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Comparations of efficacy and safety of rituximab, calcineurin inhibitors and cyclophosphamide in primary membranous nephropathy: a single-center retrospective analysis

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Abstract

Background To compare the efficacy and safety of rituximab (RTX), calcineurin inhibitor (CNI) and cyclophosphamide (CTX) plus glucocorticoids in the treatment of primary membranous nephropathy (PMN).

Methods Totally 478 biopsy-proven PMN patients in single center were retrospectively included. After 1:1 propensity score matching (PSM), 258 patients were included in RTX, CNI or CTX group (86 patients in each group).

Results After PSM, there were no differences on serum creatinine, eGFR, serum albumin, urine protein, anti-PLA2R antibody levels among groups. The follow-up duration was 12 (10.5, 18) months in CNI group, 12 (12, 18) months in CTX group and 12 (12, 18) months in RTX group. Throughout entire follow-up period, 39 patients (45.3%) in CNI group, 47 patients (54.7%) in CTX group, and 59 patients (68.6%) in RTX group achieved total remission (TR, either complete remission or partial remission). The survival curve showed a higher rate of TR in RTX group than CNI group ($p = 0.018$). A relapse occurred in 15 of 39 (38.5%) patients in CNI group, significantly higher than CTX group (4.3%, $p < 0.001$) and RTX group (3.4%, $p < 0.001$). In CNI group, 36% patients had a ≥ 25% decline in eGFR.

Conclusions RTX may be more effective than CNI in inducing remission in PMN and showed similar efficacy to CTX. CNI may have a high risk of proteinuria relapse and eGFR decline.

Keywords Primary membranous nephropathy, Calcineurin inhibitor, Cyclophosphamide, Rituximab

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Introduction

Membranous nephropathy (MN) is one of the most common causes of nephrotic syndrome in adults and accounts for approximately 20–35% of adult nephrotic syndrome cases [1]. It is pathologically characterized by diffuse thickening of glomerular basement membrane and deposition of subepithelial immune complexes. The discovery of kidney-specific podocyte antigens and its autoimmune antibodies, over the past decade, bring us new insights into the disease recognition, diagnosis, and treatment. M-type phospholipase A2 receptor (PLA2R) is the major target antigen, with the anti-PLA2R antibody detectable in approximately 75–80% of patients with primary MN (PMN) [2, 3]. The level of serum anti-PLA2R antibody and other antibodies has enabled the prediction of treatment response, recurrence, and the risk of progression to end-stage renal disease (ESRD) [4, 5].

Still, PMN is one of the leading causes of progression to ESRD in patients with primary glomerular disease. The 10-year renal survival rate is around 65–75% [6, 7]. Timing appropriate therapeutic intervention is necessary for the management of patients with PMN. Calcineurin inhibitor (CNI) alone or in combination with glucocorticoids, cyclophosphamide (CTX) plus glucocorticoids, and rituximab (RTX) are the primary therapeutic options for PMN. The choice of immunosuppressive therapy is risk-based, and adjusted for situations like relapse or resistance, according to the 2021 Kidney Disease: Improving Global Outcomes (KDIGO) Guidelines [8]. However, comparisons of the efficacy of these three immunosuppressive regimens are still a matter of debate. No previous studies have compared the efficacy of these three treatment regimens simultaneously.

In this retrospectively study, we describe our experience with the efficacy and safety of treatment for PMN patients, including RTX alone, CNI and CTX plus glucocorticoids.

Methods

This retrospective study was performed at a single center, the Kidney Disease Center of the First Affiliated Hospital, Zhejiang University School of Medicine. Adult patients with PMN who were biopsy-diagnosed between January 2017 to May 2022 were eligible for enrollment. We have obtained informed consent from all patients and received approval from the hospital's ethics committee (Ref no. 2020571). The inclusion and exclusion criteria were (1) with biopsy-proven PMN; (2) older than 18 years at the onset; (3) with initial serum creatinine < 176 μmol/L and urinary protein-to-creatinine ratio (UPCR) ≥ 3.0 g/g (4), under any of the following three treatment regimens (CNI monotherapy or CNI plus glucocorticoids, CTX plus glucocorticoids, RTX monotherapy) for at least 6 months; (5) absence of concomitant autoimmune disease or any other systemic disease, such as systemic lupus erythematosus, malignancy, and hepatitis B and C virus infection, diabetes, severe infection, etc.

The enrolled PMN patients were divided into CNI group, CTX group and RTX group. All patients received at least 6 months of medication treatment. Patients had no remission (NR) after 6 months administration were considered to change treatment regimen and censored at that point [9]. Patients in CNI group were administered either tacrolimus (TAC) or cyclosporine (CsA) alone, or in combination with glucocorticoids. There were 64 patients (74.4%) received TAC, and 22 patients (25.6%) received CsA. Oral TAC is initiated at a dose of 0.05 mg/kg/d and monitored to maintain a trough concentration level between 4 and 8 ng/ml. Oral CsA is started at a dose of 3–5 mg/kg/d and adjusted to achieve a target trough concentration level at 125–175 ng/ml. Gradually tapered after 6–9 months' administration, and maintain the minimum effective dose for at least 12 months. Some patients in CNI group were treated with a combination of CNI with oral prednisone 0.3-0.5 mg/kg/d for 4–8 weeks, followed by a reduction of 5 mg every 1–2 weeks until it reaches a long-term maintenance dose of 5–10 mg/day. Cyclophosphamide was administered in a single dose of 500–750 mg/m^2 by intravenous infusion monthly for the initial 6 months, and once every 2–3 months for the later period. All patients in CTX group received a combination of oral prednisone 0.6–0.8 mg/kg/d for 4–8 weeks, and gradually tapered. Patients in RTX group received a dose of 375mg/m^2 intravenous rituximab once every two weeks until their peripheral blood B-cell count was 0/μl. Re-administration of rituximab was determined by degree of B-cell recovery, anti-PLA2R antibodies level and remission status. Adverse events were recorded through the review of the electronic medical record (EMR) system, including laboratory results, physician notes, and other relevant clinical documentation. Additionally, patient self-reports of adverse events were also collected through telephone interviews.

The primary outcome was total remission (TR, either complete or partial remission) rate, complete remission (CR) rate at 6 month, 12 months, and end of the follow-up. The secondary outcomes included the incidence of relapse and renal function decline. CR was defined as UPCR < 0.3 g/g, normal serum albumin concentration and stable renal function [8]. Partial remission (PR) was defined as a UPCR of 0.3–3.0 g/g with at least a 50% reduction from baseline, serum albumin concentration ≥ 30 g/l and stable renal function [10, 11]. Proteinuria failed to meet the criteria of CR or PR was considered as NR. Relapse was defined as UPCR ≥ 3.0 g/g in two consecutive measurements after achieving CR or PR [12]. Renal function decline is a composite endpoint that includes progression to ESRD and a ≥ 25% decline in

eGFR compared with baseline value. ESRD was defined as a permanent drop in eGFR to <15 mL/min/1.73 m^2, or initiation of renal replacement therapy (dialysis or kidney transplantation).

Statistical analysis was performed with SPSS (version 26.0), Graph Pad Prism (version 9.3), and R Studio (version 4.1.3). Quantitative variables with normal distribution were described as mean ± standard deviation (SD), compared with T-test or one-way ANOVA. Quantitative variables with skewed distribution were described as medians (interquartile ranges), compared with the Kruskal-Wallis test or Mann-Whitney test. Categoric variables were described as frequencies (percentages), and were analyzed using the Chi-squared test or Fisher's exact test. Cumulative incidences were calculated by the Kaplan-Meier method and compared by Log-rank test. A multivariate Cox regression model with the stepwise selection of variables based on the Akaike information criterion was applied to explore potential factors that influence MN remission [13]. A p value<0.05 was considered statistically significant.

Propensity score matching (PSM) was used to obtain balance among three groups. The PSM was conducted using a 1:1 nearest-neighbor matching method without replacement and a caliper of 0.02, with CNI group as the reference. A second 1:1 PSM was performed to confirm the balance between the matched CTX and RTX groups, with unmatched data removed. The standardized mean difference (SMD) was used to evaluate the balance of confounding variables, an SMD<0.1 is deemed an indicator of ideal balance between groups. The covariates selection process was based on logistic regression analysis and experience from literature and expert opinion.

Results

A total of 1126 patients with renal biopsy-proven MN were screened. Patients lost follow-up (n=165), secondary MN (n=105), optimized supportive care only (n=179), severe renal dysfunction at the onset (n=21), non-nephrotic proteinuria (n=80), insufficient data (n=17), and less than 6 months of follow-up (n=81) were excluded. 478 patients with PMN met the criteria and their median follow-up time was 12 (12, 24) months. The study enrollment flow chart is shown in Fig. 1, with 219 patients in CNI group, 106 patients in CTX group, and 153 patients in RTX group.

To minimize the treatment options assignment bias, particularly in the CNI group, a PSM was conducted to balance confounders among the three groups. Factors such as serum creatinine, serum albumin, and UPCR were considered as independent covariates for conducting the PSM model. The covariate selection process and balance test before and after PSM can be seen in Supplementary Tables 1–5.

Baseline characteristics before and after PSM for PMN patients were presented in Table 1. Before PSM, there were no significant differences of sex, age, blood pressure, serum cholesterol, UPCR, anti-PLA2R antibody positive rate, and use of angiotensin-converting enzyme (ACEI)/angiotensin 2 receptor blocker (ARB) among three groups (p>0.05). Patients in CNI group showed lower serum creatinine (p<0.001) and higher eGFR levels (p<0.001) compared with those in CTX group and RTX group. Furthermore, statistical differences were observed in the levels of serum albumin (p=0.041) and anti-PLA2R antibody titers (p=0.035) among the three treatments.

After PSM, a total of 258 patients (86 patients in each group) were selected for further statistical analysis, and no significant difference was found among three groups in terms of serum creatinine, eGFR, serum albumin, anti-PLA2R antibody levels, and follow-up time (p>0 0.05). However, the RTX group still had a significantly higher rate of prior immunosuppressant application than the other two groups (p<0.001). Details about the previously used immunosuppressants in non-treatment-naive patients were summarized in Supplementary Table 6.

Comparison of response to treatment

All patients were successfully monitored and treated for a minimum of six months. The median follow-up duration for CNI group was 12 (10.5, 18) months, while the CTX group was monitored for 12 (12, 18) months and the RTX group for 12 (12, 18) months. During a median treatment period of 6 (1, 10.5) months, an average cumulative dose of 1.9 ± 0.1 g of rituximab was administrated. Among them, 70.9% of patients received more than two doses of rituximab treatment, with an average of 3 (2, 4) doses administered. The average cumulative dose of cyclophosphamide was 6.6 ± 0.3 g during a median treatment period of 9 (6, 12) months, while the median treatment period for the calcineurin inhibitor was 10 (6, 13) months, as shown in Table 2.

Primary and secondary outcomes were listed in Table 3. A total of 25 patients (38.5%) in CNI group, 36 patients (54.5%) in CTX group, and 51 patients (68.9%) in RTX group achieved a TR at 12 months. Throughout the entire follow-up period, 39 patients (45.3%) in the CNI group, 47 patients (54.7%) in the CTX group, and 59 patients (68.6%) in the RTX group experienced TR. Compared to the CNI group, the RTX group demonstrated a significantly higher TR rate both at 12 months (68.9% vs. 38.5%, p<0.001) and at the end of follow-up (68.6% vs. 45.3%, p=0.002). However, no difference was found at 6 months in the TR rates among three groups (p>0.05). The CR rates were similar among three groups at 6 months, 12 months, and the end of follow-up (p>0.05).

The relapses were analyzed in 145 patients who achieved TR throughout the entire follow-up period.

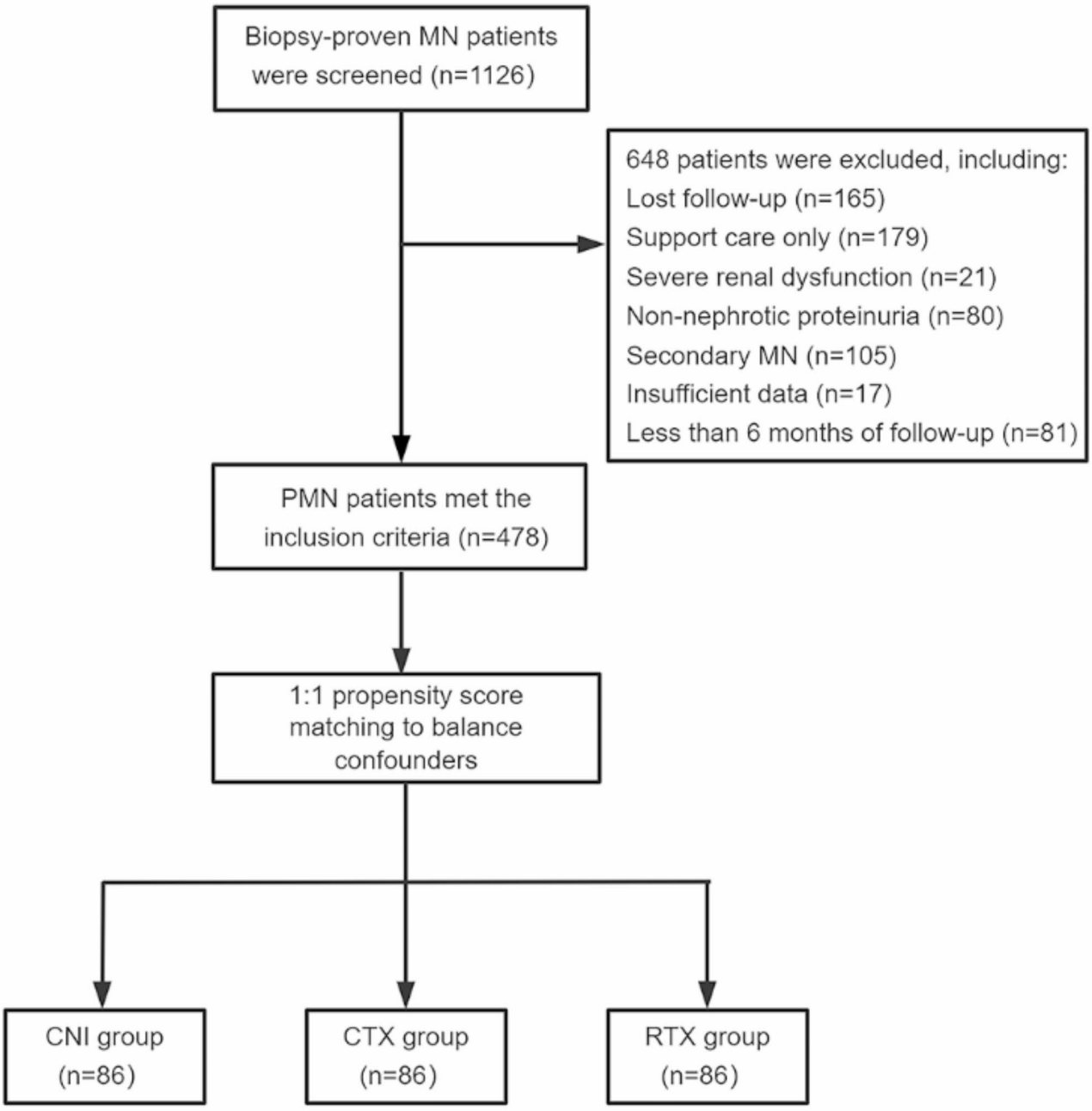

Fig. 1 Flow chart of patient enrollment

Notably, 15 patients (38.5%) experienced proteinuria relapse in CNI group, which was significantly higher than those in CTX group (2 patients, 4.3%, $p<0.001$) and in RTX group (2 patients, 3.4%, $p<0.001$). No statistical difference in relapse-free survival time was found among groups ($p>0.05$).

For renal outcomes, a decline in eGFR was more frequently observed in CNI group. A more than 25% decline in eGFR was noted in 31 patients (36%) in CNI group, which was higher than those in CTX group (14 patients, 16.3%, $p=0.003$) and in RTX group (8 patients, 9.3%, $p<0.001$). However, the risk of progressing to ESRD was comparable among three groups ($p>0.05$).

Probabilities of remission and relapse rates were shown in Fig. 2. The RTX group showed potential superiority in terms of the cumulative TR rate compared to CNI group (Log-rank HR 1.54, 95%CI 1.04–2.29, $p=0.018$). However, there was no discernible difference between CTX group and RTX group ($p>0.05$). The cumulative CR rates were comparable among three groups ($p>0.05$).

The cumulative incidence of relapse was significantly higher in CNI group compared to CTX group (Log-rank

Table 1 Baseline characteristics before and after propensity score matching

Characteristic	Before PSM				After PSM			
	CNI, *n* = 219	CTX, *n* = 106	RTX, *n* = 153	*p* value	CNI, *n* = 86	CTX, *n* = 86	RTX, *n* = 86	*p* value
Age, yr	57 (45, 64)	56 (50, 65)	56 (47, 65)	0.749	58 (45, 65)	56 (49.8, 64.3)	55 (46.5, 60)	0.074
Male, n (%)	140 (63.9)	75 (70.8)	108 (70.6)	0.293	61 (70.9)	58 (67.4)	57 (66.3)	0.793
Systolic BP, mmHg	130 (115, 141)	131 (118, 143)	130 (120, 140)	0.839	128 (114, 138)	133 (117, 143)	129 (118, 140)	0.344
Diastolic BP, mmHg	79.93 ± 14.13	81.27 ± 12.72	80.18 ± 13.44	0.699	79.31 ± 15.00	80.87 ± 13.36	79.53 ± 14.15	0.738
Serum cholesterol, mmol/L	6.89 (5.83, 8.80)	6.95 (5.50, 8.92)	6.76 (5.51, 7.92)	0.082	7.30 (5.98, 8.90)	6.85 (5.50, 8.71)	6.62 (5.40, 7.71)	0.063
Serum creatinine, μmol/L	76 (63, 88)	87 (72, 105)**	86 (71, 108)**	< 0.001	80 (68, 94)	82 (69, 92)	81 (67, 97)	0.966
Serum albumin, g/L	24.8 (21.1, 28.4)	23.4 (20.2, 26.3)	23.4 (20.0, 27.8)	0.041	24.6 ± 5.5	23.9 ± 4.8	24.8 ± 5.4	0.486
eGFR, mL/min/1.73m^2	93.9 (78.6, 105.5)	83.7 (66.3, 96.5)**	83 (62.7, 99.9)**	< 0.001	88.9 (71.1, 100.7)	90.2 (72.4, 97.7)	91.2 (71.9, 104.1)	0.604
UPCR, g/g	4.47 (3.53, 5.23)	4.23 (3.55, 6.00)	4.48 (3.60, 6.62)	0.108	4.21 (3.60, 5.26)	4.21 (3.48, 5.94)	4.26 (3.49, 5.54)	0.983
Anti-PLA2R-Ab-positive, n (%)[a]	86/108 (79.6)	66/83 (79.5)	127/146 (87)	0.203	37/48 (77.1)	60/74 (81.1)	70/81 (86.4)	0.384
Anti-PLA2R-Ab titer, RU/ml	36.6 (5.7, 113.1)	41.1 (7.3, 109.3)	68.2 (16.6, 168.7)	0.035	35.9 (3.5, 100.3)	42.8 (7.0, 112.5)	62.8 (16.8, 164.0)	0.154
ACE inhibitor/ARB use, n (%)	116 (53)	59 (55.7)	90 (58.8)	0.535	42 (48.8)	48 (55.8)	50 (58.1)	0.444
Prior immunosuppressive therapy, n (%)	14 (6.4)	41 (38.7)**	74 (48.4)**	< 0.001	5 (5.8)	30 (34.9)**	50 (58.1)**^^	< 0.001
Follow-up, month	18 (12, 24)	12 (12, 18)**	12 (12, 15)**	< 0.001	12 (10.5, 18)	12 (12, 18)	12 (12, 18)	0.939

Data were shown as n (%), median (IQR), or mean ± SD. [a]A part of patients lack baseline anti-PLA2R-Ab tests, were shown as n/N. Significance values were adjusted by the Bonferroni correction for multiple comparisons. *$p < 0.05$, **$p < 0.01$, CNI group as control; ^^$p < 0.01$, CTX group as control. ACE, angiotensin-converting enzyme; ARB, angiotensin 2 receptor blocker; BP, blood pressure; CNI, calcineurin inhibitor; CTX, cyclophosphamide; eGFR, estimated glomerular filtration rate; anti-PLA2R-Ab, anti-M-type phospholipase A2 receptor antibody; PSM, propensity score matching; RTX, rituximab; UPCR, urine protein-to-creatinine ratio

Table 2 Therapy received by groups

	CNI, *n* = 86	CTX, *n* = 86	RTX, *n* = 86
Total follow-up duration, mo	12 (10.5, 18)	12 (12, 18)	12 (12, 18)
Total treatment period, mo	10 (6, 13)	9 (6, 12)	6 (1, 10.5)
Cumulative administration dose, g	N/A	6.6 ± 0.3	1.9 ± 0.1

Data were shown as median (IQR) or mean ± SD. CNI, calcineurin inhibitor; CTX, cyclophosphamide; RTX, rituximab; N/A not applicable

Table 3 Primary and secondary endpoints

Primary and secondary endpoints	CNI, *n* = 86	CTX, *n* = 86	RTX, *n* = 86
Complete remission			
6 months, n (%)	5/86 (5.8)	3/86 (3.5)	2/86 (2.3)
12 months, n (%)	5/65 (7.7)	11/66 (16.7)	9/74 (12.2)
End of follow-up, n (%)	11/86 (12.8)	18/86 (20.9)	18/86 (20.9)
Total remission			
6 months, n (%)	27/86 (31.4)	33/86 (38.4)	31/86 (36)
12 months, n (%)	25/65 (38.5)	36/66 (54.5)	51/74 (68.9)**
End of follow-up, n (%)	39/86 (45.3)	47/86 (54.7)	59/86 (68.6)**
Relapse			
Relapse, n (%)	15/39 (38.5)	2/47 (4.3)**	2/59 (3.4)**
Relapse-free survival time, mo	11.6 ± 8.1	3, 21[a]	12, 21[a]
Renal function decline			
≥ 25% decline in eGFR, n (%)	31/86 (36)	14/86 (16.3)**	8/86 (9.3)**
ESRD/dialysis, n (%)	4/86 (4.7)	3/86 (3.5)	0

Data were shown as n/N (%) or mean ± SD. [a]Time from remission to relapse for the two patients are separately listed in the table. **$p < 0.01$, CNI group as control. CNI, calcineurin inhibitor; CTX, cyclophosphamide; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; RTX, rituximab

HR 8.91, 95% CI 3.43–23.13, $p < 0.001$) and RTX group (Log-rank HR 8.45, 95% CI 3.26–21.91, $p < 0.001$).

Factors influence the treatment response

The clinical characteristics of patients with remission and non-remission were summarized in Table 4. A total of 145 patients experienced complete or partial remission, whereas 113 patients did not respond to treatments. Factors such as age ($p = 0.003$), treatment selection ($p = 0.008$), and duration of follow-up ($p < 0.001$) may impact the response to treatments. Thus, a multivariate Cox regression model was fitted to mitigate the risk of confounding. Factors may influence the treatment response were listed in the Supplementary Table 7.

Safety profiles

Adverse events (AE) recorded in this study were shown in Table 5. At least one adverse event was observed in 14 (16.3%), 19 (22.1%), and 22 (25.6%) patients in CNI group, CTX group and RTX group respectively. About 4.6% of patients experienced serious adverse events (SAEs), including tumor detection, cardiovascular events, pneumonia hospitalization, and death. Relationship between SAEs and treatment regimens were uncertain. Seven patients in the RTX group experienced leukopenia, which was notably higher compared to CNI and CTX groups ($p = 0.001$), where only one patient reported leukopenia. It is surprising that we observed more leukopenia events in RTX group, rather than CTX group. This may be attributed to the combination use of glucocorticoid with cyclophosphamide, while RTX was in monotherapy. While the incidence of other adverse events was not statistically different among three treatments ($p > 0.05$).

Discussion

In this study, we compared the efficacy and safety of CNI, CTX, and RTX in the treatment of PMN. The studied PMN cohort included patients at varying risk of disease progression, with more than half of them belonging to the high-risk group (see Supplementary Table 8). We found that these three regimens were of similar efficacy at the outset of first 6 months. However, RTX might be more efficient in inducing proteinuria remission than CNI after 12 months. In terms of cumulative remission rates, RTX was potentially superior to CNI but comparable to CTX. Patients with CNI therapy had the highest risk of proteinuria relapse and renal function decline.

Rituximab, a monoclonal antibody targeting the CD20 antigen, effectively exhausts B cells. In recent years, an increasing number of prospective randomized controlled trials have elevated rituximab to the forefront of treatment for PMN. In this study, 68.9% of patients in RTX group achieved a complete or partial remission at the end of 12 months, which is comparable to the results of 60% in the MENTOR trial [14] and 62% in the RI-CYCLO trial [15]. Our center adopted a low-dose rituximab therapy

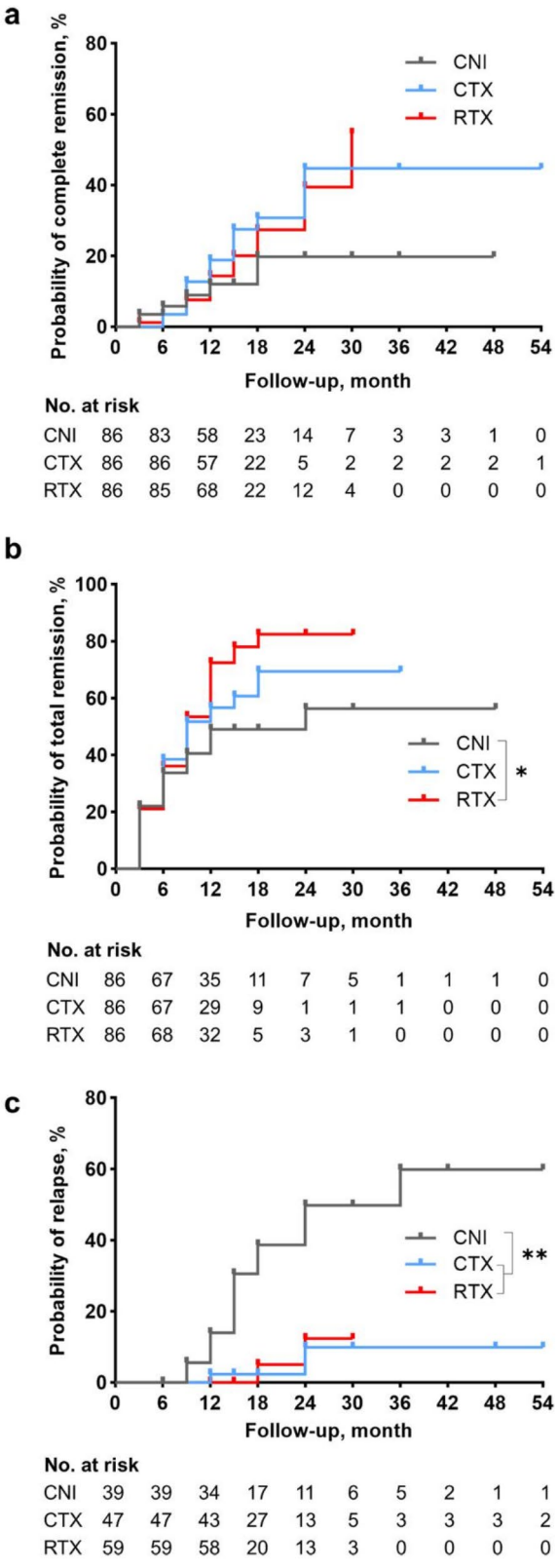

Fig. 2 Kaplan-Meier analysis of (**a**) complete remission (**b**) total remission, and (**c**) relapse rate in three regimens. *$p < 0.05$, **$p < 0.01$

Table 4 Baseline characteristics in patients with different response to treatment

Characteristic	Remission, *n* = 145	Non-remission, *n* = 113	*p* value
Age, yr	55 (45, 62)	57 (51, 67)	0.003
Male, n (%)	100 (69)	76 (67.3)	0.770
Systolic BP, mmHg	130 (113, 140)	131 (118.5, 142)	0.203
Diastolic BP, mmHg	79.65 ± 13.10	80.24 ± 15.44	0.089
Serum cholesterol, mmol/L	6.80 (5.48, 8.28)	6.89 (5.80, 8.64)	0.432
Serum creatinine, μmol/L	83 (68.5, 96.5)	80 (67, 92.5)	0.643
Serum albumin, g/L	24.9 ± 5.5	23.8 ± 4.9	0.227
eGFR, mL/min/1.73m^2	90.6 (71.3, 101.8)	90.5 (72.7, 99.3)	0.609
UPCR, g/g	4.22 (3.53, 5.68)	4.24 (3.52, 5.28)	0.796
Anti-PLA2R-Ab-positive, n (%)[a]	100/124 (80.6)	67/79 (84.8)	0.449
Anti-PLA2R-Ab titer, RU/ml	41.10 (4.56, 110.63)	70.86 (22.26, 156.34)	0.015
ACE inhibitor/ARB use, n (%)	77 (53.1)	63 (55.8)	0.672
Treatment			0.008
CNI, n (%)	39 (26.9)	47 (41.6)	0.013
CTX, n (%)	47 (32.4)	39 (34.5)	0.723
RTX, n (%)	49 (40.7)	27 (23.9)	0.005
Prior immunosuppressive therapy, n (%)	51 (35.2)	34 (30.1)	0.389
Follow-up, mo	15 (12, 24)	12 (6, 15)	< 0.001

Data were shown as n (%), median (IQR) or mean ± SD. [a]A part of patients lack baseline anti-PLA2R-Ab tests, were shown as n/N. ACE, angiotensin-converting enzyme; ARB, angiotensin 2 receptor blocker; BP, blood pressure; CNI, calcineurin inhibitor; CTX, cyclophosphamide; eGFR, estimated glomerular filtration rate; anti-PLA2R-Ab, anti-M-type phospholipase A2 receptor antibody; RTX, rituximab; UPCR, urine protein-to-creatinine ratio

Table 5 Adverse events

Adverse events	CNI, *n* = 86	CTX, *n* = 86	RTX, *n* = 86
Nausea/diarrhea	1	3	0
Allergies/rashes	1	0	2
hypertrichosis	2	0	0
Impaired glucose tolerance	1	2	1
Diabetes	3	3	1
Osteoporosis	0	0	2
Leukopenia	1	1	7**
Elevated liver enzymes	3	1	6
Pneumonia	1	3	3
Other infection	2	6	1
Cancer	3	2	0
Cardio-cerebrovascular events	3	1	0
Death	2	3	0

Data are shown as n. **$p < 0.01$

(375mg/m^2 every 2 weeks until peripheral blood B cell count was 0/μl), and 13 patients (15.1%) achieved remission after twice injections of rituximab, with a median remission time of 9 [3, 12] months. Although high-dose rituximab is more effective in inducing depletion of B cells and remission of proteinuria [16], the value of low-dose rituximab in the treatment of PMN still deserves recognition.

Cyclophosphamide in combination with glucocorticoids is the preferred treatment of choice for patients at high risk for progression of renal function. Previous non-randomized studies have reported a 12-month remission rate of approximately 50–60% with CTX therapy [17, 18]. While more promising results have arisen from recent trials. The remission rates at the end of 12 months, as reported in the STARMEN trial and the RI-CYCLO trial were around 70% [15, 19]. We reported a 12-month CR of 16.7% and a TR rate of 54.5%, which is lower than our expectation. A relatively frequent replacement of the regimen during the CTX therapy course may account for it. Although most of the patients in this study exhibited good tolerance to the regimen during their treatment, the potential toxic side effects of CTX have been a source of concern.

CNI used to be an alternative first-line treatment option for high-risk patients who declined CTX therapy or for those in whom CTX was contraindicated. Both cyclosporine and tacrolimus are effective in reducing proteinuria. Results of previous non-randomized studies have reported 12-month remission rates of approximately 70–75% for cyclosporine and 80–90% for tacrolimus [20–22]. A network meta-analysis of 72 RCTs suggests that tacrolimus and cyclosporine may be more effective than CTX in the treatment of PMN [23]. However, in this study, the 12-month TR rate of CNI was only 38.5%, making it the least effective compared to the other two regimens. A part of patients received CNI monotherapy may be the reason. So, we compared the efficacy of CNI alone and CNI plus glucocorticoids therapy, finding a difference in the complete remission rate, while the other outcomes showed comparable efficacy (Supplementary materials).

Decline in renal function is the most common adverse event of CNI therapy. Both drug nephrotoxicity and

persistent proteinuria may lead to a permanent eGFR decline, which is hard to distinguish in this retrospective study. The toxicity side effect of CNI is associated with constriction of afferent arterioles, vacuolization of tubular epithelial cells, and histological damage of the glomeruli, arterioles, and tubulointerstitium [24, 25]. Meanwhile, prolonged administration of CNI may increase the risk of nephrotoxicity [26, 27]. In this study, nearly one-third patients suffered an eGFR decline after 3-year maintenance of CNI. Timing appropriate treatment withdraw or regimen replacement may better benefit in preserving renal function.

In addition, we noticed more patients had treatment withdrawals and regimen change in the CTX and CNI group when the efficacy was not satisfactory. During their treatment course, 10 patients (11.6%) in CTX group, 23 patients (15.1%) in CNI group, and 5 patients (4.7%) in RTX group underwent regimen changes after 6 months. Reasons for regimen change including persistent proteinuria, progress in renal function, intolerance to treatment, severe infection, and discovery of malignant tumor. This kind of situation usually occurred during the 6–9 months follow-up period. However, it is noteworthy that the 6-months CR or TR rates were comparable among three groups. Patients who received rituximab were more inclined to continue their therapy rather than opt for a regimen replacement, despite the lack of remission. This could potentially attribute for the higher cumulative remission rate observed in RTX group at our center.

We acknowledge the limitations of this study. Firstly, this was a single medical center based retrospective study, with small cohort size and unequal follow-up duration. Although all patients have undergone biopsy confirmation, the baseline anti-PLA2R antibody levels were not measured in some of the patients. Secondly, the inclusion criteria placed no restrictions on the utilization of previous immunosuppressive therapy. Consequently, some patients with refractory characteristics were included, and the effectiveness of the current intervention may be influenced by the delayed impact of previous courses of therapy. Thirdly, this study spans a long period during which treatment methods and standards have changed. Additionally, heterogeneity exists among patients, and variations in physicians' experience may lead to individualized treatment plans. However, this study had mitigated the impact of confounding variables by utilizing the PSM method that allowed us to draw conclusions about the treatment of PMN in clinical practice. Large, prospective, multi-center clinical researches are still need to further confirm these results.

In conclusion, RTX appears to be more effective than CNI in inducing remission of PMN, and show similar efficacy to CTX group. CNI therapy may have a higher risk of proteinuria relapse and renal function decline.

Supplementary Information

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Supplementary Material 1

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None.

Author contributions
L.Lu and F.Han were involved in the study design, conduct of the study, data collection and interpretation. L.Lu wrote the first draft of the manuscript, and F.Han revised it. S.Cai and H.Zhu were involved in conduct of the study, data collection and data analysis. G.Liu, Y.Wang, P.Ren, L.Lan, X.Shen, L.Chen, Y.Xu, J.Cheng, X.Li, J.Chen and F.Han were involved in follow up the patients and data collection. All authors reviewed the manuscript drafts, provided approval of the final version for submission, and take the responsibility for the accuracy and integrity of the data.

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Data availability
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study passed an ethical review by the Institutional Review Board of the First Affiliated Hospital, Zhejiang University School of Medicine (Ref no. 2020571). All subject were explained the significance and purpose of the study, and signed informed consent.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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