Primary membranous nephropathy: comprehensive review and historical perspective

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ABSTRACT
Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in non-diabetic Caucasian adults over 40 years of age. It has an estimated incidence of 8–10 cases per 1 million. Fifty per cent of patients diagnosed with primary MN continue to have nephrotic syndrome and 30% of patients may progress to end-stage renal disease over 10 years. Although it was recognised as a distinct clinical-pathological entity in 1940s by immunofluorescence and electron microscopy, the pathogenesis and treatment have become more apparent only in the last decade. Discovery of M-type phospholipase A2 receptor (PLA2R) antibodies and thrombospondin type 1 domain-containing 7A antibodies has given new perspectives in understanding the pathogenesis of the disease process. Anti-PLA2R antibody is the first serologic marker that has promising evidence to be used as a tool to prognosticate the course of the disease. More importantly, therapeutic agents such as rituximab and adrenocorticotropic hormone analogues are the newer therapeutic options that should be considered in the therapy of primary MN.

HISTORY
The term membranous glomerulonephritis was proposed by Bell1 and was identified as a distinct pathologic entity in 1957 by Jones2 based on the unique glomerular basement membrane (GBM) structural changes and capillary wall thickening with periodic acid-Schiff-silver methenamine stain. These characteristic histological changes were later recognised to be due to immune complex deposits forming between the podocytes and subepithelial aspect of GBM. These deposits interfere with normal podocyte barrier function and result clinically in the nephrotic syndrome. The term membranous glomerulonephritis has been largely replaced by membranous nephropathy (MN) due to the lack of significant glomerular inflammation.

In 75%–80% of the patients, MN occurs in the absence of identifiable causes and is therefore called primary MN.3

Approximately 20%–25% of MN occurs secondarily due to a variety of other illnesses: malignancy (lung, kidney, stomach, colon), systemic lupus erythematosus, drug reactions (non-steroidal anti-inflammatory drugs, gold, penicillamine) and infection (hepatitis B, C). Immunohistology and disease course can differ considerably between primary and secondary MN.4 5

AUTOIMMUNE PATHOGENESIS
Heymann antigen
The first animal model of MN was developed by Heymann. In this model, rats developed nephrosis when immunised with crude kidney extract plus Freund’s adjuvant.6 The autoantigen in rats was later identified to be the brush-border protein called megalin. This low-density lipoprotein receptor-related protein is present in podocyte foot processes and proximal convoluted tubules. Nephrotic syndrome was secondary to the damage of podocyte foot processes due to complement fixing antibodies, which led to the loss of slit diaphragm function of podocytes. Podocyte expression of megalin seems to be unique to the rat, and it was shown not to be the antigen in human MN.

Neutral endopeptidase
Debiec et al, in 2002, described human MN in association with immunity against neutral endopeptidase (NEP). In this scenario, MN was identified in fetuses born to mothers congenitally deficient in NEP. Maternal antibodies against NEP traversed the placenta to cause MN in the fetus at birth.7 Renal biopsy showed an unusually severe form of MN where glomerular capillary tufts were collapsed in majority of glomeruli. Immunofluorescence microscopy showed epithelial deposits of IgG and C3. Electron microscopy revealed subepithelial deposits like those found in adult form of MN.

M-type phospholipase A2 receptor 1 antibody
In 2009, Beck et al identified the M-type phospholipase A2 receptor 1 (PLA2R1) as the target antigen in 70% of cases of human MN.3 This was a major breakthrough that enhanced our understanding regarding primary MN. PLA2R1 is a 185 kDa glycoprotein which belongs to the mannose receptor protein family but its function in podocytes is currently unknown.8 Receptors belonging to this family undergo endocytic recycling thereby providing constant source of surface-accessible antigen.9 10 Antibodies against PLA2R1 predominantly belong to IgG4 isotype and the presence of other subclasses of IgG on biopsy makes secondary MN more likely.11 IgG4 subtype appears to have limited ability to activate complement and therefore the presence of C3 within immune deposits remained unexplained. It may represent the activation of complement pathways other than the classical pathway.12 13

Between 70% and 80% of patients presumed to have primary MN have PLA2R1 antibodies. A small
percentage of patients with secondary MN also have positive anti-PLA2R1 antibodies. More importantly, PLA2R antibodies have also been reported to be of prognostic significance. Patients with positive PLA2R antibody titres at the time of biopsy have a lower rate of complete remission. Decreasing antibody titres suggest immunological remission which is generally followed by remission of proteinuria. Beck and Salant also observed that titres of PLA2R antibodies become undetectable prior to complete remission of proteinuria. Immunosuppressive therapies have been shown to decrease the titres of anti-PLA2R1. An excellent review by De Vriese et al summarises the utility of PLA2R antibodies and explains how its use can complement the existing management that is based only on proteinuria.

Thrombospondin type 1 domain-containing 7A antibody
More recently, Tomas et al discovered anti-thrombospondin type 1 domain-containing 7A (THSD7A) antibodies. These were noted to be positive in 3%–5% of patients with primary MN who were anti-PLA2R negative. THSD7A is 250 kDa glycoprotein expressed by podocytes. Like PLA2R, THSD7A is a transmembrane glycoprotein that triggers IgG4-predominant antibody response.

Other implicated antigens
Other proteins postulated to serve as autoantigens in MN include superoxide dismutase, aldose reductase, cationic bovine serum albumin and alpha-enolase in cases of childhood MN. However, these cases are rare.

EPIDEMIOLOGY
MN may occur in both sexes and all ethnic groups. Primary MN, however, is more common in white males after 40 years. MN is the most common cause of nephrotic syndrome in non-diabetic Caucasian adults with estimated incidence of 8–10 cases per 1 million. A genome-wide association study done by a European consortium revealed associations with single nucleotide polymorphisms in HLA-DQA1, a member of HLA class II in Caucasians, which may predispose to autoimmunity. In Chinese population, a risk locus was found within the nearby DR-B locus.

CLINICAL PRESENTATION
Primary MN has extremely heterogeneous presentation. In a case series of 116 patients with biopsy-proven primary MN, 76% of the patients presented with nephrotic range (>3 g/24 hours) proteinuria and the rest had subnephrotic proteinuria. Fifty-five per cent had microscopic haematuria. Seven patients had moderate renal failure and all of them were noted to be more than 56 years old.

Natural history and clinical course of primary MN
Clinical course of primary MN is characterised by spontaneous remissions and relapses. About 20% undergo spontaneous complete remission and another 20% undergo spontaneous partial remission. Fifteen to thirty per cent have relapses. About 50% continue to have nephrotic syndrome and 30% of them progress to end-stage renal disease (ESRD). Proteinuria, initial serum creatinine and rate of change of creatinine were most important in predicting progression to chronic kidney disease than initial proteinuria alone. Female gender and non-nephrotic proteinuria portend a better prognosis in terms of remission of proteinuria. Japanese ancestry tends to have a milder disease and a better prognosis.

Pathology
Although it is considered a standard method to study renal tissue with immunofluorescence and electron microscopy in the current times, these techniques were not developed until after 1950s. Dr Albert Coons pioneered the immunofluorescence applications for tissue processing that we use today. His publication from 1950s laid the foundations for modern immunohistology. Later came electron microscopy for tissue diagnosis. The first commercially available electron microscope was made available by Siemens in 1939. It was first applied to study glomerular pathology in 1957 by Farquhar et al who described fenestrated endothelium, GBM and podocytes in patients with nephrotic syndrome. It must be noted that only after the discovery of immunohistochemistry and electron microscopy, primary MN was identified as a distinct clinicopathological entity.

Light microscopy
Early MN shows no pathologic changes on light microscopy. Later stages are characterised by homogeneous thickening of capillary walls as seen on periodic acid-Schiff staining. Methylene silver staining of basement membrane reveals an increase in basement membrane deposition between the immune deposits giving rise to a characteristic ‘spike and hole’ configuration (figure 1). Later, lucencies may develop in GBMs as the immune deposits are resorbed. MN is characterised by a lack of GBM disruption and inflammation.

Immunohistology
Granular staining for IgG is easily recognised on immunohistochemistry. These IgG deposits are subepithelial in location and are present on outer surface of glomerular capillary wall. IgG4 is the predominant subclass. The predominance of other subclasses or a ‘full house’ pattern with additional IgA, IgM and C1q staining suggests secondary MN. Although IgG4 subclass is unable to fix complement, C3 staining is positive in active disease suggesting the activation of complement pathway other than classical pathway. Positive staining for C4d with negative C1q staining is also a feature of primary MN and is due to activation of the mannose-binding lectin pathway of complement.

Figure 1 Hole and spike formation within basement membranes seen on silver staining on light microscopy.
Electron microscopy

Electron microscopy shows electron-dense deposits in GBM in the subepithelial regions (figure 3). During the initial stages, podocyte effacement is noted with minimal to no changes in the GBM (stage 1). If the deposits persist, new basement membrane material is laid between these immune deposits giving rise to the spike formations identified on methenamine silver stains which are readily observed on electron microscopy (stage 2) (figure 3). In stage 3, these deposits are completely encircled by newly laid basement membrane. In more advanced stages, basement membranes are thickened, and the deposits become more lucent and the spikes become less apparent.

DIAGNOSIS/LAB TESTING

Diagnostic evaluation should include tests usually done to evaluate patients with nephrotic syndrome including:

► Antinuclear antibodies, complement C3, C4. These are normal in primary MN. Low complement levels are suggestive of other glomerulopathies.
► Hepatitis B and C serologies. Positive serology might suggest secondary MN.
► Age appropriate cancer screening as the malignancies are known triggers for secondary MN.
► Select patients with rapid worsening of renal function may need renal vein Dopplers, CT with contrast or MR angiography scans to look for renal vein thrombosis.
► Renal biopsy with light, immune fluorescence and electron microscopy.
► PLA2R1 antibody.

MANAGEMENT

The first step in managing MN is differentiating between primary and secondary MN as the therapy in secondary MN is directed at the underlying cause. This is followed by supportive therapy and targeted therapy with immunosuppression if the MN is primary.

Since spontaneous remission occurs in up to 30% of patients, it is not unreasonable to delay immunosuppression. However, up to 30% of patients with persistent nephrotic syndrome progress to ESRD over 10 years and need more aggressive therapy. There is evidence that treatment-induced remission is associated with improved prognosis.

In 1992, Pei et al proposed that the magnitude and duration of proteinuria be an important prognostication factor in primary MN. Our current strategy of risk stratification is based on Toronto Risk Score model proposed by Cattran et al, which has been validated in several studies. However, it must be noted that proteinuria is now known to be a late marker of disease activity. It is likely to be replaced by anti-PLA2R activity.

The Kidney Disease Improving Global Outcomes (KDIGO) summary of current opinion regarding treatment guidelines is summarised below.

Immunosuppression should be started in patients with nephrotic syndrome and one of the following:

► Proteinuria exceeding 4 g/day and remains over 50% of baseline value while on 6 months of conservative therapy involving antihypertensive and antiproteinuric agents.
► Presence of severe or life-threatening symptoms of nephrotic syndrome.
Figure 3  Subepithelial electron-dense deposits seen on electron microscopy.

- Serum creatinine has risen by 30% or more within 6–12 months from the time of diagnosis. Immunosuppression is not recommended in patients with serum creatinine >3.5 mg/dL, estimated glomerular filtration rate (eGFR) less than 30 mL/min/1.73 m².

**SUPPORTIVE THERAPY**

**Control of proteinuria**
Reduction of proteinuria in MN to less <1000 mg/day is often not possible. Partial remission of proteinuria defined as proteinuria of <3.5 mg/day and 50% reduction from peak value was independently associated with slower decline in renal function.42

**Renin-angiotensin blockade**
There is at least some evidence supporting the use of ACE inhibitor (ACEi)/angiotensin receptor II blocker (ARB) in primary MN. In a study by Polanco et al, spontaneous remission was more frequent with lower baseline proteinuria. Spontaneous remission was 37%, 26% and 21% with baseline proteinuria of <8 g/24 hours, 8–12 g/24 hours and >12 g/24 hours, respectively. In this study, 80% of patients in spontaneous remission group were on ACEi/ARB compared with the group that did not attain spontaneous remission, in which only 60% were on ACE/ARB.46

**Management of hypertension**
Hypertension management in MN is like that of other proteinuric diseases. Ideal goals for blood pressure (BP) controls are not established but are supported by data from the MDRD (modification of diet in renal disease) trial in which patients with proteinuria with lower BPs progressed more slowly than patients with higher BPs. KDIGO guidelines recommend 130/80 mm Hg for the treatment goal. Data to support a lower BP target do not exist.

**Controlling hyperlipidaemia**
Hyperlipidaemia is a part of nephrotic syndrome and the mainstay of treatment is statins. Hyperlipidaemia is managed in the context of elevated cardiovascular risk factors due to nephrotic syndrome.

**Anticoagulation**
Primary MN is a hypercoagulable state due to the hyperlipidaemia resulting from nephrotic syndrome. In a study conducted by Lionaki et al, clinically significant venous thromboembolic events occurred in about 7% of patients with MN and hypoalbuminaemia. Serum albumin <2.8 g/dL was the most significant independent predictor of venous thrombotic risk. Due to lack of randomised controlled trials (RCT), optimal approach for anticoagulation is unknown. Some experts prophylactically anticoagulate all patients with severe nephrotic syndrome and others look for an additional factor such as low ejection fraction, atrial fibrillation, prior thrombotic episode and thrombophilia to initiate anticoagulation. Duration of anticoagulation is also a topic of debate.

KDIGO guidelines recommend prophylactic anticoagulation with warfarin if serum albumin is below 2.5 g/dL.

**HISTORICAL PERSPECTIVE OF IMMUNOSUPPRESSION IN PRIMARY MN**
With its incidence rate of 8–10 per million, its waxing and waning clinical course and often indolent rate of progression,
understanding the disease and formulating an effective immunosuppressive regimen has always been a challenge. This was further compounded by the lack of histological and serological advancements that are available today.

**IMMUNOSUPPRESSIVE THERAPY**

Many studies remote and recent have been conducted with steroids, alkylating agents, calcineurin inhibitors (CNI), mycophenolate, rituximab and adrenocorticotropic hormone (ACTH) analogues. Key studies are briefly summarised as follows:

**Glucocorticoids**

In 1979, Coggins conducted the first RCT to evaluate the utility of corticosteroids in primary MN.\(^48\) Seventy-two patients with biopsy-proven primary MN were enrolled in this study. The treatment group received prednisone ranging from 100 to 150 mg every other day. Twenty-two patients in treatment group were in complete or partial remission compared with 11 patients in control group. At 36 months, eight treated patients and three controlled patients were in partial remission. A more significant finding was that the rate of decline in eGFR was 2%/year in treatment group compared with 10% in control group. A 1989 RCT by Kobayashi et al involving 18 patients\(^49\) similarly showed results favouring the use of prednisone.

On the other hand, a Canadian trial by Cattran et al in 1989 reported contradictory findings.\(^50\) In this study, 158 patients were randomised to receive alternate day prednisone (45 mg/\(m^2\) body surface area) or placebo. Outcomes with respect to complete remission of proteinuria, decline in renal function, were not significantly different. These results were supported by another randomised trial conducted by Cameron et al in 1990.\(^51\)

By the early 1990s there was no unified consensus for or against the use of corticosteroids in MN. A meta-analysis by Hogan et al in 1995,\(^52\) which included four controlled trials using steroids and three studies using alkylating agents, concluded that patients treated with alkylating agents had higher chance to enter remission compared with corticosteroids alone.

Therefore, the use of glucocorticoids as a single therapeutic agent for the treatment of primary MN fell out of practice.

**Alkylating agents**

An Italian study in 1984 by Ponticelli et al is one of the important studies that steered nephrologists towards alkylating therapy in primary MN.\(^53\) Sixty-seven patients with biopsy-proven primary MN and nephrotic range proteinuria were treated with either methylprednisolone or a combination of chlorambucil and methylprednisolone. Chlorambucil was alternated with methylprednisolone every month for 6 months such that the subjects in the study group received methylprednisolone during months 1, 3 and 5 and chlorambucil during months 2, 4 and 6. After 24 months of follow-up, 72% in the combination group were in complete or partial remission compared with 30% in the control group. More importantly, the plot of eGFR versus time remained stable in the treatment group but declined in the control group over a period of 2 years.

In 1993, a decade after the original study, Ponticelli et al reanalysed the original data and the ensuing 10-year follow-up data\(^54\) to assess the long-term change in renal function and adverse effects of chlorambucil. They found that 83% of patients who were treated with chlorambucil were in complete or partial remission compared with only 38% of the controls. Chlorambucil treatment effectively doubled chances for remission. The 10-year survival probability with functioning kidney was 92% after chlorambucil treatment and patients spent 58% of the time nephrotic. In contrast, patients treated only with corticosteroids had but a 60% chance of remaining free from kidney failure and were nephrotic 80% of the time. Ponticelli et al concluded that use of chlorambucil, alternating with steroids for a total duration of 6 months, improves remission rates, and decreases risk of dialysis or death within 10 years in about one-third of patients treated.

Given the greater familiarity of nephrologists with cyclophosphamide, Ponticelli et al designed a study to compare cyclophosphamide with chlorambucil which was published in 1998. Eighty-seven patients were randomised and followed for 1 year. Forty-four patients received methylprednisolone and chlorambucil. Forty-three received methylprednisolone and cyclophosphamide. Methylprednisolone was dosed as 1 g intravenously for 3 days followed by 0.4 mg/kg/day for 27 days. Chlorambucil was dosed at 0.2 mg/kg/day and cyclophosphamide at 2.5 mg/kg/day. Corticosteroids were alternated with alkylating agents monthly such that each subject received 3 months of steroid alternating with either of the alkylating agents. In this study, the remission rates, slope of eGFR, relapse of nephrotic syndrome was not significantly different between the two groups. The conclusions from this study are that cyclophosphamide and chlorambucil are equally efficacious but the infection profile of cyclophosphamide might be slightly better than chlorambucil, although the study was not powered to say that with confidence.

Cyclophosphamide therapy is associated with significant cancer risk (bladder and haematologic). A single course of cyclophosphamide, in a protocol similar to that outlined above in a 100 kg man, results in a cumulative dose of 22.5 g. According to one study, a cumulative dose of 20–49 g of cyclophosphamide is associated with sixfold risk of bladder cancer\(^55\) and a dose more than 50 g increases the bladder cancer risk by 14-fold.

Cyclophosphamide is also known to cause gonadal dysfunction in both women and men. Premature ovarian failure is estimated to occur in 12% of women younger than 26 years, 27% of those aged 26–30 years and 60% of those older than 30, indicating that older women are more susceptible to gonadal toxicities.\(^56\) Cyclophosphamide is also known to induce Leydig cell failure leading to oligozoospermia and azoospermia in both prepubertal and adult males.\(^57,58\)

**Calcineurin inhibitors**

**Cyclosporine**

A controlled Canadian study by Cattran et al was published in 1995.\(^59\) In this study, 64 patients with biopsy-proven MN were placed on conservative therapy including low-salt diet and antihypertensives. This was designated as part 1 of the study. Among these patients, individuals with rapid decline in creatinine clearance of 8 mL/min and persistent nephrotic range proteinuria were eligible for part 2 of the study. In due course of time, 23 patients among the 64 became eligible for part 2. Out of these 23 subjects, 17 were randomised to receive cyclosporine and the rest received placebo. Treatment and control groups were followed for 12 months. At the end of the study period, slope of decline in eGFR in treatment group improved compared with placebo group. Also, proteinuria decreased 30% in four of eight patients in treatment group compared with none of eight patients in control group. This study is the basis for use of cyclosporine as treatment for primary MN.

In 2006, a Greek study by Alexopoulos et al\(^60\) compared cyclosporine monotherapy with cyclosporine and corticosteroid combination. The dose of cyclosporine was 2 mg/kg/day.
Prednisone was started at 0.6 mg/kg/day and tapered to 10 mg/day by 6 months. The first part of this study lasted for 12 months in which 51 subjects were treated with either cyclosporine alone or a combination of cyclosporine and prednisone. After 12 months, patients with remission were placed either on low-dose cyclosporine or a combination of cyclosporine and steroid (cyclosporine: 1 mg/kg/day, prednisone: 0.6 mg/kg/day). The second part of the study was followed for 26 months. This study noted that long-term relapses were more common in the monotherapy group. This study concluded by saying that combination therapy with cyclosporine and steroid is more effective than cyclosporine alone.

These studies showed that the combination of cyclosporine with steroids was superior to steroid alone.

Tacrolimus

Another 2007 study by Praga et al looked at tacrolimus monotherapy in primary MN. This study involved only 25 patients in whom tacrolimus was studied in biopsy-proven primary MN. Remission of nephrotic syndrome occurred in 82% of patients in tacrolimus group at the end of 1 year compared with 24% in the control group. About 50% of patients relapsed 18 months after tacrolimus was stopped. Six patients in the control group compared with one patient in the tacrolimus group reached the secondary endpoint of a 50% rise in serum creatinine above baseline.

Evidence thus supports the use of tacrolimus to induce remission in MN; but no data are available on long-term effects of this agent.

CNIs versus alkylating agents

In 2010, a Chinese multicentre RCT compared tacrolimus and cyclophosphamide in primary MN. In this study, 73 subjects with primary MN were recruited. Thirty-nine received tacrolimus and prednisone and 34 received cyclophosphamide and prednisone. Tacrolimus was started at 0.1 mg/kg/day and adjusted to a 12-hour trough level of 5–10 ng/mL for 6 months and reduced to 2–5 ng/mL for the next 3 months. Cyclophosphamide was dosed at 100 mg/day for 4 months (cumulative dose of 12 g). Both these groups received prednisone 1 mg/kg/day for 4 weeks and tapered gradually to zero over the next 7 months. Although the remission rate in tacrolimus group was higher at the end of 6 months (85% vs 65%), remission rates were not significantly different between these groups at the end of 12 months (75%). However, long-term remission or relapse rates were not studied.

Therefore, CNIs can be used as an alternative to alkylating agents if the latter therapy is poorly tolerated.

Mycophenolate mofetil

Mycophenolate mofetil (MMF) is an inhibitor of inosine-monophosphate dehydrogenase, which plays a key role in guanosine nucleotide synthesis. It is an attractive immunosuppressive agent due to its lack of nephrotoxicity. There were no data available on this agent in MN until 2008 when a study was reported by Dussol et al. In this RCT, MMF monotherapy was compared with conservative therapy. Thirty-six patients were enrolled and followed for 1 year. Nineteen patients received MMF and 17 were in a control group who received supportive therapy only with antihypertensive and diuretics. At the end of study period, there was no significant difference between treatment and control groups in terms of remission of nephrotic syndrome or decline in renal function.

Lack of efficacy with MMF was also found by Branten et al. In this clinical trial with historic controls, MMF and steroids were compared with cyclophosphamide with steroids. MMF was dosed at 1 g twice daily and cyclophosphamide at 1.5 mg/kg/day for 12 months. Both groups received corticosteroids and the median follow-up was for 23 months. Remission of proteinuria was 66% in MMF group compared with 72% in cyclophosphamide group. Sixteen per cent of patients in the MMF group versus none in cyclophosphamide group had six in partial remission at the end of 1 year. This amounts to 57% remission rate which is an improvement over the natural history of the disease where spontaneous remissions occur only in up to 40% of the subjects. Remission rates were also comparable or even slightly better than most of the aforementioned agents with no evidence of nephrotoxicity. This study also noted that the B cells started to recover 3 months after the last dose of rituximab and that there was no correlation between proteinuria and the recovery of B cell population.

Rituximab

Research on monoclonal antibodies by the National Cancer Institute began in year 1975. CD20, a B cell antigen present only on mature B cells, was discovered in 1988. Anti-CD20 antibody was developed with the idea of targeting cancerous and mature B cells which express CD20. The goal was to eliminate mature lymphoma cells and spare precursor B cells to replenish the B cell population after the therapy is stopped. Rituximab, the anti-CD20 monoclonal antibody, was approved by the Food and Drug Administration (FDA) in 1998, for treatment of non-Hodgkin’s lymphoma. Around the same time, experiments on murine models suggested the involvement of B cells in the pathogenesis of primary MN. Rituximab, an anti-CD20 monoclonal antibody, therefore became a drug of research interest in the treatment of primary MN.

In 2002, Remuzzi et al conducted a pilot study in eight patients with proteinuria >8 g/day with primary MN. Patients were treated with 375 mg/m² weekly rituximab infusions for 4 weeks. At the end of 20 weeks, proteinuria decreased by slightly more than 50% from baseline. Improvement in the serum albumin was noted in 31%. The short-term risk-benefit profile of rituximab was felt to be better than other therapeutic agents. This trial laid the foundation for the use of rituximab in primary MN.

In 2008, Fervenza et al conducted a 1-year open-label prospective follow-up study on rituximab in primary MN. Fifteen patients with MN and nephrotic range proteinuria were administered rituximab 1 g, 2 weeks apart. Patients who remained nephrotic at the end of 6 months received an extra course of rituximab. Out of 14 patients who were followed, two were in complete remission and six in partial remission at the end of 1 year. This amounts to 57% remission rate which is an improvement over the natural history of the disease where spontaneous remissions occur only in up to 40% of the subjects. Remission rates were also comparable or even slightly better than most of the aforementioned agents with no evidence of nephrotoxicity. This study also noted that the B cells started to recover 3 months after the last dose of rituximab and that there was no correlation between proteinuria and the recovery of B cell population.

Another prospective 2-year study was done by Fervenza et al to further determine optimal drug dosing. In this study, 20 patients were enrolled. They were given rituximab 375 mg/m² 1 week apart for four doses. This regimen was repeated at 6 months irrespective of response. Four and 12 patients had complete and partial remission, respectively, putting remission rate at 80%. Although this regimen was more effective in suppressing B cell population, the effective decrease in proteinuria did not reach statistical significance compared with the prior study.

The Membranous Nephropathy Trial of Rituximab study was designed to test rituximab in comparison to cyclosporine in treatment of MN. Patients randomised had proteinuria of at least
5 g/day after conservative treatment for 3 months. Participants received rituximab 1 g intravenous infusion 2 weeks apart versus cyclosporine 3.5–5 mg/kg/day for 6 months. This study is now complete. Preliminary results were presented at American Society of Nephrology 2017 meeting, demonstrating that rituximab was non-inferior to cyclosporine.

**Ongoing trials with rituximab**  
The Sequential Treatment with Tacrolimus and Rituximab versus Cyclophosphamide and Corticosteroids study is currently recruiting subjects. Remission rates, relapse rates and renal function will be studied after a follow-up of 2 years.69

A head-to-head comparison between rituximab and Ponticelli regimen is another study currently in the process (NCT03018535).

**Adrenocorticotropic hormone**  
ACTH was used prior to 1950s for childhood nephrotic syndromes. Use of this compound has since been replaced by synthetic steroids. Experimental studies suggest that ACTH has anti-inflammatory, antiproteinuric effects that cannot be purely explained by its steroidogenic effects.70

In a 2006 RCT, Ponticelli et al71 compared tetracoside (synthetic ACTH analogue) with cytotoxic-steroid combination therapy. Group A received methylprednisolone (1 g intravenous for 3 days followed by 0.4 mg/kg/day orally for 27 days) followed by either chlorambucil (0.2 mg/kg/day) or cyclophosphamide (2.5 mg/kg/day). Steroids were alternated with cytotoxic drugs every other month like in the original Ponticelli regimen. Group B received tetracoside 1 mg every other week in the beginning and then increased to 1 mg twice a week. At 24 months, 75% of the subjects in group A were in remission compared with 87% in group B with no statistical difference.

Tetracoside is currently not available in USA. Acthar Gel is the only FDA-approved therapy for nephrotic syndrome. Use of this compound has since been replaced by synthetic steroids. Experimental studies suggest that ACTH has anti-inflammatory, antiproteinuric effects that cannot be purely explained by its steroidogenic effects.70 In a 2006 RCT, Ponticelli et al71 compared tetracoside (synthetic ACTH analogue) with cytotoxic-steroid combination therapy. Group A received methylprednisolone (1 g intravenous for 3 days followed by 0.4 mg/kg/day orally for 27 days) followed by either chlorambucil (0.2 mg/kg/day) or cyclophosphamide (2.5 mg/kg/day). Steroids were alternated with cytotoxic drugs every other month like in the original Ponticelli regimen. Group B received tetracoside 1 mg every other week in the beginning and then increased to 1 mg twice a week. At 24 months, 75% of the subjects in group A were in remission compared with 87% in group B with no statistical difference.

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Therefore, Acthar Gel is an acceptable alternative if alkylating agents or rituximab cannot be used. Cost remains an important prohibitive factor and more RCTs designed for longer duration are needed to study its efficacy in long term.

**Ongoing research**  
Primary MN remains an active field for pharmaceutical research. Many other agents like another novel CD20 antibody, proteasome inhibitors and B lymphocyte stimulator protein inhibitors are currently being studied.

**Contributors** KCK is the primary author. KCK, TC and VK performed literature review and drafted the manuscript. SB and LB edited and revised the final draft to completion.

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

**Patient consent for publication** Not required.

**Provenance and peer review** Not commissioned; externally peer reviewed.

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**Main Messages**

- In summary, cyclophosphamide, CNIs, rituximab and Acthar Gel are all appropriate therapies with different adverse effect profiles that can be used at present times.
- However, the new paradigm is that, clinicians must strongly consider a serology-based approach using PLA2R antibody monitoring to complement the more traditional proteinuria-based approach for better outcomes.

**Current research questions**

- Head-to-head randomised controlled trial to compare the efficacy of rituximab versus cyclophosphamide regimen.
- Prospective open-label trials to study the efficacy of rituximab in secondary membranous nephropathy.
- Long-term prospective follow-up to study remission rates and relapse rates with the use of adrenocorticotropic hormone therapy in patients who are unable to use other immunosuppressive agents.

**Key references**


**Self assessment questions**

- **Question 1.** Primary membranous nephropathy is the most common cause of nephrotic syndromes in young African-American females of age 20–40 years.
- **Question 2:** PLA2R antibody is present in all patients with primary membranous nephropathy and is always diagnostic of the disease.
- **Question 3:** C1q staining on immunofluorescence microscopy is suggestive of secondary membranous nephropathy.
- **Question 4:** Subendothelial deposits on electron microscopy is a feature of primary membranous nephropathy.
- **Question 5:** Immunosuppression either with cyclophosphamide or rituximab must be considered as soon as the diagnosis of primary membranous nephropathy with nephrotic syndrome is made.
Answers

1. False. In fact, primary membranous nephropathy is the most common cause in Caucasian males over 40 years of age.
2. False. Anti-PLA2R antibody is present in 70%–80% of primary membranous nephropathy.
3. True. C1q staining on immunofluorescence microscopy is suggestive of secondary membranous nephropathy. Causes such as hepatitis B and C, lupus, malignancies and drugs need to be considered for establishing the diagnosis.
4. False. Subendothelial, mesangial electron-dense deposits on electron microscopy is suggestive of secondary membranous nephropathy. Causes such as hepatitis B and C, lupus, malignancies and drugs need to be considered.
5. False. Immunosuppression with any agent must be considered only after a conservative management for 3–6 months with ACE/ARB therapy and diuretics as 25%–30% undergo spontaneous remission of disease.