Abstract: Crescentic glomerulonephritis (GN) in a renal biopsy is a widely accepted “critical diagnosis” in Anatomic Pathology practice. Prompt biopsy evaluation and notification of the referring physician is essential to facilitate rapid therapeutic intervention. The differential diagnostic categories of crescentic GN include pauci-immune GN, anti-glomerular basement membrane (GBM) nephritis and immune complex-mediated GN, distinguished from one another by immunofluorescence and electron microscopic study of the renal biopsy. Immune complex-mediated GN is characterized by abundant glomerular deposits and encompasses several diseases including but not limited to lupus nephritis, cryoglobulinemic GN and immunoglobulin A nephropathy. Pauci-immune GN, with paucity of deposits, correlates closely with antineutrophil cytoplasmic antibody disease due to the identifiable circulating pathogenic antineutrophil cytoplasmic antibody in most patients. Recent studies have identified other antibodies in pauci-immune GN and implicated infectious organisms in triggering autoimmunity in a susceptible host by molecular mimicry of host antigens. Anti-GBM nephritis is a rare but potentially life-threatening autoimmune disease with circulating antibodies against GBM epitopes in the α3 chain of type IV collagen. It is characterized by a linear immunoglobulin G deposition along GBM on immunofluorescence microscopy. Environmental triggers including infections and solvent exposure seem to change the tertiary structure of the type IV collagen α345 hexamer in GBM, expose neo-epitopes, and initiate autoimmunity. Even in light of advances in understanding of pathophysiology and serologic testing, renal biopsy remains the mainstay of diagnosis of crescentic GN.

Key Words: crescentic glomerulonephritis, pauci-immune, anti-GBM, ANCA


Crescentic Glomerulonephritis: An Update on Pauci-immune and Anti-GBM Diseases

Neeraja Kambham, MD

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Crescentic glomerulonephritis (GN) in a renal biopsy is a widely accepted “critical diagnosis” in Anatomic Pathology practice. Prompt biopsy evaluation and notification of the referring physician is essential to facilitate rapid therapeutic intervention. The differential diagnostic categories of crescentic GN include pauci-immune GN, anti-glomerular basement membrane (GBM) nephritis and immune complex-mediated GN, distinguished from one another by immunofluorescence and electron microscopic study of the renal biopsy. Immune complex-mediated GN is characterized by abundant glomerular deposits and encompasses several diseases including but not limited to lupus nephritis, cryoglobulinemic GN and immunoglobulin A nephropathy. Pauci-immune GN, with paucity of deposits, correlates closely with antineutrophil cytoplasmic antibody disease due to the identifiable circulating pathogenic antineutrophil cytoplasmic antibody in most patients. Recent studies have identified other antibodies in pauci-immune GN and implicated infectious organisms in triggering autoimmunity in a susceptible host by molecular mimicry of host antigens. Anti-GBM nephritis is a rare but potentially life-threatening autoimmune disease with circulating antibodies against GBM epitopes in the α3 chain of type IV collagen. It is characterized by a linear immunoglobulin G deposition along GBM on immunofluorescence microscopy. Environmental triggers including infections and solvent exposure seem to change the tertiary structure of the type IV collagen α345 hexamer in GBM, expose neo-epitopes, and initiate autoimmunity. Even in light of advances in understanding of pathophysiology and serologic testing, renal biopsy remains the mainstay of diagnosis of crescentic GN.

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distinctions are not reliable indicators of subtype of crescentic GN. In addition to clinical and serological parameters, further classification of crescentic GN rests largely on immunofluorescence microscopic (IF) evaluation of the renal biopsy, with additional diagnostic electron microscopic (EM) findings in some cases. Although all 3 categories are indeed “immune-mediated,” the immune complex-mediated GN refers to the presence of immune complex deposits visible by both IF (granular deposits) and EM. As the name suggests, pauci-immune GN has relative absence of deposits by both IF and EM. Anti-GBM nephritis is characterized by linear immunoglobulin (Ig) G staining along GBM, but no deposits are visible by EM, possibly due to uniform binding of circulating autoantibodies to GBM epitopes rather than formation of clumps of deposits.

In general, pauci-immune GN is the most frequent cause of crescentic GN in all age groups and among patients older than 60 years of age, it accounts for up to 80% of cases. Anti-GBM nephritis is characterized by linear immunoglobulin (Ig) G staining along GBM, but no deposits are visible by EM, possibly due to uniform binding of circulating autoantibodies to GBM epitopes rather than formation of clumps of deposits.

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This review will focus mainly on pauci-immune GN and anti-GBM nephritis, highlighting the recent insights gained into their pathogenic mechanisms. Detailed discussion about immune complex-mediated GN is beyond the scope of this review, but salient renal biopsy findings are listed in Table 1 and Figure 2.

**PAUCI-IMMUNE GLOMERULONEPHRITIS**

More than half of crescentic GN have complete lack of or relative paucity of immune complex deposits as demonstrated by IF and EM, and thus belong to pauci-immune GN category. A vast majority of patients with pauci-immune GN have circulating ANCA and hence, this entity is often termed as ANCA-mediated GN. Pauci-immune GN is a renal manifestation of ANCA-associated small-vessel vasculitides with involvement of small arteries, arterioles, venules, and capillaries (and thus glomeruli). ANCA-associated vasculitides involving the kidney include microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA; formerly known as Wegener’s granulomatosis), Churg-Strauss syndrome (CSS) and a renal-limited form. Necrotizing vasculitides of medium and small-sized arteries in the absence of GN or involvement of arterioles, venules, and capillaries (and thus glomeruli) are also described.

**FIGURE 1.** A, Glomerular cellular crescents with severely compressed capillary tufts preclude an adequate evaluation of mesangium and capillary lumens [Jones methenamine silver stain (JMS); ×400]. B, C, In addition to a crescent, foci of GBM rupture with extravasated fibrin (long arrows) are evident on JMS (B) and trichrome (C) stain (×400). D, Crescentic GN is often associated with prominent interstitial inflammation and acute tubular injury as seen here with loss of tubular brush borders and sloughed epithelial cells (*; ×200). E, F, Fibrocellular (arrowhead) and fibrous crescents represent subacute and chronic glomerular changes. Focal capillary loops may be seen enmeshed in a crescent (short arrow) [periodic acid-Schiff stain (PAS), ×400]. GBM indicates glomerular basement membrane; GN, glomerulonephritis.
pulmonary renal syndrome is a common presentation. 2,5,10

initiate further work up and confirm the diagnosis.

very nonspecific and a high index of suspicion is needed to

overall presentation with elevated serum creatinine can be

veals dysmorphic RBCs and frequently, RBC casts. The

tinine at presentation was 6.5 ± 4.0 mg/dL. 2 Urinalysis re-

include RPGN, and in one study, the mean serum crea-

often precede the onset of disease. The renal manifestations

symptoms such as fever, malaise, myalgias, and arthralgias

significant sex predilection. 2,5 Constitutional flu-like

presentation for pauci-immune GN is 60 years and there is no

with limited disease and conversely a limited form of GPA with

only upper and lower respiratory tract disease, without

limited disease and conversely a limited form of GPA with

vasculitis are observed in > 75% of patients. 2,5,10 Less

hemetemesis, melena, etc. 11 Overall, signs of extrarenal

involvement by vasculitis such as skin purpura (leukocy-

oclastic vasculitis), livedo reticularis, urticularia, peripheral

neuropathy (especially mononeuritis multiplex), meningitis,

mucosal involvement such as nasal septum perforation.

Localized symptoms may vary based on the organ

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common localized forms of ANCA disease include renal-

limited disease and conversely a limited form of GPA with

only upper and lower respiratory tract disease, without

renal or systemic involvement. 10,12

vasculitis is now considered to be a C3 glomerulopathy.

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deposit disease does not demonstrate glomerular crescents.

Clinical Presentation

With a worldwide prevalence, the mean age at pre-

sentation for pauci-immune GN is 60 years and there is no

significant sex predilection. 2,5 Constitutional flu-like

symptoms such as fever, malaise, myalgias, and arthralgias

often precede the onset of disease. The renal manifestations

include RPGN, and in one study, the mean serum creati-
inine at presentation was 6.5 ± 4.0 mg/dL. 2 Urinalysis re-

veals dysmorphic RBCs and frequently, RBC casts. The

overall presentation with elevated serum creatinine can be

very nonspecific and a high index of suspicion is needed to

initiate further work up and confirm the diagnosis.

ANCA disease is usually a multisystem disorder and

pulmonary renal syndrome is a common presentation. 2,5,10

The pulmonary symptoms range from mild cough and

blood tinged sputum to severe hemoptysis due to life-

threatening massive pulmonary hemorrhage. A sudden

drop in hemoglobin levels should raise concern for an in-

ternal hemorrhage, even if not otherwise clinically evident.

Bilateral nodular infiltrates and cavitary lesions may be

seen on imaging studies, especially with GPA and CSS.

Head and neck involvement is quite common in GPA and

may take the form of recurrent sinusitis, nasal septum

damage, orbital disease, or otitis media. CSS is charac-
terized by relatively less frequent renal involvement, and

these patients have allergic rhinitis, asthma, peripheral

cosinophilia, and in some instances eosinophilic pneumonia

or gastroenteritis. 2,5,10 Interestingly, the onset of asthma in

CSS can precede small-vessel vasculitides by a few years or

even decades.

Localized symptoms may vary based on the organ

involvement by vasculitis such as skin purpura (leukocy-
toclastic vasculitis), livedo reticularis, urticularia, peripheral

neuropathy (especially mononeuritis multiplex), meningitis,

hemetemesis, melena, etc. 11 Overall, signs of extrarenal

vasculitis are observed in > 75% of patients. 2,5,10 Less

common localized forms of ANCA disease include renal-

limited disease and conversely a limited form of GPA with

only upper and lower respiratory tract disease, without

renal or systemic involvement. 10,12

Laboratory Investigations

Pauci-immune GN is associated with normal se-

rum complement levels and laboratory studies to exclude
cryoglobulinemia or lupus nephritis are often indicated.

**TABLE 1. Renal Biopsy Findings in 3 Categories of Crescentic Glomerulonephritis (GN)**

<table>
<thead>
<tr>
<th>Glomerulonephritis Subtype</th>
<th>Frequency of Crescentic GN ( &gt; 50% Crescents)</th>
<th>% Cases With Vasculitis</th>
<th>Laboratory Investigations</th>
<th>IF Findings</th>
<th>EM Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pauci-immune GN</td>
<td>50%</td>
<td>20</td>
<td>Elevated ANCA, normal C3, C4</td>
<td>Absent or nonspecific staining</td>
<td>No electron-dense deposits</td>
</tr>
<tr>
<td>Anti-GBM nephritis</td>
<td>85%</td>
<td>Rare†</td>
<td>Elevated Anti-GBM antibodies, normal C3, C4</td>
<td>Linear IgG along GBM</td>
<td>No electron-dense deposits</td>
</tr>
<tr>
<td>Immune complex-mediated GN</td>
<td>Lupus nephritis (class III/IV)</td>
<td>13</td>
<td>2-3</td>
<td>“Full-house” staining of Ig and complements; extraglomerular deposits</td>
<td>Mesangial and subendothelial deposits ± subepithelial; tubuloreticular inclusions</td>
</tr>
<tr>
<td>Henoch-Schönlein purpura</td>
<td>IgA nephropathy</td>
<td>9.7</td>
<td>Rare</td>
<td>IgA ≥ IgG</td>
<td>Mesangial, GCW</td>
</tr>
<tr>
<td>Acute postinfectious GN</td>
<td>Dense deposit disease</td>
<td>3.3</td>
<td>None</td>
<td>IgA ≥ IgG</td>
<td>Mesangial, ± GCW</td>
</tr>
<tr>
<td>Cryoglobulinemic GN</td>
<td>Infrequent</td>
<td>18.8</td>
<td>None</td>
<td>Low C3, normal C4</td>
<td>Inframembranous deposits in lamina densa; mesangial deposits</td>
</tr>
<tr>
<td>“MPGN type I”*</td>
<td></td>
<td>4.6</td>
<td>Variable*</td>
<td>Low C3, sometimes C4*</td>
<td>Subendothelial deposits; organized substructure</td>
</tr>
<tr>
<td>Fibrillar GN</td>
<td></td>
<td>5</td>
<td>None</td>
<td>Normal C3, C4</td>
<td>Mesangial and subendothelial deposits*</td>
</tr>
</tbody>
</table>

*MPGN type I previously described in the literature is now recognized to encompass a spectrum of diseases including C3 glomerulopathies; dense deposit disease is now considered to be a C3 glomerulopathy.
†If present, consider coexistent pauci-immune GN.

ANCA indicates antineutrophil cytoplasmic antibodies; EM, electron microscopy; GBM, glomerular basement membrane; GCW, glomerular capillary wall; GN, glomerulonephritis; IF, immunofluorescence microscopy.
FIGURE 2. Immune complex-mediated crescentic GN is often associated with mesangial and/or endocapillary proliferation. A–C, Biopsy findings of diffuse proliferative lupus nephritis (A: PAS, ×400) with mesangial and capillary wall “full-house” staining including IgG (B), IgA, IgM, C3, and C1q. Corresponding mesangial (*) and subendothelial (arrowhead) electron-dense deposits are seen (×4000) along with tubuloreticular inclusions (inset C, ×25,000). D–F, Renal features of Henoch-Schonlein purpura. The glomerulus shows crescent and mesangial sclerosis along with increased endocapillary cellularity (JMS, ×400); IF demonstrates dominant IgA (E) staining and subendothelial deposits (arrowhead) are observed on EM (F, ×10,000). G–I, Postinfectious GN with diffuse glomerular proliferation and numerous neutrophils (exudative pattern) seen on H&E stain (G, ×400). The deposits on IF are granular and strongly positive for C3 (H) and characteristic subepithelial “humps” (arrow) are identified on EM (I, ×12,000). J–L, Cryoglobulinemic GN shows characteristic membranoproliferative features (JMS, ×400) and, on occasion, necrotizing arteritis (>). IF demonstrates IgM and C3 staining (not shown) and EM shows reduplicated GBMs (>) along with subendothelial deposits. EM indicates electron microscopy; GBM, glomerular basement membrane; GN, glomerulonephritis; H&E, hematoxylin-eosin; IF, immunofluorescence microscopy; Ig, immunoglobulin; JMS, Jones methenamine silver stain; PAS, periodic acid-Schiff.
TABLE 2. Clinicopathological Subtypes of Pauci-immune Glomerulonephritis

<table>
<thead>
<tr>
<th>Frequency of renal involvement (%)</th>
<th>Other frequently involved organs</th>
<th>Microscopic Polyangiitis (MPA)</th>
<th>Churg-Strauss Syndrome (CSS)</th>
<th>Renal-limited Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>80</td>
<td>Pulmonary, head and neck (90%)</td>
<td>Pulmonary (50%), musculoskeletal (60%)</td>
<td>Pulmonary, neurologic (70%)</td>
<td>None</td>
</tr>
<tr>
<td>90</td>
<td>Perinuclear without nuclear extension</td>
<td>Constitutional symptoms</td>
<td>Constitutional symptoms, asthma, allergic rhinitis, blood eosinophilia</td>
<td>Constitutional symptoms</td>
</tr>
<tr>
<td>95</td>
<td>Constitutional symptoms</td>
<td>70</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>25:75</td>
<td>Intestinal granulomatous inflammation</td>
<td>60:40</td>
<td>60:40</td>
<td></td>
</tr>
<tr>
<td>Additional renal biopsy findings</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
</tbody>
</table>

*Granulomatous inflammation in GPA and CSS is more often described in respiratory tract and in the case of GPA also in head and neck lesions.
†Can be even lower, ~25% in the absence of circulating ANCA.
ANCA indicates antineutrophil cytoplasmic antibody; PR3, Proteinase 3; MPO, myeloperoxidase.

Davies and colleagues first reported ANCA in pauci-immune GN patients and its significance has been confirmed by subsequent studies.\(^{13-15}\) ANCA testing can be performed either by indirect IF or by enzyme-linked immunosorbent assay (ELISA) using purified specific antigens proteinase-3 (PR3) and myeloperoxidase (MPO).\(^{10}\) Both PR3 and MPO are components of neutrophil and monocyte lysosomes. Traditionally, the IF testing involves incubation of patient’s serum with ethanol fixed human neutrophils as a substrate to detect the presence of circulating antibodies. Two patterns of ANCA staining observed on indirect IF include perinuclear with nuclear extension (p-ANCA) and cytoplasmic granular with interlobular accentuation pattern (c-ANCA).\(^{16}\) Classic c-ANCA is almost always directed against PR3, but p-ANCA may be directed against MPO or PR3. Hence, indirect IF and ELISA should test for both subcategories of IF patterns and specific antigens. The frequency of ANCA subtypes is different in various ANCA vasculitides (Table 2). In addition to classic IF patterns, atypical ANCA patterns have been recognized and these include cytoplasmic homogenous pattern (c-ANCA atypical) or perinuclear without nuclear extension (p-ANCA atypical).

In general, indirect IF is more sensitive while ELISA test is more specific, but certain caveats need to be considered.\(^{5,16}\) Nonspecific or atypical ANCA (positive by IF, but ELISA for PR3 and MPO negative) can be detected in nonvasculitic autoimmune or inflammatory conditions (eg, ulcerative colitis, autoimmune hepatitis, sclerosing cholangitis), and reportedly with cocaine abuse.\(^{17}\) The antigen targets responsible for such pattern include lactoferrin, elastase, cathepsin-G, lysozyme, and other minor antigens not routinely tested in the laboratories.\(^{8,16}\) Factors such as formalin fixation of neutrophil substrate, in-house preparation of substrate, or different commercial substrates and ELISA kits seemingly contribute to variable sensitivities and specificities of indirect IF and ELISA reported in the literature. The International Consensus Statement for ANCA testing recommends that all sera for ANCA be screened first by indirect IF followed by a positive result confirmation by ELISA.\(^{16,18}\) However, on rare occasion, ELISA may be positive in the context of negative indirect IF test and in that light, there may be a role for simultaneous IF and ELISA testing.\(^{17}\) In general, testing for ANCA by indirect IF or ELISA alone is not ideal.

The positive and negative predictive values of these tests are closely linked to the clinical presentation.\(^{19,20}\) In the setting of RPGN, the positive predictive value is approximately 95%, but negative predictive value is only 80% (and 65% in patients older than 50 years), indicating the need for pursuing further studies such as a renal biopsy despite a negative ANCA test. It is now well documented that approximately 10% to 20% of biopsy-proven pauci-immune GN patients lack indirect IF ANCA and ELISA support. CSS patients have higher frequency of false-negative ANCA, especially in the absence of renal disease, as do patients with limited form of GPA.\(^{17,19,20}\) On the other end of the spectrum, if the patient has minimally increased serum creatinine with hematuria and mild proteinuria, the negative predictive value of ANCA test is 99%, essentially ruling out ANCA disease. The positive predictive value in this clinical scenario is also low (47% to 66% depending on the age of patient). Thus, an ANCA serology result should be interpreted with caution and always in the context of clinical presentation. Although active pauci-immune disease usually has high ANCA levels, there are no standard ANCA units and the levels of antibody reported are laboratory specific. Of note, several medications and infections can induce ANCA, but this does not necessarily represent active disease with vasculitis.\(^{16}\)

Pathology

Given the long turnaround times for ELISA results, a renal biopsy is of immense value in the diagnosis of clinically suspected pauci-immune GN. Even in patients with predominantly pulmonary symptoms, relative to lung sampling, a renal biopsy is associated with ease of IF interpretation, higher yield of definitive diagnosis, and a lower procedural risk.\(^{21}\) The biopsy findings of pauci-immune GN are represented in Figure 3.
FIGURE 3. Biopsy findings of pauci-immune GN. A, B, Cellular crescents are seen in both (JMS, × 400) and note the lack of mesangial or endocapillary proliferation in intact capillary loops. GBM rupture site is also seen (B). C, Necrotizing arteritis with transmural inflammation (arrowhead) is more frequently seen in ANCA disease when compared with immune complex-mediated GN (PAS, × 200). D, Medullary features observed in pauci-immune GN include capillaritis with extravasated RBCs (>). Tubular RBC casts are also identified (long arrow) (H&E, × 400). E, Eosinophil-rich interstitial inflammation is not specific for Churg-Strauss syndrome (CSS) and may be observed in all subtypes of pauci-immune GN (H&E, × 400). F, Interstitial granulomas (short arrow) are described in granulomatous with polyangiitis (GPA) and CSS, but are rather infrequent in renal biopsy (H&E, × 400). G, ANCA disease is associated with paucity of deposits and may show low-intensity, nonspecific staining for IgM and C3 (G), especially in areas of necrosis or crescents. H, No electron-dense deposits are seen on EM. Fibrin material (*) adjacent to foci of glomerular damage should not be interpreted as deposits. ANCA indicates antineutrophil cytoplasmic antibody; GBM, glomerular basement membrane; GN, glomerulonephritis; JMS, Jones methenamine silver stain; PAS, periodic acid-Schiff; RBC, red blood cell.
Light Microscopy

The cellular crescents can be segmental or circumferential, often with disrupted Bowman’s capsules. In addition, a variable number of glomeruli have areas of necrosis, karyorrhexis, or fibrin extravasation. A GBM rupture site may be evident on periodic acid-Schiff or silver stain and fibrin is brightly fuchsinophilic (red) on trichrome stain. Foci of necrosis and crescents often have associated inflammatory cells, but there is usually no evidence of mesangial or endocapillary proliferation in uninvolved segments of glomeruli. The biopsy may demonstrate uniformly acute lesions or may have variable combination of subacute or chronic lesions such as fibrocellular/fibrous crescents and Bowman’s capsular synechiae. These changes correspond to the rapidity of onset and duration of ANCA disease. Global glomerulosclerosis and chronic tubulointerstitial damage identified can be attributed to either progression of ANCA GN or preexisting disease such as hypertensive nephrosclerosis and careful examination of sclerosed glomeruli is helpful in distinguishing between the two possibilities. Acute tubular injury is often observed with loss of brush borders and sloughed epithelial cells. Occasionally, no necrotizing or crescentic lesions are identified despite a clinical scenario of RPGN and positive ANCA serology. If the biopsy sample in such cases is limited by glomerular paucity, consideration should be given to unsampled ANCA-related crescents, especially if RBC casts are identified in the biopsy or urine microscopy.

Tubulointerstitial and periglomerular inflammation tends to be prominent in ANCA disease and is likely within the spectrum of disease rather than just a secondary phenomenon due to glomerular damage. Multinucleated giant cells seen on occasion within periglomerular inflammation may be misinterpreted as interstitial granulomas. Although described in GPA and CSS, interstitial granulomas are rather infrequent in kidney (2% to 4% of cases). In contrast, granulomatous inflammation in GPA is relatively common in pulmonary and head and neck lesions. Although eosinophil-rich interstitial inflammation is considered a hallmark of CSS, it is not a helpful feature as eosinophils can be quite prominent in all ANCA diseases. In essence, the subtype of ANCA vasculitis is often difficult to discern on renal biopsy alone. However, there seem to be differences in the pathology seen in MPO versus PR3-ANCA-associated disease. Although extra renal disease is more frequent, the extent of glomerular disease and chronic renal damage appears to be less in PR3-ANCA disease when compared with MPO-ANCA disease.

Vasculitis can be observed in up to a third of renal biopsies, characterized by fibrinoid necrosis and transmural infiltrates of lymphocytes and neutrophils. The disrupted elastic lamina in arteries may be evident on silver stain. The affected blood vessels are usually of small caliber and include interlobular arteries, arterioles and medullary vasa recta. When present, medullary capillaritis results in interstitial hemorrhage and RBC extravasation admixed with apoptotic bodies. Other medullary-specific lesions described in ANCA disease include necrotizing arteriolitis, granulomas and papillary tip necrosis.

Immunofluorescence Microscopy

By definition, there is a paucity of immune deposits on IF. Necrotic foci are highlighted by fibrinogen and may demonstrate entrapped C3 and IgM. However, no linear or granular deposits of Ig or complements of $\geq 2^+$ (scale of 0 to 4) intensity should be observed in unaffected glomeruli. Although occasional cases have non-specific-associated immune staining, prominent deposits in “normal” glomeruli should raise concern for superimposed immune complex-mediated disease.

Electron Microscopy

Similar to IF, no electron-dense deposits are observed on ultrastructural examination. Electron-dense fibrin material in the vicinity of necrosis should not be misinterpreted as deposits. On occasion, the ruptured site of GBM may be visible in the area of fibrinoid necrosis or cellular crescent. The adjacent endothelial cell usually has features of injury and cytoplasmic swelling.

Pathological Classification

A recently proposed histological classification categorizes the glomerular lesions as focal (>$50\%$ normal glomeruli), crescentic (>$50\%$ glomeruli with cellular or fibrocellular crescents), sclerotic (>50% glomeruli are globally sclerosed), and mixed (heterogenous lesions, none involving >50% of glomeruli). A normal glomerulus is defined as one with no segmental sclerosis, GBM double contours, or extensive ischemic change. This classification is prognostically relevant in that the 5-year renal survival rates for focal, crescentic, mixed, and sclerotic categories are 93%, 76%, 61%, and 50%, respectively. The tubulointerstitial parameters did not provide independent predictive value. The study results reinforce the significance of describing the extent and distribution of active and chronic glomerular lesions in the pathology report.

Pathogenesis

Despite absence of visible immune deposits, the pathogenic mechanisms in ANCA disease are evidently immune mediated with development of autoantibodies. ANCA have been extensively studied in various in vitro experiments and animal models. Passive transfer of MPO-ANCA IgG induces pauci-immune GN and vasculitis in mice and as per one report, transplacental transfer of these maternal antibodies caused human neonatal renal pulmonary syndrome, confirming the pivotal role of ANCA. Although abundant in vitro evidence exists for pathogenic role of MPO-ANCA, convincing animal models are not available for PR3-ANCA GN.

Upon binding with the target antigens PR3 and MPO, ANCs activate neutrophils, promote neutrophil-endothelial cell binding, and cause release of reactive oxygen species and tissue-degrading proteolytic enzymes from neutrophils with resultant vessel wall injury. In vitro studies have demonstrated that ANCA-mediated neutrophil activation is mediated by binding of Fab’2 and Fc receptors with subsequent initiation of signal induction pathways and activation of alternative complement pathway.

PR3 and MPO are intracellular enzymes within neutrophil and monocyte granules. For the ANCA-mediated injury mechanisms to kick in, these antigens have to be exposed on the cell surfaces as in activated state of neutrophils and monocytes. The milieu of proinflammatory cytokines in the setting of infection is very conducive to this cell priming. The cytokines released by degranulating neutrophils and monocytes also, in turn, exacerbate this autoimmune injury. The fenestrated endothelium in glomerulus and negatively charged GBM may facilitate trapping of activated neutrophils and promote the renal predilection for injury.
Antibodies other than ANCA have been studied in patients with pauci-immune GN. Recently, antibodies to lyosomal membrane protein-2 (LAMP-2) were detected in patients with pauci-immune GN at prevalence rates significantly greater than that of MPO-ANCA and PR3-ANCA combined. The pathogenicity of LAMP-2 antibodies was confirmed in in vitro and in vivo studies. LAMP-2 is a membrane surface protein expressed on neutrophils and endothelial cells and unlike ANCA, is readily accessible to the circulating antibodies. LAMP-2 is involved in cell adhesion and homeostasis. Although it is an attractive alternative to intracellular ANCA antigens, a subsequent study could not confirm the significance of LAMP-2 antibodies. Anti-plasminogen antibodies were detected in approximately 25% of ANCA patients, possibly triggered by sequence homology between plasminogen and complementary PR3 (see below) and thus, contributing to deep vein thrombosis.

What triggers the formation of these autoantibodies? Several lines of evidence point to infectious organisms and their successful mimicry of host molecules in initiating autoimmune. Previous observations that GPA patients often are chronic nasal carriers of Staphylococcus aureus and relapses in them can be reduced with antimicrobial therapy support this hypothesis. Seasonal variation in the incidence of pauci-immune GN also suggests a correlation with microbial infection. Toll-like receptors that recognize infectious agents can exacerbate anti-MPO GN in experimental models. ANCA-positive serology has been reported in many infections including supplicative lung disease, Pseudomonas infection in cystic fibrosis and subacute bacterial endocarditis. More recent evidence includes identification of a LAMP-2 epitope with 100% homology to fimbrial adhesin (FimH) seen in gram-negative bacteria. It has been hypothesized that immune response to FimH as in uroepithelial tissue in anti-LAMP-2 antibodies due to molecular mimicry.

Neutrophils can kill bacterial organisms such as S. aureus extracellularly by undergoing respiratory burst and forming a web of released DNA called “neutrophil extracellular traps.” These traps, demonstrated in ANCA vasculitis, contain PR3 and MPO antigens, and are thus capable of initiating and exacerbating autoimmune disease. Ross river virus, S. aureus, and Entamoeba histolytica have sequences similar to complementary PR3 peptide. Experimental evidence shows that complementary PR3 peptide (generated by antisense DNA strand of PR3) is capable of inducing antibodies in turn generate a second round of antibodies (anti-idiotypic antibodies) that have affinity to PR3 antigen. This cascade of antibody production continues when a third generation of antibodies are produced by these anti-idiotypic antibodies with binding properties to complementary PR3. Although this theory of autoantigen complementarity generated much enthusiasm, other laboratories are yet to confirm these observations.

In addition to infections, other environmental factors can induce ANCA formation. These include silica exposure, drugs such as propothiouracil, hydralazine, penicillamine, cefotaxime, clozapine, indomethacin, isoniazid, rifampicin, and minocycline. Patients respond to discontinuation of the offending drug, but ANCA levels may persist despite disease remission. There seems to be an association between development of CSS and use of leukotriene receptor antagonist in treatment of bronchial asthma. Recent reports have implicated antitumor necrosis factor α (anti-TNFα) used in the treatment of rheumatoid arthritis in inducing ANCA and in a smaller fraction of patients, ANCA-associated renal disease. The detection of circulating MPO or PR3-ANCA was temporally related to anti-TNFα treatment and at least in some cases, the patients responded to drug withdrawal.

Obviously, not all patients exposed to bacterial infections, drugs, or other environmental factors develop pauci-immune GN. There seems to be a role for genetic susceptibility. Differences in surface expression of PR3/MPO-ANCA on neutrophils due to loss of epigenetic silencing, gene polymorphisms such as defective z-1 antitrypsin allele (a physiological inhibitor of PR3), leptin receptor gene, CTLA-4 and CD226 are some examples accounting for increased risk. The presence of MHC class II gene HLA-DRB1*0401 is associated with higher risk of developing GPA and HLA-DRB4 with susceptibility to CSS.

In addition to autoantibodies, T-cell–mediated immune responses also have a significant role in pauci-immune GN, especially CSS. As is widely known, CD4+ helper T cells with interferon-γ secretion are involved in granuloma formation. Patients with pauci-immune GN have T-cell abnormalities such as dysregulated T-helper cells, defective T-regulatory cells, and a preferential Th17 response. Th17 cells are increasingly being implicated in autoimmunity including ANCA disease. Neutrophil activation by cytokines synthesized by Th17 cells may contribute to exacerbation of GN. Studies have demonstrated that Th17 inducing toll-like receptors may be a potential link between infection and autoimmunity. In addition to autoantibody responses, B cells can present antigens to T cells and may modulate T-cell responses in small-vessel vasculitis. Eosinophil activation with subsequent tissue damage has been shown to be an important component of pathogenesis in CSS.

Treatment and Prognosis

In the setting of highly suspicious and life-threatening clinical picture of ANCA, empiric therapy may be initiated despite pending serological or biopsy results. The treatment is similar irrespective of the subtype of pauci-immune GN and includes induction and maintenance immunosuppression. Induction of remission is achieved with steroids and cyclophosphamide. Plasmapheresis to remove the circulating antibody is beneficial for patients with pulmonary hemorrhage and severe kidney disease. Mycophenolate mofetil and azathiopeine are usually used in maintenance of remission. The efficacy and safety of B-cell depletion therapies are also being investigated. Anti-TNFα therapy has been considered because of its ability to interfere with immunological pathways involved in ANCA disease. In light of reports of ANCA disease in rheumatoid arthritis patients treated with anti-TNFα, the role of this agent is unclear. Approximately a third of patients with ANCA disease relapse and overall, 5-year patient and renal survival rates are in the range of 60% to 70%. The subtype of ANCA (MPO vs. PR3) is a better predictor of outcomes and relapse rates than the specific clinicopathological subtype such as GPA versus MPA. For example, PR3-ANCA and lung disease are associated with higher relapse rates. A repeat biopsy may be justified in some to determine the extent of chronic changes and to evaluate the options for therapeutic modulation.

ANCA serological titers do correlate with disease activity and a new negative result in a patient with ANCA portends complete remission. It is generally suggested that the patient be in clinical remission before transplantation. However, persistently positive ANCA is not predictive of...
relapse and renal transplantation may be successful despite a positive test. There are no good predictors of disease recurrence after transplantation, which, based on pooled analysis of multiple studies, is estimated to be 17%. In most instances, such relapse in a renal allograft occurs more than 1 year after transplantation.

**ANTIGLOMERULAR BASEMENT MEMBRANE NEPHRITIS**

Anti-GBM nephritis is a rare autoimmune disorder characterized by autoantibodies to noncollagenous (NC1) domain of α3 chain of type IV collagen, an integral component of GBM. The clinical presentation is RPGN with biopsy findings of severe crescentic GN and a linear deposition of IgG along the GBM as evidenced by IF. When it is accompanied by pulmonary involvement, it is referred to as anti-GBM disease or “Goodpasture syndrome,” after Ernest Goodpasture who first described the index case with pulmonary renal syndrome in 1919. Anti-GBM disease is by far the most severe form of crescentic GN both in terms of clinical presentation and extent of glomerular involvement by crescents.

**Clinical Presentation**

Frequently seen in whites, the incidence of anti-GBM disease shows a bimodal age distribution, the first peak being at 20 to 30 years of age with a male preponderance and a second peak at 50 to 70 years, mainly in females. The overall incidence in the United States is < 1 case per million population per year. It is quite rare in children, presumably due to structural transition that occurs from pediatric to adult GBM. Renal pulmonary syndrome is a frequent presentation, especially in younger individuals. About a third of patients, primarily older individuals, present with isolated GN. Isolated pulmonary hemorrhage is, however, uncommon in anti-GBM nephritis.

General malaise and weakness are usual systemic manifestations, likely related to anemia and renal disease. Many patients develop flu-like illness before the onset of disease and some report exposure to hydrocarbons. Although RPGN is the most frequent presentation, older patients may present with milder kidney damage and less frequent pulmonary disease. On rare occasion, a patient may have normal renal function despite severe pulmonary hemorrhage with the only evidence of anti-GBM nephritis being linear IgG along GBM. RBC casts on urine microscopy confirm the glomerular origin of bleeding and the proteinuria is usually subnephrotic.

**Laboratory Investigations**

The diagnosis of anti-GBM nephritis requires the demonstration of anti-GBM antibodies, usually IgG class, in the patient’s serum or tissue. The circulating antibodies are detected by an ELISA test using human or animal GBM or recombinant antigen as a substrate. Although commercially available kits vary in their sensitivity, ELISA testing is considered most sensitive and specific method of anti-GBM antibody detection. The anti-GBM antibody levels also seem to correlate with renal disease activity.

However, the standard ELISA test can be negative in approximately 10% to 15% of biopsy-proven anti-GBM nephritis, and in some of these cases, the autoantibodies are detectable only by specialized biosensor analysis. A proportion of these cases may have circulating antibodies with specificity to α3 chain determinants not routinely detected by ELISA substrate or have specificity to other GBM components such as entactin. Rarely, the circulating autoantibodies belong to IgA class (rather than IgG) and can be directed against novel epitopes within GBM including α1/2 chains or NC1 domains of α5 and α6 chains of type IV collagen.

**Pathology**

**Light Microscopy**

Anti-GBM nephritis is a severe form of crescentic GN with widespread crescent formation evident on biopsy in most patients (Fig. 4). More than 80% of the patients have ≥ 50% glomerular crescents at the time of diagnosis. On occasion, the renal biopsy may demonstrate only focal and segmental glomerular necrosis. The crescents range from segmental to circumferential and tend to be similar in acuity, reflecting the abrupt onset of GN. The Bowman’s capsules are often disrupted extensively and periglomerular inflammation may appear granuloma-like with occasional multinucleated giant cells. Although infiltrating leukocytes and granulocytes accompany the crescents and fibrinoid necrosis, the unaffected segments of glomeruli are normocellular, similar to pauci-immune GN. But in some cases, the extensive circumferential crescents with compressed capillary loops preclude evaluation of mesangium or capillary lumens. The GBM rupture may be evident on periodic acid-Schiff or silver stain and the extravasated fibrin within the urinary space and crescent is highlighted by trichrome stain. Extensive tubulointerstitial inflammation, interstitial edema, and acute tubular injury often accompany the glomerular change. The interstitial infiltrate is usually mixed and may contain several eosinophils. Vasculitis, if present, should raise suspicion for combined anti-GBM and ANCA disease and a serological confirmation is recommended. Based on duration of disease before biopsy, subacute and chronic changes can be observed with glomerular fibrocellular and fibrous crescents and chronic tubulointerstitial damage.

**Immunofluorescence Microscopy**

Diffuse linear IgG staining, intensity ≥ 2 (on a scale of 0 to 4), along the GBM is diagnostic of anti-GBM nephritis, especially in the setting of clinical RPGN or crescents on biopsy. Nonspecific or low-intensity IgG staining may be observed in elderly individuals, patients with diabetes mellitus and obesity, allograft biopsies, and autopsy specimens. Hence, clinicopathological correlation is of great relevance in interpreting these IF results. Also, when in doubt, comparing the intensity of linear staining of IgG with that of albumin is helpful as anti-GBM nephritis is associated with IgG intensity greater than that of albumin. If the crescents are extensive, linear IgG staining may be difficult to identify or may be limited to a few fragments of remnant GBM in the glomerulus. Linear, but less-intense κ and λ staining (and on occasion IgA and IgM) is also observed in anti-GBM nephritis, whereas C3, if present, tends to be patchy and granular. Distal tubular basement membranes and Bowman’s capsules that express α3 chain of type IV collagen occasionally have linear IgG staining too. Antisera to fibrin highlight foci of necrosis and cellular crescents. In rare patients with circulating IgA anti-GBM antibodies, the linear GBM staining is observed with antisera to IgA rather than IgG. Linear Ig heavy and/or light chains may be noted in patients with diabetic
Anti-GBM nephritis is usually a severe form of crescentic GN. A, Unaffected capillary loops, if present, are devoid of proliferation (JMS, ×400). B, Severe destruction of glomerulus and Bowman’s capsule is frequent. Multinucleated cells (arrowhead) and periglomerular palisading of inflammatory cells (long arrow) should not be misinterpreted as granulomatous inflammation (H&E, ×400). C, The IF is diagnostic with linear IgG staining of GBM. D, Fibrinogen stain highlights the cellular crescent of a different glomerulus. E, F, On EM, the GBM of the affected glomerulus is collapsed (*) and fibrin (short arrow) is seen admixed with the cellular component of crescent. But preserved capillary loops (F) are entirely normal (×4000). EM indicates electron microscopy; GBM, glomerular basement membrane; GN, glomerulonephritis; IF, immunofluorescence microscopy; Ig, immunoglobulin; JMS, Jones methenamine silver stain;
glomerulosclerosis, idiopathic nodular sclerosis, and monoclonal Ig deposition disease, but light microscopy is distinctly different in these entities with nodular mesangial sclerosis rather than glomerular crescents. In rare cases of anti-GBM nephritis with coexistent membranous nephropathy, granular capillary wall IgG staining may be barely discernable in the presence of linear pattern and ultrastructural confirmation is needed.

Electron Microscopy

Other than confirming the lack of electron-dense deposits, ultrastructural features in anti-GBM nephritis are diagnostically nonspecific and show crescents and GBM breaks in the acute phase. Some of the changes observed adjacent to necrotic foci include subendothelial expansion by ECM material, margination of neutrophils and monocytes in capillary lumens, and fibrin tactoids in crescents. Increasing fibrosis and collagen deposition is seen as the lesions evolve into chronic phase with fibrous crescents. These EM findings are similar to pauci-immune GN. In the setting of concurrent membranous nephropathy, subepithelial electron-dense deposits are observed.

A renal biopsy is invaluable in the diagnosis of anti-GBM nephritis due to the ease of interpretation and a short turnaround time. In contrast, confirmatory serological tests typically take a few days. Even in cases with predominant pulmonary involvement, a renal biopsy is preferred over transtracheal biopsies that have a significant rate of false-negative IF results. Lung biopsy IF interpretation is rather difficult due to high background, autofluorescence, and irregular poorly localized pattern of IgG staining in anti-GBM disease.

Pathogenesis

The pathogenicity of anti-GBM antibodies was first demonstrated by Lerner et al, by passive transfer of disease in monkeys by injecting antibodies from patients with linear IgG-positive glomerulonephritis. Subsequent animal studies have confirmed causative role of anti-GBM antibodies. Temporal associations between relapse and re-emergence of antibodies and prognostic value of anti-GBM levels also support this hypothesis. The susceptibility for autoantibody generation may be genetically determined as supported by strong association between HLA DR and DQ antigens and anti-GBM disease. Despite geographic variations, patients with HLA-DRB1*1501 alleles seem prone to develop anti-GBM nephritis. Environmental triggers such as viral infections, exposure to organic solvents (glue and solvent sniffing), and cigarette smoke probably are “second hits” to developing autoimmune. It is well known that respiratory infections precede anti-GBM nephritis causing flu-like symptoms and miniepisodes of anti-GBM nephritis have been reported.

In vast majority of cases, anti-GBM antibodies target the NC1 domain, specifically epitopes within E3 and E4 regions of α3 chain of type IV collagen. This antigen, also referred to as “Goodpasture antigen,” is an important constituent of the kidney GBM as well as alveolar capillary basement membranes, choroid plexus, and eye. Normal GBM is composed of α3, 4, and 5 chains of type IV collagen assembled as triple helical protomers. Two triple helical protomers are associated together at the C-terminal NC1 domain forming a hexamer that is in turn stabilized by sulfilimine bonds. In the presence of intact sulfilimine bonds, anti-GBM antibodies cannot bind to its hidden target antigen. It is postulated that triggering events may change the conformation of these hexamers, expose neoepitopes, elicit autoantibody production, and facilitate antigen-antibody binding. Some possible triggers include enzymatic and nonenzymatic modifications (oxidation, nitrosylation, glycation), rise in body temperature, and proteolytic cleavage. Environmental exposure to cigarette smoke or organic solvents may inhibit the enzymes that catalyze the sulfilimine bond formation and alter the quaternary structure of the hexamer. These events may also initiate epitope spreading and in fact, many patients with anti-GBM nephritis have circulating autoantibodies to α3 NC1 monomer in addition to expected anti-α3 NC1 antibodies. The specificity of these antibodies is similar irrespective of involvement of either lung or kidney.

In addition to autoantibodies, T cells and macrophages have a role in exacerbating the inflammatory process and glomerular injury in anti-GBM disease. Antigen-antibody binding activates complement and recruitment of neutrophils and monocytes which facilitate GBM rupture and crescent formation.

Treatment and Prognosis

Early diagnosis and treatment is of paramount importance for improved outcomes in anti-GBM nephritis. High-dose corticosteroids and cyclophosphamide therapy need to be instituted rapidly and plasmapheresis is useful in removal of circulating antibodies. Severe renal disease requiring dialysis at presentation and serum creatinine > 5 mg/dL is associated with poor prognosis. In general, anti-GBM nephritis has worse prognosis than pauci-immune GN or immune complex-mediated GN. Relapse of anti-GBM nephritis as an active disease is quite infrequent, but can occur even after several years. Anti-GBM nephritis recurrence in renal allografts is uncommon, especially if the antibody titers remain low.

De novo anti-GBM nephritis can occur in approximately 3% to 5% of patients with Alport syndrome who undergo renal transplantation. It is most frequently seen in males with X-linked mutation in genes encoding type IV collagen. In the absence of α5 chain, these patients fail to assemble the α345 triple helix in the GBM. Subsequent to renal transplantation, they develop antibodies against α5 NC1 of the intact hexamer in the allograft, a novel protein in the recipient. Unlike native anti-GBM nephritis, the antigenic epitope appears to be on the surface of the hexamer and thus can elicit antibody response without the triggering factors needed for hexamer dissociation.

Concurrent Disease

Positive ANCA serology, especially anti-MPO has been identified in approximately a third of the patients with anti-GBM disease. ANCA can be detected either before or after the appearance of anti-GBM antibodies. In addition to glomerular crescents, the renal biopsies from these patients likely demonstrate vasculitis. Despite previous reports, the prognosis of dual-positive patients is comparable to patients with isolated anti-GBM nephritis. However, similar to isolated ANCA disease patients, these dual-positive cases also have higher frequency of active relapses. This necessitates close clinical follow-up of such patients for early detection of relapses. It is presumed that development of one disease, either anti-GBM or pauci-immune disease
GN causes exposure of hidden antigens, thus precipitating the concurrent disease process.\textsuperscript{8,9,12}

A recent study identified naturally occurring low-titer anti-MPO and anti-GBM antibodies in healthy individuals that are evidently nonpathogenic.\textsuperscript{93} Although such naturally occurring low-avidity antibodies suggest incomplete immune tolerance, they may have an important role in defense against microbial infections, removal of apoptotic or neoplastic cells. Geographic variation in the prevalence of such antibodies may be related to frequency of exposure to infectious pathogens.\textsuperscript{93} It is speculated that dysregulated immunological mechanisms result in higher pathogenic titers of antibody. Although the discussion of immunological theories is beyond the scope of this review, it seems that idiotypic and anti-idiotypic antibodies may play a role in both anti-GBM and ANCA diseases.\textsuperscript{43,94}

Rarely, anti-GBM nephritis and membranous nephropathy can occur simultaneously or one can precede the other.\textsuperscript{95} Although this concurrence can be entirely coincidental, epitope spreading or release of damaged GBM antigens into circulation can be triggered by one disease, precipitating the occurrence of a second glomerular disease.\textsuperscript{96,97} IF can be challenging to interpret, but EM confirms the presence of subepithelial membranous deposits.

**CONCLUSIONS**

Despite advances in understanding the pathophysiology of ANCA GN and anti-GBM nephritis, and the availability of serologic laboratory studies with reasonable sensitivity and specificity, renal biopsy remains the mainstay of diagnosis for patients presenting with the clinical syndrome of RPGN. IF, together with light microscopy and EM, allow distinction of ANCA GN, anti-GBM disease, and immune complex-mediated GN, including overlap syndromes and mimics. Clinical correlation and close communication with the clinical team remains critical for rapid initiation of appropriate therapy in these critically ill patients.

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