Primary Membranous Nephropathy

William G. Couser

Abstract
Membranous nephropathy (MN) is a unique glomerular lesion that is the most common cause of idiopathic nephrotic syndrome in nondiabetic white adults. About 80% of cases are renal limited (primary MN, PMN) and 20% are associated with other systemic diseases or exposures (secondary MN). This review focuses only on PMN. Most cases of PMN have circulating IgG4 autoantibody to the podocyte membrane antigen PLA2R (70%), biopsy evidence PLA2R staining indicating recent immunologic disease activity despite negative serum antibody levels (15%), or serum anti-THSD7A (3%–5%). The remaining 10% without demonstrable anti-PLA2R/THSD7A antibody or antigen likely have PMN probably secondary to a different, still unidentified, anti-podocyte antibody. Considerable clinical and experimental data now suggests these antibodies are pathogenic. Clinically, 80% of patients with PMN present with nephrotic syndrome and 20% with non-nephrotic proteinuria. Untreated, about one third undergo spontaneous remission, especially those with absent or low anti-PLA2R levels, one-third progress to ESRD over 10 years, and the remainder develop nonprogressive CKD. Proteinuria can persist for months after circulating anti-PLA2R/THSD7A antibody is no longer detectable (immunologic remission). All patients with PMN should be treated with supportive care from the time of diagnosis to minimize protein excretion. Patients with elevated anti-PLA2R/THSD7A levels and proteinuria >3.5 g/d at diagnosis, and those who fail to reduce proteinuria to <3.5 g after 6 months of supportive care or have complications of nephrotic syndrome, should be considered for immunosuppressive therapy. Accepted regimens include steroids/cyclophosphamide, calcineurin inhibitors, and B cell depletion. With proper management, only 10% or less will develop ESRD over the subsequent 10 years.


Introduction
About 20% of all cases of membranous nephropathy (MN) are associated with other diseases or exposures (secondary MN) that are listed in Table 1. Secondary MN is not discussed further in this review. Primary membranous nephropathy (PMN) is a kidney-specific, autoimmune glomerular disease that presents with increased protein in the urine associated with a pathognomonic pattern of injury in glomeruli (Figures 1–3). Both clinical and pathogenetic aspects of the disease have been recently reviewed elsewhere (1–8). PMN is the commonest cause of idiopathic nephrotic syndrome in nondiabetic adults worldwide, representing between 20% and 37% in most series and rising to as high as 40% in adults over 60 (1,2,7). MN is rare in children (1%–7% of biopsies) (3). Most PMN is mediated by antibodies to the M-type phospholipase A2 receptor (anti-PLA2R) (85%), thrombospondin type 1 domain containing 7A (THSD7A) (3%–5%), or by other as yet unidentified mechanisms (10%) (1,2,4–8). The recognition that PMN is an autoimmune disease has dramatically altered both the diagnostic and therapeutic approach to what was previously called idiopathic MN. Patients with immunologically active disease can now be separated from those with inactive disease and therapeutic initiatives in active disease can be adjusted to the presence and levels of the pathogenic antibody causing the disease rather than relying empirically on clinical consequences of immune injury to the glomerulus such as proteinuria or reduced GFR (1,4–7).

Epidemiology
In the United States, the incidence of MN is estimated at about 12/million per year with a mean age between 50 and 60 and a 2:1 male predominance (1–4). The incidence of ESRD due to MN in the United States is about 1.9/million per year (1). Because only 10%–20% of patients with PMN currently progress to ESRD, the real incidence may be as high as 20/million per year. PMN is most common in whites followed by Asians, blacks, and Hispanics (1,2).

Pathogenesis
Studies in the past decade have dramatically improved understanding of the pathogenesis of PMN (1,2,4–8). Current concepts derive in large part from earlier studies carried out in the Heymann models of MN in rats which revealed that the pathognomonic, exclusively subepithelial deposits of IgG resulted from in situ immune complex formation involving megalin, a rat podocyte membrane antigen, and that the associated proteinuria was mediated primarily by complement through the membrane attack complex
C5b-9 (9). The first confirmation that PMN in man involved an analogous mechanism came from Debiec et al. in Paris in 2002, who showed that alloimmune MN in babies of neutral endoproteinase (NEP)–deficient mothers was mediated by maternal anti-NEP antibody that formed immune complexes in situ with NEP on the podocyte membranes of the infant (10). In 2009, a seminal paper from Beck et al. in Boston reported that about 70% of adult patients with PMN have IgG4 antibodies to podocyte-expressed PLA2R that are present in the circulation and also deposited in glomeruli (11), a finding since confirmed, with a range of 52%–78%, by many other laboratories (1,4–8).

A second IgG4 antibody specific for THSD7A, another podocyte membrane antigen with similar properties to PLA2R, was later identified in a smaller number of patients with PMN (2%–5%) (Table 2) (12). About 10% of patients with typical PMN are negative for both antibodies, making it probable that more autoantibodies to podocyte antigens will be found. Dual expression of antibodies to both PLA2R and THSD7A has been reported but is rare (13).

Most statements in this review are assumed to apply to patients with either antibody, designated in this paper as anti-PLA2R/THSD7A, unless otherwise specified. The only significant clinical difference identified so far is a female predominance and a higher frequency of associated malignancies with THSD7A (14,15). THSD7A is expressed in those tumors most frequently associated with PMN (16). Twenty percent of THSD7A-positive patients in one series had coexistent malignancy, usually detected within 3 months (14,17). The observation that THSD7A was expressed in the tumor in two cases suggests one potential mechanism for the well established association between MN and malignancy (14,17,18). The pathogenicity of anti-PLA2R has not yet been confirmed because PLA2R is not expressed in rodents. However, human anti-THSD7A has recently been shown to transfer MN with proteinuria in mice (19).

Table 1. Recognized causes of anti-PLA2R/THSD7A–negative secondary membranous nephropathy

<table>
<thead>
<tr>
<th>Cause</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infections</td>
<td>bHBV, HCV, HIV, parasites (filariasis, schistosomiasis, malaria), leprosy, syphilis, hydatid disease, sarcoïd</td>
</tr>
<tr>
<td>Malignancy</td>
<td>bSolid tumors (lung 26%, prostate 15%, hematologic [plasma cell dyscrasias, non-Hodgkin lymphoma, CLL] 14%, colon 11%), mesothelioma, melanoma, pheochromocytoma; some benign tumors</td>
</tr>
<tr>
<td>Autoimmune diseases</td>
<td>bSLE (class V), thyroiditis, diabetes, rheumatoid arthritis, Sjogren syndrome, dermatomyositis, mixed connective tissue disease,ankylosing spondylitis, retroperitoneal fibrosis, renal allografts Anti-GBM disease, IgAN, ANCA-associated vasculitis</td>
</tr>
<tr>
<td>Alloimmune diseases</td>
<td>IgG4 disease Membranous-like glomerulopathy with masked IgGκ deposits (90)</td>
</tr>
<tr>
<td>Drugs/toxins</td>
<td>bNSAIDs and cyclooxygenase-2 inhibitors, gold, d-penicillamine, bucillamine, captopril, probenecid, sulindac, anti-TNFα, thiola, trimetadione, tiopronin</td>
</tr>
<tr>
<td></td>
<td>Mercury, lithium, hydrocarbons, formaldehyde, benvironmental air pollution (China)</td>
</tr>
<tr>
<td></td>
<td>Cationic BSA (infants)</td>
</tr>
</tbody>
</table>

HBV, hepatitis B; HCV, hepatitis C; CLL, chronic lymphocytic leukemia; MN, membranous nephropathy; NSAIDs, non-steroidal anti-inflammatory drugs.

aMost of these associations are on the basis of multiple case reports or small series. Causative roles are implied but generally not proven.

bCommon.

Figure 1. Glomerulus from a patient with primary membranous nephropathy showing the pathognomonic “spikes” of basement membrane projecting from the outer surface of the glomerular basement membrane (arrows) when stained with silver-methenamine (original magnification, ×40). (Provided by Dr. Charles Alpers, Department of Pathology, University of Washington, Seattle, WA.)
with IgG4 in glomerular deposits (Figure 2B) (17,20–25). Staining persists for weeks to months after antibody disappears (5–9,19,20). Most antibody-positive, and about 70% of antibody-negative, patients have positive PLA2R/THSD7A staining in glomeruli, suggesting that up to 85%–90% of all cases of PMN are anti-PLA2R/THSD7A-mediated (21) (Table 2). However, occasional anti-PLA2R-positive, or presumed positive, patients, especially early in the course, have been reported without staining for PLA2R antigen in glomeruli (23–25). As summarized in Table 2, patients presenting with PMN include those who are free of systemic disease and are anti-PLA2R (70%) or THSD7A

Figure 2. | Immunofluorescence microscopy in primary membranous nephropathy (PMN). (A) Finely granular staining for IgG, predominately IgG4, present uniformly in a subepithelial distribution in all glomeruli in a patient with PLA2R-associated PMN (original magnification, ×40) (generously provided by Dr. Charles Alpers, Department of Pathology, University of Washington, Seattle, WA). (B) Finely granular staining for PLA2R antigen that colocalizes with IgG4 in a patient with PMN. The presence of PLA2R indicates that anti-PLA2R antibody was present and forming deposits in glomeruli at the time of biopsy or within the past several weeks. (original magnification, ×40) (generously provided by Dr. Charles Alpers, Department of Pathology, University of Washington, Seattle, WA). (C) Finely granular staining for the complement membrane attack complex, C5b-9, in a patient with active PMN (original magnification, ×40). Reprinted from reference 89, with permission.

Figure 3. | Electron micrograph of chronic primary membranous nephropathy showing discontinuous, electron-dense deposits representing aggregates of PLA2R–anti-PLA2R immune complexes formed in situ along the outer surface of the glomerular capillary wall beneath a layer of effaced podocyte foot processes (arrows). BM, basement membrane; CL, capillary lumen. Original photomicrograph generously provided by Dr. Charles Alpers, Department of Pathology, University of Washington, Seattle, WA.
Table 2. Interpretation of serum anti-podocyte antibody and glomerular antigen staining in primary membranous nephropathy (4,21,23–26)

<table>
<thead>
<tr>
<th>Serum Antibody (±)</th>
<th>Glomerular Antigen (±)</th>
<th>Percent of Patients Who Underwent Biopsy, %</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-PLA2R (+)</td>
<td>PLA2R (+)</td>
<td>70</td>
<td>PLA2R-mediated PMN (active)</td>
</tr>
<tr>
<td>Anti-PLA2R (−)</td>
<td>PLA2R (+)</td>
<td>15</td>
<td>PLA2R-mediated PMN (inactive)</td>
</tr>
<tr>
<td>Anti-THSD7A (+)</td>
<td>THSD7A (+)</td>
<td>3–5</td>
<td>THSD7A-mediated PMN (active)</td>
</tr>
<tr>
<td>Anti-THSD7A (−)</td>
<td>THSD7A (+)</td>
<td>Unknown</td>
<td>THSD7A-mediated PMN (inactive)</td>
</tr>
<tr>
<td>Anti-PLA2R/THSD7A (−)</td>
<td>PLA2R/THSD7A (−)</td>
<td>10</td>
<td>Non-PLA2R/THSD7A-mediated (pathogenesis unknown)</td>
</tr>
</tbody>
</table>

+, positive; PMN, primary membranous nephropathy; −, negative.

*Patients with nephrotic syndrome due to PMN without evidence of PLA2R/THSD7A antibody or glomerular staining are presumed to have autoimmune PMN mediated by a different, still unidentified, anti-podocyte antibody.

(3%–5%) positive, those who are anti-PLA2R/THSD7A negative but have positive glomerular staining for PLA2R/THSD7A (another 15%), and those without PLA2R/THSD7A antibody or glomerular staining (10%) who may: (1) develop detectable anti-PLA2R/THSD7A antibody later, (2) have disease mediated by a different anti-podocyte antibody, or (3) develop another autoimmune disease to which the MN might be considered secondary.

Initiation of an antibody response likely precedes development of proteinuria in PMN by weeks or months (preclinical disease) (22,26) and may occur following several etiologic events in the presence of immunogenetic risk alleles (see below). There is one report of homology between genes for PLA2R and the LTLENCK domain in some gram-positive bacterial enzymes (27). Viral infections including hepatitis B virus, hepatitis C virus, and HIV have been reported in association with anti-PLA2R/THSD7A, but these may represent patients who have coincidental MN (4–8,22) (Table 1). Other potential etiologic factors, e.g., exposures to environmental toxins such as drugs, mercury, formaldehyde, and air pollutants, have been identified (Table 1) but generally the anti-PLA2R/THSD7A status in these patients is not known. For example, a recent report from China correlated the rising incidence of MN in that country with increasing levels of air pollution (28).

Over time, a threshold quantity of IgG4 and C5b-9 deposition is reached in some patients that is sufficient to cause enough podocyte injury/activation to increase urine protein excretion and lead to nephrotic syndrome (5–7,22,26). Just as appearance of proteinuria lags behind initial antibody production by weeks/months, so resolution of proteinuria (clinical remission) also lags behind antibody disappearance (immunologic remission) by week/months. This offset between immunologic and clinical remissions reflects the prolonged time required to form sufficient deposits to cause proteinuria initially and the time required to clear subepithelial deposits, repair podocyte and capillary wall damage, and restore glomerular permselectivity (5). Thus, proteinuria is a poor clinical biomarker for the pathogenetic disease processes that are targeted by current immunosuppressive therapies (IST).

C activation leading to sublytic C5b-9 attack on podocytes has been established to be the primary mediator of anti-podocyte antibody–induced cellular injury and proteinuria in most studies of the Heymann rat models (5,9,29–31). Currently available serologic and immunohistochemical data in PMN are most consistent with complement activation by under-glycosylated IgG4 through the mannose-binding lectin pathway, but roles for the classic and alternate pathways have not been entirely excluded (29–31). In the rat models, the complement effect involves a sublytic agonistic effect of C5b-9 insertion on the podocyte membrane to activate several signaling pathways that lead collectively to increased production of oxidants, proteases, growth factors, and extracellular matrix components as well as to slit diaphragm disruption, apoptosis, autophagy, remodeling of the actin cytoskeleton, DNA damage with cell cycle arrest, and detachment of damaged cells (29,30). A similar role for complement in human PMN is suggested by the facts that C3, C4d, and C5b-9 are prominent in glomerular deposits; complement activation products are elevated in the serum (M.H. Zhao, personal communication); and serum and urine C5b-9 seem to parallel disease activity (28–30). However, proteinuria in some human PMN may also be C-independent as suggested by some studies of the Heymann models by Hall and colleagues (32), the transfer of anti-NEP alloimmune MN without complement activation (10), and an in vitro transfer study with human anti-THSD7A where heterologous phase proteinuria appears to precede detectable complement deposition (19).

Genetics of PMN

Genetic findings in PMN are reviewed in more detail elsewhere (4–8,33–35). Familial MN is rare and usually seen in children (3,33,34). Genome-wide association studies (GWAS) implicate risk alleles in HLA genes, particularly HLA–DQA1, that increase the risk for PMN three-fold in
white patients (27). GWAS studies have also identified single nucleotide polymorphisms in noncoding regions of the PLA2R gene (27,36–38). Homozygosity for high-risk alleles in both HLA and PLA2R genes increases the odds ratio for PMN almost 80-fold in white patients and ten-fold in Chinese patients and is associated with higher levels of antibody production, strongly suggesting interaction between HLA and PLA2R genes (36–38). Two recent GWAS studies in Chinese patients have identified additional independent HLA risk alleles including DRB1*1501/DRB1*0301 (37) and DRB3*02:02 (38) and suggested that DRB1 may be more important in generating the HLA signal than DQA1 in that population. Ninety-nine percent of PLA2R-positive patients carry at least one of these HLA risk alleles, and the presence of one HLA risk allele increases the odds ratio for developing PMN almost 100-fold (35) However, risk alleles identified so far are common in the general population, and studies to date are also consistent with a predisposition to autoimmunity conferred by HLA genes and an environmental trigger rather than any unique coding variant in PLA2R genes (33).

Structure of the PLA2R Antigen

PLA2R is a transmembrane glycoprotein member of the mannose receptor family, which has a conserved extracellular structure consisting of the cysteine-rich (Ricin B) domain (Cys-R), a fibronectin II domain, and a tandem repeat of 8 C-type lectin domains (CDLD 1–8) (1,4,7,39,40). Anti-PLA2R-reactive epitopes are conformation-dependent and have been identified in three domains: Cys-R, CTLD1, and CTLD7 (1,4,39,40). The potential for these different epitopes to be of clinical significance is suggested by the finding that patients with anti-PLA2R directed at the Cys-R epitope, which is recognized by 100% of anti-PLA2R antibodies, may have less severe disease and undergo more spontaneous remissions than those with antibodies primarily reactive with the CDL1 and CDL7 domains, and that epitope spreading beyond the Cys-R domain may confer a worse prognosis, but this observation requires confirmation (36). The small contact residues that bind the antibody, and would be essential to developing specific peptide-based immunotherapies, have not yet been identified.

Pathology

In most centers a diagnostic renal biopsy remains the standard of care, even in anti-PLA2R/THSD7A-positive nephrotic patients. The characteristic morphologic changes in PMN are described elsewhere (41) and are illustrated in Figures 1–3. By light microscopy, glomeruli may appear entirely normal in early disease despite nephrotic-range proteinuria. With time, changes in basement membrane, with thickening and formation of subepithelial “spikes” of basement membrane on the outer surface of the capillary wall, become apparent using an extracellular matrix stain such as silver methenamine (Figure 1). Immunofluorescence microscopy (Figure 2) in anti-PLA2R/THSD7A-positive patients usually reveals diffuse, uniform, finely granular deposits of IgG4 along the outer surfaces of all capillary walls (Figure 2A) (41). Lesser amounts of IgG1 and IgG3 may also be seen, particularly in early disease (21,22,26). The antigen PLA2R or THSD7A (Figure 2B) colocalized with IgG4 may be seen by immunofluorescence microscopy with appropriate antigen enhancement techniques and persist for weeks to months after serum antibody is undetectable and immune complex formation has ceased (4–8,20–23). Complement components including C3, C4d, and C5b-9 (Figure 2C) are also commonly present, but not C1q (29,30,41). Although these findings describe the typical case, they are not universal, and occasional patients with IgG4-dominant deposits but without detectable PLA2R/THSD7A antibody or staining have been described (20,21).

Electron microscopy in PMN confirms the exclusively subepithelial localization of electron-dense deposits produced by capping and shedding of immune complex lattices formed on the podocyte membrane, which then accumulate beneath slit pores (Figure 3). Glomerular basement membrane thickening is seen with progression, and the deposits are gradually incorporated within new glomerular basement membrane and become more electron-lucent as they are resorbed before eventually disappearing in patients with earlier complete remissions (41).

Additional biopsy findings that should prompt careful search for secondary causes (Table 1) include electron-dense deposits in subendothelial or mesangial locations; significant mesangial or endothelial cell proliferation; crescents; tubular basement membrane staining; dominant deposition of IgG1/IgG3, IgM, IgA, or C1q; and endothelial tubuloreticular inclusions by electron microscopy (1,2,4–7,21,22,41). One report has described an association between MN with intraglomerular inflammatory cell infiltrates and cancer (18).

Clinical Manifestations

All adult patients with idiopathic nephrotic syndrome should be screened initially for anti-PLA2R/THSD7A antibodies as well as for the common causes of secondary MN including hepatitis B and C, lupus, and sarcoid (Figure 4, Table 1). Although the specificity of the anti-PLA2R assay for PMN is essentially 100%, this finding has somewhat blurred the distinction between primary and secondary disease because some patients with secondary diseases such as hepatitis B and C, cancer, and sarcoid have been found to be anti-PLA2R-positive suggesting the coincidental presence of PMN in some patients with an unrelated systemic disease rather than MN as a manifestation of, or secondary to, the systemic disease (4–8). Observational correlations between the anti-PLA2R/THSD7A pathogenic mechanisms discussed above and clinical features of PMN that now support incorporating anti-PLA2R/THSD7A into clinical decision-making are summarized in Table 3.

The most common clinical features at onset and during the course of PMN are presented in Table 4. The most prominent is nephrotic syndrome and its associated manifestations including various degrees of edema, hypoalbuminemia, and hyperlipidemia (1,2,42–45) (Table 4). About 80% of patients with PMN present with nephrotic-range proteinuria (>3.5 g/d) and the remaining 20% have subnephrotic proteinuria, but 61% of these latter patients later become nephrotic, usually within the first year, and especially if anti-PLA2R antibody is present (1,26,27,43–45). Although the peak incidence is between ages 50 and
Renal function is normal at presentation in 90% (242–45) (Table 4).

Spontaneous remissions occur in about 32% in an average of 14 months and up to 62% by 5 years, and occur more commonly in patients with low anti-PLA2R/THSD7A levels (46–48). Anti-PLA2R/THSD7A levels generally correlate with proteinuria, clinical course, and outcomes (Table 3) (4–8,22,46–51).

The clinical consequences of PMN can be considered as both short and long term. In the short term, they include:

- Figure 4. Antibody-guided diagnosis and treatment algorithm for primary membranous nephropathy (PMN).

Patients who undergo biopsies for proteinuria of uncertain cause who have MN should initially be classified as anti-PLA2R/THSD7A–positive (active disease) or negative (Table 2). Patients who are antibody-negative should have the absence of a PLA2R/THSD7A–related mechanism further excluded by the absence of PLA2R/THSD7A staining in glomeruli and usually the dominance of IgG1–3 in the biopsy sample. Most of these latter patients have secondary MN and require other tests to identify the cause. They are treated with supportive care and therapy for their underlying systemic disease. Patients who are negative for anti-PLA2R/THSD7A in the serum but have PLA2R or THSD7A antigen staining in the biopsy sample, and usually predominately IgG4 deposition in glomeruli, have inactive anti-PLA2R/THSD7A–mediated PMN and should be treated with supportive care and monitoring for anti-PLA2R levels for 4–6 months. They would be considered for IST only if anti-PLA2R becomes positive or proteinuria >3.5 g/d persists after 6 months of supportive care. Patients with elevated anti-PLA2R levels (with positive PLA2R staining and (usually) predominately IgG4 in the biopsy sample) and proteinuria <3.5 g/d have active anti-PLA2R/THSD7A–mediated PMN but would receive supportive care with monthly anti-PLA2R monitoring because most of these patients will undergo spontaneous remission. If patients have, or develop, elevated anti-PLA2R levels and >3.5 g/d of proteinuria they have active, anti-PLA2R/THSD7A–mediated PMN and would be considered for immediate IST. IST options would be selected on the basis of characteristics of individual patients with dose and duration of therapy (Table 6) guided by regular monitoring of anti-PLA2R levels. About 10% of patients with anti-PLA2R/THSD7A–negative antibody and glomerular staining have PMN presumably mediated by a different anti-podocyte antibody rather than secondary MN and would be treated with traditional restrictive care (4,52,53). Occasional patients with negative anti-PLA2R antibody but dominant IgG4 in the biopsy sample have also been reported and should be monitored for later development of positive circulating anti-PLA2R antibody. ANA, anti-nuclear antibody; HBV, hepatitis B; HCV, hepatitis C; IST, immunosuppressive therapy; MN, membranous nephropathy. Modified from other schemas in references 1 and 4, with permission.

60, 23% or more are over 60 (243–45). Renal function is normal at presentation in >90% (242–45) (Table 4). Spontaneous remissions occur in about 32% in an average of 14 months and up to 62% by 5 years, and occur more commonly in patients with low anti-PLA2R/THSD7A levels (46–48). Anti-PLA2R/THSD7A levels generally correlate with proteinuria, clinical course, and outcomes (Table 3) (4–8,22,46–51).

The clinical consequences of PMN can be considered as both short and long term. In the short term, they include...
complications of nephrotic syndrome such as development of thrombotic and thromboembolic events that are proportional to the degree of hypoalbuminemia and increase significantly below albumin levels of about 2.8 g/L (1,2,42,52–54) (Table 4). There is also an increased risk of infection, due primarily to urinary loss of Igs, and of cardiovascular disease (42,49). An association with malignancies is well documented (55). Cancer may be seen within 3 years in up to 20% of patients over 60 and may be more common in the anti-THSD7A group where up to 20% have had a malignancy detected within 3 months (14–16).

The most feared long-term consequence of MN is progression of renal function as occurs in 60% of untreated patients with about 35% eventually developing ESRD within 10 years (1,2,4,44–46). Patients who never become nephrotic virtually never progress (1,2,4,43–45). Other established risk factors for progression include age, male sex, decreased GFR on presentation, increased excretion of some low molecular weight markers such as β2 microglobulin, persistent elevation of anti-PLA2R levels after therapy, C3 staining in the biopsy sample, and increased urinary excretion of C3dg and C5b-9 (1,2,43–45).

### Treatment

**Selection of patients for IST**

Traditional approaches to treatment of PMN begin with supportive care alone and withhold IST until the patient meets certain criteria that predict progression (restrictive therapy). Supportive care should be initiated in all patients at the time of diagnosis and continued for the course of the disease. It includes careful BP control, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker therapy to minimize proteinuria and enhance chances of a spontaneous remission, statins for hyperlipidemia, salt restriction and diuretics to control edema, and a low protein diet allowing for replacement of urinary protein losses (1,2,4,56–60) Some patients are also anticoagulated if serum albumin is <2.5 g/L in the presence of other risk factors and a favorable risk/benefit ratio as defined by online calculators (54).

Current criteria for adding IST to supportive care (SC) are based on either evidence of progressive loss of GFR (usually >50% increase in serum creatinine or a level >1.5 mg/dl) or proteinuria refractory to 6 months of SC as defined by the Toronto risk score (56). The latter approach divides patients after 6 months of supportive care into three categories: low risk (<4 g/d, stable GFR), moderate risk (4–8 g/d with stable GFR), or high risk (>8 g/d, <50% decrease from baseline or >30% decline in GFR since baseline) categories. Initiation of IST is recommended in most patients in the moderate- and high-risk categories unless factors that reduce the chance of a good response are present, such as GFR <30 ml/min, serum creatinine >3.5 mg/dl, small fibrotic kidneys, or an abundance (>50%) of sclerotic glomeruli. Other situations that would dictate early initiation of IST would include proteinuria >10 g/d or failure to reduce proteinuria below 8 g/d after 3 months, complications of nephrotic syndrome such as...

### Table 3. Clinical and translational correlates of circulating levels of anti-PLA2R

<table>
<thead>
<tr>
<th>Condition</th>
<th>Circulating Levels of Anti-PLA2R</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMN with anti-PLA2R/THSD7A antibody</td>
<td>70%–80%</td>
</tr>
<tr>
<td>Anti-PLA2R antibody sensitivity</td>
<td>About 80%</td>
</tr>
<tr>
<td>Anti-PLA2R antibody specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Anti-PLA2R antibody duration</td>
<td>Can be present for many months</td>
</tr>
<tr>
<td>Patients over 60</td>
<td>Common in the anti-THSD7A group</td>
</tr>
<tr>
<td>Malignancies</td>
<td>Can be seen within 3 years</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>Well documented</td>
</tr>
<tr>
<td>Cancer</td>
<td>Common in patients over 60</td>
</tr>
</tbody>
</table>

---

PMN, primary membranous nephropathy; HBV, hepatitis B; HCV, hepatitis C.

*Although established in only a few cases, most of these associations are likely similar in anti-THSDA–mediated PMN.*
Definitions of terms conventionally used to classify responses to therapy of PMN are presented in Table 5 (4,52,53). Goals of therapy would be complete or partial remission. Thompson et al. have recently presented evidence supporting the validity of considering complete and partial remissions of proteinuria in PMN as surrogate markers of good outcomes and consequently as valid goals of therapy or end points for therapeutic trials (43).

For decades, clinicians have treated PMN with nephrotic syndrome with potentially toxic IST using the above proteinuria/GFR-based guidelines, because there was no way to distinguish patients with immunologically active disease from those with inactive disease who have persistent proteinuria despite immunologic remission. Table 6 provides an overview of IST regimens of established benefit in preserving renal function in PMN.

Figure 4, and the legend to Figure 4, outline a different approach to IST applicable to all patients with proteinuria >3.5 g/d, normal or stable GFR and active disease, defined as ongoing glomerular immune deposit formation indicated by elevated circulating levels of anti-PLA2R/THSD7A. Based on the data reviewed above, such patients have active immunologic disease and should be considered for immediate IST without waiting 6 months on supportive care alone as prescribed by restrictive therapy protocols. Although much remains to be learned about translating anti-PLA2R/THSD7A levels into predictive clinical algorithms, patients with anti-PLA2R levels in the highest tertile have only a 4% chance of undergoing spontaneous immunologic remission while being treated with SC alone (47,48,50,51,59,60). Informed consent should always be obtained before IST is initiated.

Both supportive care and IST should continue with antibody monitoring every 1–2 months until anti-PLA2R/THSD7A...
leaks become undetectable (or fall below some as yet undefined level below which progression is unlikely), recognizing that a response in proteinuria (clinical remission) may only occur several weeks to months after an immunologic remission is achieved (4,6,7). Considering the current high cost of anti-PLA2R assays, obtaining levels at the time of diagnosis and when a clinical decision point is reached (e.g., after 6 months of IST) would be acceptable if cost to the patient is a limiting factor. However, monitoring levels initially every 1–2 months may justify shorter courses of therapy and better predict remissions and relapses. In most anti-PLA2R/THSD7A-positive patients, circulating antibody disappears after 4–6 months of IST, which should then be tapered and discontinued even if some proteinuria persists (4,6,56–60). If antibody levels persist but show a downward trajectory after 4–6 months, the IST regimen can be maintained until antibody disappears (immunologic remission). If no substantial reduction in antibody levels is seen after 4–6 months or GFR falls (>30% increase in serum creatinine on two determinations) and nephrotic-range proteinuria persists at >50% of baseline, changing to an alternate IST regimen would be justified (Figure 4).

The quantitative ELISA assay for PLA2R (Euroimmune AG, Luebeck, Germany), or the recently developed cell-based ALBIA assay (Mitogen Advanced Diagnostics Laboratory, Calgary, Canada) should be used for monitoring antibody levels (4–7). As we move more toward antibody-guided therapy, it is essential that measurements be standardized between commercial laboratories and that the threshold antibody level below which IST does not provide sufficient benefit to offset potential risks be defined. In the ELISA assay, levels >20 RU/ml are considered positive, and available data indicates a relationship between anti-PLA2R levels and clinical outcomes with patients in the lowest tertile frequently undergoing spontaneous remission and levels in the highest tertile associated with progression and rarely (<5%) with spontaneous remission (4,47,50,51).

**Table 5. Definitions of clinical responses in primary membranous nephropathy (4,56–58)**

<table>
<thead>
<tr>
<th>Clinical Response</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete remission</td>
<td>Proteinuria &lt;0.3 g/d</td>
</tr>
<tr>
<td>Partial remission</td>
<td>&gt;50% reduction from baseline and between 0.3 and 3.5 g/d</td>
</tr>
</tbody>
</table>
| No remission | With stable GFR
| Relapse | <50% reduction or >3.5 g/d |
| ESRD | Recurrence of >3.5 g/d after remission |

*Measurements of proteinuria for clinical decision-making should be done on 24-hr collections. Overnight or random samples can be used for monitoring.

**IST regimens in PMN**

Table 6 presents an overview of the IST regimens of established benefit in PMN. Current data do not permit an evidence-based choice between these IST protocols on the basis of differences in their efficacy in suppressing anti-PLA2R/THSD7A production. The three most utilized regimens (cyclophosphamide/steroids, calcineurin inhibitors [CNIs]/steroids, and rituximab) appear similar in their effect on anti-PLA2R levels (1,4,59–63). Small studies have shown that a combination of a CNI and rituximab was more effective in suppressing antibody than cyclophosphamide/steroids (63), that cyclophosphamide/steroids was more effective than mycophenolate mofetil (MMF) (64), and that tacrolimus (TAC) and cyclophosphamide/steroids were of similar efficacy (62). However, each of these studies is small and short-term. All three regimens lead to marked reductions in circulating antibody in most patients within 3–4 months, followed by disappearance of antibody within 6–9 months, and remission of proteinuria in 12–24 months in >80% of patients (4,63). Thus, IST prolonged for 6 months may not be necessary in all patients, whereas longer courses might be required in others.

Most current therapeutic guidelines, on the basis of older randomized controlled trials (RCTs), recommend initiating IST in patients with proteinuria resistant to supportive care after 6 months utilizing a modified Ponticelli regimen of 6 months of alternating pulse steroids and cyclophosphamide (56–60,65). This leads to remissions of proteinuria in about 50%–60% of patients at 1 year and 70%–80% at 2–3 years, versus about 30% of controls treated with supportive care only (2,56–60,65). Development of ESRD 10 years later is reduced from 30%–40% to 10% or less when all patients with PMN are treated at the time of diagnosis with alkylating agents/steroids (65). Proteinuric relapses, seen in about 25% of patients, are not predicted by any clinical parameter, but usually follow the return of anti-PLA2R/THSD7A antibody and are treated by repeating the same therapy that induced the initial remission (1,2,56–60). A course of cyclophosphamide should be repeated only once because cumulative doses >36 g are associated with an increased incidence of malignancy (55), although increased incidence ratios for malignancy have been reported in PMN at all levels of cumulative cyclophosphamide dose (66). Advantages of the Ponticelli regimen include the well established efficacy,
including reduction in ESRD, lower relapse rate (25%), and considerable experience with its use (4,59,60,62). Disadvantages include a relatively high adverse event rate (25%) that includes infection, need for close monitoring of hematologic parameters, infertility, and later malignancy (56,57,62,66).

CNIs (cyclosporin [CSA] or tacrolimus [TAC]), used either as monotherapy or combined with low-dose steroids, which is thought to improve response and reduce nephrotoxicity, have also been shown to decrease proteinuria, reduce the rate of loss of renal function, and decrease anti-PLA2R levels in PMN (1,2,4,56–60). In the United States, many clinicians prefer to initiate therapy with CNIs to avoid the more severe adverse events associated with alkylating agents and higher doses of steroids. Most studies show about equal efficacy between TAC and CSA (1,4,56–60). Advantages of CNIs include the lower incidence of infection and malignancy compared with cytotoxic drugs and the efficacy of monotherapy if steroids are not used (1,56,58). Disadvantages include long-term nephrotoxicity with consequent need to closely monitor drug levels, increased incidence of hypertension and diabetes, especially with TAC, and some recent concerns about whether CNIs are effective at all in preventing ESRD in the long term (67). The relapse rate with CNIs (40%–50%) is also higher than with cyclophosphamide (25%) but may diminish with longer periods of therapy (1,59,60), and some have advocated using low-dose CSA as maintenance therapy to reduce or prevent relapses. CNIs have been shown to not only reduce anti-PLA2R levels (62,63) but also to

<table>
<thead>
<tr>
<th>Table 6. Summary of the most common IST protocols for treating patients with primary membranous nephropathy (56–60,93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IST Regimen</td>
</tr>
<tr>
<td>-------------</td>
</tr>
<tr>
<td><strong>Cytotoxic drugs</strong></td>
</tr>
<tr>
<td>Modified Ponticelli</td>
</tr>
<tr>
<td>Dutch protocol</td>
</tr>
<tr>
<td><strong>CNIs</strong></td>
</tr>
<tr>
<td>Cyclosporin</td>
</tr>
<tr>
<td>Tacrolimus</td>
</tr>
<tr>
<td><strong>B cell depletion</strong></td>
</tr>
<tr>
<td>Rituximab</td>
</tr>
<tr>
<td><strong>ACTH</strong></td>
</tr>
<tr>
<td>Tetracosactrin (Synacthen) (synthetic)</td>
</tr>
<tr>
<td>Corticotropin (ACTHAR) (purified)</td>
</tr>
</tbody>
</table>

IST, immunosuppressive therapy; KDIGO, Kidney Disease Improving Global Outcomes; WBC, white blood cells; MP, methylprednisolone; CNI, calcineurin inhibitor; alt, alternate; RCT, randomized controlled trial; ACTH, adrenocorticotropic hormone; IM, intramuscular.
have a direct effect to stabilize the podocyte actin cytoskeleton, which reduces protein filtration (68). CNIs induce partial or complete remissions in up to 80% of cases of PMN within 12 months and could be employed if cyclophosphamide/steroid treatment fails, previous cumulative doses of cyclophosphamide approach 36 g, there is inability to tolerate cytotoxic drugs, or issues such as osteoporosis or preservation of fertility are present (1,56–60). However, CNIs have not yet been established by proper RCTs to reduce the long-term development of ESRD, although a strong case can be made that complete and partial remissions of proteinuria can serve as surrogate markers of failure to progress to ESRD in PMN (44). The limited data comparing cytotoxic drug therapy directly to CNIs suggests they have similar short-term clinical efficacy (1,44,56–60). However, in one recent RCT, the response to CNIs was not different from the response to SC alone (67). An excellent initial response rate to CNIs (80%) has been reported in some patients who failed the Ponticelli regimen and, conversely, some patients who have failed CNIs may respond to the Ponticelli regimen (59,60,69).

MMF monotherapy has not been established to be effective in PMN (64). In one study only 44% of patients receiving MMF were in remission at 23 months versus 75% in the cyclophosphamide group (63).

B cell depletion using the anti-CD19/20 monoclonal rituximab as monotherapy has recently emerged as a less toxic approach, with efficacy equivalent to cytotoxic drugs and CNIs but with a significantly lower adverse event rate (58,70–72). Rituximab was initially given weekly for 4 weeks as doses of 375 mg/m² intravenously (iv) or, more recently, as two iv doses of 1000 mg, or 375 mg/m², 15 days apart (71). Rituximab, 375 mg/m² as a single dose repeated only when B cell counts return (B cell–driven therapy), has also been established to reduce anti-PLA2R antibody levels and to induce remission of proteinuria in about 64% of patients in a mean of 7 months, but that figure increases over 3–4 years and the safety profile is better than that of cyclophosphamide or CNIs (1,70–72). Also, similar effects on proteinuria have been reported in PLA2R/THSD7A antibody–negative patients, suggesting that these patients too may have an antibody-mediated disease (71). Advantages of B cell–driven therapy include the freedom from steroids, low adverse event rate, ability to monitor B cell levels to assess efficacy and predict remission and relapse, and modest cost when only a single dose is given (71).

Disadvantages include the lack of confirmation that early reductions in proteinuria predict a lower incidence of ESRD (with the caveat mentioned above that remissions of proteinuria in PMN may be acceptable surrogate markers for ESRD in PMN [44]). The response rate closely parallels CD 19/20 B cell counts and anti-PLA2R levels, and seems similar in patients treated initially and those in whom rituximab was used later as rescue therapy (70–72). The relapse rate in rituximab responders is about 30%, associated with return of circulating B cells and anti-PLA2R antibody, and relapse can sometimes be associated with development of anti-rituximab antibodies (72). Most patients who relapse respond to another dose (70–72). A recent RCT (Evaluate Rituximab Treatment for Idiopathic Membranous Nephropathy Study) comparing supportive care alone to supportive care plus rituximab, 375 mg/m² iv on days 0 and 8 in patients with persistent nephrotic syndrome after 6 months of supportive care, demonstrated much greater efficacy of rituximab in reducing anti-PLA2R levels (56% versus 4% at 3 months, P<0.05) and more complete and partial remissions of proteinuria at a mean of 17 months in the rituximab group (65% versus 34%, P<0.01), with short-term adverse event rates similar to those in the group receiving SC alone (72). Analysis of the cost of therapy over the course of the disease using a Markov decision analysis model suggests it is lower than the cost of cyclophosphamide/steroids (73). Thus, experience to date supports considering rituximab monotherapy as a third option for induction therapy, as well as a popular choice for maintenance or rescue therapy (71).

Adrenocorticotropic hormone monotherapy, usually given as 1 mg twice a week for a year (Table 6), has also been shown in one small randomized controlled study to reduce anti-PLA2R levels and produce results (>80% remission at 6 months) equivalent to cyclophosphamide/steroids, with minimal adverse events (Table 6) (74–75). However, another study comparing the two using historical controls favored cyclophosphamide (75). The cost of adrenocorticotropic hormone is very high, and its place in the therapeutic armamentarium for PMN remains to be established (76).

Another treatment option is to utilize combinations of drugs in lower doses (multidrug therapy), usually rituximab plus a cytotoxic drug or CNI to retain efficacy and reduce adverse events, especially those due to steroids. For example, a recent observational study using rituximab with low-dose cyclophosphamide and an accelerated taper of steroids reported a 100% remission rate over a mean follow-up of 37 months (77). Another, using a combination of rituximab and CSA, achieved remissions in 92% and antibody depletion in 100% in 9 months (78), and a combination of rituximab and plasma exchange showed promise in a third small study (79). These studies all require confirmation, and RCTs comparing these approaches to conventional IST reviewed above are necessary before multidrug therapy can be recommended as an established approach to initial therapy in PMN.

Transplantation in PMN

Renal transplantation is effective in the 10%–20% of patients who do reach ESRD from PMN (1,4,75–78). In anti-PLA2R–positive patients, subepithelial deposits can appear in the allograft within 6 days (70,80,81). As deposits increase, clinical recurrence (proteinuria) is seen within 13–15 months in about 40%–50% of allografts and can diminish allograft survival (70,80–85). The recurrence rate of subepithelial deposits in patients positive for anti-PLA2R antibodies at the time of transplantation may approach 90% (70,80,81). Delaying transplantation until anti-PLA2R/THSD7A is no longer detectable seems advisable unless there are clinical reasons for greater urgency. Anti-PLA2R–negative de novo MN is also a common cause of transplant nephrotic syndrome, affecting about 2% of all renal transplant recipients whose original disease was not MN, and is about equal in frequency to recurrent MN in patients with PMN (82–85). In these patients, IgG1 deposits often predominate, anti-PLA2R/THSD7A is negative, and the mechanism(s) involved are not yet known, although the
Gaps and Shortfalls in Current Therapy

Despite the numerous translational observations that have already been made (Table 3), many questions remain unanswered (86). Some of the more clinically relevant ones include: What is the best way to measure anti-PLA2R, and, in the future, anti-THSD7A, antibody? How do antibody levels translate into risk of progression and are there levels below which IST is not worthwhile? If therapy is antibody-directed, will this achieve better clinical outcomes or fewer adverse events in patients with active disease compared with current restrictive therapy regimens? Is it important for prognosis or treatment to know the pathogenic epitopes and molecular configurations of the PLA2R peptides against which antibodies in individual patients are directed? Does a biopsy contribute to improved outcomes in typical anti-PLA2R/THSD7A-positive MN with nephrotic syndrome? If a patient has apparent secondary MN with another systemic disease, but also has elevated anti-PLA2R/THSD7A antibody or positive glomerular staining, should such a patient be treated for PMN? If a biopsy is done in antibody-negative patients, is positive glomerular staining for IgG4 and PLA2R/THSD7A sufficient to establish anti-PLA2R/THSD7A-related MN and exclude secondary causes?

Can multidrug therapy protocols combining currently available drugs reduce adverse events without sacrificing efficacy as has been done in lupus nephritis? Can outcomes be further improved if complement inhibitors are added to current IST protocols, especially during the interval of active disease between initiation of IST and disappearance of the antibody? Can routine maintenance therapy reduce relapses and the need for retreatment?

New Therapies on the Horizon

Current knowledge of the roles of autoantibody IgG and complement in the pathogenesis of MN makes better B cell–depleting agents and complement inhibitors of particular interest. New therapeutic approaches to suppress antibody production or interfere with antibody-induced podocyte injury include improved B cell–depleting agents, B cell depletion targeted specifically to anti-PLA2R reactive cells, and suppressors of B cell activation. A recent pilot study of belimumab, an inhibitor of B cell activation, in 11 anti-PLA2R-positive patients reported a 90% reduction in anti-PLA2R levels and a (delayed) 70% reduction in proteinuria in patients receiving monthly iv doses of the drug over a period of 28 weeks (87). Other approaches under development include antibody traps or decoys and efforts to directly protect the podocyte itself from consequences of immune injury such as endoplasmic reticulum stress, autophagy, and oxidant injury (88). Pending identification of PLA2R peptides that neutralize antibody, peptide-blocking agents will likely also be developed. Although one trial of the C5 inhibitor eculizumab was negative in MN, adequate complement -inhibiting doses were not used, and other trials with newer complement inhibitors, including oral agents, recombinant complement regulatory proteins, small molecules, new monoclonal antibodies, small interfering RNA agents, and approaches that upregulate natural complement inhibitors, are in progress or under development (31).


Published online ahead of print. Publication date available at www.cjasn.org.
Correction

Due to author error, two doses were incorrectly reported in Table 6 of the above referenced article. The doses in the text are correct. The correct doses for Table 6 are:

1. The dose of Rituximab is 375 mg/M².
2. The dose of Prednisone with Cyclosporin is 5–10 mg.

Published online ahead of print. Publication date available at www.cjasn.org.

<table>
<thead>
<tr>
<th>Table 6. Summary of the most common IST protocols for treating patients with primary membranous nephropathy (56–60,93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IST Regimen</td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td><strong>Cytotoxic drugs</strong></td>
</tr>
<tr>
<td>Modified Ponticelli</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Dutch protocol</td>
</tr>
<tr>
<td><strong>CNIs</strong></td>
</tr>
<tr>
<td>Cyclosporin</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Tacrolimus</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>B cell depletion</strong></td>
</tr>
<tr>
<td>Rituximab</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>ACTH</td>
</tr>
<tr>
<td>Tetracosactrin (Synacthen) (synthetic)</td>
</tr>
<tr>
<td>Coricortropin (ACTHAR) (purified)</td>
</tr>
</tbody>
</table>

IST, immunosuppressive therapy; KDIGO, Kidney Disease Improving Global Outcomes; WBC, white blood cells; MP, methylprednisolone; CNI, calcineurin inhibitor; alt, alternate; RCT, randomized controlled trial; ACTH, adrenocorticotrophic hormone; IM, intramuscular.