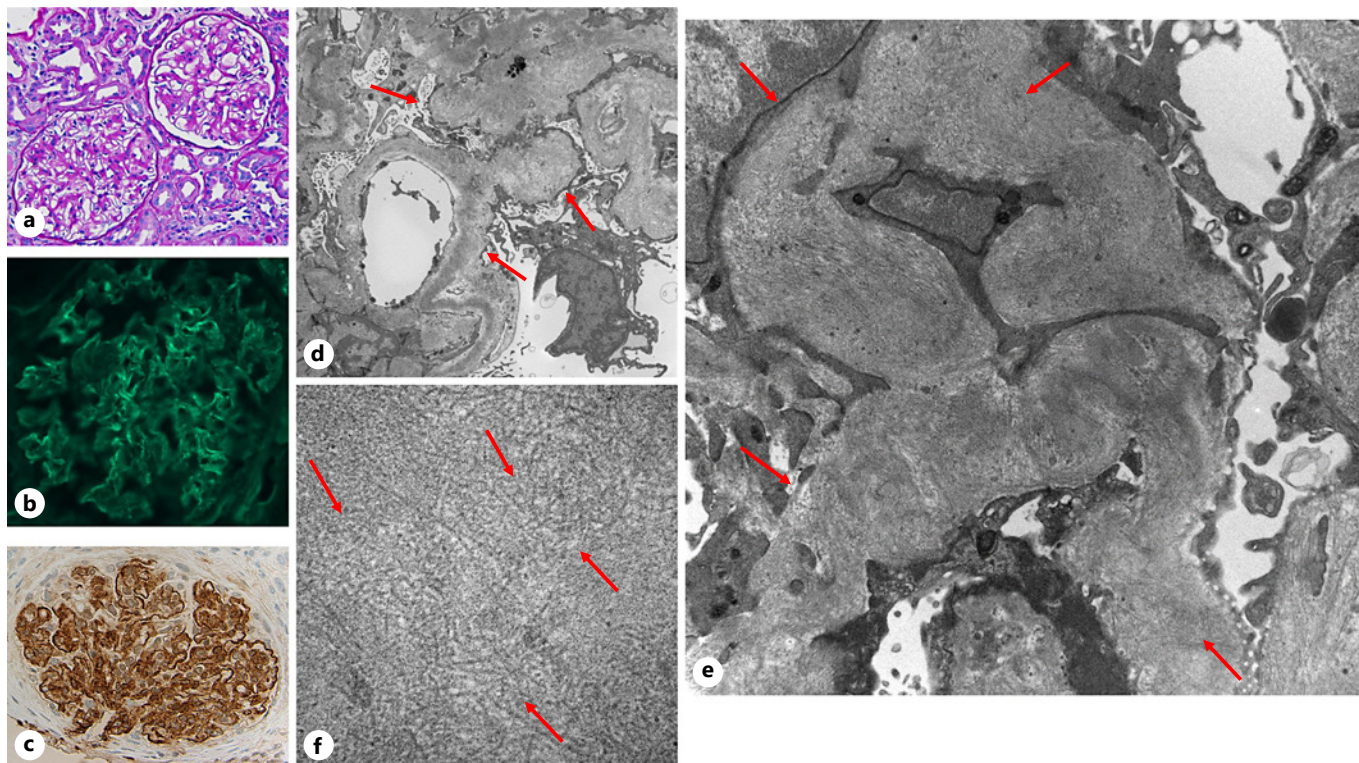


Fig. 12. Recurrent FGN. This is a 55-year-old woman transplanted with a deceased donor kidney due to unknown ESKD 1 year prior to biopsy. The renal function was stable (creatinine of 0.9 mg/dL) with subnephrotic proteinuria (1.5 g/24 h). **a** The glomeruli appear mild to moderately enlarged showing focal mild mesangial expansion without significant hypercellularity. The peripheral capillary walls appear somewhat thickened with no apparent irregularity (PAS, $\times 400$). **b** IF disclosed positive relatively low intensity of polyclonal IgG staining in a pseudo-linear pattern within the capillaries and focally in the mesangial areas (IgG, $\times 400$). **c** Immunohistochemistry performed on paraffin-embedded tissue shows strongly positive, granular staining for DNAJB9 proteins within the deposits along the glomerular capillary walls and mesangial areas (PAP, $\times 400$). **d, e** EM displayed almost nonuniform full-thickness infil-

tration of the extracellular matrix by loose pale deposits, showing a fibrillar configuration (arrows), effacing much of the lamina densa of the glomerular capillary walls ($\times 600$ (**d**), $\times 15,000$ (**e**)). **f** High-resolution magnification of these fibrillar deposits display randomly arranged, straight fibrils having the solid cross section (arrows) and ranging from 18 to 23 nm in diameter, infiltrating a background of focal finely granular matrix ($\times 70,000$). These deposits have to be differentiated from other forms of organized deposits within the glomeruli by their appearance on LM, staining properties, composition, and morphology by EM. FGN, fibrillary glomerulonephritis; ESKD, end-stage kidney disease; PAS, periodic acid-Schiff; IF, immunofluorescence; EM, electron microscopy; LM, light microscopy.

phy and interstitial fibrosis with vascular sclerosis. Since significant information regarding the pathological details of recurrent FGN are not available in the reported literature, the frequency and the types of the glomerular lesions occurring in the allograft are not known. It could be assumed that the glomerular lesions may be milder with mesangial and capillary wall thickening initially without significant proliferative features (as seen in our case at 1 year posttransplant with proteinuria of 2 g/24 h) (Fig. 12a), probably related to ongoing immunosuppressive therapy. Recently, DNAJB9, a member of the heat shock protein family, has been exclusively localized within the fibrillary deposits by immunohistochemistry (Fig. 12c). This may

serve as a marker of fibrillary GN in suspected cases [27], when EM facility or adequate tissue is not available for confirmation by EM. Although most cases are negative for Congo red stain, a small number of cases with Congo red-stained fibrillar deposits are reported [27].

IF and EM

As in the native FGN cases, there is polyclonal dominant IgG with strong C3 staining of the immune deposits with lesser intensities of IgA and IgM, and sometimes trace to 1 + C1q, demonstrating a smudgy, rather homogeneous/semilinear staining pattern, in the mesangial areas and capillary walls (Fig. 12b). The diagnosis of FGN

is essentially made by EM, where the deposits typically appear mostly fibrillar, infiltrating the extracellular matrix in the mesangial areas and subepithelial and intramembranous locations. The fibrils are of variable length, randomly arranged and straight, with a solid cross section, often displaying an irregular or a stellate shape, ranging from 15 to 25 nm in thickness (Fig. 12d–f). The foot process effacement is widespread and depends on the extent of basement membrane involvement. Although extraglomerular fibrillar deposits may be found in tubular basement membrane and microvasculature, in some of the variants of native kidney FGN, no information is available in the recurrent cases.

Paraprotein-Related Renal Lesions

Monoclonal Immunoglobulin Deposition Disease

Definition and Background

Monoclonal immunoglobulin deposition disease (MIDD) is a systemic disease as a result of deposition of abnormal, monoclonal light and/or heavy chains originating from an overt or subclinical form plasma cell dyscrasia or sometimes B-cell malignancies or monoclonal gammopathy of renal significance (MGRS), mostly affecting adults. The kidney is a major target of the monoclonal proteins and MIDD, producing a spectrum of glomerular lesions and tubulointerstitial and vascular disease due to a unique pattern of monoclonal deposits, with a tendency to progress to end stage within 24–36 months. Due to the advances in the treatment of myeloma and other monoclonal disorders leading to longer survival of patients, renal transplant has been used as replacement therapy. The prospect of renal transplantation is challenging as to what level of hematological remission to be achieved and when to transplant in such patients. Although data regarding the posttransplant course of the disease are scant, MIDD tends to recur at a high rate and rapidly in renal transplants, within the first 6 months to 3 years, with persistent or incompletely controlled hematological disease. This can affect the allograft almost immediately following transplantation, producing pathological lesions akin to the original disease in the native kidney [29].

Clinical Features

The clinical renal disease following transplantation in the setting of MIDD may present with initial low level of proteinuria and progressive rise in creatinine. If the glomerular lesions develop an active proliferative lesion, a

more nephritic picture may develop. Regular hematological monitoring of the disease is recommended, where monoclonal serum markers are available for detection. Complement levels in these cases are normal. The detection of serum or urine monoclonal protein is less predictable in cases of MIDD, due to the absence of significant bone marrow disease, and low levels of monoclonal protein produced by smaller clones, as described in MGRS. The course of posttransplant MIDD and graft survival following recurrence, which spans 7–25 years, depending on the hematological response to treatment.

Light Microscopy

Since only a few studies are available addressing transplant recurrence of MIDD, the range of glomerular lesions is not completely known. In the earlier lesions, the deposits may not elicit a significant response as in our case showing only minimal focal mesangial prominence and capillary wall thickening that is PAS positive (Fig. 13a). The other lesions are mesangial proliferative GN and some progression to sclerosing changes or nodular sclerosis resembling diabetic glomerulopathy in the later stages. The tubular basement membranes and small arterial walls appear relatively of normal thickness in the earlier lesions but may show increased thickening, expansion, and layering, which is strongly PAS positive.

IF Microscopy

As in the native kidney disease, there is diffuse linear staining along the glomerular capillary basement membranes in the mesangial areas and all tubular basement membranes for monoclonal kappa (Fig. 13b) or lambda light chains, or with IgG heavy chains. Similar staining is also visible within the small arterial vessels in advanced cases. No localization of other immunoglobulins or complement components is noted.

Electron Microscopy

The EM findings are similar to those observed in the native kidney disease, though more milder forms of glomerular lesions or deposits may occur, probably altered by immunosuppression in transplantation. The fine particulate electron-dense deposits localized as a narrow band-like pattern mainly along the inner aspect or the lamina rara interna of the GBMs in an attenuated or global diffuse manner (Fig. 13c, d). This is a typical or unique feature of MIDD, not seen in other forms of paraproteinemia-associated renal lesions. There may be focal involvement of the lamina densa. Similar deposits may be seen in the mesangial matrix (Fig. 13d). The tubular

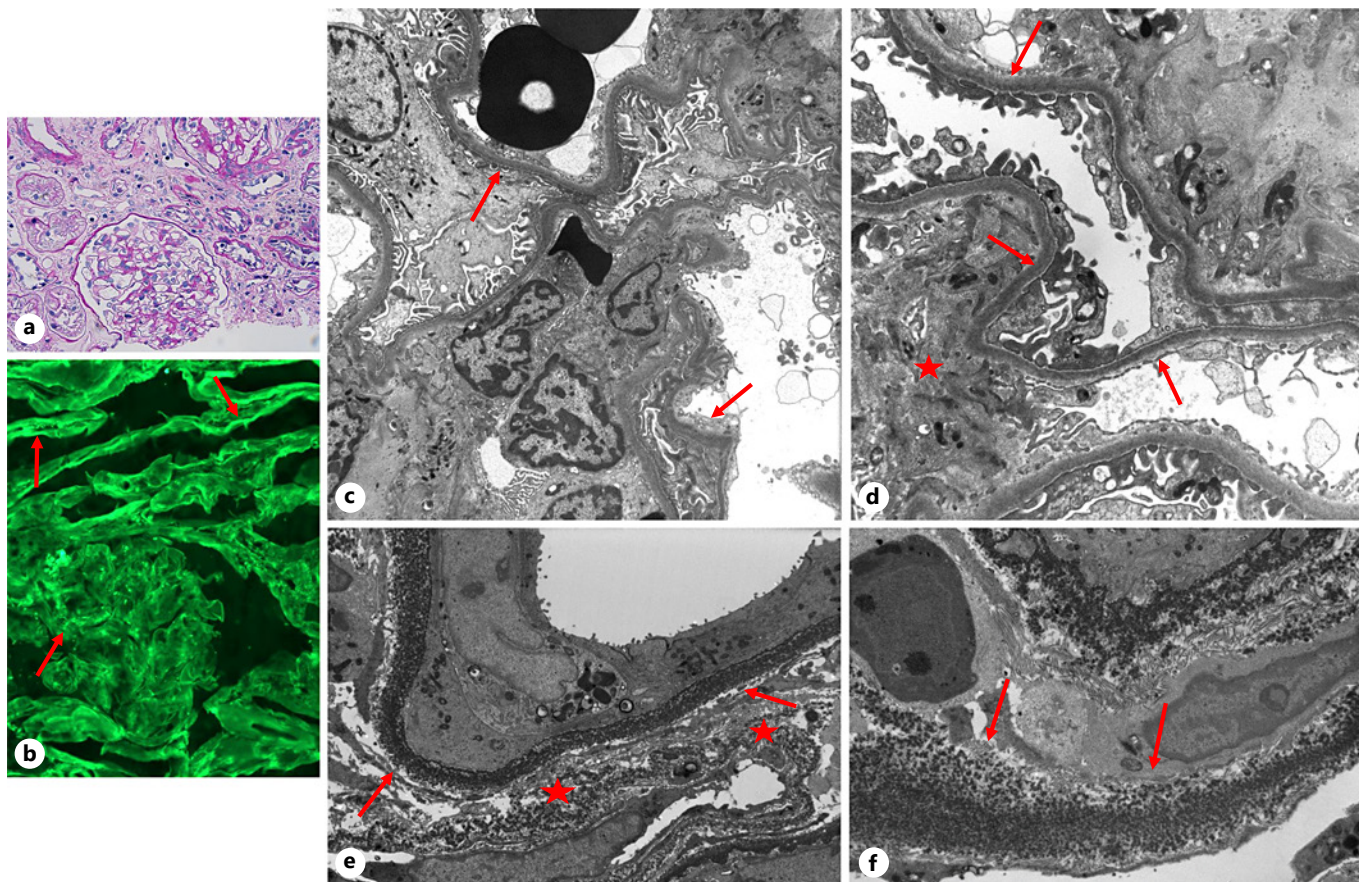


Fig. 13. Recurrent MIDD. This case had a prior history of ESKD disease as a result of MIDD and presented with subnephrotic proteinuria and stable renal function 8 months posttransplantation. **a** The glomeruli show no significant changes surrounded by a relatively preserved tubulointerstitial compartment (PAS, $\times 400$). **b** IF shows the characteristic, strong linear staining for exclusively kappa light chains along the tubular basement membranes, Bowman capsule, and within the glomerular capillary walls (arrows) (kappa light chains, $\times 400$). **c, d** In the absence of other glomerular changes, by EM the capillary basement membranes appear somewhat mottled with dark, fine electron-dense deposits that appear to be stronger in the subendothelial areas (arrows), while the foot processes are generally preserved. In image **d**, focal mesangial den-

ties suggest monoclonal deposits (asterisk) ($\times 6,000$ (**c, d**)). **e, f** These images depict high magnification of tubular basement membranes showing dark, coarsely granular, or “peppery” deposits on the outer aspect, infiltrating the thickness of the basement membranes. In image **e**, a peritubular capillary also shows deposits along the basal lamina of similar texture (asterisks). The finding of these characteristic deposits along the tubular basement membranes with or without glomerular involvement is a universal feature of MIDD ($\times 8,000$ (**e**), $\times 10,000$ (**f**)). MIDD, monoclonal immunoglobulin deposition disease; ESKD, end-stage kidney disease; PAS, periodic acid-Schiff; IF, immunofluorescence; EM, electron microscopy.

basement membranes display these coarsely granular to “peppery” deposits on the outer aspect or partly incorporated into the superficial layers of the matrix with time (Fig. 13e, f). Depending on the duration of disease and abundance, they may be found in the peritubular capillary basement membranes and in the interstitial space (Fig. 13e).

Proliferative Glomerulonephritis with Monoclonal IgG Deposits

Definition

Proliferative GN with monoclonal IgG deposits is defined as a form of glomerular lesion developed as a result of deposition of an intact monoclonal IgG with light chain restriction and a single subtype, commonly IgG3, having a granular texture by EM, found in the glomerular subendothelial zones of the capillary basement mem-

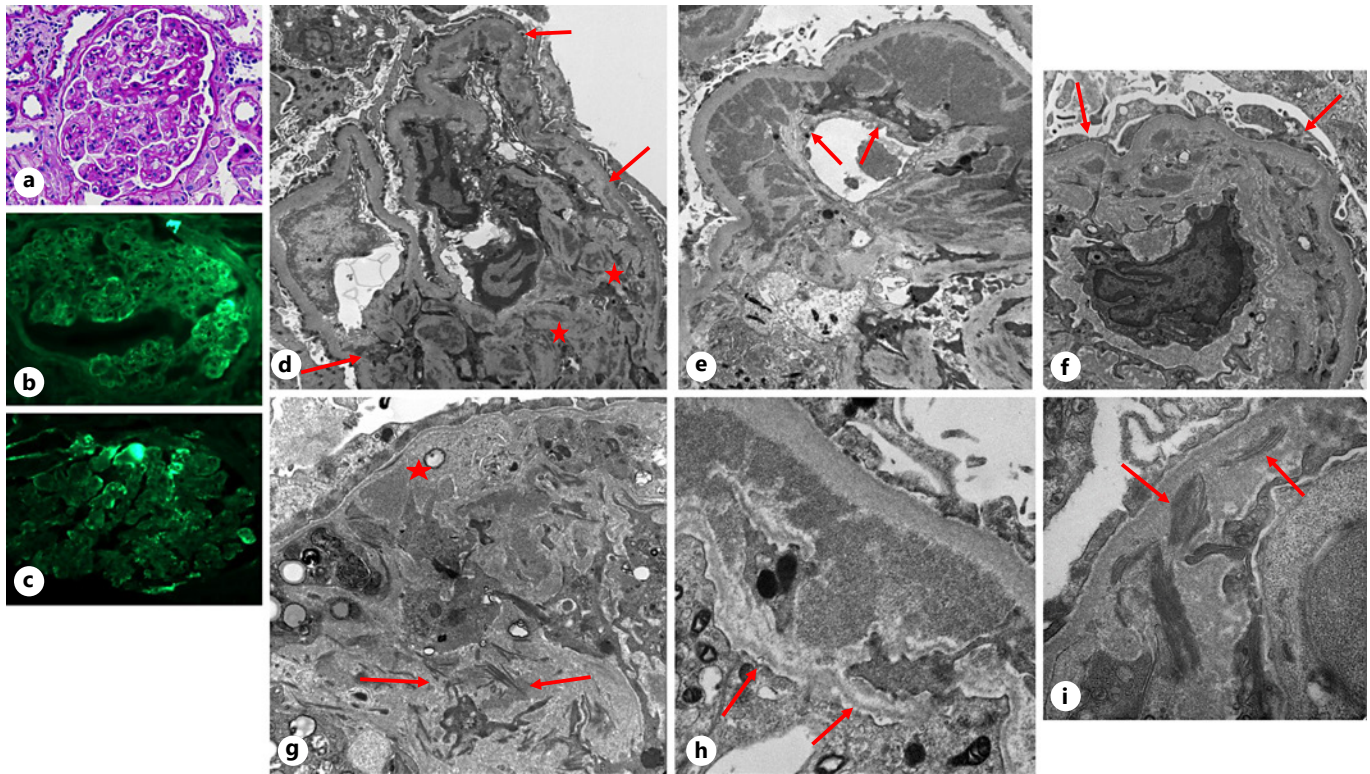


Fig. 14. PGNMID generally mimics the usual range of patterns of proliferative glomerular lesions of IC type but contains monoclonal immunoglobulins. **a** The glomerulus exhibits mild lobular pattern with mesangial and focal endocapillary hypercellularity as well as marked thickening of the capillary walls with frequent double contours and cellular interposition (PAS, $\times 400$). **b, c** There is granular focally strong staining for IgG and C3 within the glomerular capillary tufts (B-IgG, C-C3, $\times 400$). **d-f** Glomerular capillary basement membranes showing focal to segmental subendothelial and mesangial (asterisk) mainly finely granular electron-dense deposits. There is intact lamina densa with segmental to circumfer-

ential formation of new layers of basement membrane material in the subendothelial area with focal cellular interposition (arrows) ($\times 6,000$ (**d, e**), $\times 150,000$ (**f**)). **g-i** High-magnification images of the glomerular capillary wall showing subendothelial expansion with mostly finely granular electron-dense deposits, interspersed by focal, small clusters of organized parallel arranged fibrillar deposits (**g, i**) (arrows). Focal cellular interposition and new basement membrane formation are also observed (**h**) ($\times 15,000$ (**g**), $25,000$ (**h**), $\times 40,000$ (**i**)). PGNMID, proliferative glomerulonephritis with monoclonal immunoglobulin deposition disease; IC, immune complex; PAS, periodic acid-Schiff.

brane and mesangial areas, giving rise to endocapillary proliferative or MPGN.

Clinical Features

This is a disease commonly occurring in the older age groups although some young adults have been affected as well with a slight predominance of females, mostly Caucasian. The initial recurrent clinical renal disease may begin within a few days to weeks following transplantation, characterized by subnephrotic or nephrotic range proteinuria and progressive renal insufficiency. Cases of proliferative GN with monoclonal IgG deposits have shown a high potential for rapid recurrence in $>90\%$ of patients, as seen in protocol biopsies [30, 31]. No established serum markers sensitive to detect monoclonal protein are

currently available in majority of the cases, and some fall under the category of MGRS. Another important feature is the presence of hypocomplementemia and detectable monoclonal protein in the serum in a small proportion of patients. Since the magnitude of proteinuria and longer time from transplant to diagnosis have emerged as predictors of graft loss, which nears 50% in 3 years, appropriate immunosuppressive therapy to reduce proteinuria has resulted in improvement clinically and of the pathological lesions.

Light Microscopy

Unlike the fully developed global endocapillary proliferative, MPGN or rarely MGN often seen in the native kidney disease, an earlier lesion may manifest itself with

proteinuria in the posttransplant setting. This may appear as a mild form of mesangial proliferative or focal proliferative GN with minimal or moderate hypercellularity and without significant tubulointerstitial disease. These lesions can progress with time when appropriate therapeutic intervention is not in place. They can also develop global proliferative changes with exudative features of infiltrating neutrophils or MPGN pattern revealing segmental to circumferential capillary wall interposition resulting in double contours by PAS or silver stains with infiltrating capillary mononuclear leucocytes, particularly macrophages (Fig. 14a), following which segmental to global glomerulosclerosis may ensue accompanied by interstitial inflammation and chronic tubulointerstitial scarring.

IF Microscopy

When prior native disease is known, the IF testing panel can be adjusted to detect monoclonal deposits. IgG deposits with a kappa or lambda light chain restriction primarily involve glomeruli, along the capillary walls and mesangial areas in a global distribution with mainly granular pattern of staining (Fig. 14b). These deposits also almost always stain strongly for C3 (Fig. 14c) and to some extent for C1q. When suspected of a recurrence of a monoclonal disease, IgG subtyping can be performed using specific antibodies to IgG1–4 subtypes to confirm the monotypic nature of the IgG deposits.

Electron Microscopy

EM generally demonstrates granular subendothelial and mesangial deposits with segmental capillary wall interposition (Fig. 14d–i), without involvement of the extraglomerular structures, such as the Bowman capsule, tubular basement membranes, arterial vessels, and adjacent microvasculature, a feature of this entity. On occasion, small clusters of organized fibrils may be present amid mostly granular deposits, as a few short bundles measuring 20–25 nm in thickness in the subendothelial and mesangial zones (Fig. 14g, i).

Amyloidosis

Definition and Background

Recurrent amyloidosis is defined as the appearance of amyloid deposits in the allograft, having similar composition prior to ESKD. With the emerging newer therapies to improve and control certain specific types of this multisystem disease and increasing methods of early diagnosis of amyloidosis, renal transplantation has become a viable option to more patients now. Most forms of amyloi-

dosis (systemic light chain AL type, systemic amyloid A-AA type that are secondary to familial Mediterranean fever, other chronic inflammatory states, and certain hereditary types) have been found to recur with various frequencies. The availability of novel biologics in AA amyloidosis and newer chemotherapeutic agents including anti-plasma cell therapies and stem cell transplantation for AL amyloid have contributed significantly to minimize the precursor proteins in these forms [32]. Known cases of specific type of amyloidosis (AL or AA type) with ESKD are usually worked up for eligibility of renal transplantation. The main criteria for patient selection include complete remission or very good partial response of underlying hematological disease, absence of heart involvement (AL type), or elimination of the chronic infectious process or quiescence of the inflammatory disorder (AA type), thus controlling the production of precursor protein and recurrence of disease posttransplantation for a low rate of graft loss or patient mortality. While those with elimination of the chronic infectious process may benefit from long survival of the graft, those with an incomplete remission or relapse of the hematologic malignancy are at risk for recurrence within 5 years [32–34].

Clinical Features

The earliest clinical renal finding of recurrence is detection of subnephrotic proteinuria on routine testing without significant renal dysfunction. Alternately, they may present with new-onset nephrotic syndrome, particularly in the absence of other transplant-related processes. Rarely, extrarenal involvement may become apparent clinically. In view of the favorable impact on graft function, it is usually assessed as the rate of graft survival rather than graft loss. The overall long-term (5–10 years) graft survival following recurrence of amyloidosis of most types has been documented as >50% or higher, provided the underlying diseases are appropriately managed.

Renal Pathology

A kidney biopsy performed for the investigation of new onset of proteinuria will help in making the definitive diagnosis of recurrent amyloidosis of any stage and exclude other causes of chronic posttransplant renal parenchymal changes that may cause proteinuric states. In the initial stages only, minimal glomerular changes may be evident by LM with pale staining amorphous deposits accumulating progressively in the glomeruli, tubulointerstitial compartment, or vascular walls in the later stages, confirmed by routine Congo red staining (Fig. 15a–c). IF, immunohistochemistry, or mass spectrometry may be

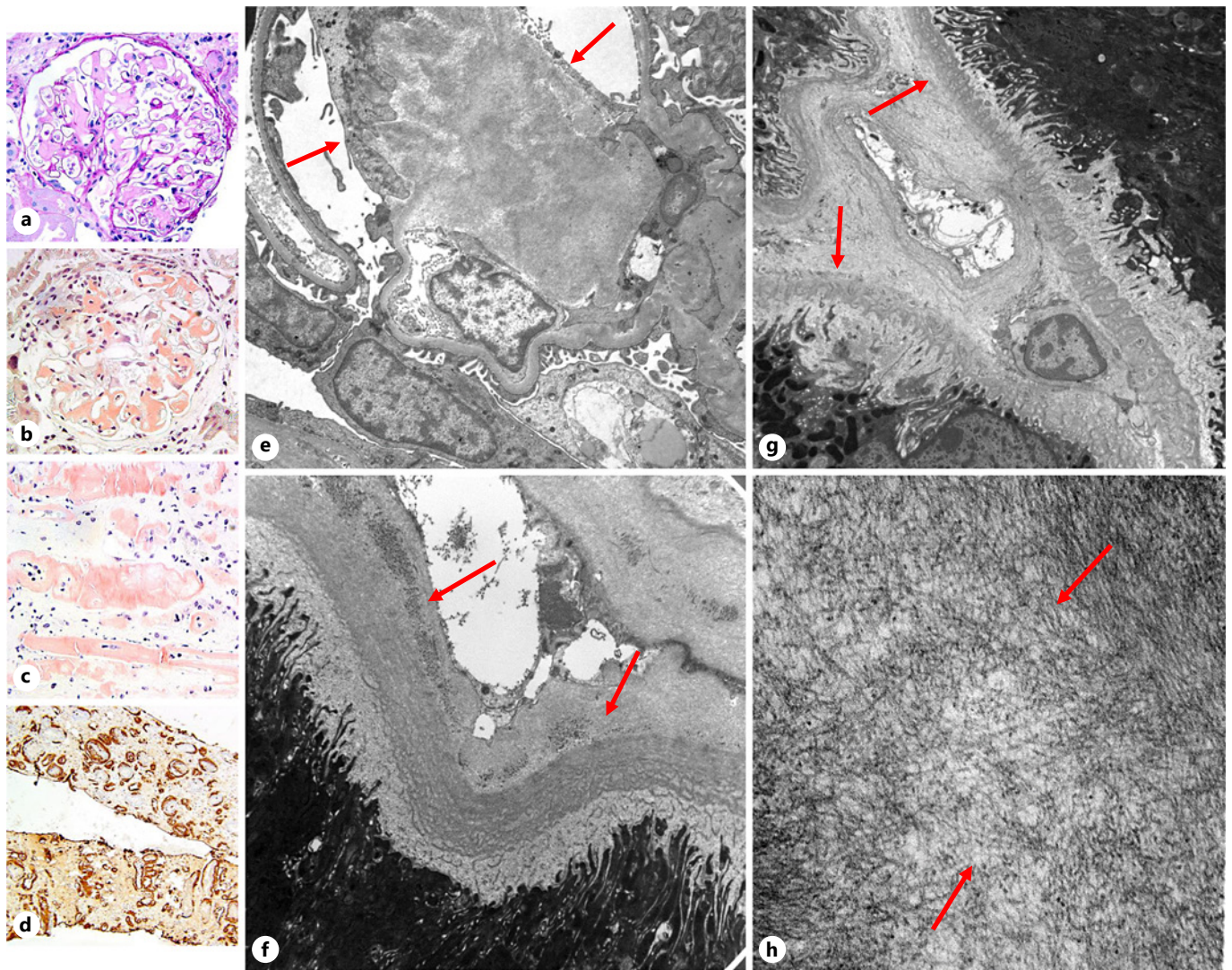


Fig. 15. Recurrent renal amyloidosis, AA type. This is a patient who developed ESKD as a result of advanced AA type amyloidosis, secondary to familial Mediterranean fever, who had several other affected family members. **a–c** The glomeruli show a bland architecture with mainly pale mesangial expansion involved by amyloid deposits as well as along tubular basement membranes, both of which are confirmed by Congo red staining (–PAS, $\times 400$ (**a**); Congo red, $\times 400$ (**b**, **c**)). **d** Immunoperoxidase stain on paraffin-embedded tissue is strongly positive for amyloid A protein along the tubular basement membranes, no glomerulus is present in this field (PAP, $\times 200$). **e** The glomerulus shows marked mesangial expansion by loose fibrillar deposits, extending into the overlying basement membrane with focal spicule formation covered by ef-

–faced epithelial foot processes (arrows), while other capillary basement membranes are uninvolved with preserved foot processes ($\times 6,000$). **f**, **g** The tubular basement membranes are markedly and irregularly expanded by full-thickness infiltration by loose, fine fibrillar deposits, lifting the overlying tubular epithelial cells and forming new layers of basement membrane material. A peritubular capillary (**g**) also shows full-thickness involvement and replacement by fibrillar deposits ($\times 6,000$ (**f**, **g**)). **h** High magnification of the glomerular fibrillar deposits reveal randomly arranged rigid fibrils with a solid cross section, ranging from 9 to 12 nm in diameter, diagnostic of amyloid deposits ($\times 100,000$). ESKD, end-stage kidney disease; PAS, periodic acid-Schiff.

used to localize and type the amyloid protein deposits, correlating with the original type of amyloid deposits at end stage (Fig. 15d). While EM is seldom pursued in many institutions for confirmation, the classic morphol-

ogy and size of fibrillar deposits are identified within the various locations of the glomeruli, tubulointerstitium, and microvasculature, depending on the predilection and type of amyloid involved (Fig. 15e–g). The fibrils are ran-

domly arranged, rigid with a solid cross section, ranging from 8 to 12 nm in diameter (Fig. 15h).

Diabetic Kidney Disease

Definition and Background

DKD constitutes a major cause of ESKD in most countries. Recurrence of diabetes kidney disease is defined as the appearance of renal histological features of diabetes in a nondiabetic allograft at 2–3 years posttransplantation, with or without initial overt clinical renal disease or mild proteinuria, indicating onset of diabetic glomerular disease. Although these changes are milder, they have to be distinguished from preexisting donor-related diabetic renal disease by way of comparison to preimplantation or postimplantation protocol biopsy, when available. Based on the natural history of the renal disease, generally it may require at least 5 years or more to fully develop the typical glomerular, tubulointerstitial, and vascular lesions. However, it has been noted that the time to develop histological evidence of recurrent DKD may be more rapid than usually anticipated ($6.68 + 3.86$ years) [35]. Risk factors of recurrence are suggested as inadequate posttransplant glycemic control that may be enhanced by immunosuppressive therapies containing calcineurin inhibitors (e.g., tacrolimus) and mTOR inhibitors (e.g., rapamycin) [36], although the clinical predictors of DKD or those related to transplantation have not been predictive of recurrences [35].

Clinical Features

It is imperative that posttransplant diabetes is in good control since DKD develops during a persistent mild or moderate clinical diabetic state. Recurrence rate may be as high as 25–40% with graft loss in 5–10 years, comparable to those with other diseases. There may be minimal to moderate or nephrotic-range proteinuria. Progressive loss of renal function with rise in creatinine takes place with advanced parenchymal disease and vascular sclerosis even in the absence of rejection of infection-associated changes.

Light Microscopy

A renal biopsy is needed to establish early recurrence and record the advancement of the diabetic lesion in the renal allograft. The subtle glomerular alterations may begin to appear within 6 months, as arteriolar hyalinosis and moderate glomerulomegaly with minimal or no mesangial expansion by matrix, and may be attributed to compensatory hypertrophy following transplantation. These early changes are followed by progressive thicken-

ing of glomerular and tubular basement membranes and a mild to moderate increase in mesangial matrix, spanning 2–3 years (Fig. 16a, b). Late changes, in addition to the above, include a marked increase in mesangial matrix, with or without nodular configuration after 5–10 years, without significant hypercellularity (average 8 years) (Fig. 16c). Varying extent of tubular atrophy and interstitial fibrosis along with progressive arteriosclerosis and diffuse arteriolar intimal hyalinosis, often circumferential, with microvascular obliterative features are recognized in advanced cases.

IF Microscopy

Linear positive staining of IgG and albumin is seen in all glomerular and tubular basement membranes (Fig. 16d) in a diffuse distribution as part of the alteration of the extracellular matrix by the hyperglycemic state. No IC-type electron-dense deposits are detected.

Electron Microscopy

The ultrastructural examination is most useful in the early diagnosis of evolving DKD, where the mildest alterations of the basement membrane matrix are best visible. This is followed by mild to moderate mesangial expansion with increasing nodular matrix, which may be nonuniform in all the glomeruli examined as well as from one lobule to another in the same glomerulus (Fig. 16e–f). The foot processes are preserved early, with mild flattening and distortion or focal to partial effacement (Fig. 16h), as the diabetic lesion advances with striking thickening of the basement membranes reaching up to 1,200 nm. The GBMs generally have a regular contour and uniform texture in those without sclerosing changes. Progressive thickening of the tubular basement membranes is also observed (Fig. 16i) concurrently and may help to differentiate other conditions of isolated GBM thickening. Although IC-type deposits are not present, focal capillary and mesangial finely granular insudative protein/hyaline deposits may be seen. On rare occasion, another primary or secondary IC-mediated glomerular lesion may be superimposed, for example, recurrent/de novo MGN (Fig. 16j) or infection-related GN with characteristic capillary wall and/or mesangial deposits.

Differential Diagnosis

Some of the earliest features by EM of recurrent diabetic glomerular disease may be nonspecific, such as GBM thickening and mild mesangial expansion with matrix. Similar findings may be seen in hyperfiltration-related GBM injury such as from metabolic disease, obesity,

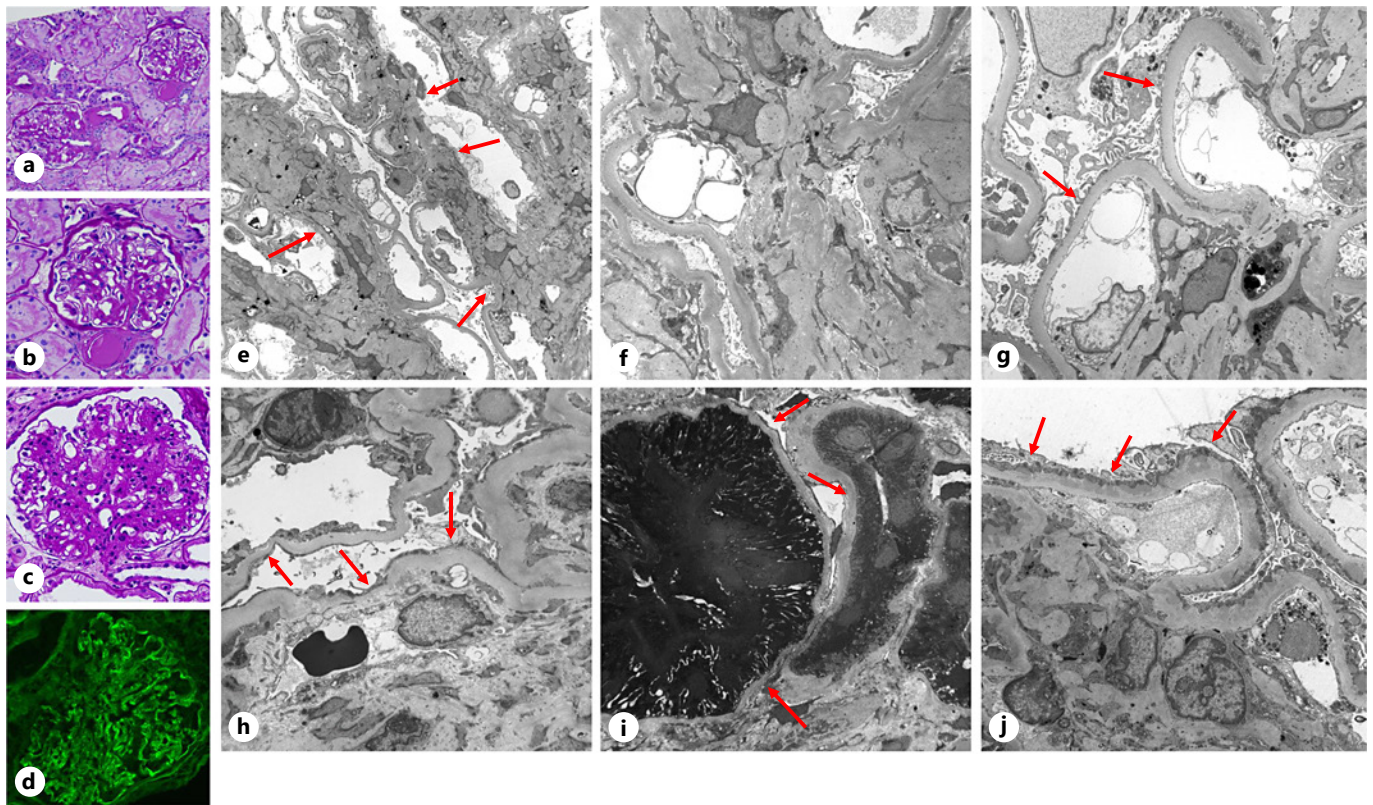


Fig. 16. Recurrent DKD. **a, b** This patient had a history of diabetic nephropathy with end-stage, developed subnephrotic proteinuria of 1.2 g per 24 h, 28 months posttransplantation. The glomeruli show mild mesangial expansion with mainly increased matrix and minimal to mild peripheral capillary wall thickening suggesting diabetic glomerulopathy. While most of the tubulointerstitial compartment is relatively preserved with small foci of tubular atrophy, many of the hilar arterioles exhibit moderate to marked hyalinosis, indicating development of recurrent DKD (PAS, $\times 200$, $\times 400$). **c** Glomerulus in a more advanced stage of DKD and glomerulopathy discloses global, moderate to marked mesangial expansion with mainly matrix encroaching on the peripheral capillary lumina, with focal nodular configuration (PAS, $\times 400$). **d** Linear positive IgG staining is noted in the glomeruli as well as along the tubular basement membranes (IgG, $\times 400$). **e–g** Diabetic glomerulopathy may begin with mild glomerular mesangial widening by nodular increase in matrix at the expense of cells and minimal to mild capillary basement membrane thickening. Progression of disease shows increasing mesangial expansion (arrows) and thickening of capillary basement membranes, which can attain 2 or 3

times the normal thickness with relatively preserved foot processes (arrows) ($\times 1,800$ (**e**), $\times 60,000$ (**f**, **g**)). **h** In some cases, particularly in those with increasing proteinuric states, significant podocyte injury and foot process effacement (arrows) may also develop in the setting of diabetic glomerulopathy without a superimposed primary glomerular podocytopathy ($\times 6,000$). **i** The tubular basement membranes also progressively increase in thickness (arrows) and may or may not follow the same pace as GBMs ($\times 1,800$). **j** Another case of recurrent diabetic glomerulosclerosis, now presenting with new-onset nephrotic syndrome as a result of a superimposed de novo primary MGN (PLA2R+) 6 years following transplantation. In addition to diabetic glomerular changes, this image also shows numerous subepithelial electron-dense deposits interspersed by spikes (arrows) with total foot process effacement. While there is nodular mesangial expansion of the matrix with a few cells, no deposits are identified ($\times 6,000$). DKD, diabetic kidney disease; PAS, periodic acid-Schiff; GBM, glomerular basement membrane; MGN, membranous glomerulonephritis; PLA2R, phospholipase A2 receptor.

hypertension, prolonged smoking history, or long-standing solitary kidney, as in a renal allograft. Patients with HIV, autoimmune disease, or a donor with underlying genetic disease such as APOL1 high-risk alleles may also manifest thickening of the GBM. It is useful to obtain clinical information for a relevant clinicopathologic correlation in these cases.

Antineutrophil Cytoplasmic Antibody-Associated Vasculitis and Crescentic GN

Definition and Background

Renal involvement is known to occur in antineutrophil cytoplasmic antibody (ANCA)-associated systemic SVV that include microscopic polyangiitis, granulomatosis with polyangiitis, and eosinophilic granulomatosis

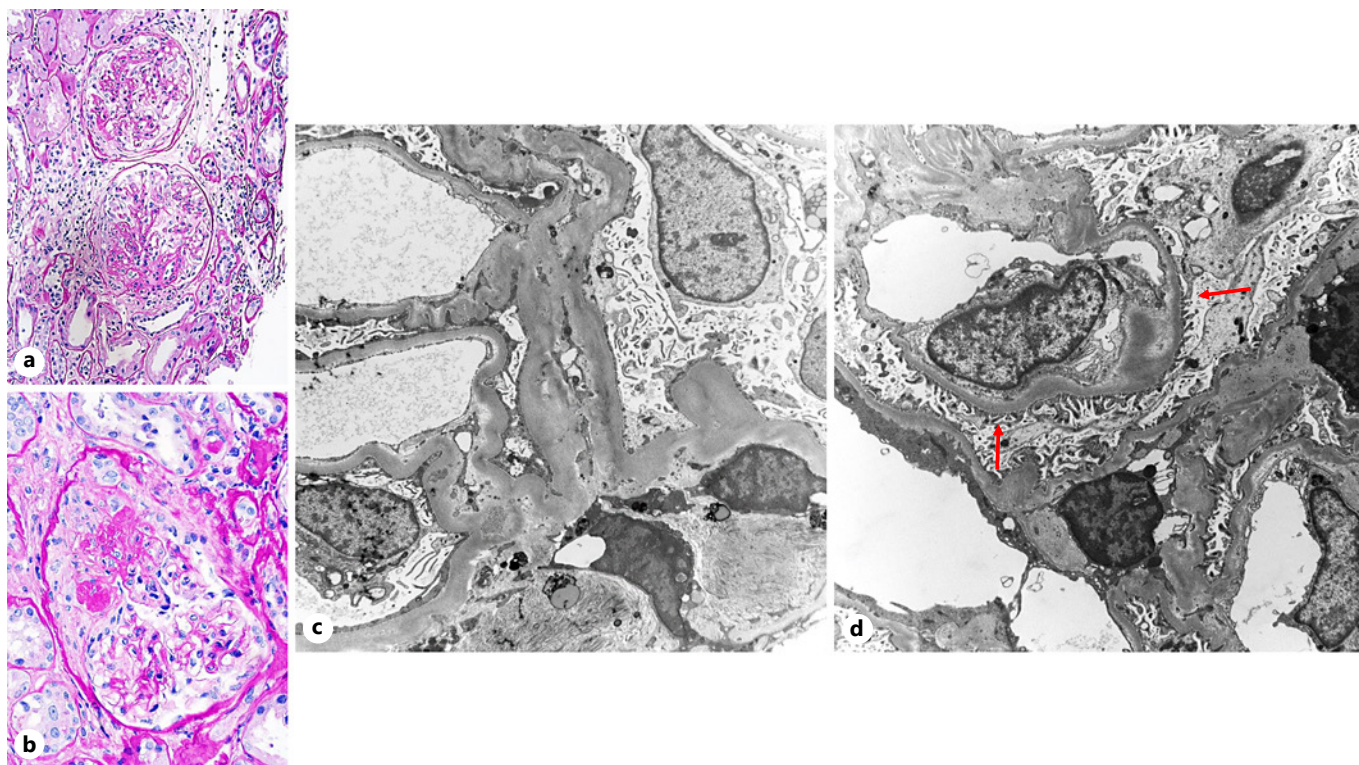


Fig. 17. Recurrent crescentic GN, pauci-immune type, ANCA associated. This is a patient with known ESKD as a result of ANCA-associated crescentic GN presented with a nephritic picture and elevated creatinine 1 year following renal transplantation. **a, b** The glomeruli show both active and subacute segmental glomerulonephritis with small cellular or fibrocellular crescents in the Bowman space, while the rest of the glomerular capillary tufts are generally unremarkable. This is surrounded by mild active and chronic interstitial inflammation and focal tubular atrophy with evidence of

tubular injury (PAS, $\times 200$ – 400). **c, d** In the absence of immune deposits, the glomerular findings by EM are nonspecific and show normal morphology of the capillary basement membranes and mesangial areas with relatively preserved foot processes (arrows). EM is pursued in cases with glomerular crescents to exclude other underlying IC glomerular lesions or rejection-related changes ($\times 6,000$). GN, glomerulonephritis; ANCA, antineutrophil cytoplasmic antibody; ESKD, end-stage kidney disease; PAS, periodic acid-Schiff; EM, electron microscopy; IC, immune complex.

with polyangiitis. These may develop ESKD despite adequate immunosuppressive therapy in nearly 25% of cases in a few years. Recurrence of ANCA-mediated SVV in nearly 20% renal allografts is defined by the appearance of segmental necrotizing lesions with crescents and rarely arteriolitis and small vessel arteritis, presenting as acute renal failure, similar to the native kidney disease [37].

Clinical Features

The recurrence can occur as early as within the first week of transplantation or several years later. It may not always be dependent on serological evidence of ANCA (although it is detected in most cases), the specificity of the ANCA (MPO vs. PR3), or the type of donor used (living related vs. unrelated vs. deceased). The onset is relatively rapid with hematuria, active urine sediment, rise in

creatinine, and minimal proteinuria. Sometimes, the recurrence could affect other organs instead of the kidney, which is more common in patients with PR3-ANCA than MPO-ANCA. As the response to the conventional therapy is generally favorable, graft loss directly as a result of the recurrence is relatively low, as 2–3% in 10 years.

Renal Pathology

The morphological findings of crescentic GN are similar to those seen in the native kidney, showing segmental necrotizing lesion with cellular crescents or sclerosing changes with fibrocellular crescents (Fig. 17a, b) surrounded by active interstitial inflammation. Rarely, SVV is found. No immune deposits are localized by IF. EM findings are usually nonspecific with no alterations of the

glomerular capillary basement membranes thickness or epithelial foot processes and absence of electron-dense deposits (Fig. 17c, d).

Complement-Mediated TMA/Also Known as Atypical Hemolytic Uremic Syndrome

Definition and Background

TMA in the renal transplant is morphologically defined by the presence of glomerular and microvascular endothelial injury and noninflammatory intracapillary or microvascular thrombosis presenting with hypertension and acute renal failure. This can be renal limited or manifest clinically with thrombocytopenia and microangiopathic hemolytic anemia. While recurrence of other forms of TMA (Shiga toxin-associated hemolytic uremic syndrome or thrombotic thrombocytopenic purpura) is uncommon, complement-mediated TMA (also known as atypical hemolytic uremic syndrome) is by far the most severe form of TMA, with a high rate of recurrence [38]. TMA involving the renal allograft, similar to the native kidney pathology, may have a wide range of etiologies, and it is important to perform a clinicopathologic correlation considering evaluation for antibody-mediated rejection, immunosuppressive medication (calcineurin inhibitor)-induced TMA, or de novo infections, as well. This is in addition to entities that could be recurrent such as genetic susceptibility causing complement dysregulation, autoimmune diseases, monoclonal gammopathy-associated autoantibodies, or continuation of endothelial toxic medications, or a combination of more than one of these factors. Here, we focus on recurrent diseases, particularly with an underlying genetic trigger for complement abnormalities.

Clinical Features

Recurrence of complement-mediated TMA, a non-diarrhea-associated TMA, depending on the frequency and the quality of the detectable genetic abnormalities of complement factors or complement regulatory protein activation/dysregulation (e.g., complement factor H [CFH], complement factor I, membrane cofactor protein, C3 gene variant, CFH receptor 3-1, and complement factor B) can develop both in children and adults, ranging from 30 to 80%. This can become apparent as early as within a few days after renal transplantation leading to delayed graft function, within a few weeks to months later. The clinical features vary from relatively asymptomatic expressing minimal hematologic changes to rapid renal graft dysfunction, with peripheral smear schistocytes, thrombocytopenia and microangiopathic

hemolytic anemia, elevation of LDH and low haptoglobin, as well as moderate to severe hypertension. The triggers/risks proposed for the initiation of endothelial injury in complement-mediated TMA are therapeutic interventions such as transplant surgery, ischemia reperfusion injury, immunosuppressive medications (tacrolimus and mTOR inhibitors), rejection, or infections. The 1-year graft loss is significantly high, with over 90% in 5 years. Another important cause of recurrent acute or chronic micro- and macrovascular thrombotic complications is antiphospholipid antibody syndrome with a high rate of graft loss. Often a high degree of suspicion is necessary with any prior history or information of ESKD, since these patients may not be amenable for an immediate biopsy. In addition, causes of de novo TMA should also be considered in the posttransplant period secondary to rejection, immunosuppressive medications, and infections. The poor outcome of renal transplantation is observed in those patients who have developed ESKD with CFH and complement factor I mutations within 2 years than those with membrane cofactor protein mutation [39]. Several management strategies are used to either prevent or treat recurrent complement-mediated TMA [40].

LM and IF Microscopy

The LM findings can appear focal, mild to severe, ranging from merely glomerular congestion, endothelial swelling, or ischemic collapse to prominent microthrombi obstructing the capillaries and focal margination of neutrophils (Fig. 18a, b). Endothelial separation, trapping of fragmented RBCs, and mesangiolysis can also be seen by H&E, PAS, and silver stains. The hilar arterioles and small arteries may display similar features of severe endothelial swelling, myxoid intimal expansion, with or without fibrinoid change, and intraluminal thrombi (Fig. 18a). As a result, there is widespread tubular injury and focal necrosis contributing to the persistent rise in creatinine, sometimes causing anuria. In rare instances, preexisting donor TMA can also show similar microscopic features causing delayed graft function. The more glomerular chronic changes with peripheral double contours can resemble those features seen in transplant glomerulopathy (Table 3). Both the small arteries and arterioles heal by fibrointimal sclerosis causing marked luminal narrowing. IF is generally noncontributory, except for strong staining for fibrin deposits in the early and active stages (Fig. 18c). In the absence of antibody-mediated rejection, C4d is usually negative.

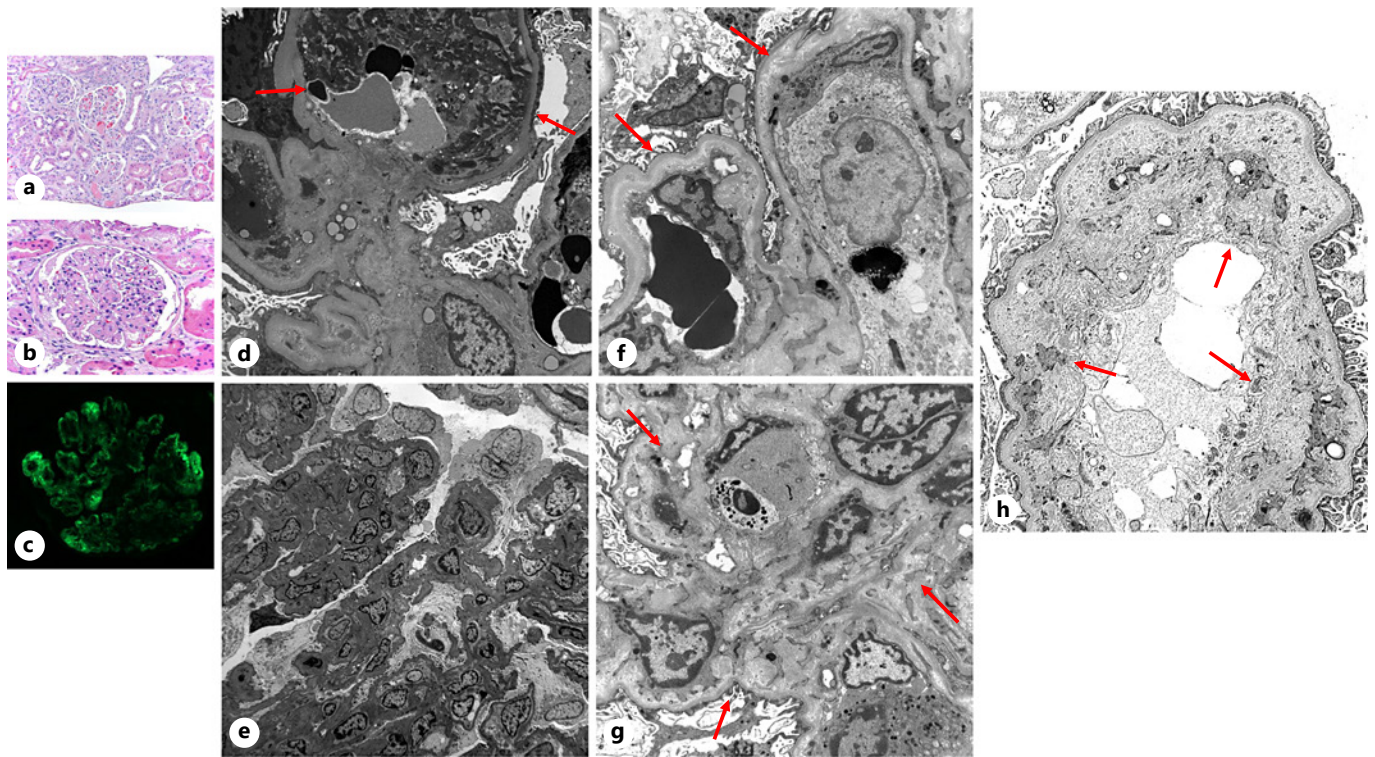


Fig. 18. Recurrent TMA-aHUS. This is a patient who developed ESKD of unknown cause in childhood now requiring renal transplantation. She developed acute renal failure along with clinical TMA features of microangiopathic hemolytic anemia and mild thrombocytopenia within 1 month of transplantation. **a, b** The low and high magnification of the light microscopic images show glomerular and hilar arteriolar microthrombi, mild to moderate ischemic glomerular changes, and focal glomerular capillary wall thickening due to endothelial swelling (**b**) (hematoxylin and eosin, $\times 200$, $\times 400$). **c** Glomerular capillary wall and luminal staining for fibrinogen is noted (fibrinogen, $\times 200$). **d** Glomerular capillaries showing intraluminal thrombus with fibrin tactoids admixed with RBCs and platelets (arrows) ($\times 6,000$). **e** Extensive wrinkling and ischemic collapse of the glomerular capillary tufts as a result of hilar arteriolar thrombus ($\times 1,800$). **f** Glomerular capillary wall changes demonstrating mild to moderate subendothelial expansion having a lucent space containing fine “fluffy” or particulate proteinaceous material (arrows), endothelial swelling with total loss of fenestrations, and margination of RBCs or circulating mac-

rophages ($\times 6,000$). **g** Within the same glomerulus as (**f**), the mesangium shows considerable expansion with loose matrix also known as “mesangiolysis” that extends into the subendothelial space (arrows) with mild degenerative changes of the cells ($\times 6,000$). **h** This is a case of chronic TMA in a renal allograft biopsy, where a single glomerular capillary loop demonstrates subendothelial expansion, now showing significant organization, trapping of cells, cellular interposition, and new layers of basement membrane material in the subendothelial area, lined by endothelial cells, giving rise to a membranoproliferative pattern of glomerular injury. However, no IC-type electron-dense deposits are identified in these areas ($\times 6,000$). Such lesions have to be differentiated from chronic transplant glomerulopathy, which may have a similar appearance, commonly seen secondary to chronic antibody-mediated rejection or other recurrent or de novo MPGN with immune complex deposits (Table 3). TMA-aHUS, thrombotic microangiopathy-atypical hemolytic uremic syndrome; ESKD, end-stage kidney disease; IC, immune complex; MPGN, membranoproliferative glomerulonephritis.

Electron Microscopy

Although in overt cases of TMA, the diagnosis is made by LM, EM can be particularly useful in the early or unsuspected cases. The findings include glomerular endothelial injury, swelling with loss of fenestrations and detachment, and fibrin tactoids within the subendothelial space and capillary lumina along with capillary microthrombi and ischemic glomerular collapse (Fig. 18d–f).

The foot processes may be preserved in milder forms but are partially to totally effaced in severe TMA. Mild to severe mesangiolysis is an accompanying feature in acute cases (Fig. 18g), which may be focal in distribution, sometimes merging with the subendothelial space with fine granular protein precipitate and occasional trapped RBCs. IC type of deposits are not found. These changes in the healing phases lead to reorganization of the matrix

Table 4. Rates of recurrence, posttransplant interval, and graft outcomes of common recurrent glomerular diseases

Diagnosis	Rate of recurrence, %	Time interval, months	Graft loss, %
FSGS	25–55 (mean 40) (80% in second graft)	0–8	15–20 in 10 years
MGN	10–40	4–18, rarely within 1 month	20–50 in 5–10 years
IgA nephropathy	10–50 (mean 30), higher in protocol biopsies	6–12	10–20 in 10 years
MPGN type 1	30–70	>24–36	25–30 in 5 years
C3GN	30–50	6–12	>50 in 5 years
DDD	>80–100	<12	15–25 in 5 years
LN	30–50, symptomatic 10	12–72	5–10 in 10 years
Fibrillary GN	30–50	12–24	50 in 5–10 years
MIDD	50	6–36	50 in 5–10 years depends on hematologic disease
PGNMID	90	1–4	50 in 3 years
Amyloidosis, AL	10–30	>12	35 in 3 years
Amyloidosis, AA	15–20	8–20 years	<5 in 10 years
Diabetic nephropathy	>50	24–36	40–60 in 10 years
ANCA-associated crescentic GN	0–20	1–60	10 in 10 years

FSGS, focal segmental glomerulosclerosis; MGN, membranous glomerulonephritis; C3GN, C3 glomerulonephritis; DDD, dense deposit disease; LN, lupus nephritis; GN, glomerulonephritis; ANCA, antineutrophil cytoplasmic antibodies; MIDD, monoclonal immunoglobulin deposition disease; PGNMID, proliferative GN with monoclonal IgG deposits.

with new layers of basement membrane material and sub-endothelial cellular interposition, mimicking MPGN pattern or chronic transplant glomerulopathy (Fig. 18h).

Conclusions

Recurrent/de novo renal diseases in transplantation account for the third highest cause of allograft dysfunction and graft loss based on large studies and transplant registries. Although many glomerular, tubulointerstitial, and vascular diseases have a potential to develop in the kidney transplant, recurrent glomerular lesions appear to constitute the vast majority of diseases in the allograft. For a proper diagnosis of these diseases in the transplant setting, knowledge of prior kidney disease before end stage, routine clinical urine testing for proteinuria and hematuria, and a transplant kidney biopsy that are subjected to LM, IF, and EM are essential. The rate and time of recurrence posttransplantation as well as graft loss are dependent on the specific disease process and its underlying pathogenetic mechanisms (Table 4). Often the histologic or ultrastructural findings may be modified as a result of ongoing immunosuppressive therapy, inherent donor and recipient characteristics, as well as the stage and severity of the disease process. The most common forms of recurrent diseases are focal segmental sclerosis, IgA nephropathy, MGN, MPGN along with C3 glomeru-

lopathies, paraproteinemia-associated diseases, and complement mediated TMA, with impact on graft function and graft loss. EM is necessary in most cases of glomerular diseases for proper identification, staging, and diagnosis of the lesions.

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

S.V.S. and S.P.S. participated in preparation of the manuscript and assembling images and references.

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