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Retrospective Study

Successful Treatment of Patients With Refractory PLAR-Associated Membranous Nephropathy With low-dose Rituximab: A Single-center Experience

Refractory Membranous Nephropathy; low-dose Rituximab

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Abstract

BACKGROUND

The recognition of idiopathic membranous nephropathy (IMN) as an autoimmune disease has paved the way for the use of B-cell depleting agents such as Rituximab (RTX), which is now a first-line drug for IMN patients with proven safety and efficacy. Nevertheless, RTX for the treatment of refractory IMN patients remains controversial and challenging.

AIM

To evaluate the efficacy and safety of a new low-dose RTX regimen for the treatment of refractory IMN patients.

METHODS

A retrospective Study was performed on refractory IMN patients that accepted Low-dose RTX regimen (RTX, 200mg, once a month for five months) in Xiyuan Hospital of Chinese Academy of Chinese Medical Sciences' Department of Nephrology from October 2019 to December 2021. To assess clinical and immune remission data, we collected the 24-hour urinary protein quantitation (24 h UTP), albumin (ALB), serum creatinine (Scr), PLA2R antibody titer, and $CD19^+$ B cell count every 3 months.

RESULTS

A total of 9 refractory IMN patients were analyzed. At 12 mo of follow-up data from baseline, the 24 h UTP was decreased from 8.14 ± 6.05 g/d to 1.24 ± 1.34 g/d ($P < 0.05$), and the ALB was improved from 28.06 ± 8.42 g/L to 40.93 ± 5.85 g/L ($P < 0.01$). Notably, after administering RTX for 6 months, the Scr decreased from 78.13 ± 16.49 umol/L to 109.67 ± 40.87 umol/L ($P < 0.05$). All of the 9 patients were serum anti-PLA2R positive at the beginning, and 4 patients had a normal anti-PLA2R titer at six months. The level of $CD19^+$ B cells decreased to 0 at three months, and the count of $CD19^+$ B cells lasted to 0 until six months of follow-up.

CONCLUSION

Our low-dose RTX regimen appears to be a promising treatment strategy for refractory PLA2R-associated MN.

Key Words: Refractory nephrotic syndrome; Idiopathic membranous nephropathy; Low-dose rituximab

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Core Tip: According to the Kidney Disease Improving Global Outcomes 2021 guidelines, RTX is now the first-line therapy for patients with IMN. However, the use of RTX for the treatment of patients with refractory IMN remains challenging. We conducted a retrospective study on nine patients with refractory phospholipase A2 receptor (PLA2R)-associated MN to explore the efficacy and safety of a new low-dose RTX regimen (RTX, 200 mg, once a month for five months), and conclude that our low-dose RTX regimen is a promising treatment strategy for refractory PLA2R-associated IMN.

INTRODUCTION

Idiopathic membranous nephropathy (IMN) is a common pathological type of glomerular disease, and its incidence rate has continuously increased yearly[1]. In the past 10 years, the proportion of adult patients with IMN who have experienced renal puncture has risen from 12.2–24.9%, ranking second among primary glomerular diseases[2]. Currently, IMN is considered to be an autoimmune disease[3]. The M-type phospholipase A2 receptor (PLA2R) on the cell surface of podocytes is the major target antigen in IMN and can be found in 70–80% of IMN patients[4]. Recently, new antigens

such as thrombospondin type-1 domain-containing 7A (THSD7A), neural epidermal growth factor-like 1 protein, and semaphorin 3b have also been discovered[5, 6]. These findings have rationalized the use of B cell-depleting agents.

Rituximab (RTX) is an anti-CD20 monoclonal antibody that is used to treat several autoimmune disorders[7]. Since 2004, some reports have explored the use of RTX in patients with refractory nephrotic syndrome, and the preliminary results showed that RTX could lead to remission and reduce immunosuppressive drug use[8, 9]. According to the Kidney Disease Improving Global Outcomes (KDIGO) 2021 guidelines, RTX is now the first-line therapy for patients with IMN, and the remission rate can reach 60–80% at 12 mo[10]. However, 35–40% of IMN patients still showed no response to RTX. Refractory IMN is characterized by recurrence or resistance to RTX therapy and traditional immunosuppressive therapy, including prednisone (Pre), cyclophosphamide (CTX), and calcineurin inhibitors (CNIs)[11], etc.,. In Asia, 5–14% of refractory patients progress to end-stage renal disease (ESRD) within 10–15 years[12]. Therefore, new therapies are urgently required to treat IMN.

When introduced in the IMN treatment, RTX standard doses (375 mg/m^2 every week for four weeks or 1 g fixed-dose with a repeat dose in two weeks) were drawn from other autoimmune diseases, such as anti-neutrophil cytoplasmic antibody-associated vasculitis, rheumatoid arthritis (RA), autoimmune cytopenia, and focal segmental glomerulosclerosis[13]. Recently, some case series studies have proven the effectiveness of low-dose but repeated injection RTX regimens in treating patients with refractory IMN and showed that low-dose RTX could effectively improve the remission rate and peripheral blood B-cell elimination[14]. Currently, for the treatment of RA, the low-dose RTX regimen (500 mg twice) has replaced the original dose (1000 mg twice) and has become the new standard. Kurosu *et al*[15] reported a case of steroid-resistant NS, and the kidney pathology showed minimal changes in glomerulopathy. The patient achieved complete remission (CR) with a single dose of RTX of 375 mg/m^2. Wang *et al*[16] also reported the case of a 51-year-old man diagnosed with refractory IMN. The patient received a single dose of 100 mg RTX, and then the B cells declined rapidly, and

a gradual reduction was also observed in proteinuria. Takayuki *et al*[17] reported three patients with refractory IMN treated with single-dose RTX (500–600 mg), and two of the patients achieved complete or incomplete remission.

Based on previous studies, we evaluated the efficacy and safety of low-dose RTX in patients with refractory IMN. The regimen was 200 mg RTX once a month for five months. Compared to traditional regimens, our regimen appears to be a promising treatment strategy for refractory PLA2R-associated IMN.

MATERIALS AND METHODS

Patients. This was a retrospective case series study that included patients with refractory IMN at Xiyuan Hospital of the Chinese Academy of Chinese Medical Science Department of Nephrology from October 2019 to December 2021 (n = 9). The study protocol was approved by the ethics review board of Xiyuan Hospital, China's Academy of Chinese Medical Sciences (Beijing, China, approved No. 2022XLA130-2). All of the procedures were performed in accordance with the Declaration of Helsinki and relevant policies in China.

Acceptance and discharge standards. The inclusion criteria were: (1) patients with histologically proven IMN and PLA2R antibody-positive (Anti-PLA2R titer>20RU/mL); (2) patients diagnosed with refractory IMN, broadly defined, who remained in nephrotic syndrome after six months of regular corticosteroid and immunosuppressive therapy, such as Pre, CTX, cyclosporine (CsA), tacrolimus (TAC), mycophenolate mofetil (MMF), and RTX[10]; (3) patients who signed the informed consent form for the low-dose RTX regimen and fully understood the risks of treatment; and (4) a follow-up period > 1 year with an interval between visits of <3 mo or >4 visits per year. Patients had complete clinical data, including routine blood tests, liver and kidney function tests, 24-hour urinary protein quantitation (24 h UTP), serum anti-PLA2R antibodies, and $CD19^+$ B cells. The exclusion criteria were: (1) secondary membranous nephropathy, such as lupus nephritis, hepatitis B virus, hepatitis C, and tumors; (2) glomerular diseases combined with other types; (3) severe infections; and (3) allergic constitution.

Intervention. The low-dose RTX regimen was administered at 200 mg once a month for five months. Notably, after the patients were treated with RTX, the dosage of the primary therapeutic immunosuppressant was gradually decreased in all patients. If the patient's blood pressure remained >130/80 mmHg, an appropriate angiotensin receptor blocker was added to stabilize blood pressure.

Every three months, clinical data such as 24 h UTP, serum albumin (ALB), serum creatinine (Scr), PLA2R antibody titer, and $CD19^+$ B cell count were evaluated.

Efficacy evaluation criteria. The primary outcome was clinical complete remission. A composite remission was defined as a complete response (CR) or partial response (PR). Proteinuria of <0.3 g/d is defined as CR, while PR is defined as a 24 h UTP of 0.3 g to 3.5 g/d, or 24 h UTP decreased by more than 50% compared to baseline. No reaction (NR) was defined as a decrease in proteinuria of less than 30% or renal function deterioration.

The secondary outcomes were 24 h UTP, ALB, Scr, Anti-PLA2R titer, $CD19^+$ B cell count, adverse events, and a composite endpoint of 40% reduction in eGFR, doubling of serum creatinine, ESRD, and death.

Statistical methods. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, N.Y., USA). Data is presented as the mean ± standard deviation (SD) unless otherwise indicated. The comparison of each indicator before and after treatment was performed using a paired sample t-test, and unsatisfactory results were expressed as the median. The paired rank-sum test was used to compare before and after the treatment. Qualitative data was expressed as percentages (%). Comparisons between the groups were performed using the chi-square test, and the test level was 0.05.

RESULTS

Baseline characteristics. Nine patients with refractory IMN were treated with low-dose RTX at our center between October 2019 and December 2021. The baseline features of the patients are listed in Table 1. There were five males and four females, with an

average age of 44.0 ± 11.7 years. Of these, six patients were diagnosed with IMN by kidney biopsy, and the other three patients were diagnosed with a serum anti-PLA2R titer >100 RU/mL. Furthermore, all nine (100%) patients were serum anti-PLA2R positive at the beginning of the trial. Before the administration of RTX, all nine patients had received regular corticosteroid and immunosuppressive therapy for at least six months and remained in nephrotic syndrome; therefore, they were diagnosed with refractory IMN. Of these, six patients were steroid-resistant and three patients were steroid-dependent.

Furthermore, at baseline, all nine patients had adverse reactions triggered by the above treatment, including two with elevated serum glucose, one with hypertension, one with Cushing syndrome; one combined with steroid-induced diabetes, hypertension, and abnormal liver function; one combined with hypertension and Cushing syndrome; one combined with steroid-induced diabetes and Cushing syndrome; and one combined with steroid-induced diabetes, abnormal liver function, and abnormal liver function. **Clinical outcomes during follow-up.** The clinical outcomes of the nine patients during 12 mo of follow-up are listed in Table 2 and Supplementary Table 1. The proportion of patients who achieved complete or partial remission (PR) over time and the trend of remission rate, 24 h UTP ALB, and Scr levels are shown in Figure 1. The PR and CR rates gradually increased with increasing treatment time. The remission rates were 56% (5/9; PR 5) at three months, 67% at six months (6/9; PR 6), 89% at nine and twelve months (8/9; CR 3 and PR 5), and one patient did not complete the course of treatment due to infusion reactions.

The comparison data of the clinical outcomes during 12 mo of follow-up is shown in Table 3. In the eight patients treated with a low dose of RTX for six months, the 24 h UTP and ALB showed no significant changes compared with those before treatment. Nine months later, the 24 h UTP decreased from 8.14 ± 6.05 g/d to 1.74 ± 1.81 g/d ($P < 0.05$), and the ALB improved from 28.06 ± 8.42 g/L to 36.84 ± 6.74 g/L ($P < 0.05$). At the end of the 12-month follow-up, 24 h UTP decreased to 1.24 ± 1.34, and ALB improved to 40.93 ± 5.85 g/L, a significant difference compared with those before treatment ($P < 0.05$,

P <0.01). This means that the longer the follow-up period, the more remission may be observed. Notably, after administering RTX, there was a significant difference in Scr levels compared with those before treatment ($P < 0.05$).

Immunologic remission during follow-up. We used serum PLA2R antibodies and $CD19^+$ B cells to assess immune remission. As shown in Table 4, all nine patients had elevated serum PLA2R antibody titers at the beginning of the trial. Concerning the anti-PLA2R titers before RTX administration, three patients had low titers of anti-PLA2R (≤50 IU/L), two patients had medium titers of anti-PLA2R (50-150 IU/L), and four patients had a high titer of anti-PLA2R (> 150 IU/L). Of them, eight patients were followed up for six months, and the titers of anti-PLA2R decreased after administering RTX. Furthermore, one patient (no. 6) PLA2R antibody was negative at three months; at the six-month follow-up, four patients (no. 1, 6, 8, and 9) had negative PLA2R antibody. The level of $CD19^+$ B cells decreased to 0 at three months, and the count of $CD19^+$ B cells lasted to 0 until six months of follow-up.

Adverse events. During the median (8.7 ± 3.7) months of follow-up with the nine patients with adverse reactions, there was one patient with shivering. Shivering occurred during the first infusion of RTX, but no dyspnea or fever occurred. Symptoms were relieved 10 minutes after terminating RTX treatment, and no RTX was infused afterward, so the patient did not complete the treatment. Two patients had a fever. Fever occurred within 24 hours after the infusion of RTX. The body temperature was 37.5–38.5 °C but returned to normal within 48 h without antibiotics, and the fever did not recur.

DISCUSSION

IMN is the most common cause of primary nephrotic adults. The prognosis of patients with IMN varies greatly, with around 1/3 of the patients progressing to ESRD within 10–15 years, and another 1/3 will be relieved[3]. To date, alkylating agents are the only drugs with proven efficacy in preventing the development of ESRD[18]. Therefore, corticosteroid therapy combined with alkylating agents has been recognized as the

treatment of choice for decades. Other immunosuppressive agents[19], such as CNIs, have been tested only in trials using proteinuria reduction as an alternative endpoint.

Although an effective immunosuppressive treatment scheme for IMN has been established clinically, 20–30% of IMN patients are resistant to standard immunosuppressive therapy or often relapse[20]. These patients were diagnosed with refractory IMN. In Asia, 5–14% of refractory IMN patients will progress to ESRD. Therefore, effective and safe therapeutic strategies for refractory IMN should be explored. During the past decade, significant advances in understanding of IMN have established that it is an autoantibody-driven disease[21, 22]. As a result, there is a clear choice for treating B cell depletion. Currently, RTX is the most necessary immunosuppressive treatment for IMN. The results of the MENTOR trial provide the basis for RTX as the first-line treatment for patients with IMN[23].

Although the results of RTX are promising, it should be noted that approximately 30–40% of cases face treatment failure, which means that other treatment regimes are needed. Currently, some studies have focused on the paradigm shift from RTX to new alternatives or combined drugs in treating patients with refractory IMN to overcome drug resistance. According to the 2021 KDIGO guidelines, if the treatment is ineffective and the estimated glomerular filtration rate (eGFR) remains stable, if eGFR is decreasing, the addition of CNI for treatment could choose CTX. However, the second course of RTX may achieve remission even in the setting of resistant RTX after the first course[24].

However, the natural course of IMN varies greatly, so it is not suitable for all patients to receive a unified treatment. Therefore, the optimal RTX dose of RTX in the treatment of IMN remains controversial. Different RTX application regimens were used across various studies, ranging from a single dose of 375 mg/m^2 to a repeated dose of 375 mg/m^2 for four weeks after six months. A prospective clinical study evaluated the low-dose RTX regimen (375 mg/m^2, once) compared with the standard RTX regimen (375 mg/m^2, four times)[25]. If the consumption of B cells is insufficient, the same dose can be used in the low-dose RTX group. The results showed that of 12 patients

administered low-dose RTX, only one needed a second dose to achieve complete B-cell depletion, and at one year, the remission rate of the two groups was the same. They also concluded that a single-dose RTX regimen was extremely cost-effective and may limit the production of antichimeric antibodies, lowering the risk of adverse reactions. Similarly, another retrospective study compared 42 patients administered low-dose RTX (375 mg/m^2)[26]. The control group was treated with steroid hormones combined with alkylating agents or a standard RTX regimen (375 mg/m^2, four times). At 24 mo, there was no significant difference in clinical outcomes between the two groups. All patients treated with RTX showed complete B-cell depletion in the first month, but B-cell recovery occurred earlier in the low-dose group than in the standard group. Recently, some case reports and case series studies have proven the effectiveness of low-dose but repeated injection RTX regimens in treating patients with refractory IMN. These regimens seem to extend the inhibition of B cells. We explored a new RTX regimen (200 mg once a month for five months) for treating patients with refractory IMN in our hospital.

Nine patients with refractory PLAR-associated MN were included in this analysis. In the data provided 12 mo after baseline, the remission rate was 56% (5/9; PR 5) at three months, 67% at six months (6/9; PR 6), and 89% at nine and twelve months (8/9; CR 3 and PR 5). Another patient did not complete the treatment because of infusion reactions. In a review of currently available studies, the overall remission rates (complete and partial) of RTX at 12 mo of treatment for patients with IMN consistently ranged from 44–85%. Some studies have reported that among patients with refractory IMN, the efficacy rate of RTX can only reach 40%. The remission rate of the low-dose RTX regimen at our center was significantly higher than that reported in previous studies.

In addition, we observed that clinical remission in patients with refractory IMN was slower than in patients with immune remission. We used serum PLA2R antibodies and CD19$^+$ B cells to assess immune remission. The level of CD19$^+$ B cells decreased to 0 at three months, and the count of CD19$^+$ B cells lasted to 0 until six

months of follow-up. At the beginning of the trial, all nine patients were positive for the PLA2R antibody. Of these, the titers of anti-PLA2R all decreased after administering low-dose RTX, and one patient (no. 6) had a normal anti-PLA2R titer at the time of three months, and at the six-month follow-up, four patients (no. 1, 6, 8, and 9) had a normal anti-PLA2R titer. However, compared with the previous situation, the 24 h UTP and ALB levels showed no significant changes at six months. Until nine months ago, 24 h UTP decreased from 8.14 ± 6.05g/d to 1.74 ± 1.81 g/d ($P < 0.05$), while ALB has increased from 28.06 ± 8.42 g/L to 36.84 ± 6.74 g/L ($P < 0.05$). At the end of the 12-month follow-up period, the clinical outcomes were significantly enhanced compared with those before treatment. This means that the longer the follow-up time, the more remission was observed. Notably, none of the eight patients had a recurrence until the submission date.

Compared with the standardized dose (375 mg/m^2 every week for four weeks, or 1 g fixed-dose with a repeat dose in two weeks), our low-dose RTX regimen lasted for B cells at a lower level for a longer time, so that the disease seemed less likely to relapse. Furthermore, compared with the high-dose RTX regimen and traditional immunosuppressive regimen, our low-dose RTX regimen also has advantages in terms of security. Although effective immunosuppressive regimens for IMN have been established clinically, the preceding therapeutic options have significant disadvantages, which limit their application, especially in patients with renal insufficiency. At the beginning of the trial, eight patients had a side reaction associated with previous immunosuppressive treatment. During the 12-month follow-up concerning adverse reactions, there was only one case of shivering. Notably, after administering RTX for six months, the Scr level decreased from 78.13 ± 16.49 umol/L to 109.67 ± 40.87 umol/L ($P < 0.05$). In this study, we found that our low-dose RTX regimen for the treatment of refractory IMN significantly improved clinical outcomes.

However, it is worth noting that this research also has some limits that should be mentioned. First of all, our study was retrospective and the sample size was small that only 9 patients, the statistical analysis of data may be biased inadequacies. Secondly, the

clinical outcomes average follow up period was 1 year, but the data of Immunological indicators included serum anti-PLA2R titer and CD 19^{+}B cell count were only 6-month follow-up data. The long-term prognosis of our low-dose RTX regimen for refractory IMN patients remaining to be researched. To confirm these results, we need to conduct large-scale clinical trials and further clarify the mechanism of action of RTX to deepen the interpretation.

CONCLUSION

RTX for the treatment of refractory IMN patients remains controversial and challenging. Conventional immunosuppressive treatment strategies have been used for many years and have clinically established therapeutic efficacy. In conclusion, we found that our low-dose RTX regimen for the treatment of refractory IMN significantly improved clinical outcomes and further increased the remission rate. Moreover, treatment with our low-dose RTX regimen is more cost-effective than high-dose RTX.

ARTICLE HIGHLIGHTS

Research background

The recognition of idiopathic membranous nephropathy (IMN) as an autoimmune disease has paved the way for the use of B-cell depleting agents such as Rituximab (RTX), which is now a first-line drug for IMN patients with proven safety and efficacy.

Research motivation

RTX for the treatment of refractory IMN patients remains controversial and challenging.

Research objectives

To evaluate the efficacy and safety of a new low-dose RTX regimen for the treatment of refractory IMN patients.

Research methods

A retrospective Study was performed on refractory IMN patients that accepted Low-dose RTX regimen (RTX, 200mg, once a month for five months).

Research results

A total of 9 refractory IMN patients were analyzed. At 12 mo of follow-up data from baseline, the 24 h UTP was decreased from 8.14 ± 6.05 g/d to 1.24 ± 1.34 g/d ($P < 0.05$), and the ALB was improved from 28.06 ± 8.42 g/L to 40.93 ± 5.85 g/L ($P < 0.01$). Notably, after administering RTX for 6 months, the Scr decreased from 78.13 ± 16.49 umol/L to 109.67 ± 40.87 umol/L ($P < 0.05$). All of the 9 patients were serum anti-PLA2R positive at the beginning, and 4 patients had a normal anti-PLA2R titer at six months. The level of $CD19^+$ B cells decreased to 0 at three months, and the count of $CD19^+$ B cells lasted to 0 until six months of follow-up.

Research conclusions

We found that our low-dose RTX regimen for the treatment of refractory IMN significantly improved clinical outcomes and further increased the remission rate.

Research perspectives

Treatment with our low-dose RTX regimen is more cost-effective than high-dose RTX.

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