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Observational study of immunosuppressive treatment patterns and outcomes in primary membranous nephropathy: a multicenter retrospective analysis

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Abstract

Background We evaluated the efficacy of different immunosuppressive regimens in patients with primary membranous nephropathy in a large national cohort.

Methods In this registry study, 558 patients from 47 centers who were treated with at least one immunosuppressive agent and had adequate follow-up data were included. Primary outcome was defined as complete (CR) or partial remission (PR). Secondary composite outcome was at least a 50% reduction in estimated glomerular filtration (eGFR), initiation of kidney replacement therapies, development of stage 5 chronic kidney disease, or death.

Results Median age at diagnosis was 48 (IQR: 37–57) years, and 358 (64.2%) were male. Patients were followed for a median of 24 (IQR: 12–60) months. Calcineurin inhibitors (CNIs) with or without glucocorticoids were the most commonly used regimen (43.4%), followed by glucocorticoids and cyclophosphamide (GC-CYC) (39.6%), glucocorticoid monotherapy (25.8%), and rituximab (RTX) (9.1%). Overall remission rate was 66.1% (CR 26.7%, PR 39.4%), and 59 (10.6%) patients reached secondary composite outcome. Multivariate logistic regression showed that baseline eGFR (OR 1.011, 95% CI: 1.003–1.019, $p=0.007$), serum albumin (OR 1.682, 95% CI: 1.269–2.231, $p<0.001$), and use of RTX (OR 0.296, 95% CI: 0.157–0.557, $p<0.001$) were associated with remission rates; whereas only lower baseline hemoglobin was significantly associated with secondary composite outcome (OR: 0.843, 95% CI: 0.715–0.993, $p=0.041$). CYC use was significantly associated with higher remission (OR 1.534, 95% CI: 1.027–2.290, $p=0.036$).

Conclusions Higher baseline eGFR and serum albumin levels correlated with increased remission rates. Remission rates were lower in patients treated with RTX, while those on GC-CYC showed higher rates of remission. Due to the study's retrospective nature and multiple treatments used, caution is warranted in interpreting these findings.

Keywords Membranous nephropathy, Immunosuppression, Chronic kidney disease, Remission

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Introduction

Primary membranous nephropathy (pMN) is a main cause of nephrotic syndrome in non-diabetic adults [1]. The prevalence of pMN in Turkey is similar to that in European countries, comprising 25.6% of all primary glomerulonephritides, according to a recent nationwide study among 4399 patients [2]. The most common presentation of pMN is nephrotic syndrome [3]. The disease follows a variable course, with one-third of patients entering spontaneous remission, but 60% of untreated patients progress to chronic kidney disease (CKD), and 35% of patients who remain nephrotic ultimately develop end-stage kidney disease (ESKD) within 10 years [3, 4]. Many advances have been made in managing pMN alongside our growing understanding of the etiopathogenesis of the disease and the role of autoimmunity, particularly with the discovery of anti-phospholipase-2 receptor antibodies (anti-PLA2R) [5].

The 2021 Kidney Disease: Improving Global Outcomes (KDIGO) Guidelines for the Management of Glomerular Diseases involve important changes regarding pMN treatment compared to those published in 2012 [6]. The 2021 guidelines recommend that patients with pMN and at least one risk factor for disease progression be treated with "rituximab (RTX) or cyclophosphamide (CYC) and alternate month glucocorticoids for six months (GC-CYC), or tacrolimus (Tac)-based therapy for ≥6 months, depending on the estimate of risk". Also, the guidelines advocate GC-CYC for patients at very high risk, including life-threatening nephrotic syndrome or rapidly deteriorating kidney functions [7].

The updated recommendations of the current KDIGO guidelines are based on the findings of several studies that examined the efficacy and safety of the newer agent RTX against non-immunosuppressive therapy, cyclosporine (CsA), and GC-CYC [8–11]. STARMEN and RI-CYCLO trials confirmed that the GC-CYC regimen is still effective in high-risk patients under current standards of supportive care [10, 11]. RTX is shown to be superior to non-immunosuppressive therapy and CsA [8, 9] and provides a safe and effective treatment option [12]. Recent studies also suggest that the unfavorable effect on kidney functions and high relapse risk limit the use of calcineurin inhibitors (CNIs) to treat pMN. In many observational and the aforementioned randomized trials, a superiority of RTX on GC-CYC regimen regarding efficacy and safety has not been shown. The short observation period and low RTX doses might have contributed to the lower remission rate compared to GC-CYC [13].

Accordingly, there are still knowledge gaps regarding the optimal immunosuppressive regimen and dosing protocols, especially when using RTX. Moreover, the possible consequences of the GC-CYC regimen, including myelosuppression and cancer risk, require further investigation. Furthermore, different immunosuppressive treatments fail to provide adequate control in some patients with nephrotic syndrome. Clinical and laboratory markers for recognizing this specific group of patients are needed.

The primary aim of this study is to describe the general characteristics of a multicenter cohort of pMN patients and evaluate the outcomes of different immunosuppressive regimens, with particular focus on CYC, RTX, and CNI based regimens, by analyzing the rate of complete and partial remission and changes in kidney function. The secondary aim is to determine the possible predictors for remission failure and loss of kidney function.

Methods

Study population

For the purposes of this nationwide retrospective multicenter study, data were obtained from the registry of the Glomerular Diseases Working Group of the Turkish Society of Nephrology (TSN-GOLD) [14]. We analyzed 558 adult patients with biopsy-proven pMN who received immunosuppressive therapy in 47 centers in Turkey from May 2000 to January 2022. All patients were meticulously screened for secondary membranous nephropathy (MN) to exclude potential contributing factors, such as infectious and rheumatologic diseases, drugs, and cancers. This comprehensive screening involved age-appropriate cancer screening, assessment for hepatitis, measurement of C-reactive protein levels to investigate any potential infections, and examination for anti-nuclear antibodies and complement levels to rule out rheumatologic diseases. All patients included in the study received the maximum tolerated dose of renin-angiotensin-aldosterone system (RAAS) inhibition as supportive care. Patients were eligible for inclusion based on persistent nephrotic syndrome despite ongoing RAAS inhibitor therapy, which was continued concurrently with immunosuppressive treatment. Patients with secondary MN and pMN patients only on supportive treatment or with insufficient laboratory data, as well as those with spontaneous remission after six months of RAAS inhibition, were excluded.

The patients' demographic, clinical, and laboratory characteristics were collected and recorded to the registry by an attending nephrologist at every center. Histopathological details were obtained from the kidney biopsy reports. Hypertension was defined as systolic blood pressure (BP) ≥ 140 mm Hg or diastolic BP ≥ 90 mm Hg or using antihypertensive agents. Proteinuria was measured by urinary protein-to-creatinine ratio in the first morning specimens during the follow-up, and 24-hour urine collection was used in case of any discrepancies arising from

the spot urine assessment. Nephrotic-range proteinuria was defined as proteinuria level of ≥3.5 g/24 h in the absence of nephrotic syndrome.

Histopathological examination

At each center, a nephropathologist evaluated individual kidney biopsies. In general, adequate kidney biopsy specimens with at least eight glomeruli were assessed using light and immunofluorescence microscopy. Immunofluorescence staining was conducted for IgG, IgM, IgA, C1q, C3, and fibrinogen. Staining was graded from 0 to 3 (0, negative; 1, weak; 2, moderate; 3, strong). Routine stains for light microscopy included hematoxylin and eosin, periodic acid-Schiff, methenamine silver-periodic acid, Masson trichrome, and Congo red [14]. Diagnosis of membranous nephropathy involved identifying thickening and a spike appearance of the glomerular basement membrane (GBM) between the immune deposits on light microscopy, along with granular subepithelial IgG and variable C3 staining along the GBM on immunofluorescence [15]. Interstitial fibrosis (IF) and tubular atrophy (TA) were graded on a scale from 0 to 3: 0, normal; 1 (mild), < 25% of interstitium; 2 (moderate), 25-50%; and 3 (severe), > 50% [16].

Treatment modalities

Treatment regimens were decided according to the nephrologist's discretion at each center. After 2012, treatment decisions were made based on the Kidney Disease: Improving Global Outcomes guidelines [6, 7].

All patients included in the study received at least one treatment regimen. The different treatment regimens evaluated are as follows: GC-CYC, CNIs with or without glucocorticoids, glucocorticoid monotherapy, RTX, mycophenolic acid analogs (MPA) with or without glucocorticoids, and azathioprine (AZA) with or without glucocorticoids. Patients with refractory nephrotic syndrome were switched to a different regimen. CNIs were discontinued gradually if withdrawal of these agents were necessary. Remission assessment was conducted based on the last treatment administered. Subsequent and tertiary treatments were only considered if the initial treatment(s) failed to achieve a response or relapsed after the first treatment.

Additionally, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers were started in all patients except in patients with stage 4 or 5 CKD, and these agents were maintained as long as the patients tolerated.

Follow-up

Patients were hospitalized to perform kidney biopsies and followed up on outpatient visits afterwards. Baseline data before immunosuppressive therapy and data at 3, 6, and 12 months, and every six months thereafter were collected and included serum creatinine, quantitative proteinuria, estimated glomerular filtration rate (eGFR) calculated by CKD Epidemiology Collaboration (CKD-EPI) 2009 formula [17], serum albumin, and hemoglobin. Patients were evaluated for adverse events at every follow-up visit. Infectious complications and thromboembolism were recorded.

Outcomes

Primary outcome was the occurrence of partial or complete remission, according to KDIGO [7]. Complete remission (CR) was decided to be achieved when proteinuria decreased to ≤0.3 g/24 h with normal serum albumin and creatinine concentrations, while partial remission (PR) was considered as a proteinuria reduction of ≥50% (and a proteinuria value of < 3.5 g/24 h in patients with nephrotic syndrome or nephrotic-range proteinuria at baseline) and stabilization or improvement in kidney function. Secondary composite outcome was defined as at least a 50% reduction in eGFR, initiation of kidney replacement therapies (KRT), development of stage 5 CKD (eGFR < 15 ml/min/1.73 m^2), or death. Associations of clinical, laboratory, and histopathological features with study outcomes were also evaluated.

Statistical analyses

Statistical analyses were performed using SPSS for Windows (SPSS version 26.0, IBM Corp., Armonk, NY). Normally distributed data were reported as mean ± standard deviation (SD), and non-normally distributed data as median [interquartile range (IQR)]. Categorical variables are shown as frequencies (%). Comparisons of continuous variables of all patients before and after treatment were evaluated with the paired *t*-test or the Wilcoxon signed-rank test according to the distribution pattern. Three-group comparisons were calculated by using analysis of variance (ANOVA) or Kruskal-Wallis tests. Sex, age at diagnosis, preexisting hypertension (HT), preexisting diabetes mellitus (DM), percentage of sclerotic glomeruli, IF, TA, levels of hemoglobin, eGFR, serum albumin, and proteinuria at the baseline, use of CYC, CNI-based regimens, RTX or glucocorticoid monotherapy were included in univariate logistic regression for primary and secondary outcomes. For IF and TA, none and mild were considered as the reference, and moderate and severe as the risk factor. Variables with p values < 0.10 in univariate analyses were selected for the multivariate logistic regression models with enter method. Also, sex and age at diagnosis were included. Results of the regression models were demonstrated as odds ratios (ORs) and 95%

confidence intervals (CIs). All analyses were two-sided, and a p-value of ≤ 0.05 is considered as significant.

Ethical approval

Included patients provided informed consent to extract their data to the registry. TSN-GOLD registry and the studies derived from its data were approved by Istanbul University Istanbul Faculty of Medicine Ethical Committee (2011/1164), and complied with the Declaration of Helsinki and its later amendments.

Results

Baseline characteristics

Median age of patients at diagnosis was 48 (IQR: 37-57) years, and 358 (64.2%) were male. One hundred eighty-nine (33.9%) patients had preexisting HT, while 66 (11.8%) had DM (type 2 DM: 62, type 1 DM: 4) at the time of the diagnosis of pMN. Median serum creatinine, eGFR, serum albumin, and proteinuria before treatment initiation were 0.8 mg/dl (IQR: 0.6-1.1), 100.6 (IQR: 76.2-117.1) ml/min/1/73m^2, 2.5 g/dl (IQR: 2-3), and 7100 mg/day (IQR: 4500-10593), respectively. On histopathologic examination, chronicity features were evaluated in detail. Median percentage of sclerotic glomeruli was 4.35%. Also, most patients had either absent or mild IF and TA. Baseline demographic, laboratory, and histologic characteristics of the patients are summarized in Table 1.

Table 1 Baseline characteristics at the time of diagnosis of all patients

Characteristics	Data ($n=558$)
Male sex, n (%)	358 (64.2)
Age, median (IQR)	48 (37–57)
Clinical and laboratory features	
Systolic BP (mmHg), median (IQR)	130 (120–140)
Diastolic BP (mmHg), median (IQR)	80 (75–90)
Hemoglobin (g/dl), mean ± SD	13.2 ± 1.9
Serum creatinine (mg/dl), median (IQR)	0.8 (0.6–1.1)
eGFR (ml/min/1.73 m^2), median (IQR)	100.6 (76.2-117.1)
Serum albumin (g/dl), median (IQR)	2.5 (2–3)
Total cholesterol (mg/dl), median (IQR)	294.5 (239–369)
Proteinuria (mg/day), median (IQR)	7100 (4500–10593)
Pathological features	
Percentage of sclerotic glomeruli, median (IQR)	4.35 (0-13.04)
Interstitial fibrosis, n (%)	
None	320 (57.3)
Mild	201 (36)
Moderate	27 (4.8)
Severe	10 (1.8)
Tubular atrophy, n (%)	
None	302 (54.1)
Mild	220 (39.4)
Moderate	27 (4.8)
Severe	9 (1.6)

BP Blood pressure, *eGFR* Estimated glomerular filtration rate, *IQR* Interquartile range, *SD* Standard deviation

Follow-up and treatment regimens

Patients were followed up for a median of 24 (IQR: 12-60) months. CNIs with or without glucocorticoids were the most commonly used treatment regimen (43.4%), followed by GC-CYC (39.6%) and glucocorticoid monotherapy (25.8%). RTX, MPA, and AZA with or without glucocorticoids comprised 9.1%, 4.5%, and 4.3% of the administered treatments, respectively. Treatment regimens used during the follow-up are detailed in Table 2.

Study outcomes

Overall remission rate was 66.1% (n=369). One hundred and forty-nine (26.7%) patients achieved CR, while an additional 220 (39.4%) patients attained PR at the time of last follow-up. Since many patients underwent more than one immunosuppressive treatment regimen, we found that 115 out of 558 patients (20.6%) remained refractory to their initial immunosuppressive regimen. Fifty-nine (10.6%) patients reached the secondary composite outcome. Among them, 13 (2.3%) started KRT, seven (1.3%) reached stage 5 CKD without commencing KRT, 25 (4.5%) experienced at least a 50% reduction in eGFR, and 14 (2.5%) died. In 10 patients, causes of death remained unknown; however, three died of cardiovascular disease, and one due to gastric perforation (Table 3).

A rise in serum creatinine value from baseline to the end of the follow-up was noted (0.8mg/dl. vs 0.9mg/dl, p<0.001). Median proteinuria decreased significantly from 7100 to 1100 mg (p<0.001). A significant increase in serum albumin was observed accordingly, and median serum albumin normalized at the end of the study increasing from 2.5 (IQR 2-3) g/dl to 3.9 (IQR: 3.3-4.3) g/dl (p<0.001) (Table4). Thirty-five (6.3%) patients experienced infectious complications and 28 (5%) suffered from thromboembolic disease.

Multivariate logistic regression model for the primary outcome showed that higher baseline eGFR (OR 1.011, 95% CI: 1.003-1.019, p=0.007) and serum albumin (OR 1.682, 95% CI: 1.269-2.231, p<0.001) were associated with increased CR and PR. Use of RTX (OR 0.296, 95% CI: 0.157-0.557, p<0.001) was associated with decreased CR and PR rates. On the other hand, CYC use was significantly associated with improved rates of CR and PR (OR 1.534, 95% CI: 1.027-2.290, p=0.036) (Table 5). In addition, further comparison of patients who were treated with GC-CYC, RTX or both showed that baseline clinical features of these patients were generally similar (Supplementary Table 1). A second multivariate logistic

Table 2 Treatment regimens during the follow-up

Treatment	Patients with primary MN (*n*=558)
Cyclophosphamide and glucocorticoids, n (%)	221 (39.6)
Calcineurin inhibitors with or without glucocorticoids, n (%)	242 (43.4)
Glucocorticoid monotherapy, n (%)	144 (25.8)
Rituximab, n (%)	51 (9.1)
Mycophenolic acid analogs with or without glucocorticoids, n (%)	25 (4.5)
Azathioprine with or without glucocorticoids, n (%)	24 (4.3)

Table 3 Study outcomes

Outcomes	Patients with primary MN (*n* = 558)
PRIMARY OUTCOME, N (%)	369 (66.1)
Complete remission, n (%)	149 (26.7)
Partial remission, n (%)	220 (39.4)
SECONDARY COMPOSITE OUTCOME, N (%)	59 (10.6)
Initiation of kidney replacement therapies, n (%)	13 (2.3)
eGFR < 15 ml/min/1.73 m^2, n (%)	7 (1.3)
≥50% reduction in eGFR, n (%)	25 (4.5)
Death, n (%)	14 (2.5)

eGFR Estimated glomerular filtration rate

regression analysis was used to identify the possible predictors of the secondary composite outcome. It showed that lower baseline hemoglobin levels were significantly associated with the worsening of kidney functions (OR 0.843, 95% CI: 0.715-0.993, p=0.041); however, other variables, including sex, age, preexisting HT, percentage of sclerotic glomeruli, baseline eGFR, and use of immunosuppression were not associated with the secondary outcome (Table 6).

Discussion

This study examined the effectiveness of immunosuppressive therapy and the impact of other clinical, laboratory, and pathologic variables on remission rates and loss of kidney function. We demonstrated that 66% of the patients achieved some form of remission through immunosuppressive treatment, consistent with the findings of recent significant randomized controlled trials in pMN [9, 10, 18, 19]. Our study also revealed that 2.3% of patients underwent KRT. This number may seem relatively low compared to the current literature, but the limited follow-up time may have contributed to this finding [20].

Our study showed that higher baseline albumin and eGFR predicted higher remission rates. The remission of proteinuria is a surrogate for favorable kidney outcomes in patients with pMN [21]. Similar to our findings, a study from 2023 indicated that baseline albumin value is associated with rapid improvement of proteinuria [22]. Furthermore, severe baseline hypoalbuminemia and lower eGFR have been shown to be associated with worse kidney functions in various studies of glomerular diseases [23, 24]. Our study cohort had moderately low baseline serum albumin levels and normal kidney functions. This may explain the absence of any effect of serum albumin and creatinine on the secondary outcome. However, low serum albumin levels and eGFR may serve as a warning signal for a decreased remission rate and probably unfavorable kidney outcomes.

GC-CYC represents the primary treatment option for patients with very high risk for progression or worsening kidney functions [7].The GC-CYC regimen has been demonstrated to be associated with high remission rates [10, 11, 25, 26]. Our findings align with these studies,

Table 4 Laboratory features of patients at the baseline and the last follow-up

Characteristics	Baseline	Last Follow-up	*p*
Serum creatinine (mg/dl), median (IQR)	0.8 (0.6–1.1)	0.9 (0.7–1.2)	**< 0.001**
eGFR (ml/min/1.73 m^2), median (IQR)	100.6 (76.2-117.1)	92.3 (59.8-108.6)	**< 0.001**
Serum albumin (g/dl), median (IQR)	2.5 (2–3)	3.9 (3.3–4.3)	**< 0.001**
Proteinuria (mg/day), median (IQR)	7100 (4500–10593)	1100 (266–3514)	**< 0.001**

eGFR Estimated glomerular filtration rate, *IQR* Interquartile range

Table 5 Univariate and multivariate logistic regression analyses for the primary outcome

Variable	Univariate		Multivariate	
	OR (95% CIs)	*p*	OR (95% CIs)	*p*
Male sex	0.708 (0.487–1.028)	0.070	0.853 (0.561–1.297)	0.458
Age at diagnosis	0.994 (0.981–1.006)	0.315	1.009 (0.992–1.026)	0.300
Preexisting hypertension	0.754 (0.523–1.088)	0.132	-	-
Preexisting diabetes mellitus	0.660 (0.391–1.114)	0.120	-	-
Percentage of sclerotic glomeruli	0.981 (0.969–0.994)	0.004	0.986 (0.972–1.001)	0.062
Moderate or severe interstitial fibrosis	0.652 (0.332–1.282)	0.215	-	-
Moderate or severe tubular atrophy	0.620 (0.313–1.226)	0.169	-	-
Baseline hemoglobin	1.017 (0.926–1.117)	0.721	-	-
Baseline eGFR	1.010 (1.005–1.016)	< 0.001	1.011 (1.003–1.019)	**0.007**
Baseline serum albumin	1.563 (1.212–2.014)	0.001	1.682 (1.269–2.231)	**< 0.001**
Baseline proteinuria	1.000 (1.000–1.000)	0.243	-	**-**
Use of cyclophosphamide	1.498 (1.039–2.160)	0.031	1.534 (1.027–2.290)	**0.036**
Use of calcineurin inhibitors	0.796 (0.559–1.133)	0.205	-	**-**
Use of rituximab	0.266 (0.146–0.484)	< 0.001	0.296 (0.157–0.557)	**< 0.001**
Use of glucocorticoid monotherapy	0.744 (0.502–1.102)	0.140	-	**-**

CI Confidence interval, *eGFR* Estimated glomerular filtration rate, *OR* Odds ratio

Table 6 Univariate and multivariate logistic regression analyses for the secondary composite outcome

Variable	Univariate		Multivariate	
	OR (95% CIs)	*p*	OR (95% CIs)	*p*
Male sex	1.198 (0.674–2.131)	0.538	1.421 (0.714–2.831)	0.317
Age at diagnosis	1.014 (0.995–1.034)	0.156	1.005 (0.978–1.032)	0.732
Preexisting hypertension	2.048 (1.189–3.528)	0.010	1.402 (0.708–2.776)	0.332
Preexisting diabetes mellitus	1.193 (0.539–2.639)	0.664	-	-
Percentage of sclerotic glomeruli	1.015 (0.997–1.034)	0.094	1.010 (0.990–1.031)	0.336
Moderate or severe interstitial fibrosis	1.709 (0.682–4.285)	0.253	-	-
Moderate or severe tubular atrophy	1.398 (0.522–3.746)	0.505	-	-
Baseline hemoglobin	0.863 (0.751–0.991)	0.037	0.843 (0.715–0.993)	**0.041**
Baseline eGFR	0.992 (0.984–1.001)	0.075	1.002 (0.990–1.014)	0.775
Baseline serum albumin	0.758 (0.515–1.115)	0.159	-	-
Baseline proteinuria	1.000 (1.000–1.000)	0.936	-	-
Use of cyclophosphamide	0.971 (0.559–1.689)	0.918	-	-
Use of calcineurin inhibitors	0.956 (0.554–1.649)	0.870	-	-
Use of rituximab	1.959 (0.901–4.259)	0.090	1.723 (0.746–3.977)	0.203
Use of glucocorticoid monotherapy	1.186 (0.652–2.157)	0.577	-	-

CI Confidence interval, *eGFR* Estimated glomerular filtration rate, *OR* Odds ratio

revealing that the use of CYC is positively correlated with remission. Conversely, we found that the use of RTX was associated with a lower treatment response, which contradicts the current literature. The RI-CYCLO trial demonstrated that RTX has comparable efficacy to GC-CYC during long-term follow-up [11] . Several factors warrant cautious interpretation of this finding. First, the response to RTX can be delayed, as RTX targets antibody-producing cells and gradually leads to the resolution of subepithelial deposits over time [27]. Although the follow-up period in our study was quite long, it may still have been insufficient to observe this gradual efficacy in some patients treated with RTX. Secondly, RTX was not the initial treatment choice for most of our patients, suggesting that those who were resistant to previous treatments were more likely to receive rituximab. Additionally, the absence of routine anti-PLA2R testing limits our ability to categorize patients definitively. Finally, the

small number of patients treated with rituximab poses another challenge in interpreting these results.

Another notable finding of our study is that CNIs that we administered for at least six months, with discontinuation achieved through gradual dose reduction had no discernible effect on primary and secondary outcomes. While CNIs have been a central treatment option for low to moderate-risk patients since the early 2000s [28], emerging concerns about their lower long-term efficacy compared to GC-CYC and RTX, along with the risk of nephrotoxicity and a high likelihood of relapses after withdrawal may seem to limit their future use [29].

Lower baseline hemoglobin levels were significantly correlated with the loss of kidney function. Similar to our findings, previous studies in IgA nephropathy also indicated increased rates of kidney failure among patients with anemia [30, 31]. Anemia may contribute to the hypoxic environment in the tubulointerstitium during kidney damage, and the hypoxia may exacerbate kidney fibrosis, leading to accelerated injury and eventual failure [32]. However, despite the potential significance of this observation, definitive conclusions remain elusive due to the absence of comprehensive data on the duration of low hemoglobin levels, the correction of anemia, and the timing of any interventions.

On the contrary, immunosuppressive treatment had no efficacy on the secondary composite outcome of a 50% reduction in eGFR, initiation of KRT, development of stage 5 CKD, or death. The approach to immunosuppressive treatment in pMN has evolved through the repurposed use of various drugs and the diversification of regimens. Initial pivotal studies demonstrated that alkylating agents reduced proteinuria and stabilized kidney function [33, 34]. Subsequent studies indicated a persistent long-term protective effect, but it is essential to consider the age of the studies as well as the limited number of participants [26, 35]. Over the past decade, four landmark randomized controlled trials have compared different immunosuppressive regimens in pMN with remission rates as primary outcomes. However, the information regarding the preservation of kidney function remained largely inconclusive. Notably, regimens involving CNIs were associated with eGFR decline [9]. No RCT has examined the long-term effect of immunosuppressive treatment on the progression of kidney disease as the primary outcome yet. Furthermore, two meta-analyses, one from 2019 and another from 2022, reported that immunosuppressive treatment failed to prevent ESKD [36, 37]. In contrast, a 2017 meta-analysis suggested that only CYC and chlorambucil significantly decreased the risk of mortality or ESKD. It is worth noting that RTX was not included in this particular meta-analysis [38]. Therefore, the success of immunosuppressive treatment in preserving kidney functions still remains a matter of intense debate, particularly when considering the high rate of spontaneous remission in pMN. This reason for the contrast between the effectiveness of immunosuppression in inducing remission and its failure to improve kidney outcomes may stem from the studies' limitations. Most studies' relatively short follow-up time may be insufficient in detecting ESKD due to the long interval between the diagnosis and the development of ESKD.

We have demonstrated a significant decrease in proteinuria and a compatible increase in serum albumin levels after the treatment compared to baseline, possibly related to the substantial rate of CR and PR. On the contrary, serum creatinine increased, and eGFR values decreased significantly. This may be explained by other covariates on kidney function, like interruptive relapses and some other diseases, which may have gone unnoticed due to the nature of our study design. Also, the time elapsed could have contributed to the eGFR decline.

Chronic histopathologic changes were relatively infrequent in our study, with more than 90% of patients exhibiting either no or only mild IF and TA, and a median percentage of glomerular sclerosis of only 4.35%. Typically, factors such as older age, HT, and DM drive IF, TA, and glomerular sclerosis [39]. The scarcity of these chronic alterations in our study can be attributed to several factors: the relatively young age of our participants, effective blood pressure control in hypertensive patients, preserved kidney function, and a small number of patients with DM. A recent study demonstrated that these chronic histopathologic alterations on kidney biopsy were associated with progression among a diverse group of kidney diseases, including MN patients [40]. However, in our study, we could not show an association between these chronic changes and kidney outcomes. One reason for this could be the occurrence of chronic changes in a relatively small number of patients. Furthermore, since kidney biopsies in our study were performed at baseline, we lack information on whether refractory patients underwent re-biopsy, which would have probably shown a worsening of the degree of IF, TA, and glomerular sclerosis. Therefore, although our findings regarding chronic histopathological changes do not predict kidney outcomes, it is not possible to generalize and draw definitive conclusions from this finding.

Arterial and venous thromboembolic events are among the prominent complications of pMN. The highest risk is within 6-12 months after diagnosis, and prophylactic anticoagulation should be considered in patients with serum albumin levels below 2.5 g/dl [41]. Accordingly, 28 (6.3%) patients in our cohort suffered from thromboembolism. This incidence is lower than that of a current study from 2021, reporting an event rate of 20%. We

suggest that our study cohort's moderately low serum albumin levels and higher remission rate may have contributed to this finding. Also, since we used registry data, it should be kept in mind that it may have been underreported. Infections are a consequence of both nephrotic syndrome and its treatment. Thirty-five (6.3%) patients in our cohort experienced infectious complications. This was in line with a recent meta-analysis reporting an infection rate of 7.9% in 21 studies analyzed [38].

This study has suffered from several limitations, the most significant being its retrospective and observational nature. Limited follow-up time, lack of anti-PLA2R measurement, absence of data on PLA2R staining in the recent biopsies, heterogeneous treatment regimens, lack of relapse rates, and lack of data regarding treatment details, particularly dosing and duration of specific treatments, are among the challenges in deriving definitive conclusions from the study's outcomes. Also, data regarding adverse events were not detailed. Additionally, it is important to note that our study's findings may have limitations when applied to populations outside of Turkey due to differences in epidemiological data. However, the study also has some strengths. The data were derived from a multicenter large cohort. Furthermore, the inclusion of histopathologic findings has broadened the study's scope.

In conclusion, higher baseline eGFR and serum albumin values predicted higher rates of remission. Regarding immunosuppressive treatment, while use of GC-CYC but not RTX was associated with higher remission rates, it is important to emphasize that this study was not designed to evaluate the superiority of treatment regimens. Given its retrospective observational nature and the frequent use of multiple treatments by patients, these findings regarding RTX should be interpreted cautiously without definitive conclusions.

Abbreviations

Anti-PLA2R	Anti-phospholipase-2 receptor antibodies
AZA	Azathioprine
BP	Blood Pressure
CI	Confidence intervals
CKD	Chronic kidney disease
CKD-EPI	CKD Epidemiology Collaboration formula
CNIs	Calcineurin inhibitors
CR	Complete remission
CsA	Cyclosporine
CYC	Cyclophosphamide
DM	Diabetes mellitus
eGFR	Estimated glomerular filtration
ESKD	End-stage kidney disease
GBM	Glomerular basement membrane
GC-CYC	Glucocorticoids and cyclophosphamide
HT	Hypertension
IF	Interstitial fibrosis
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
KRT	Kidney replacement therapies
MPA	Mycophenolic acid analogs
MN	Membranous nephropathy
PMN	Primary membranous nephropathy
OR	Odds ratio
PR	Partial remission
RAAS	Renin angiotensin aldosterone system
RTX	Rituximab
SD	Standard deviation
TAC	Tacrolimus
TA	Tubular atrophy
TSN-GOLD	Glomerular Diseases Working Group of the Turkish Society of Nephrology

Supplementary Information

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Supplementary Material 1.

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Authors' contributions
SO designed the study. SM performed the statistical analyses. ASA drafted the first version of the manuscript. All authors were involved in revising it critically for important intellectual content, and all authors approved the final version to be submitted.

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Declarations

Competing interests
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