ORIGINAL ARTICLE

Low-dose Rituximab therapy in resistant idiopathic membranous nephropathy: single-center experience

Soumita Bagchi[1], Arun Kumar Subbiah[1], Dipankar Bhowmik[1], Sandeep Mahajan[1], Raj Kanwar Yadav[1], Mani Kalaivani[2], Geetika Singh[3], Amit Dinda[3] and Sanjay Kumar Agarwal[1]

[1]Department of Nephrology, All India Institute of Medical Sciences, New Delhi, India, [2]Department of Biostatistics, All India Institute of Medical Sciences, New Delhi, India and [3]Department of Pathology, All India Institute of Medical Sciences, New Delhi, India

Correspondence and offprint requests to: Soumita Bagchi; E-mail: soumita_bagchi@yahoo.co.in

Abstract

Background: Persistent significant proteinuria has been associated with increased risk of progression to end-stage kidney disease in patients with idiopathic membranous nephropathy (IMN). Rituximab (RTX) therapy has given encouraging results in IMN, but most of the studies have used a higher dose, which is limited by the high cost as well as a potential increased risk of infections. Our study aimed to assess the efficacy and safety of low-dose RTX in patients with immunosuppression-resistant IMN.

Methods: A total of 21 patients with treatment-resistant IMN treated with RTX from 2015 to 2016 at our center were included in the study. They received two doses of RTX (500 mg each) infusion 7 days apart. CD19 count was performed after 4 weeks. A single dose of RTX was repeated after 4–6 weeks if CD19 count was not depleted.

Results: The mean standard deviation age of patients was 33.3 ± 12.3 years and 33.3% were females. Mean proteinuria before RTX therapy was 6.2 ± 2.2 g/day, serum creatinine was 0.9 ± 0.3 mg/dL and estimated glomerular filtration rate (eGFR) was 95.8 ± 26.9 mL/min/1.73 m^2. All the patients were non-responders to prior immunosuppressive treatment. Twenty (95.2%) patients achieved targeted CD19 depletion with two doses of RTX. One patient required one additional RTX dose due to inadequate B-cell suppression. A total of 13 (61.9%) patients achieved remission with RTX therapy: 4 (19.0%) complete and 9 (42.9%) partial remission. Patients who did not respond to RTX had a significantly lower baseline eGFR compared with those who achieved remission ($P = 0.022$). One patient developed respiratory tract infection following RTX during the follow-up, which responded to a course of oral antibiotics. During median follow-up of 13.1 (10–23.9) months, four (19%) patients had deterioration in renal function and one patient relapsed after achieving partial remission. Renal survival was significantly better in patients who responded to RTX therapy as compared with those who did not achieve remission ($P = 0.0037$).

Conclusion: Low-dose RTX therapy is effective and safe in immunosuppression-resistant IMN.

Key words: immunosuppression, membranous nephropathy, outcome, proteinuria, rituximab

Introduction

Idiopathic membranous nephropathy (IMN) is a common cause of proteinuria and nephrotic syndrome (NS) in adults [1]. In a large multicentric retrospective study, ~32% of patients with IMN were found to achieve spontaneous remission ~14 months after diagnosis [2]. Persistent proteinuria due to IMN is associated with progression to end-stage kidney disease in 10 years with increased risk of mortality [3]. Therefore, in patients with significant proteinuria, who do not achieve spontaneous remission with conservative management, the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (2012) recommended the modified Ponticelli regimen (MPR) comprising alternating courses of corticosteroids and cyclophosphamide as the first-line therapy [4]. Calcineurin inhibitors (CNIs), that is, tacrolimus or cyclosporine, were suggested as alternative therapy in patients resistant/intolerant to the MPR. However, MPR [5, 6] and CNIs [7, 8] achieve remission in ~60–70% patients. MPR is limited by the high incidence of adverse effects requiring withdrawal or hospitalization [9], while the use of CNI is associated with high-relapse rates and decline in renal function [7–9].

Rituximab (RTX) is a monoclonal antibody against CD20 expressed on B cells, which was initially used for the treatment of lymphoma. With the identification of auto-antibodies to podocyte antigens, phospholipase A2 receptor (PLA2R) [10] and now thrombospondin type-1 domain containing 7 A (THSD7A) [11] in IMN, the use of RTX has emerged as an important therapeutic option in these patients [12].

There are multiple studies using RTX as first-line therapy as well as in patients resistant to other immunosuppressive regimens in Western populations since 2002 [13]. However, there is a paucity of data from Asia [14]. Also, though the available data about RTX in IMN is encouraging, there is no consensus about the optimum dose. Most centers [15–19] have used 375 mg/m^2 weekly × 4 doses or 1 g on Days 1 and 15, sometimes repeated after 6 months [16]. This dosing regimen is limited by the high cost. There is also a potential risk of infections with use of biologic agents like RTX [20], especially in patients who have been previously treated with other immunosuppressive drugs. Higher doses of RTX will increase the cumulative immunosuppression exposure in such patients, which is especially significant in low- and middle-income countries like India where the infectious disease burden in the community is very high.

Studies [21–23] using lower doses of RTX have reported conflicting outcomes. Our study aimed to assess the efficacy and safety of low-dose RTX in patients with immunosuppression-resistant IMN.

Materials and methods

In this retrospective study, we included patients who had resistant IMN and were treated with low-dose RTX during 2015–16 in our nephrology department. Resistant IMN was defined as persistent NS or persistent edema with significant proteinuria despite completion of MPR (6 months) with an additional follow-up of 6 months and/or tacrolimus therapy for at least 6 months with tacrolimus trough level of 5–10 ng/mL. NS was defined as 24-h urine protein ≥3.5 g/day with serum albumin <3 g/dL and edema. Patients with 24-h urinary protein >1.5 g/day with significant edema were also considered to have significant proteinuria and treated with immunosuppression as is the practice in our center. Low-dose RTX was defined as two doses of 500 mg of RTX. All these patients had been screened for

secondary causes of MN including hepatitis B and C, malignancy and systemic lupus erythematosus. Serum anti-PLA2R antibody was assessed by ELISA before giving RTX to patients when possible.

All patients were screened for infections with complete blood count, chest, X-ray and urinalysis before giving RTX. Therapy comprised of two doses (500 mg each) given as intravenous infusion 7–10 days apart. All patients were given premedication with hydrocortisone and paracetamol. CD19 count was done 4 weeks after the second dose of RTX. B-cell depletion was defined as CD19 count <1%. Complete blood counts, serum creatinine, 24-h urinary protein and creatinine and serum albumin were assessed at the time of initiation of treatment and repeated every 4 weeks. Estimated glomerular filtration rate (eGFR) was calculated using the four-variable Modification of Diet in Renal Disease (MDRD) formula.

Additional doses of RTX (one to two) were planned after 4–6 weeks if CD19 count did not show adequate depletion. Co-trimoxazole prophylaxis was given to all patients for at least 6 months after RTX therapy.

Complete remission was defined as 24-h urinary protein <500 mg/day and partial remission as 24-h urinary protein between 500 mg and 3.5 g/day with at least 50% reduction in proteinuria from the time of initiation of therapy with eGFR maintained within 25% of the baseline. Non-responders were defined by <50% decrease in proteinuria with or without sustained decline in eGFR. Renal deterioration was defined as sustained decline in eGFR ≥50% from baseline documented at least twice.

The study was approved by the Institute Ethics Committee of our hospital.

Statistical methods

Statistical analysis was carried out using STATA 12.0 (College Station, TX, USA). Data were summarized as frequency (%) or mean ± standard deviation (SD)/median (range) as appropriate. The baseline categorical and continuous variables were compared between those who achieved remission and were non-responders using the chi-square test/Fisher's exact test and independent *t*-test/Wilcoxon rank sum test, respectively. The impact of remission on renal survival was assessed using the Kaplan–Meier method and log-rank test for comparisons. A P-value of <0.05 was considered statistically significant.

Results

A total of 23 patients with biopsy-proven resistant IMN received low-dose RTX therapy during the study period. Two patients were lost to follow-up within 3 months and thus 21 were included in the final analysis. Their characteristics are shown in Table 1. The mean age of patients was 33.3 ± 12.3 years and 33.3% were females. Two patients had associated diabetes and six had hypertension. Mean proteinuria before RTX therapy was 6.2 ± 2.2 g/day. As shown in Table 2, two patients had less than nephrotic range proteinuria (3.4 g/day) but were symptomatic with edema and responded to treatment subsequently. Mean serum creatinine level was 0.9 ± 0.3 mg/dL and mean eGFR was 95.8 ± 26.9 mL/min/1.73 m^2. Two patients had eGFR <60 mL/min/1.73 m^2.

Immunosuppression received before RTX by these patients were (Table 2): six MPR, two tacrolimus, eight MPR and tacrolimus, two tacrolimus and mycophenolate mofetil (MMF),

Table 1. Characteristics of patients with and without response to RTX therapy

	Total (n = 21)	Remission (n = 13)	No remission (n = 8)	P
Age (years), mean ± SD, median (range)	33.3 ± 12.3, 32 (15–62)	32.6 ± 14.1, 32 (15–62)	34.5 ± 9.5, 35.5 (22–46)	0.446
Gender (females) [n (%)]	7 (33.3)	6 (85.7)	1 (14.3)	0.133
Baseline serum creatinine (mg/dL), mean ± SD	0.9 ± 0.3	0.8 ± 0.3	1.1 ± 0.2	0.033
Baseline eGFR (mL/min/1.73m^2), mean ± SD	95.8 ± 26.9	104.6 ± 28.8	81.5 ± 16.4	0.022
Baseline urinary protein (g/day), mean ± SD	6.2 ± 2.2	5.9 ± 2.6	6.7 ± 1.6	0.157
Baseline serum albumin (g/dL), mean±SD	2.5 ± 0.5	2.5 ± 0.6	2.4 ± 0.5	0.423
ACEi/ARB [n (%)]	19 (90.5)	11 (84.6)	8 (100)	0.371
Worsening renal function [n (%)]	4 (19.0)	0	4 (50%)	0.012

Table 2. Treatment details of individual patients

No.	Age (years)	Gender[a]	SCr 1 (mg/dL)	SAlb 1 (g/dL)	Urinary protein 1 (g/day)	Prior IS before RTX	Serum anti-PLA2R antibody before RTX therapy	Follow-up after RTX therapy (months)	SCr 2 (mg/dL)	SAlb 2 (g/dL)	Urinary protein 2 (g/day)	Status at last follow-up
1	19	M	0.8	1.8	12.2	MPR	ND	20.5	0.6	4.3	0.2	CR
2	32	M	0.8	3.1	8.8	MPR	POS	12.8	0.8	5	0.3	CR
3	43	F	0.6	2.8	7.1	MPR/TAC	POS	11.1	0.5	4.7	0.4	CR
4	18	M	0.8	1.6	6	TAC	NEG	11.2	0.7	3.8	0.2	CR
5	20	M	1.5	2.5	6.5	MPR/TAC	POS	22	1.4	5	2.2	PR
6	32	M	0.7	2.4	6	MPR	ND	23.9	0.8	4.8	0.8	PR
7	15	F	0.6	2.1	3.6	MPR	NEG	18.6	0.6	3.8	1.8	PR–relapsed during follow-up, responded to TAC
8	21	F	0.7	2.6	3.7	MPR/MMF/TAC	POS	18	0.6	3.6	1.2	PR
9	51	M	0.8	3.1	3.4	MPR/TAC/MMF	ND	16.5	0.8	4.7	1.3	PR
10	62	F	0.5	2.6	6.2	MPR	NEG	13.1	0.5	4.2	1.8	PR
11	37	F	0.8	3	3.4	TAC/MMF	ND	17.4	0.7	4.5	1.2	PR
12	34	M	0.8	2.4	6.2	MPR/TAC	POS	12.6	0.8	4.2	1.0	PR
13	40	F	1.5	3.1	3.6	MPR/TAC	POS	10	1.5	4.1	1.5	PR
14	40	M	0.9	1.6	4.7	TAC	ND	10.8	3.4	3.8	4.5	NR
15	46	M	1	2.8	7	MPR/TAC	POS	18.8	3.3	2.9	10.0	NR
16	33	M	1.3	2.4	5	MPR/TAC	NEG	14.3	2	3.6	11.0	NR
17	46	M	1.1	2.9	9.6	MPR/TAC	POS	12.2	1.2	1.7	15.4	NR
18	22	F	0.7	2.6	6.3	MPR/TAC/MMF	POS	13.8	0.5	2.2	6.0	NR
19	27	M	1	1.8	8	MPR	ND	12.4	5	2	6.3	NR
20	24	M	1	2.5	7.1	MPR/TAC	NEG	11.7	1.2	3.9	7.7	NR
21	38	M	1.4	2.9	6.4	TAC/MMF	POS	10.3	2.9	2.9	7.3	NR

[a]M, male; F, female.
SCr 1, SAlb 1, urinary protein 1—before giving RTX, TAC; SCr 2, SAlb 2, urinary protein 2—at last follow-up; CR, complete remission; PR, partial remission; NR, no response; ND, not done; POS, positive; NEG, negative; TAC, tacrolimus; IS, immunosuppressive therapy; SCr, serum creatinine; SAlb, serum albumin.

and three MPR, tacrolimus and MMF. All the patients had not responded to the prior immunosuppressive treatment.

Pre-treatment serum anti-PLA2R antibody levels were available in 15 patients, of which 10 were positive. Five patients who were PLA2R-negative did not have any evidence of secondary cause and were therefore considered as IMN. Nineteen (90.5%) patients were on angiotensin-converting enzyme inhibitors (ACEi)/angiotensin receptor blockers (ARBs). Patients who did not respond to RTX had a significantly lower baseline eGFR compared with those who achieved remission (P = 0.022). All other baseline characteristics were similar in the two groups.

A total of 13 (61.9%) patients achieved remission with RTX therapy: 4 (19.0%) complete and 9 (42.9%) partial remission. Twenty (95.2%) patients achieved targeted CD19 depletion with two doses of RTX. One patient required one additional RTX dose due to inadequate B-cell suppression after two doses and

subsequently developed partial remission. Median time to remission after last dose of RTX was 2.7 months (1.2–7 months). One patient developed respiratory tract infection following RTX during the follow-up, which responded to a course of oral antibiotics. No other adverse effect was reported in any other patient.

During a median follow-up of 13.1 (10–23.9) months, four (19%) patients had deterioration in renal function. Renal survival was significantly better in patients who responded to RTX therapy as compared with those who did not achieve remission (Figure 1, P = 0.0037). Of eight patients who did not respond to RTX, six received additional doses (one or two) of RTX and two patients received subcutaneous adrenocortical hormone therapy but with no response. Four patients who had not responded to RTX therapy underwent repeat kidney biopsy, of which three showed increase in interstitial fibrosis and tubular atrophy. One
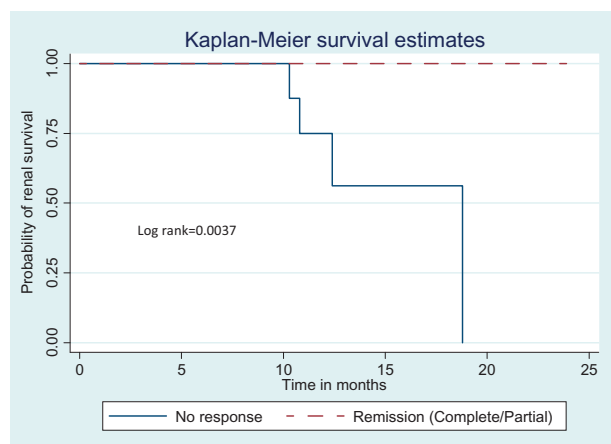
Fig. 1. Renal survival in patients with and without remission following RTX therapy.

patient who had achieved initial remission relapsed during the study period.

Discussion

There has been a paradigm shift in the management of IMN with the discovery of the pathogenic role of PLA2R antibodies and the use of RTX therapy. MPR and CNIs have conventionally been used for treating persistent NS due to IMN. These drugs, though effective, have been associated with significant adverse effects [7–9] and high-relapse rates. In this context, RTX is an attractive treatment option, considering its ease of administration and minimal risk of noncompliance. Initial experience with RTX in IMN has been positive with multiple studies using it as first as well as second-line immunosuppressive therapy in these patients [15–19, 21]. However, ambiguity about the optimum dose of RTX continues. Initial studies [15–19] have used the conventional higher doses of RTX. This is limited by the high cost and the potential risk of infections, which are especially relevant in low- and middle-income countries. Subsequently, lower doses of RTX have been used using CD19 counts to decide the dose and monitor response.

We analyzed our experience with low-dose RTX in 21 patients with resistant IMN. All these patients had initially received MPR and/or tacrolimus, which are currently considered to be the standard immunosuppressive therapy offered to patients of IMN with persistent NS. Two doses of RTX achieved B-cell depletion in all except one patient, who required one additional dose. Other studies have also reported adequate B-cell depletion with two doses of RTX [21–23]. In a study evaluating the efficacy of B-cell-driven therapy, even a single dose of RTX (375 mg/m^2) was sufficient to achieve full CD19 depletion [20].

In our study, 61.9% patients achieved remission during a median follow-up of 13 months. All our patients had received prior immunosuppressive therapy with no response, thus they had difficult-to-treat disease. Despite this, our response rate was similar to other studies, which have shown remission in 60–70% of patients [15–19, 21, 23]. The time to remission after last dose of RTX was 2.7 months (1.2–7 months) in our study. This response time is shorter than that reported from other studies, where patients have responded even after 12 months [18, 19, 23]. Whether more patients will ultimately achieve remission with longer follow-up in our cohort needs to be seen.

There are conflicting data about the efficacy of low-dose RTX in IMN. Cravedi *et al.* [21] found low-dose RTX therapy titrated based on B-cell depletion to be equally effective as standard protocol of four weekly doses of 375 mg/m^2, with a remission rate of >60%. In a cohort of 100 patients, Ruggenenti *et al.* reported remission in 65% following RTX therapy [18]. The patients in this study had received four doses of RTX (375 mg/m^2) until 2005 and subsequently lower doses were given using a B-cell-driven protocol. The GEMRITUX trial [23], which randomized patients with IMN and NS to two doses of RTX (375 m/m^2) versus no immunosuppressive therapy after an adequate period of conservative management, did not show any significant difference between the two groups at 6 months, but with extended follow-up the remission rate was significantly higher in the RTX group (64.9% versus 34.2%, P < 0.01) with no significant adverse effects compared with the untreated patients. However, the study by Moroni *et al.* [22] concluded that low-dose RTX was less efficacious in IMN. In this study, RTX was given to 34 patients with IMN (19 as first-line therapy, 15 had previously received immunosuppressive therapy with resistant or relapsing disease). At 6 and 12 months, 44.1% patients had achieved remission, which was lower than other studies though all their patients had achieved adequate B-cell depletion. Though this was a prospective study, 18 patients had received a single dose and 16 had received two doses of RTX. The authors have not mentioned the criteria based on which the dose was decided for each patient. In their cohort, 41% patients had eGFR <60 mL/min/1.73 m^2, suggesting higher chronicity. We had only 14.3% of patients with eGFR <60 mL/min/1.73 m^2 in our study. We also observed that our patients who did not respond to RTX therapy had a significantly lower baseline eGFR compared with those who achieved remission. Some of these patients had features of chronicity on repeat biopsy, which may explain the lack of response to immunosuppression [10, 24]. There were no significant adverse effects observed in our study barring an episode of lower respiratory tract infection in one patient. The absence of cardiovascular events, as observed in other studies [18, 22, 23], following RTX infusion may be attributed to the younger age of our patients.

Our study, being retrospective, has some limitations. We did not have serum anti-PLA2R levels for all the patients. The follow-up period of 13 months was short and the response rate may improve with longer follow-up. Since the use of low-dose RTX in Caucasian populations has shown variable results, further studies are needed to determine whether the Indian patients *per se* require lower doses of RTX for B-cell depletion.

To conclude, low-dose RTX is safe and efficacious for the treatment of resistant IMN. Further studies with longer follow-up and more frequent anti-PLA2R antibody and CD19 count monitoring are needed to determine the lowest possible effective dose of this antibody.

Conflict of interest statement

None declared.

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