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REVIEW

Treatment of primary membranous nephropathy: where are we now?

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Abstract In the last 10 years, basic science and clinical research have made important contributions to the understanding and management of primary membranous nephropathy (MN). The identification of antibodies directed against the M-type phospholipase A_2 receptor (PLA2R) and thrombospondin type-1 domain-containing 7A protein have added a new perspective on diagnosis, monitoring the immunological activity, predicting prognosis and guiding therapy in patients with primary MN. Mounting evidence suggests that quantification and follow-up of antiPLA2R Abs levels can help in assessing prognosis and evaluate the response to treatment. The kidney disease improving global outcomes guidelines published in 2012 have not been updated. New data on the use of rituximab suggest it should be considered as a potential initial therapy in the treatment of patients with primary MN.

Keywords Membranous glomerulonephritis · Immunosuppression · Renal diseases · Glomerulopathies

Introduction

Membranous nephropathy (MN) is the most common cause of nephrotic syndrome in Caucasian adults. Considering the incidence of the glomerulopathies in Italy, MN is relatively frequent, representing 44.1% of patients diagnosed with nephrotic syndrome [1].

Initial studies performed in rat models (Heymann's nephritis) documented that subepithelial immune deposits were formed in situ in the basal surface of podocytes following the autoimmune targeting of megalin, a protein not expressed in human podocytes [2]. The first proof of concept that the same pathogenic mechanism may occur in humans came from Debiec et al. describing in situ formation of immune deposits in a neonate born to a mother deficient in neutral endopeptidase (NEP), a protein expressed in the podocytes [3]. During previous pregnancies, the mother developed antibodies against NEP, which crossed the placenta and bound to the protein in the fetal podocytes, leading to the development of primary MN. However, these cases are extremely rare. A major breakthrough came when Beck et al. identified circulating autoantibodies against the M-type phospholipase A_2 receptor (antiPLA2R Abs) that are present in the serum of approximately 70% of patients with MN [4].

Subsequent studies by Tomas et al. identified new circulating autoantibodies against thrombospondin Type-1 domain-containing 7A (anti-THSD7A Abs) in 5–10% of

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patients with MN who are anti-PLA2R negative [5]. As such, in approximately 80% of the cases an autoantibody can be identified and the disease is considered primary MN. In the majority of the remaining cases, an underlying cause such as drugs, infections, malignancies or systemic autoimmune disease can be identified and the disease is considered secondary MN [6]. Antibodies to not yet recognized podocytes' antigens are likely to account for the remaining cases of 'idiopathic' MN.

In this review, we summarize the prognosis and the several lines of treatment proposed in the literature for primary MN.

Anti-PLA2R antibodies as diagnostic tools

Among the blood tests currently available, the indirect immunofluorescence test (IIF) holds the best sensitivity for anti-PLA2R Abs; its semi-quantitative nature, however, makes it less useful in monitoring disease activity and response to immunosuppressive therapy. Enzyme-linked immunosorbent assay (ELISA) tests are less sensitive than IIF but are less time consuming and allow for accurate quantitation of the circulating anti-PLA2R Abs levels. On the other hand, the use of a high threshold for seropositivity in commercial ELISA tests (>14 RU/ml), may lead to consider low-titer samples falsely negative.

On renal biopsy the diagnosis of PLA2R related MN can be made by demonstrating PLA2R staining overlapping the granular pattern of immunoglobulin (Ig)G deposits (IgG4 dominant). This "enhanced" pattern must be differentiated from the weak background positivity of the PLA2R Ag which can be detected in normal kidney. Approximately 30% of patients with PLA2R Ag positive deposits on renal biopsy may nave negative anti-PLA2R in the serum [7]. This scenario can be explained by two different mechanisms. In the first one anti-PLA2R Abs negativity reflects an immunologic remission (spontaneous or induced by immunosuppressive therapy) that precedes immunodeposits clearance and clinical remission. The second mechanism could be explained by a phenomenon called "kidney as a sink": anti-PLA2R Abs are cleared from the blood by the bind to target antigens in podocyte and they become detectable in the blood only when their rate of production exceeds the buffering capacity of the kidney [8].

The positivity of either serology or histology defines PLA2R-associated MN. In the serum, a positive anti-PLA2R Abs titer is extremely specific for MN (almost 100%), since circulating antibodies have not been detected in healthy individuals or in the setting of other autoimmune disorders [9]. Accordingly, some authors proposed that kidney biopsy can be avoided in patients who are antiPLA2R Abs positive, have normal renal function and do not have evidence of secondary MN, e.g. hepatitis B/C, systemic lupus erythematosus (SLE), malignancy, etc. [8–10]. In the case of negative serology for antiPLA2R Abs, however, a renal biopsy is mandatory to establish the diagnosis [11]. Of note, several cases of PLA2R- associated MN concomitant with hepatitis B, C, sarcoidosis and less frequently malignancies, have been reported [12–16]. It is unclear if MN is concomitant or secondary to the coexisting disease [8].

Similarly, recent evidence shows a prevalence of malignancies of up to 20% among patients with THSDA7A-related MN, particularly endometrial and gallbladder carcinomas, suggesting the need for an aggressive screening for cancer when these antibodies are detected [17]. A semi-quantitative indirect immunofluorescence test has been recently developed to detect circulating THSDA7A antibodies [18]. Theoretically, if both circulating PLA2R antibodies and PLA2R antigens on IF microscopy are negative, and immune deposits appear IgG4 dominant, a THSD7A-related MN should be ruled out. However, there are some additional elements that may help to differentiate cancer- vs. non cancer-related MN [19].

Anti-PLA2R antibodies level to monitor disease activity and predict prognosis in primary MN

The natural history of the disease is heterogeneous. Approximately 20–30% of patients will develop spontaneous complete remission [20] and 30–40% of patients will progress to end-stage renal disease (ESRD) [8]. In the remaining patients mild to moderate proteinuria, with stable renal function, persists over time. Predicting disease progression is important in order to restrict immunosuppressive therapy to those patients with significant risk of progression to ESRD.

Traditionally, several factors have been suggested as predictors of poor prognosis for developing renal insufficiency in primary MN, including male gender, age >50 years, hypertension, histological markers, and proteinuria and creatinine at presentation [21]. Among these risk factors, persistent proteinuria, initial creatinine clearance and change in creatinine clearance over time have shown the best predictive value in identifying patients at increased risk of disease progression [22]. According to the Toronto Risk Score which has been validated in several countries three groups of risk were recognized:

1. a "low risk" group, defined by patients with normal serum creatinine/creatinine clearance and proteinuria consistently ≤4 g/24 h over a 6-month observation period,
2. a "medium risk" group, defined by patients with normal and stable kidney function and with proteinuria

>4 ≤ 8 g/24 h over 6 months of observation (55% probability of developing ESRD within 10 years),
3. a "high risk" group, defined as patients with persistent proteinuria >8 g/24 h, independent of the degree of kidney function impairment (66–80% probability of progression to ESRD in 10 years).

The Toronto Risk Score predicts with an 80–90% accuracy the risk of progression of patients affected by primary MN. However, a long period of observation (at least 6 months) is needed to calculate the risk score with a prolonged delay for the start of treatment [14, 23].

Since the discovery of anti-PLA2R Abs, an increasing body of evidence supports the quantification of these autoantibodies as a reliable tool to predict spontaneous remission, disease progression and monitor the response to treatment. The occurrence of spontaneous remission is more common in patients with low antibody titers compared to those with high antibody titers [24, 25]. Anti-PLA2R Abs levels may predict progression from sub-nephrotic proteinuria to full nephrotic syndrome [26]. High titers of anti-PLA2R antibodies have also been associated with the rate of relapses, lower response to immunosuppressive therapy and longer time to remission [27]. Furthermore, high antibody levels are associated with high rapid loss of kidney function [28].

The evolution of anti-PLA2R Abs levels in response to immunosuppression reliably predicts outcomes. Relapse rate also correlates with the level of antibodies at the time of clinical remission, with patients who become anti-PLA2R Abs negative having lower relapse rates than patients who remain anti-PLA2R Abs positive at the end of immunosuppression.

Hosfra et al. analyzed a cohort of 82 patients with primary MN, and showed an inverse correlation between anti-PLA2R Abs levels and rate of spontaneous remission (38 vs. 4% in the lowest and highest tertiles, respectively) [29]. High anti-PLA2R Abs levels can predict a more rapid loss of renal function [30]. The titer of autoantibodies was directly correlated with proteinuria and serum creatinine, strong predictors of disease activity [24].

In patients treated with rituximab, Beck et al. demonstrated that 88% of patients with a drop in antibody levels developed complete or partial remission by 24 months vs. 33% for those with no significant immunologic response to rituximab treatment [27]. Of note, decline in anti-PLA2R almost always preceded the decline in proteinuria by months. Subsequent studies showed that antibody levels decrease independently of the type of immunosuppressive therapy [28].

In 2015 Ruggenenti et al. published a series of 132 patients with primary MN treated with rituximab, showing that all 25 complete remissions were preceded by anti-PLA2R Abs depletion, and lower baseline anti-PLA2R Abs level strongly predicted remission [31]. As initially demonstrated by Beck et al., a 50% reduction of anti-PLA2R Abs titer anticipated an equivalent reduction of proteinuria by 10 months and re-emergence of circulating antibodies predicted relapses of the disease [31]. Furthermore, a study performed by Bech et al. included 48 patients with primary MN and high risk of progression treated with cyclophosphamide or mycophenolate mofetil in combination with corticosteroids for 12 months, with 33 patients positive for anti-PLA2R Abs. Although in this study the levels of autoantibodies at baseline did not predict the initial response, the anti-PLA2R Abs titer at the end of the therapy predicted long-term outcomes. After 5 years of follow-up, 14 of 24 patients with a negative titer of autoantibodies at the end of treatment were in persistent remission compared to 0 of 9 patients with persistent circulating anti-PLA2R Abs [27].

The prognosis for patients with primary MN and no circulating antibodies seems to be less clear; recently Hoxha et al. described a high proportion of spontaneous remission in anti-PLA2R Abs and anti-THSD7A Abs-negative patients [25]. Therefore, maximizing support therapy may be a reasonable initial approach in these cases. Serial anti-PLA2R Abs assessment is required in those patients with positive PLA2R Ag staining in renal biopsy, who are seronegative for anti-PLA2R Abs.

Supportive therapy

Supportive therapy involves restricting dietary sodium to less than 2 g/day, restricting protein intake (0.8–1 g/kg/day), and controlling blood pressure (blood pressure targeted to 125/75 mmHg), hyperlipidemia, and edema. This approach is recommended by KDIGO guidelines for all patients with MN and nephrotic syndrome [32].

Angiotensin-converting enzyme inhibitors (ACEi) or angiotensin receptor blockers (ARBs) should be the first line of therapy in all cases, due to their antiproteinuric effect. However, it should be considered that in patients with primary MN their antiproteinuric effect is modest (<30% decrease from baseline), in part because the majority of these patients are not hypertensive. Moreover, the antiproteinuric effect has been mainly observed in those patients with a lower degree of proteinuria [20] and use of ACEi or ARB therapy has not shown to be related to prognosis in patients with primary MN [33]. Dual blockade of the renin-angiotensin system can be used in patients who do not have diabetes or significant cardiovascular disease and can tolerate it.

In addition, supportive therapy must be directed to improve hyperlipidemia and lessen the risk of thromboembolism in patients with primary MN.

The effect of statins on cardiovascular disease in patients with primary MN has not been demonstrated in clinical trials. However, most clinicians consider hyperlipidemia as a significant risk factor for cardiovascular disease in patients with nephrotic syndrome.

The use of anticoagulant therapy should be considered in cases of primary MN with a high risk for thrombotic events (in particular: proteinuria >10 g/day, positive family history, previous thrombotic events, serum albumin less than 2 g/dl, obesity or physical inactivity). A recent study evaluating 898 patients with primary MN showed that venous thromboembolic events occurred in approximately 7% of patients, with an incidence rate significantly higher than for other causes of nephrotic syndrome. A serum albumin level less than 2.8 g/dl was the only independent predictor of venous thromboembolism [34].

Immunosuppressive therapy

We will now review the most commonly used therapeutic approaches in clinical practice (Table 1) as well as the degree of evidence supporting such practices (Table 2).

Corticosteroid monotherapy

In 1979, the first randomized controlled trial in patients with primary MN showed that a short course of high-dose prednisone monotherapy (125 mg every other day for 8 weeks) was associated with a reduced rate of deterioration in renal function when compared to placebo [35]. Nevertheless, this study was criticized because of the really poor rate of renal survival in the control group. Two subsequent studies failed to show a beneficial effect of corticosteroid monotherapy on survival, proteinuria and renal function in primary MN [36, 37]. Moreover, meta-analyses did not demonstrate a benefit on renal survival/death and complete remission rates [38, 39]. As such, the KDIGO guidelines do not recommend corticosteroid monotherapy in patients with primary MN [32].

Alkylating agents

The combination of corticosteroids and cyclophosphamide or chlorambucil has improved substantially the prognosis of patients with primary MN (Table 2) [38–40]. In 1984, Ponticelli's group demonstrated the efficacy of a 6-month schedule of alternate monthly courses of glucocorticoids and chlorambucil in patients with primary MN, nephrotic syndrome and normal renal function. During a mean follow-up of 31 months, the intervention group showed a 72% rate of complete or partial remission compared to 30% in the placebo group [41]. A 10-year follow-up revealed a higher rate of remission and dialysis-free survival in the treated group than placebo group (62 vs. 33 and 92 vs. 60%, respectively) [42]. In a subsequent study, chlorambucil was compared with cyclophosphamide (CYC) as an alkylating agent. Patients assigned to chlorambucil treatment received steroids on months 1, 3 and 5 (methylprednisolone 1 g iv on three consecutive days followed by prednisone 0.4 mg/kg/day for 27 days) and oral chlorambucil on months 2, 4 and 6 (0.2 mg/kg/day); in the CYC group, chlorambucil was substituted by oral CYC (2.5 mg/kg/day). The two groups did not show significant differences in remission rate (82% in the chlorambucil group and 93% in the CYC group after 1-year follow-up) and relapse rate (30.5 vs. 25% after 30 months of follow-up). The safety profile was more favorable in the CYC group, with lesser patients who had to stop treatment because of side effects (5% in CYC vs. 14% in the chlorambucil group) [43].

Similar results were found by Jha et al. in an open-label, randomized study comparing treatment with a CYC-based Ponticelli regimen (methylprednisolone 1 g/day for three consecutive days followed by oral prednisolone 0.5 mg/kg/day for 27 days on months 1, 3 and 5 and oral cyclophosphamide at 2 mg/kg/day on months 2, 4 and 6) versus supportive therapy, in adults with nephrotic syndrome and biopsy-proven primary MN. This study confirmed the efficacy of cytotoxic therapy in inducing remission (72 vs. 34% in the control group), leading to a superior long-term renal survival (89 vs. 65%, after a mean follow-up of 11 years) [44].

More recently, the UK Membranous trial attempted to identify the best approach for patients considered at high risk of progression (defined as a decline of estimated glomerular filtration rate (eGFR) ≥20% during the previous 2 years). A cohort of 108 patients was randomized to treatment with cyclosporine A (CSA) monotherapy (12 months), chlorambucil-based Ponticelli schedule (6 months) or supportive therapy alone. The risk of a further 20% decline in eGFR was significantly lower in the chlorambucil group, but not in the CSA group compared with supportive therapy alone. The rate of progression in the chlorambucil group (58%) was greater than in other randomized controlled trials (5–8%), raising concerns about the lesser effectiveness of late treatment in primary MN. The surrogate renal endpoint (20% reduction in eGFR) could be inappropriate, since several factors such as diuretic use or lowering of blood pressure might all contribute to slight changes in serum creatinine levels [45].

Although CYC and chlorambucil are equally effective in inducing remission, CYC has a better tolerability profile and is associated with less short and long-term side effects, in particular bone marrow suppression and hematological malignancies [43, 46]. For practical purpose, chlorambucil is no longer used as an alkylating agent in the treatment of

Table 1 List of the most common regimens applied in clinical practice to treat primary MN

Regimen	References	Class of risk to ESRD[a]	Agents	Dose/levels	Timing	Line of approach
Chlorambucil-based "Ponticelli schedule"	Ponticelli et al. KI [42] (*RCT*) Reichert et al. Ann Intern Med 1994 (*RCT*)	→ Moderate → High[b]	Chlorambucil + Prednisolone + Methylprednisolone	0.2 mg/kg/day 0.5 mg/kg/day 1000 mg iv	Months 2, 4, 6 Months 1, 3, 5 Months 1, 3, 5. Three pulses	First-line
Cyclophosphamide-based "Ponticelli schedule"	Ponticelli et al. JASN [43] (*RCT*) Jha et al. JASN 2007 (*RCT*) Howman et al. Lancet [45] (*RCT*) Reichert LJ et al. Ann Intern Med 1994 (*RCT*)	→ Moderate → High	Cyclophosphamide + Prednisolone + Methylprednisolone	2.5 mg/kg/day 0.5 mg/kg/day 1000 mg iv	Months 2, 4, 6 Months 1, 3, 5 Months 1, 3, 5. Three pulses	First-line
Cyclosporine and steroids	Cattran et al. KI [49] (*RCT*) Alexopoulos et al. NDT [52] (*RCT*) Cattran et al. KI [48] (*RCT*)	→ Moderate → High	Cyclosporine + Prednisolone or methylprednisolone	3.5 mg/kg/day (divided in two daily doses) – serum target: 150–200 ng/ml 0.15 mg/kg/day, max. 15 mg 0.4 mg/kg/day	For 26 weeks, then taper CyA dose by 25% per month; continue treatment at 50% of dose until 12 months, then taper to lowest possible maintenance dose	First-line
Tacrolimus	Praga et al. KI [50] (*RCT*)	→ Moderate	Tacrolimus	0.05 mg/kg/day (divided in two daily doses)—serum target 3–5 mcg/l or 5–8 if unresponsive after 2 months	Induction phase: 12 months, then 6-month taper	First-line
Rituximab	Dahan et al. JASN [65] (*RCT*)	→ Moderate	Rituximab	375 mg/m^2—recommended CD19 + target: 0 cells	Induction phase: a single infusion every week (total: 2 infusions)	Resistant disease
Rituximab (lymphoma schedule)	Ruggenenti et al. JASN [63] (*Observational*)	→ Moderate	Rituximab	375 mg/m^2—recommended CD19+ target: 0 cells	Induction phase: a single infusion every week (total: 4 infusions)	Resistant disease
Rituximab (rheumatoid arthritis schedule)	Fervenza et al. cJASN [62] (*Observational*)	→ Moderate	Rituximab	1000 mg—recommended CD19 + target: 0 cells	Induction phase: a single infusion at time 0 and after 15 days (total: 2 infusions)	Resistant disease
Synthetic ACTH	Ponticelli et al. AJKD [83] (*RCT*)	→ Moderate	Synthetic ACTH	Starting dose of 1 mg/week, increasing to 1 mg twice weekly	Taper after 6–9 months	Resistant disease
Mycophenolate mofetil and steroids	Chan et al. Nephrology (Carlton) 2007 (*RCT*) Senthil Nayagam et al. NDT 2008 (*RCT*)	→ Moderate	Mycophenolate mofetil + prednisone	2.0 g/day 0.5 mg/kg/day	For 6 months For 12 weeks	Resistant disease

ESRD end-stage renal disease, *RCT* randomized controlled trial, *CYC* cyclophosphamide, *ACTH* adrenocorticotropic hormone CyA

[a]According to Cattran et al., Kidney Int. 1997

[b]RCTs on chlorambucil in high risk patients are not available, but efficacy can be assumed from CYC trials and meta-analyses

Table 2 In this table, we compared the most common regimens in literature against placebo/no treatment/no immunosuppressive therapy or other treatments, according to the results of the three most significant meta-analyses in the literature [37–39]

Comparison		References	Complete remission		Complete/partial remission		ESRD/death	
Agent 1	Agent 2	\	Effect size RR (95% CI)	Patients, n (RCT)	Effect size RR (95% CI)	Trials, n	Effect size RR (95% CI)	Trials, n
Immunosuppression	No treatment or ACEi/ARBs	Chen et al. [38]	1.59 (0.87, 2.88)	761 (15)	**1.31** (1.01 to 1.70)	864 (16)	**0.58** (0.36 to 0.95)	791 (15)
Steroids	Placebo No treatment Non-immunosuppressive therapy	Chen et al. [38] Hogan et al. [39]	At 24 months 5.33 (0.70, 40.54) 1.55 (0.99, 2.44)	31 (1) 351 (4)	At 24 months 2.4 (0.93, 6.17) /	31 (1) /	0.75 (0.34, 1.63) /	295 (3) /
CYC	Placebo No treatment Non-immunosuppressive therapy	Chen et al. [38]	At 24 months 0.36 (0.02, 8.05)	25 (1)	At 24 months 2.17 (0.87, 5.37)	25 (1)	0.33 (0.01, 7.84)	102 (3)
Alkylating agents + steroids	Placebo No treatment Non-immunosuppressive therapy Steroids	Chen et al. [38] Imperiale et al. [40][a] Hogan et al. [39][a]	At 24 months **13.86** (2.70, 71.21) **3.0** (1.5–11.0) **4.8** (1.44, 15.96)	172 (2) 110 (3) 142 (3)	At 24 months **4.02** (2.38, 6.77) **2.2** (1.4, 3.3) /	172 (2) 110 (3) /	**0.44** (0.26, 0.75) / /	448 (8) / /
CYC + steroids	Chlorambucil Steroids	Chen et al. [38]	*At 32–39 months* 2.52 (0.38, 16.81)	119 (2)	*At 32–39 months* 2.12 (0.48, 9.31)	119 (2)	1.17 (0.15, 9.36)	147 (3)
CSA	Other treatments	Chen et al. [38]	At 9–60 months 1.03 (0.52, 2.03)	185 (5)	At 9–60 months 1.03 (0.73, 1.44)	185 (5)	1.00 (0.47, 2.15)	202 (6)
TAC	Other treatments	Chen et al. [38]	At 12–30 months 0.91 (0.47, 1.74)	121 (2)	At 12–30 months 1.19 (0.91, 1.55)	121 (2)	0.31 (0.01, 7.20)	121 (2)
MMF	Other treatments	Chen et al. [38]	At 12–24 months 0.99 (0.35, 2.82)	77 (3)	At 12–24 months 0.88 (0.58, 1.35)	77 (3)	/	/
ACTH	Other treatments	Chen et al. [38]	At 21–22 months 3.81 (0.75, 19.27)	62 (2)	At 21–22 months 2.55 (0.45, 14.55)	62 (2)	/	/

Effect size is expressed as relative risk (RR), if available

CYC cyclophosphamide, *CSA* cyclosporine A, *TAC* tacrolimus, *MMF* mycophenolate mofetil, *ACTH* adrenocorticotropic hormone, *ACEi* angiotensin-converting-enzyme inhibitor, *ARB* angiotensin receptor blockers, *RCT* randomized controlled trial

Numbers in bold indicate statistical significance ($p < 0.05$)

Study design of the referenced trial are reported in italics

[a]Trials with alkylating agents without steroids were included

primary MN. However, serious adverse events secondary to CYC exposure have been widely reported (in particular following cumulative doses superior to 36 g), such as malignancies, infertility and severe bone marrow suppression. In a cohort of 293 patients with granulomatosis with polyangiitis, a cumulative CYC dose over 36 g was associated with an increased risk of cancer (bladder cancer and acute myeloid leukemia). Moreover, risk of gonadic toxicity has been reported with cumulative doses of 10–15 g in female patients and 200–250 mg/kg in male patients [6]. Taking into account that a patient of 80 kg treated with a course of CYC 2.5 mg/kg per day for 3 months would receive a cumulative dose of 18 g, CYC appears of limited utility in relapse treatment and its choice as first-line treatment should take into consideration the high risk of infertility [6].

Several cases of *Pneumocystis jirovecii* pneumonia have been reported in patients exposed to alkylating agents; therefore, prophylactic treatment with trimethoprim-sulfamethoxazole is recommended [47].

Calcineurin inhibitors (CNI)

CSA and tacrolimus (TAC) can be used to induce remission in patients affected with primary MN with preserved renal function (creatinine clearance >60 ml/min); these drugs are not mutually exclusive, and resistance to CSA does not mean resistance to TAC or vice versa [45]. According to the KDIGO guidelines (2012), these drugs are considered an alternative therapy when alkylating agents combined with corticosteroids are contraindicated or refused by the patient [32].

Support for CNI use comes from two randomized controlled trials (RCTs) from Canada. The first study involved patients diagnosed with progressive primary MN, identified by an absolute decrease in creatinine clearance ≥8 ml/min and persistent nephrotic range proteinuria after 12 months of supportive therapy. The patients were randomly assigned to CSA (initial dose: 3.5 mg/kg/day, desired 12-h trough level between 110 and 170 µg/l for 12 months) or placebo. After 12 months, the CSA group showed a greater improvement in creatinine clearance slope and proteinuria; these results were sustained in 75% of CSA patients [48]. A subsequent study compared CSA (3.5 mg/kg/day adjusted according to serum levels between 125 and 225 µg/l for 6 months) plus low dose steroids (prednisone 0.15 mg/kg/day up to a maximum daily dose of 15 mg) to low dose steroids alone in 51 patients with corticosteroid-resistant disease. After 26 weeks of treatment, the CSA arm achieved 75% of remissions compared to 22% in the placebo arm. The relatively short course of CSA was associated with a high rate of relapses of 43% at week 52 and 48% at week 78 [49].

Current KDIGO guidelines recommend that in patients who achieved remission, CSA treatment should be continued for at least 12 months before considering discontinuation [32].

TAC was introduced after a successful clinical trial from Spain, which expanded the experience inherited from renal transplantation. A total of 48 patients were randomly assigned to receive either supportive therapy and TAC monotherapy (0.05 mg/kg/day for 12 months and then tapered over 6 months, with a desired trough level of 3–5 ng/ml) or supportive therapy alone. After 18 months, 76% of the patients treated with TAC achieved complete or partial remission vs. 30% in the control group. As observed in CSA, 47% of patients had a relapse within 18 months of TAC withdrawal (mean time to relapse 4.2 months) [50]. The remission rates of the Spanish study were recently confirmed in a multicenter retrospective study, where primary MN patients (34% of whom previously treated with other regimens) achieved 84% of complete or partial remission; as previously described, 44% of the responders relapsed after the treatment was tapered [51].

Hypertension and nephrotoxicity are the major side effects seen in patients treated with CNI. This is particularly important since many responders will become CNI dependent. Worsening in renal function in these patients may be managed by decreasing the daily dose of CNI [52].

Mycophenolate mofetil (MMF)

MMF is a potent, selective and reversible inhibitor of inosine monophosphate dehydrogenase, a crucial enzyme of the *de novo* pathway of guanosine nucleotide synthesis. T- and B-lymphocytes are highly dependent on this pathway and its inhibition results in a significant cytostatic effect. This immunosuppressive agent presents a better safety profile compared to CYC and is widely used for the treatment of glomerular diseases, in particular lupus nephritis.

Initials reports were published about the clinical efficacy of MMF monotherapy for patients with primary MN resistant to CNI and cytotoxic agents. A case series described 16 patients resistant to multiple therapeutic regimens who were treated with an initial dose range from 500 to 2000 mg/day for a mean 8 months. Partial or complete remission occurred in eight patients (50%) [53]. A subsequent randomized controlled trial involved 19 patients-naive treated with MMF monotherapy (2 g per day) for 12 months and 17 patients with conservative therapy. After 1 year of follow-up, no differences were found between groups in terms of remission rate (37% in the intervention group vs. 41 in the control group) and proteinuria [54].

A Dutch study compared 32 patients treated with MMF (1 g twice a day) with 32 historic controls treated with CYC

(1.5 mg/kg/day) for 12 months; both groups also received pulses of methylprednisolone (3 g iv on months 1, 3 and 5) and alternate-day prednisone (0.5 mg/kg every other day). The median follow-up of the MMF group was 23 months. MMF combined with high-dose steroids was as effective as CYC in increasing serum albumin and decreasing proteinuria through a period of 12 months, with a remission rate of 66% in the MMF group vs. 72% in the CYC group. However, MMF-treated patients showed a higher rate of primary non response and more frequently experienced relapses 2 years after the end of treatment (70 vs. 20% in the CYC group) [55].

For these reasons, although the combination of MMF with a high dose of corticosteroids appears effective, KDIGO guidelines do not recommend MMF as the first line of treatment for patients with primary MN [32].

Azathioprine (AZA)

AZA was considered as a low side-effect alternative to alkylating agents. Some case series described the efficacy of an association of AZA and corticosteroids [56], but a prospective observational study showed no benefits in rates of remission of proteinuria (51 vs. 58%, p = NS) and progression to ESRD (21 vs. 18%, p = NS) in patients treated with corticosteroids plus AZA compared to the control group in the long-term period (10 years) [57].

Rituximab (RTX)

RTX is a chimeric monoclonal antibody directed against the membrane protein CD20 expressed on the surface of mature B-lymphocytes, but not on hematopoietic stem cells and plasma cells. Since it has been approved in 1997 by the US Food and Drug Administration (FDA) for the treatment of non-Hodgkin's lymphoma, the use of RTX has expanded into the field of immune-mediated glomerular disease, such as anti-neutrophil cytoplasmic antibodies-associated vasculitis, lupus nephritis, minimal change disease, focal segmental glomerulosclerosis and primary MN [58].

The first study of RTX in primary MN reported a case series of 8 patients treated with the 'lymphoma protocol' (375 mg/m^2 weekly for four doses). Sixteen weeks after the end of the treatment, two patients achieved complete remission and three patients partial remission [59]. After 12 months of follow up, all five patients maintained remission and RTX revealed also an antiproteinuric effect in the remaining three patients, with a reduction of more than 40% compared to baseline proteinuria. No major side effects were reported, only three adverse events with the first infusion [60].

Afterwards, in an open-label pilot trial from Mayo Clinic, 15 patients with primary MN were treated with 1 g of RTX at time 0 and repeated after 15 days for a total of two doses ('rheumatoid arthritis' protocol). After a follow-up of 6 months, proteinuria decreased by 50%; at 1-year follow-up, two and six patients achieved complete and partial remission, respectively [61]. A subsequent study was performed, by the same group, using the lymphoma protocol in a cohort of 20 patients. After 1 year, the remission rate was 50%, increasing to 80% after 2 years, with four patients in complete remission and 12 in partial remission; moreover, only one patient (5%) relapsed [62]. These studies showed a more effective depletion of CD20+ cells with the lymphoma regimen, but no differences in the effectiveness were demonstrated. Moreover, in both studies, no relationship was found between the response and number of B-cells in the blood, CD20+ cells in renal tissue, degree of tubulointerstitial fibrosis, starting proteinuria or creatinine values [62].

A subsequent case series from Mario Negri's group described RTX treatment in 100 patients with primary MN and nephrotic syndrome resistant to inhibitors of the renin-angiotensin system or other immunosuppressive regimens (32%). The majority received only a single dose of RTX (according a B-cell titrated protocol); complete or partial remission was achieved in 65% of cases and proteinuria decreased over time from 9.1 to 4, 2 and 1.5 g/day at 1, 2 and 3 years of follow-up, respectively [63]. Similarly, another case series of 12 patients with primary MN resistant to other immunosuppressive regimens (CSA or alkylating agents) underwent a RTX-lymphoma regimen with 12 months of follow-up. Complete and partial remission was achieved in 21.4 and 50%, respectively [64]. No treatment-related serious adverse events were reported in either study.

More recently, a multicenter randomized trial of RTX performed by the GEMRITUX study group compared RTX (375 mg/m^2, day 1 and 8) plus conservative therapy vs. conservative therapy alone. At 6 months, the study group did not find any difference in complete plus partial remission rates (35.1% in RTX group vs. 21.1% in the conservative therapy group). However, post-RCT observation after a median follow-up of 17 months revealed a higher remission rate in the RTX group (64.9%) than the conservative group (34.2%) [65].

Although RTX and alkylating agents have not been directly compared in a single RCT, RTX has shown an excellent side-effect profile. The most common adverse effects are infusion related [61, 63] and can be significantly reduced by pretreatment with acetaminophen, methylprednisolone and antihistamine. As for alkylating agents, pneumocystis prophylaxis should be considered. However, RTX is not devoid of potential severe side effects. In 2006, the FDA issued a Drug Safety newsletter regarding two cases of fatal progressive multifocal leukoencephalopathy (PML) in patients affected

with non-Hodgkin's lymphoma who had been treated with RTX [66]. These patients received high-dose chemotherapy before or in association with RTX. Other cases related to PML have been reported in patients with hematological disorders or rheumatologic disorders (including SLE) [67]. Remarkably, to the best of our knowledge, no such case has been reported in patients treated with RTX monotherapy.

Fatal fulminating hepatitis due to reactivation of hepatitis B virus after RTX has been reported [68]. As such, treatment with RTX in patients with hepatitis B should be carefully evaluated and requires the use of antiviral prophylaxis [69].

Data about risk of malignancies in RTX-treated patients mainly come from the experience in treatment of hematologic malignancies. Two different studies showed that RTX-containing regimens were not associated to an increased risk of malignancy compared to non-RTX containing regimens in B-cell non Hodgkin lymphoma [70, 71]. An increase in solid tumors were, instead, reported when RTX was used with high-dose chemotherapy (HDT) and autologous stem cell transplantation [72]. However, it should be stressed that long-term data on risk for malignancies are not available for RTX monotherapy.

Considering the toxicity of the alkylating agents, particularly in patients with relapsing disease, the available evidence suggests that RTX could be considered as a first line of therapy in primary MN. Unfortunately, RTX is expensive: the cost of a 'rheumatoid arthritis schedule' is around €8268. On the other hand, a single dose of 375 mg/m^2 (as envisaged in the B-cell driven protocol) costs €3200 for an average 70 kg patient [73, 74] compared to a cost of €450 for a corticosteroid-based regimen with CYC for 6 months at the currently recommended doses [74]. However, if we consider the low rate of moderate to severe side effects (as discussed above) as well as the low rate of relapse, the cost-gap of RTX-based regimens appears to narrow when compared with the standard of care. In fact, hospitalization in Italy costs around € 800/day (excluding therapeutic interventions) [75]. These considerations closely match those with other experiences in immunology [75, 76] and hematology [77].

Three randomized controlled trials with RTX are ongoing:

1. The MEmbranous Nephropathy Trial Of Rituximab (MENTOR) study (NCT01180036) is an open-label RCT designed to evaluate RTX (1000 mg, iv day 1 and 15, repeated after 6 months if the reduction of proteinuria is >25%) versus CSA (3.5–5 mg/kg/day; target levels 125–175 ng/dl) for 6 months, and continued for an additional 6 months if proteinuria reduction is >25%, in maintenance of remission at 24 months from randomization [78]. Results are anticipated in late 2017.
2. The Sequential treatment with Tacrolimus–Rituximab vs. steroids plus cyclophosphamide in patients with primary MEmbranous Nephropathy (STARMEN) trial (NCT01955187) is now in the recruitment phase and will compare a TAC-RXT treatment (experimental group) with Ponticelli's schedule (control group). The experimental group will receive TAC for 9 months (initial dose of 0.05 mg/kg/day, adjusted to achieve blood trough levels between 5–7 ng/ml, maintained for 6 months and then progressively tapered) plus RTX 1 g iv at month 6. The rates of remission, relapse, preservation of renal function and adverse effects will be evaluated in a 2-year follow-up [79].
3. The RI-CYCLO trial (NCT03018535) is recruiting patients from Italian facilities to provide a head-to-head comparison between RTX (1000 mg, day 1 and 15) and Ponticelli's schedule (month 1, 3 and 5: 1000 mg iv methylprednisolone daily × 3 followed by prednisone 0.5/mg/kg/day; month 2, 4 and 6: oral cyclophosphamide 2.0 mg/kg/day), since RCTs are still missing.

Adrenocorticotropic hormone (ACTH)

ACTH is physiologically produced by the pituitary gland and stimulates the production of endogenous glucocorticoids and indirectly activates the melanocortin receptors, which play a role in various physiologic functions, including melanin synthesis, immunomodulation, anti-inflammation, lipolysis stimulation and modulation of exocrine function [80]. Basic research in rodents identified gene expression of these receptors in podocytes, glomerular endothelial cells, mesangial cells and tubular epithelial cells. In animal models of primary MN treated with a specific melanocortin receptor agonist, it significantly reduced proteinuria, oxidative stress and improved podocyte morphology [81].

The first pilot study evaluated 14 patients with primary MN who were treated with synacthen, a synthetic version of ACTH. Patients received synacthen starting at 1 mg intramuscularly every other week with the dose increased to 1 mg intramuscularly 3 times/week for 8 weeks. There was a 90% reduction in proteinuria at the end of the treatment. A subgroup of five patients, who were severely nephrotic and non-responsive to previous therapies, underwent a total treatment period of 12 months: 18 months after the end of treatment, all of them were still in remission [82]. These data were confirmed in an Italian RCT showing no differences between patients treated with cyclic steroids (methylprednisolone 1 g iv on three consecutive days, then 0.4 mg/kg/day orally for 27 days on month 1, 3 and 5) + CYC (2.5 mg/kg/day orally on month 2,4 and 6) versus synacthen (initially 1 mg intramuscularly every other week then increased up to 1 mg twice weekly for a total treatment period of 1 year), with remission rates of 94 and 88%, respectively [83]. A second open-label, prospective study demonstrated beneficial

effects of natural ACTH (ACTHar gel 80 IU subcutaneously twice weekly) in resistant glomerular diseases, including five patients with primary MN and significant reduction in renal function (eGFR < 45 ml/min) who were resistant to other immunosuppressive agents: three of these patients achieved immunologic remission with a negative anti-PLA2R Abs titer after 4 months of treatment, with a clinical partial remission occurring in two of them [84].

In 2014, Hladunewich et al. published a prospective open label study in 20 patients with primary MN and eGFR > 40 ml/min, receiving subcutaneous ACTHar gel at a dose of 40 or 80 IU twice weekly for a total of 12 weeks. Results showed an improvement in proteinuria (decrease ≥50% from baseline) in 65% of patients after 12 months of follow-up, with two complete remissions and ten partial remissions. An improvement in serum albumin and lipid profile was also demonstrated. Lower doses of ACTH (40 IU twice weekly) were associated with a poor response with no significant improvement in proteinuria after 12 weeks of treatment. Five patients who were initially treated with 40 IU twice weekly were continued on ACTHar gel 80 IU twice weekly for an additional 12 weeks. Interestingly, these patients showed a significant improvement in proteinuria after 12 months of follow-up; these results suggest that the effect of ACTH is dose dependent. The drug was well tolerated and safe [85]. The most frequent side effects were weight gain and hyperglycemia [83, 84].

Novel therapeutic perspectives

Ofatumumab is an anti-CD20 monoclonal antibody, which differs from RTX in terms of the different target-epitope: it showed efficacy in the treatment of B-cell lymphomas and other hematological malignancies which had previously not responded to RTX [86, 87]. Ruggenenti et al. recently described two cases of clinical remission of primary MN in patients who developed primary or secondary resistance to RTX [74]; these patients belong to a larger unpublished cohort of nephrotic patients with normal renal function successfully treated with ofatumumab as rescue therapy after failure of RTX. Resistance to RTX in these cases, is likely due to a change in the CD20 antigen conformation, which prevents B cell-RTX binding and the consequent B-cell depletion [74].

The B lymphocyte stimulator protein (BLyS, also known as BAFF) is a soluble member of the tumor necrosis factor family, which plays a fundamental role in B lymphocyte activation and differentiation [88]. Belimumab, a monoclonal antibody directed against BLyS, has been tested in 14 proteinuric patients with antiPLA2R-positive primary MN from six different centers in UK [89]. The treatment protocol consisted in 10 mg/kg iv belimumab every 4 weeks for at least 28 weeks up to week 100 (frequency increased to every 2 weeks if urine protein-creatinine ratio >1000 mg/mmol) and supportive therapy. After 12 weeks, the anti-PLA2R antibody titer showed a significant reduction (−46%) and the urine protein-creatinine ratio decreased by 38% after 38 weeks. BLyS inhibition seems to induce apoptosis and depletion of autoreactive B cells: this mechanism of action has been proposed as the main cause of the slower effect of belimumab with respect to rituximab, which likely induces complement-mediated cytolysis of CD20-positive cells [74].

There is also increasing interest in the use of anti-plasma cell agents as rescue treatment in resistant primary MN. The rationale for this approach is related to the hypothesis that in some cases of MN resistant to anti-CD20 treatments autoantibodies are produced by long-lived memory plasma cells CD20 negative.

Proteasome inhibitor bortezomib induced complete remission in a nephrotic patient resistant to high-dose steroid treatment [90] and in a case of post-transplantation recurrent MN resistant to RTX [91]. The development of a second generation of proteasome inhibitor, which presents a better safety profile with respect to bortezomib, could open new perspectives for the use of anti-plasma cell treatment in resistant MN. Another class of anti-plasma cell drugs with potential to be useful in MN treatment is represented by monoclonal antibodies, such as daratumumab or isatuximab, which target CD38, a multifunctional cell surface protein highly expressed by plasma cells but not in other blood cells or solid tissues [92].

The experience regarding the use of these new agents in MN treatment is still limited and, therefore, more data about their efficacy and safety is needed. New studies are necessary for a better understanding of the role that new anti-CD20 agents, belimumab and anti-plasma cells treatment can play in resistant disease.

A serological approach to treatment

As discussed above, the traditional approach for the clinical decision regarding immunosuppressive therapy has been based on the Toronto Risk Score [22]. The advantage of this model is that it only requires an assessment of kidney function and proteinuria, and the risk can be calculated repeatedly during the period of follow-up. The problem with this model is that that proteinuria and serum creatinine may not accurately reflect disease activity nor discriminate between immunologically active disease and irreversible structural glomerular damage. On the other hand, changes in anti-PLA2R levels typically precede reduction of proteinuria by several months. In patients with high anti-PLA2R levels, the observation period required using the proteinuria model may delay treatment resulting in significant kidney damage.

As such, an individualized serology-based approach that complements and refines the traditional proteinuria-driven approach has been recently proposed [8]. Patients with low or decreasing anti-PLA2R antibody levels are more likely to go into spontaneous remission and thus should be treated conservatively. On the other hand, high baseline or increasing anti-PLA2R antibody levels are associated with nephrotic syndrome and progressive loss of kidney function, favoring prompt initiation of immunosuppressive therapy. Monitoring serum anti-PLA2R antibody levels reliably predicts the response to therapy, and levels at completion of therapy may forecast the long-term outcome. Re-emergence of or increase in antibody titers precedes a clinical relapse.

The hope is that such an approach will improve prognostic accuracy and provide for an individualized treatment of patients with PLA2R-associated MN while limiting unnecessary exposure to immunosuppressive therapy. An RCT comparing the serology-based with the traditional approach is needed to confirm that such an approach is valid and applicable in clinical practice [8].

Conclusions

The discovery of anti PLA2R Ab has revolutionized our approach to MN. With some exceptions, we now have the tools to definitively identify an aspecific histological lesion with a specific etiology, limiting the previously wide grey zone called "idiopathic MN". The availability of anti-PLA2R Abs assays helps clinicians in the timing and monitoring of immunosuppressive therapy and in some selected cases could even allow to avoid renal biopsy, especially in cases of increased risk of complication.

Today, our therapeutic panel against primary MN has been enriched with RTX-based regimens which have been widely used in numerous case series and reports. Consistent evidence in terms of efficacy and side effects has been published. Two RCTs still ongoing should further elucidate whether treatment with RTX is as effective as conventional therapies in inducing remission of proteinuria in patients with MN as well as in maintaining these patients in remission long-term, while showing a favorable side-effect profile.

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