Monthly Mini-Dose Rituximab for Primary Membranous Nephropathy: A Different Approach

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Abstract

Background

The dose of rituximab for primary membranous nephropathy is as high as that for lymphoma. This study assessed the efficacy of monthly mini-dose rituximab monotherapy in patients with primary membranous nephropathy.

Methods

This retrospective study included 32 patients with primary membranous nephropathy treated at Peking University Third Hospital between March 2019 and July 2022. All patients were anti-phospholipase A2 receptor (PLA2R) antibody-positive and received rituximab 100 mg intravenously monthly for at least 3 months without other immunosuppressive therapy.

Results

The baseline parameters included: proteinuria, 8.5 ± 3.6 g/day; serum albumin, 24.8 ± 3.4 g/L; and anti-PLA2R antibody, 160 (20-2659) RU/mL. B-cell depletion was achieved in 87.5% patients after the first dose of rituximab 100 mg and in 100% after the second equivalent dose. Twenty-five (78%) patients achieved remission; 8 (25%) patients achieved complete remission at 12 months. Patients were stratified into the low-titer (< 150 RU/mL, n = 17) and high-titer groups (≥ 150 RU/mL, n = 15) based on the anti-PLA2R titer. Sex, age, urinary proteins, serum albumin, and estimated glomerular filtration rate at baseline did not differ significantly between the two groups. At 12 months, the rituximab dose (847 ± 217 vs 659 ± 255 mg, p = 0.033), and urinary proteins were higher (2.7 ± 2.5 vs 1.0 ± 1.2 g/day, p = 0.028), while serum albumin (33.4 ± 6.8 vs 39.6 ± 3.9 g/L, p = 0.007) and the clinical remission rate (60% vs 94%, p = 0.020) were lower in the high-titer group compared to the low-titer group.

Conclusions

Monthly rituximab 100 mg is effective for treating anti-PLA2R-associated primary membranous nephropathy. The lower the anti-PLA2R titer, the lower the rituximab dose required to achieve remission.

Trial registration:

A retrospective study, registered at ChiCTR (ChiCTR2200057381) on March 10, 2022.

Introduction
Primary membranous nephropathy (PMN) is among the most common causes of nephrotic syndrome (NS) in non-diabetic adults.\textsuperscript{1–2} Ten-year follow-up data from 2 independent trials demonstrate a 35%-40% rate of reaching kidney failure in patients treated conservatively, compared with an 8%-11% rate in patients treated with an alkylating agent/corticosteroid regimen\textsuperscript{1}. Sustained massive proteinuria is an important risk factor for progression to ESRD. Amelioration or complete inhibition of proteinuria can greatly retard the occurrence of ESRD in patients with PMN. Clinicians have always aimed to devise methods to derive the maximal clinical benefit from a given therapeutic modality with the minimal medical cost. Rituximab, a human/murine, chimeric anti-CD20 monoclonal antibody that induces rapid and long-term depletion of CD20 + B cells, has been used for the treatment of PMN since 2002; studies have demonstrated its efficacy in inducing remission in approximately two-thirds of patients and superior safety profile over cyclophosphamide or calcineurin inhibitors after observation for more than 12 months.\textsuperscript{3–8}

Most studies that investigated the efficacy of rituximab for PMN utilized the same strategy as that for lymphoma, i.e., intravenous administration at a dose of 375 mg/m\textsuperscript{2}/week for 4 consecutive weeks, or 1000 mg on days 1 and 15.\textsuperscript{3–8} As the number and activity of B lymphocytes in PMN are considerably lower than those in lymphoma, a larger dosing regimen is relatively expensive and can increase the risk of infections.\textsuperscript{9–10} This forms the rationale for the reduction in the rituximab dose to achieve the same therapeutic effect in PMN. In our clinical experience, we found that rituximab 100 mg could achieve B-cell depletion in most patients, which could be sustained for more than one month. We postulated that monthly intermittent dosing could maintain B-cell exhaustion, such that the criterion for immunological remission of PMN would be met [i.e., the titer of anti-phospholipase A2 receptor (PLA2R) antibody, a specific antibody in PMN, would gradually become negative], and eventually result in clinical remission. Herein, we reported the efficacy of rituximab 100 mg administered monthly to anti-PLA2R-positive PMN patients with NS.

**Methods**

Patients with PMN who received rituximab therapy between March 2019 and July 2022 were enrolled in the study. The inclusion criteria were as follows: (1) age more than 18 years, (2) PMN proven by biopsy, (3) NS defined as proteinuria ≥ 3.5 g/day and serum albumin (ALB) < 30 g/L with or without hyperlipidemia or edema, (4) estimated glomerular filtration rate (eGFR) ≥ 30 mL/min/1.73 m\textsuperscript{2}, and (5) serum anti-PLA2R antibody > 20 RU/mL. The exclusion criteria were as follows: (1) secondary membranous nephropathy, (2) absence of NS at baseline, (3) concurrent use of glucocorticoids and/or any other immunosuppressive agent at baseline, (4) patients who received a single rituximab infusion of more than 100 mg, and (5) loss to follow-up or follow-up for less than 12 months. The flowchart of the study selection process is depicted in Fig. 1.

This retrospective study was approved by the Research Ethics Committee of Peking University Third Hospital Medical Science (M2022060) on March 2, 2022, and registered at ChiCTR (ChiCTR2200057381)
Rituximab Treatment Protocol

The initial dose of rituximab 100 mg was administered by intravenous injection (in conjunction with dexamethasone 5 mg, paracetamol 0.6 g, and promethazine 5 mg). Subsequent doses of rituximab 100 mg were administered after an interval of 4 ± 2 weeks, until remission of NS or a minimum serum anti-PLA2R titer ≥ 2 RU/mL was achieved. Patients with infections were screened and excluded prior to each rituximab administration. Interruptions in the monthly rituximab regimen were allowed due to the outbreak of the coronavirus disease (COVID-19) epidemic and control policy in China that prevented patients from visiting the hospital, incidence of any infection, patients’ unwillingness, etc. The frequency of infusion was reduced in some patients after partial remission of NS.

Flow cytometry was used to determine CD19 + and CD20 + B cell counts in peripheral blood. The serum anti-PLA2R titer was assessed using an enzyme-linked immunosorbent assay (ELISA) kit purchased from Euroimmune (Lubeck, Germany). A titer ≥ 20 RU/mL was defined as positive according to the manufacturer’s protocol and that < 2 RU/mL was defined as negative according to literature. The serum rituximab concentration was measured using an ELISA kit purchased from Abcam (Cambridge, MA, U.S.A.). The concentrations of serum creatinine, 24-h urinary protein, serum ALB, immunoglobulins, and complements were assessed routinely. The eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration formula.

Complete remission (CR) was defined as a urinary protein excretion < 0.3 g/day, serum ALB ≥ 35 g/L, and stable renal function. Partial remission was defined as a 24-h urinary protein excretion between 0.3–3.5 g and at least 50% reduction from baseline, ALB ≥ 30 g/L, and stable renal function. Patients who did not meet the above-mentioned criteria were considered to be in non-remission. Relapse was defined as the resurgence of proteinuria > 3.5 g/day and/or ALB < 30 g/L during at least two consecutive visits in patients who had previously achieved a partial or complete response. B-cell depletion was defined as an absolute CD19 + cell count < 5/mm³ in peripheral blood.

Statistical analysis was performed using SPSS software (SPSS 23.0. Armonk, NY: IBM Corp). Data were summarized as frequency (%), mean ± standard deviation (SD), or median (range), as appropriate. The baseline numerical variables and follow-up data were compared using a paired sample t-test. Comparisons between two groups were performed using an independent sample t-test. The two rates were compared using the chi-squared test. Statistical significance was set at p < 0.05.

Results
A total of 32 patients met the selection criteria, of which 25 were men and 7 were women. The participants’ average age was 55 ± 15 years (range, 19–76). Frequent or important comorbidities at baseline included hypertension (n = 19), diabetes (n = 4), coronary heart disease (n = 1), chronic obstructive pulmonary disease (n = 1), and hepatitis B virus carriers (n = 2). All patients had used renin-angiotensin-system inhibitors and 11 had used them for more than 3 months before rituximab treatment. The values of the parameters at baseline were as follows: urinary protein, 8.5 ± 3.6 g/day; serum ALB, 24.8 ± 3.4 g/L; and eGFR, 88 ± 25 mL/min/1.73 m². The median follow-up period was 17 (12–29) months. Patients were divided into two groups based on the anti-PLA2R titer, viz. the low-titer group (anti-PLA2R titer < 150 RU/mL, n = 17) and high-titer group (anti-PLA2R titer ≥ 150 RU/mL, n = 15). There were no significant differences in sex, age, urinary protein, serum ALB, or eGFR levels between the two groups at baseline (p > 0.05).

B-cell Depletion

Our data showed that 87.5% (28/32) of patients achieved B-cell depletion before the second dose (one month after the first dose of rituximab 100 mg). All patients achieved B-cell depletion one month after the second dose of rituximab 100 mg (Fig. 2A-B). The B cell count maintained at < 5/mm³ as long as rituximab administration was continued and recovered a few months after rituximab cessation. There was no significant difference in B-cell depletion between the two groups (Table 1).

Anti-pla2r Titer

The baseline anti-PLA2R concentration in the low- and high-titer groups was 62 ± 39 and 611 ± 637 RU/mL, respectively (p = 0.005). Most individuals showed a progressive decline after two doses of rituximab (Fig. 2C). All but one patient in the low-titer group and 66.7% (10/15) of patients in the high-titer group exhibited anti-PLA2R titers ≤ 2 RU/mL by 12 months. The high-titer group required a greater number of rituximab doses (750 ± 427 vs. 335 ± 169 mg; p = 0.007) to attain negative conversion of anti-PLA2R (Table 1) compared to the low-titer group, owing to the same declining trend.

Clinical Remission Of Ns

The urinary protein levels decreased and serum ALB levels increased gradually after rituximab treatment (Fig. 3A-B). The remission rate was 46.9% (15 patients) with a CR rate of 6.3% (2 patients) at six months, while that at 12 months was 78.1% (25 patients) with a 25% CR rate (8 patients). By 12 months, eGFR was not significantly different between the two groups (p = 0.115, Fig. 3C), while the urinary protein
concentration (2.7 ± 2.5 vs 1.0 ± 1.2 g/day, p = 0.028) and cumulative dose of rituximab (847 ± 217 vs 659 ± 255 mg, p = 0.033) were higher, serum ALB (33.4 ± 6.8 vs 39.6 ± 3.9 g/L, p = 0.007) and the clinical remission rate (60% vs 94%, p = 0.020) were lower in the high-titer group compared to the low-titer group (Fig. 3D). The anti-PLA2R antibody concentration was 2 RU/mL in all but one patient who achieved clinical remission, but not all patients with anti-PLA2R antibodies 2 RU/mL achieved clinical remission.

3A. Decrease in urinary protein excretion; 3B. Increase in serum albumin; 3C. Changes in the estimated glomerular filtration rate; 3D. Overall remission rate.

Blue line: low anti-PLA2R titer group, red line: high anti-PLA2R titer group

PLA2R: phospholipase A2 receptor. *p < 0.05, ** p < 0.01
Table 1
Baseline characteristics and follow-up data of the two groups

<table>
<thead>
<tr>
<th></th>
<th>All (N = 32)</th>
<th>Low anti-PLA2R group (n = 17)</th>
<th>High anti-PLA2R group (n = 15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>55 ± 15</td>
<td>52 ± 13</td>
<td>59 ± 16</td>
<td>0.178</td>
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<tr>
<td>Sex (M/F)</td>
<td>25/7</td>
<td>13/4</td>
<td>12/3</td>
<td>0.261</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Urinary protein (g/24h)</td>
<td>8.5 ± 3.6</td>
<td>8.1 ± 3.4</td>
<td>8.9 ± 3.9</td>
<td>0.509</td>
</tr>
<tr>
<td>Serum albumin (g/L)</td>
<td>24.8 ± 3.4</td>
<td>25.8 ± 2.9</td>
<td>23.6 ± 3.6</td>
<td>0.069</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>88 ± 25</td>
<td>91 ± 24</td>
<td>85 ± 27</td>
<td>0.491</td>
</tr>
<tr>
<td>anti-PLA2R titer (RU/mL)</td>
<td>320 ± 511</td>
<td>62 ± 39</td>
<td>611 ± 637</td>
<td>0.005</td>
</tr>
<tr>
<td>CD19 + B cell (/mm³)</td>
<td>226 ± 128</td>
<td>211 ± 117</td>
<td>237 ± 141</td>
<td>0.642</td>
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<tr>
<td>Immunoglobulin G (g/L)</td>
<td>6.1 ± 2.5</td>
<td>5.4 ± 1.4</td>
<td>6.8 ± 3.1</td>
<td>0.109</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Urinary protein (g/24h)</td>
<td>3.7 ± 3.0</td>
<td>3.4 ± 3.2</td>
<td>4.1 ± 2.9</td>
<td>0.530</td>
</tr>
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<td>Serum albumin (g/L)</td>
<td>32.2 ± 5.2</td>
<td>33.5 ± 4.5</td>
<td>30.8 ± 5.7</td>
<td>0.172</td>
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<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>85 ± 26</td>
<td>95 ± 25</td>
<td>74 ± 23</td>
<td>0.023</td>
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<tr>
<td>anti-PLA2R titer (RU/mL)</td>
<td>69 ± 264</td>
<td>4 ± 6</td>
<td>135 ± 367</td>
<td>0.203</td>
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<tr>
<td>CD19 + B cell (/mm³)</td>
<td>14 ± 30</td>
<td>21 ± 40</td>
<td>8 ± 15</td>
<td>0.294</td>
</tr>
<tr>
<td>Immunoglobulin G (g/L)</td>
<td>7.7 ± 2.4</td>
<td>7.3 ± 2.1</td>
<td>8.0 ± 2.6</td>
<td>0.507</td>
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<td>rituximab cumulative dose (mg)</td>
<td>525 ± 139</td>
<td>476 ± 130</td>
<td>580 ± 108</td>
<td>0.021</td>
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<tr>
<td>12 months</td>
<td></td>
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<tr>
<td>Urinary protein (g/24h)</td>
<td>1.8 ± 2.1</td>
<td>1.0 ± 1.2</td>
<td>2.7 ± 2.5</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) Abbreviations: eGFR, estimated glomerular filtration rate; anti-PLA2R, anti-phospholipase A2 receptor antibody; PR, partial remission; CR, complete remission
<table>
<thead>
<tr>
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<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum albumin (g/L)</td>
<td>36.7 ± 6.2</td>
<td>39.6 ± 3.9</td>
<td>33.4 ± 6.8</td>
<td>0.007</td>
</tr>
<tr>
<td>eGFR (mL/min/1.73m²)</td>
<td>82 ± 25</td>
<td>88 ± 24</td>
<td>74 ± 25</td>
<td>0.115</td>
</tr>
<tr>
<td>anti-PLA2R titer (RU/ml)</td>
<td>8 ± 33</td>
<td>1 ± 2</td>
<td>17 ± 49</td>
<td>0.279</td>
</tr>
<tr>
<td>CD19 + B cell (/mm³)</td>
<td>33 ± 70</td>
<td>48 ± 89</td>
<td>14 ± 22</td>
<td>0.246</td>
</tr>
<tr>
<td>Immunoglobulin G (g/L)</td>
<td>9.9 ± 3.2</td>
<td>10.6 ± 3.3</td>
<td>9.1 ± 3.0</td>
<td>0.298</td>
</tr>
<tr>
<td>rituximab cumulative dose (mg)</td>
<td>747 ± 253</td>
<td>659 ± 255</td>
<td>847 ± 217</td>
<td>0.033</td>
</tr>
<tr>
<td>B-cell depletion</td>
<td></td>
<td></td>
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<tr>
<td>Time for B cell depletion (m)</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>1.1 ± 0.4</td>
<td>0.994</td>
</tr>
<tr>
<td>rituximab dose for B cell depletion (mg)</td>
<td>113 ± 34</td>
<td>112 ± 33</td>
<td>107 ± 26</td>
<td>0.898</td>
</tr>
<tr>
<td>Time to anti-PLA2R titer decrease (m)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>≥ 50%</td>
<td>1.4 ± 0.8</td>
<td>1.1 ± 0.5</td>
<td>1.7 ± 1.0</td>
<td>0.062</td>
</tr>
<tr>
<td>≥ 90%</td>
<td>4.1 ± 3.5</td>
<td>3.5 ± 3.4</td>
<td>4.8 ± 3.5</td>
<td>0.308</td>
</tr>
<tr>
<td>2 (RU/mL)</td>
<td>7.0 ± 5.0</td>
<td>5.8 ± 5.1</td>
<td>8.8 ± 4.6</td>
<td>0.126</td>
</tr>
<tr>
<td>rituximab cumulative dose for anti-PLA2R titer decrease (mg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 50%</td>
<td>137 ± 55</td>
<td>124 ± 44</td>
<td>153 ± 64</td>
<td>0.142</td>
</tr>
<tr>
<td>≥ 90%</td>
<td>341 ± 251</td>
<td>253 ± 128</td>
<td>440 ± 318</td>
<td>0.033</td>
</tr>
<tr>
<td>2 (RU/mL)</td>
<td>507 ± 362</td>
<td>335 ± 169</td>
<td>750 ± 427</td>
<td>0.007</td>
</tr>
<tr>
<td>Remission</td>
<td>25</td>
<td>16</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>PR + CR, n (%)</td>
<td>25 (78)</td>
<td>16 (94)</td>
<td>9 (60)</td>
<td>0.020</td>
</tr>
<tr>
<td>CR, n (%)</td>
<td>8 (25)</td>
<td>6 (19)</td>
<td>2 (6)</td>
<td>0.152</td>
</tr>
</tbody>
</table>

Mean ± standard deviation (SD) Abbreviations: eGFR, estimated glomerular filtration rate; anti-PLA2R, anti-phospholipase A2 receptor antibody; PR, partial remission; CR, complete remission
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<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Time to PR (m)</td>
<td>5.4 ± 3.7</td>
<td>5.1 ± 3.6</td>
<td>6.0 ± 4.0</td>
</tr>
<tr>
<td>Time to CR (m)</td>
<td>9.8 ± 2.1</td>
<td>10.1 ± 1.8</td>
<td>8.5 ± 3.5</td>
</tr>
</tbody>
</table>

Mean Â ± standard deviation (SD) Abbreviations: eGFR, estimated glomerular filtration rate; anti-PLA2R, anti-phospholipase A2 receptor antibody; PR, partial remission; CR, complete remission

**Peak And Trough Concentration Of Circulating Rituximab**

The peak and trough concentrations of rituximab were measured thrice at different treatment cycles for all patients (Fig. 2D). The peak rituximab samples were obtained on the next morning after rituximab infusion. The three peak rituximab concentrations were 21.3 ± 6.0, 21.8 ± 5.0, and 20.4 ± 3.9 µg/mL, which did not exhibit any statistically significant difference (p > 0.05). The trough rituximab samples were acquired a couple of days before the next rituximab treatment. The three trough concentrations were 0.9 ± 1.1, 1.4 ± 2.1, and 0.9 ± 0.6 µg/mL respectively for all patients, which did not exhibit any statistically significant difference (p > 0.05). From the peak and trough rituximab concentration fluctuations, we concluded that each rituximab dose of 100 mg was exhausted in one month and additional administration was needed to raise the serum level of rituximab to an effective concentration.

**Adverse Reactions**

Seven instances of mild infusion reactions occurred for a total of 241 injections. Eight episodes of infection occurred in 6 patients. All patients recovered soon after receiving antibiotics. No other serious adverse reactions were observed.

**Discussion**

Our study showed that monthly mini-dose rituximab monotherapy was successful in PLA2R-associated PMN. The NS remission rate after one year was 78% at an average dose of rituximab 750 mg (range, 300–1200 mg). The remission rate was lower in the high anti-PLA2R titer group than that in the low anti-PLA2R titer group, even though the number of required rituximab doses was higher in the former.

Rapid and long-lasting B cell depletion is essential to achieve a good therapeutic effect with B cell-targeting treatments. We set out to investigate whether rituximab 100 mg could achieve rapid B-cell depletion. Our data showed that a single dose of rituximab 100 mg could achieve B-cell depletion in 87% of individuals, which could be maintained for at least one month. We speculated that the rationale underlying this observation was as follows. First, the B cell count in peripheral blood was within the normal range in patients with PMN. Therefore, a lower dose of rituximab was needed to achieve B-cell
exhaustion compared to lymphoma. Ramachandran et al. also achieved CD19 depletion with a single dose of rituximab 100 mg.\textsuperscript{13}

Long-lasting B cell depletion is beneficial for the negative conversion of anti-PLA2R antibodies, which could be accomplished by a regular dose of rituximab with a high peak, resulting in a strong depletion of B cells initially, followed by a gradual decline.\textsuperscript{14} However, we adopted a different approach by administering rituximab monthly. Seitz-Polski et al. compared the NICE and GEMRITUX studies and found that the initial dose of rituximab administration and remission rate were higher in the former (1 g on days 1 and 15). They found that the residual rituximab level after 3 months was greater in the NICE cohort than that in the GEMRITUX cohort, which is an important factor impacting the choice of dosing regimen. Three months after administration, the serum rituximab concentration was measurable in about half of the patients in the NICE study, but undetectable in almost all patients in the GEMRITUX study (rituximab 375/m\textsuperscript{2} on days 1 and 8).\textsuperscript{15} Our regimen of monthly administration of rituximab 100 mg guaranteed a more effective rituximab concentration compared to the expected residual rituximab level at 3 months.

We think that a lower degree of loss via urine was another advantage of our mini-dose rituximab regimen. The pharmacokinetics of rituximab in PMN are unknown; some studies report that it differs substantially from that in follicular lymphoma and other autoimmune diseases.\textsuperscript{14,16} The half-life of rituximab was shorter in PMN (approximately 11.5 days) than that in lymphoma (approximately 20 days),\textsuperscript{14} probably because of the gross loss in urine.\textsuperscript{17} Thus, the standard rituximab protocol could result in greater loss via urine during the gross proteinuria period, while our mini-dose rituximab regimen resulted in lesser loss, which declined even further in the following months because of the amelioration of proteinuria.

Although the precise minimum effective dose of rituximab required for B-cell depletion is unknown, it warrants discussion. Considering the peak concentration observed in our study, we speculated that rituximab 20 + µg/mL approximated the minimum effective serum concentration. There is some evidence to support this speculation. First, it was demonstrated to be effective in our study. Second, Iijima et al. reported that almost no recurrence occurred after 3 months with an average serum rituximab concentration of 28.8 µg/mL in steroid-resistant children with nephrotic syndrome (minimal change disease or focal segmental glomerulosclerosis).\textsuperscript{18} Third, the residual concentration of rituximab (approximately 20 + µg/mL) at three months contributed to the higher remission rate in the NICE cohort compared to undetectable residual rituximab at three months with a lower remission rate in the GEMRITUX study.\textsuperscript{15} These data strengthen our claim that rituximab 20 + µg/mL is an effective serum concentration, and may approximate the minimal effective dose of rituximab.

We were unsure about the suitable interval for next dose after the initial administration of rituximab 100 mg. Based on the monthly cyclophosphamide experience, we administered rituximab 100 mg at monthly intervals. One month after rituximab 100 mg administration, the average trough rituximab concentration was 1.0 µg/mL, and the B cells were still in a state of exhaustion. We deliberated whether an additional dose of rituximab should be given or if it was more appropriate to wait for B cell replenishment. We could not find any study that utilized a similar rituximab concentration in patients with PMN, although one
study that investigated minimal change disease reported that the rate of recurrence at 3 months was significant when the average rituximab concentration was 2.3 µg/mL. Thus, we think the trough concentration of rituximab in our study was not effective and supplementation was necessary, despite the persistence of B-cell exhaustion. If the pathogenic factors causing PMN persist, B cell regeneration may lead to a resurgence or elevation in anti-PLA2R antibodies, which would be remedied by monthly rituximab supplementation. Although the metabolism of rituximab in patients with PMN is incompletely understood, a monthly intermittent mini-dose regimen could maintain the rituximab concentration between the peak-to-trough fluctuations; moreover, continuous B-cell depletion was observed, which blocked anti-PLAR antibody production, eventually achieving an immunological target response of ≤ 2 RU/mL.

The NS remission rate with the mini-dose rituximab regimen was non-inferior to the regular rituximab strategy. Our remission rate was 46.9% with a 6.3% CR rate at six months, which is not inferior to the results of previous studies. The six-month remission rate in the renowned randomized controlled trials (RCTs) was as follows: 35% in the GEMRITUX study, 435% in the MENTOR study, 544% in the STARMAN study, and 51% in the RI-CYCLO study. Our remission rate was 78% with 25% CR at twelve months (average cumulative dose of rituximab 750 mg), which was also non-inferior to the results of previous studies. The twelve-month remission rates were 60% in the Mentor study, 51% in the STARMAN study, and 62% in the RI-CYCLO study. Ramachandran et al. reported 50% remission in six patients with PLA2R-related refractory PMN with 2–4 doses of rituximab 100 mg at six months, which bore close resemblance to our results.

Single or multiple infusion strategies for rituximab 100 mg, which yielded positive results, have been reported for other autoimmune diseases, including ABO-incompatible living-donor kidney transplantation, de novo donor-specific anti-HLA antibody-associated renal transplantation, steroid-dependent minimal change NS, and steroid-refractory thrombocytopenia due to systemic lupus erythematosus. Our treatment course was relatively flexible including cumulative doses and dosing intervals. The cumulative doses varied individually according to the anti-PLA2R levels and remission. In fact, the high anti-PLA2R group required higher cumulative rituximab doses and longer treatment durations. The dosing intervals were not very strict, a delay or advance of one or two weeks was acceptable. There was a gap of up to 2–3 months in the treatment regimens of some patients due to the COVID-19 epidemic and control measures in China, visible/potential infection, or other events that were deemed more important than PMN.

A progressive decline in serum anti-PLA2R antibodies after two doses of rituximab was observed in most individuals in our study. Since well-differentiated plasma cells may continue producing anti-PLA2R antibodies, the serum anti-PLA2R antibody levels may continue to rise even after the first rituximab dose, and decrease only upon exhaustion of the existing plasma cells. This steady decline was indicative of the onset of the effect of rituximab. As long as the B cells were in a state of depletion, the decline in the anti-PLA2R antibody levels was sustained. The rate of decline of the anti-PLA2R antibodies was similar; thus,
the low-titer group was more likely to reach the target of 2 RU/mL than the high-titer group, which means that a higher dose of rituximab would be needed in the latter. Recurrence will probably occur in the future because of the small total amount of rituximab administered in our study. However, the median follow-up duration was 17 months (range, 12–29), and no recurrence was observed. This may be attributed to frequent administration and longer total effective rituximab period.

Rituximab is a B cell-targeted therapy. It has already been recommended as first-line treatment for PMN by the 2021 KDIGO guidelines. The guidelines recommend two standard treatment regimens for rituximab; however, clinically, there is controversy regarding the specific dosage and interval. Fenoglio et al. reported that the effects of rituximab 375 mg/m² administered once and four times were the same for PMN. We believe that the specific dosing regimen should be adjusted according to the patient’s age, primary disease, comorbidities, and immune status. Mini-dose rituximab may be more suitable for the “vulnerable” subset of the PMN population (such as the elderly, patients susceptible to infection, those who have newly recovered from a severe infection, or patients with very low serum immunoglobulin, and low anti-PLA2R antibody titer). The KDIGO guidelines consider an anti-PLA2R antibody > 50 RU/mL among the additional conditions for high risk of progression to ESRD. Previous studies have shown that patients with high titers of PLA2R antibodies have low rates of spontaneous remission. Therefore, we inferred that patients with PMN with high titers of anti-PLA2R antibodies treated with optimal supportive care and observation for 6 months are not optimal candidates for the mini-dose regimen. Immediate initiation of immunosuppressive therapy in conjunction with maximal supportive care is a rational regimen if clinicians consider the patient to be at a high or very high risk of progression to ESRD. From the perspective of health economics, rituximab is not covered by medical insurance in China. We achieved a non-inferior response rate compared to standard therapy using approximately one-fourth the conventional dose, which also reduced the medical cost. Our mini-dose, frequent-administration regimen makes rituximab affordable for patients with limited financial resources.

We were also concerned that continuous maintenance of B-cell depletion may affect normal immunoglobulin expression and lead to persistent humoral immune dysfunction. However, our data showed that immunoglobulin G levels at six and twelve months were significantly higher than those at baseline. Moreover, serum immunoglobulin G levels in the remission group were significantly higher than those in the non-remission group at six and twelve months, which suggested that the faster the remission of NS, the earlier the recovery of humoral immune function. A total of eight episodes of infections occurred in six patients in our cohort, all of whom recovered rapidly, confirming the safety of monthly administration of mini-dose rituximab.

Our study has some limitations. This real-world retrospective study was conducted at a single center with a small sample size. Further multicenter RCTs comparing low-dose rituximab with standard doses of rituximab are needed to confirm the efficacy and safety of this strategy.

In summary, our data suggested that monthly mini-dose rituximab monotherapy was effective in inducing CD20+ B-cell depletion and decreasing the anti-PLA2R titer, leading to remission of NS. It is probably
suitable for a certain subset of patients with PMN, such as the elderly, those susceptible to infection, those with a low anti-PLA2R antibody titer, and patients with limited financial resources.

**Abbreviations**

ALB: albumin

anti-PLA2R: anti-phospholipase A2 receptor

COVID-19: Corona Virus Disease 2019

CR: complete remission

eGFR: estimated glomerular filtration rate

ESRD: end-stage renal disease

GEMRITUX: Evaluate Rituximab Treatment for Idiopathic Membranous Nephropathy

KDIGO: Kidney Disease: Improving Global Outcomes

MENTOR: Membranous Nephropathy Trial of Rituximab

NICE: primary membranous nephropathy in the Department of Nephrology at Pasteur Hospital in Nice

NS: nephrotic syndrome

PMN: primary membranous nephropathy

PR: partial remission

RCT: randomized controlled trials

RI-CYCLO: Rituximab versus Steroids and Cyclophosphamide in the Treatment of Idiopathic Membranous Nephropathy

STARMAN: Sequential treatment with TAcrolimus-Rituximab versus steroids plus cyclophosphamide in patients with primary MEembranous Nephropathy

**Declarations**

**Ethics approval and consent to participate**

This study was conducted in accordance with the principles of the Declaration of Helsinki. This retrospective study was approved by the Peking University Third Hospital Medical Science Research
Ethics Committee (M2022060) on March 2, 2022, and registered at ChiCTR (ChiCTR2200057381) on March 10, 2022.

Consent for publication

Not applicable.

Availability of data and materials

The raw datasets analyzed in this study are available from the corresponding author upon reasonable request and with permission from the institutional review board.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Data were collected and analyzed by Song Wang, Zhenling Deng, Yue W, and Danxia Zheng, with suggestions from the other authors. Song Wang and Danxia Zheng wrote the main manuscript text and prepared figures 1 and table 1. Zhenling Deng prepared figures 2-3. Danxia Zheng and Yue Wang revised the manuscript. All authors reviewed the manuscript and approved for publication.

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References


Figures
PMN received RTX from March 2019 to July 2022 at Peking University Third Hospital, n=75

- Anti-PLA2R negative, n=6
- No nephrotic syndrome, n=3
  - RTX combined with Glucocorticoids and/or any other immunosuppressive agent, n=28
  - Single RTX dose more than 100 mg, n=3
  - Lost follow-up or follow-up less than 12 month, n=3

Enroll in the study, n=32

Figure 1. Flowchart of enrollment.

Figure 1

See image above for figure legend.
Figure 2

Immunological changes in individual patient before and after monthly mini-dose rituximab treatment.
Figure 3

Comparison of the clinical data between different anti-PLA2R titer groups before and after monthly mini-dose rituximab treatment.