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Long-Term Prognosis of Adult Patients with Steroid-Dependent Minimal Change Nephrotic Syndrome Following Rituximab Treatment

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Abstract: This study was to evaluate the long-term efficacy and safety of a single-dose rituximab regimen rituximab treatment in adult patients with steroid-dependent minimal change nephrotic syndrome (MCNS).

We conducted a prospective cohort study with historical controls to evaluate the effect of single-dose infusions of rituximab at 375 mg/m^2 BSA per dose administered at intervals of 6 months for a period of 24 months. At the end of the 24-month period, the patients were divided into the treatment continuation (n = 20) and treatment discontinuation (n = 5) groups according to their intention to continue/discontinue the treatment.

A significant reduction in the total number of relapses was observed during the 24-month period after the first rituximab infusion as compared with that during the 24-month period before the first rituximab infusion (108 vs. 8, $P < 0.001$). Complete remission was induced/maintained in all patients from 12 to 24 months after the first rituximab infusion. In regard to the clinical course after 24 months, 4 of the 20 patients in the treatment continuation group discontinued the rituximab treatment after the fifth infusion and 2 patients discontinued the treatment after the sixth infusion. However, complete remission was maintained in all the 20 patients of this group during the 12-month observation period after the first four single-dose rituximab infusions. On the other hand, 1 of the 5 patients in the treatment discontinuation group developed relapse during the observation period after the first four rituximab infusions, and the rituximab treatment was resumed.

In our trial, rituximab therapy was associated with maintenance of complete remission. Complete remission was maintained even in most of the patients who showed B-cell repletion after discontinuation of rituximab therapy. Thus, rituximab may be considered as a radical therapeutic agent for patients with steroid-dependent MCNS.

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Abbreviations: CyA = cyclosporine, CYC = cyclophosphamide, MCNS = minimal change nephrotic syndrome, MMF = mycophenolate mofetil, MZ = mizoribine, RRED = prednisolone, TAC = tacrolimus.

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INTRODUCTION

Steroid-dependent minimal change nephrotic syndrome (MCNS) necessitates the administration of prolonged courses of treatment with prednisolone (PRED). Therefore, most of these patients require the addition of another immunosuppressive drug(s), such as cyclosporine (CyA), tacrolimus (TAC), mycophenolate mofetil (MMF), cyclophosphamide (CYC) or mizoribine (MZ), to reduce the number of relapses and prevent the major side effects of steroid treatment.[1] However, these immunosuppressive medications may be unable to induce remission and may also have significant adverse effects of their own.[2,3] Recently, a number of publications have reported the usefulness of rituximab for the treatment of MCNS in pediatric patients,[4–13] while there are fewer reports, including our previous reports, of treatment in the adult setting.[14] The rituximab doses used for the treatment of steroid-dependent MCNS in these studies vary greatly, from a single flat dose of 500 or 1000 mg at 1 or 2 time-points, to 375 mg/m^2 BSA once weekly for 4 weeks. It is difficult to draw any robust conclusions about the optimal dosing schedule of rituximab from these previous reports.

We published the results of a prospective trial of the effects of a single dose of rituximab administered twice at an interval of 6 months in 25 steroid-dependent MCNS patients.[14] Herein, we report the results of our prospective study carried out to examine the long-term efficacy and safety of single-dose administrations of rituximab at intervals of 6 months for a period 24-months, and also the clinical courses of the patients after the rituximab treatment for 24 months in patients with steroid-dependent MCNS.

METHODS

Patient Population

Patients fulfilling the following criteria were enrolled in this study: Patients with steroid-dependent nephrotic syndrome, defined as the occurrence of relapse during the tapering down or within 2 weeks of discontinuation of PRED. Nephrotic syndrome was defined as urinary protein excretion of ≥3.5 g/day, serum albumin of <3.0 g/dL, edema, and hyperlipidemia. Relapse was defined as recurrence of massive proteinuria (daily urinary protein excretion of ≥3.5 g/day or 3+ or 4+ result of the urine albumin dipstick test for albumin); biopsy-proven diagnosis of minimal-change disease; no known associated systemic disease, including negative serology for hepatitis B and C, HIV and antinuclear antibodies, and no positive family history; no

previous history of rituximab treatment. The study was conducted with the approval of the Research Ethics Board of Tokyo Women's Medical University. All the patients gave written informed consent for participation in the study. Between March 2008 and December 2013, 25 patients (21 male and 4 female) with steroid-dependent MCNS were enrolled for the study at our department.

The mean time period from the diagnosis until the start of rituximab treatment was 13 ± 8 years. All of the patients had experienced relapses before, with a maximum of 129 relapses during the 24-month period prior to the first rituximab injection. Seven patients (28%) had nephrotic syndrome, 3 patients (12%) were in partial remission, and 15 patients (60%) were in complete remission at the time of start of the rituximab treatment. ''Complete remission'' was defined as clinical improvement or a daily urinary protein excretion of <0.3 g as judged by a trace or negative result of the urine dipstick test for albumin. ''Partial remission'' was defined by a daily urinary protein excretion of <3.5 g, below nephrotic range, with a relative increase of the serum albumin. The mean age of the patients at the time of start of rituximab treatment was 29 ± 12 years. The clinical data were examined according to the presence/absence of a history of treatment with steroids and/or conventional immunosuppressant drugs. Previously used immunosuppressant drugs in the patients, besides the steroid, included CyA ($n = 20$), MZ ($n = 6$), MMF ($n = 1$), and TAC ($n = 1$); 28 patients were receiving a steroid (PRED 24.2 ± 12.4 mg/day; dose range 5–60 mg/day) at the time of start of the rituximab treatment.

Treatment

The first rituximab dose was administered by intravenous infusion at a single dose of 375 mg/m^2 BSA. In order to minimize the infusion reactions, we administered 4 mg of betamethasone, 20 mg of monoammonium glycyrrhizinate, and 200 mg of acetaminophen to the patients 30 minute prior to the rituximab infusion. The rituximab infusion was repeated 4 times at 6-month intervals, that is, at 6 months, 12 months, and 18 months after the first infusion (a biannual, single-dose rituximab regimen $\times 24$ months, Figure 1). Thereafter, the patients were divided into two groups according to their intention to continue/discontinue the treatment, namely, the treatment continuation group ($n = 20$) and the treatment discontinuation group ($n = 5$). The patients of the treatment continuation group continued to receive the rituximab infusions at 6-month intervals according to the same dosing protocol. None of the patients received concomitant medications, including prophylaxis for Pneumocystis jiroveci (trimethoprim/sulfamethoxazole).

Follow-Up

The following laboratory parameters were examined in all the patients. An attempt was made to taper the steroid dose/discontinue the steroid by 24 months after the first rituximab injection, although no precise protocol was set for tapering the steroid dose. There were no restrictive protocols for discontinuation of either the immunosuppressants or PRED in this trial. Patients were followed up for at least 36 months after the first rituximab injection. B-cell depletion was defined as a peripheral blood CD19 count of <10/mm^3 and B-cell repletion was defined as a peripheral blood CD19 count of >10 mm^3. In addition, the cumulative dose of PRED, number of patients requiring PRED, CyA, MMF, or MZ, bone density (Bone Mineral Density [BMD] and Z score), and total number of relapses were evaluated at the baseline, during the 24-month period before the first rituximab infusion, and during the 24-month period after the first rituximab infusion. We also compared the clinical courses of the patients after the first 24 months in the treatment continuation and treatment discontinuation groups. The primary endpoints were the number of relapses

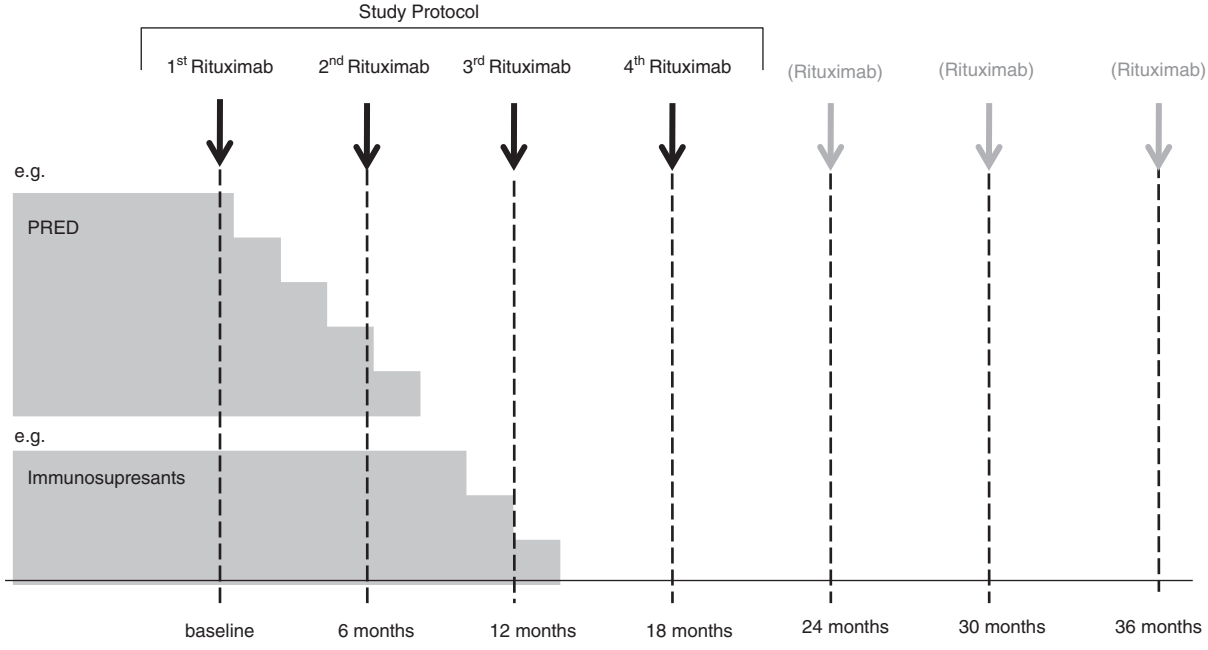

FIGURE 1. Study protocol. Rituximab was administered by intravenous injection at the dose of 375 mg/m^2 BSA at the baseline, and at 6, 12, and 18 months after the first dose. Thereafter, rituximab treatment was continued or discontinued. No precise protocol was set for tapering the steroid dose. There were no restrictive protocols for discontinuation of either the immunosuppressants or PRED.

and number of patients requiring PRED and/or immunosuppressant drugs. The second endpoints were the frequency and severity of the adverse events of PRED, including osteoporosis, and those of rituximab.

Statistical Analysis

Data were expressed as means ± standard deviation (SD). All analyzed variables were tested for distribution. The *t* test was used for samples with a normal distribution and the Mann–Whitney *U* test for samples with a skewed distribution, to analyze the differences in the laboratory data recorded between the baseline and at 1 month and 6 months after the first rituximab injection. Categorical data were analyzed by the χ^2 test. All the statistical analyses were performed using the JMP 9 software (SAS Institute, Cary, NC). Statistical significance was set at $P < 0.05$.

RESULTS

Rituximab Treatment (Biannual, Single-Dose Rituximab Regimen ×24 months)

The characteristics of the patients at the baseline (at the time of the first rituximab injection) and the clinical courses of the patients after the first rituximab injection are shown in Table 1. Complete remission was achieved within 1 month of the first rituximab infusion in all the 7 patients who had nephrotic syndrome at the baseline. Six relapses occurred during the first 6 months of follow-up (between the first and second rituximab doses), and 2 relapses occurred during the 6- to 12-month period after the first rituximab dose (between the second and third rituximab doses), however, no relapses occurred during the 1-year period from 12 to 24 months after the first rituximab infusion. Comparison of the total number of relapses between the 24-month period before the first rituximab injection and the 24-month period after the first rituximab injection revealed a significantly lower number of relapses during the latter period (108 vs. 8; $P < 0.01$) (Figure 2).

Significant decreases of the urinary protein excretion, serum total cholesterol and CD19/CD20 counts, and significant increase of the serum albumin were found at all the measurement points during the 24-month period after the first rituximab dose, as compared to the values recorded at the baseline. No significant changes in the serum creatinine levels were noted during the trial period.

The steroid could be discontinued by 24 months after the first rituximab administration in all the patients who were receiving a steroid at the time of the first rituximab infusion. In all of the patients, tapering of the PRED dose could be begun after the first administration of rituximab. The number of patients requiring PRED and/or immunosuppressant drugs was compared between the baseline and at the end of 24 months after the first rituximab infusion. The number of patients requiring PRED as well as the mean daily PRED dose was significantly lower (number of patients requiring PRED, 25 vs. 4; $P < 0.001$; mean daily PRED dose, 24.2 ± 12.4 vs. 0.6 ± 1.3; $P < 0.001$) at the end of 24 months after the first rituximab administration. The numbers of patients requiring CyA and MZ

TABLE 1. Change in Clinical Parameters in Steroid Dependent Minimal Change Nephrotic Syndrome

Parameters	Baseline (1st Rituximab)	1 Month	6 Month (2nd Rituximab)	12 Month (3rd Rituximab)	18 Month (4th Rituximab)	24 Month	36 Month
Sex (female:men)	4:21						
Age (years)	30.1 ± 11.9						
Systolic blood pressure (mm Hg)	123 ± 13	117 ± 12$^+$	119 ± 12$^+$	117 ± 12$^+$	118 ± 15	112 ± 11$^+$	117 ± 14$^+$
Diastolic blood pressure (mm Hg)	76 ± 11	70 ± 11$^+$	70 ± 12$^+$	71 ± 13$^+$	73 ± 14$^+$	68 ± 10$^+$	69 ± 14$^+$
Urinary protein (g/day)	2.5 ± 4.9	0.1 ± 0.3$^+$	1.8 ± 4.0$^+$	1.3 ± 4.8	0 ± 0$^+$	0 ± 0$^+$	0 ± 0$^+$
Serum albumin (g/dL)	3.6 ± 0.8	4.0 ± 0.6$^+$	4.0 ± 0.7$^+$	4.3 ± 0.7$^+$	4.5 ± 0.3$^+$	4.6 ± 0.3$^+$	4.5 ± 0.3$^+$
Total-cholesterol (mg/dL)	262 ± 79	212 ± 55$^+$	212 ± 70$^+$	196 ± 51$^+$	191 ± 95$^+$	179 ± 41$^+$	166 ± 30$^+$
Serum creatinine (mg/dL)	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.2	0.7 ± 0.2	0.7 ± 0.1	0.7 ± 0.1	0.7 ± 0.1
IgG (mg/dL)	705 ± 274	847 ± 233$^+$	990 ± 232$^+$	984 ± 257$^+$	1028 ± 248$^+$	1010 ± 261$^+$	928 ± 264
CD19 (/mm^3)	98 ± 104	2.5 ± 2.6$^+$	26 ± 37$^+$	31 ± 89$^+$	13 ± 29$^+$	11 ± 17$^+$	21 ± 45$^+$
CD20 (/mm^3)	101 ± 97	1.8 ± 1.2$^+$	27 ± 36$^+$	33 ± 78$^+$	13 ± 36$^+$	11 ± 19$^+$	22 ± 51$^+$
The depletion of B cell (n)	0	25	18	18	22	21	21
CD4/8	1.1 ± 0.4	1.0 ± 0.5	1.2 ± 0.4	1.3 ± 0.4	1.5 ± 0.4$^+$	1.4 ± 0.4$^+$	1.4 ± 0.4$^+$
IL-4	13 ± 15	30 ± 62	27 ± 55	28 ± 71	25 ± 60	30 ± 70	37 ± 104
TNF-a	1.1 ± 0.3	1.0 ± 0.4	1.1 ± 0.4	1.4 ± 1.8	1.7 ± 2.4	1.2 ± 0.4	1.3 ± 0.4
Th1/Th2	40 ± 88	43 ± 85	43 ± 144	12 ± 14	13 ± 15	13 ± 21	11 ± 12
Nephrotic syndrome (n)	7	0	6	2	0	0	0
PRED (mg/dL)	24.0 (5–60)	13.8 + (0–40)	5.6 + (0–20)	4.5 + (0–40)	1.3 + (0–5)	0.6 + (0–2.5)	0.6 + (0–5)
PRED (n)	25	22	13	8	6	4	5
CyA (n)	20	19	16	11	7	5	3
MMF (n)	1	1	1	3	2	2	1
MZ (n)	6	6	5	1	0	0	0
TAC (n)	1	1	1	0	0	0	0
BMD (g/cm^2)	0.84 ± 0.20					0.95 ± 0.10$^+$	
Z-score	−1.7 ± 1.5					−0.7 ± 1.0$^+$	

BMD = bone mineral density, CyA = cyclosporine, MZ = mizoribine, PRED = prednisolone, TAC = tacrolimus.
P^+ value: significantly different from baseline. Values are expressed as mean ± SD. $P < 0.05$.

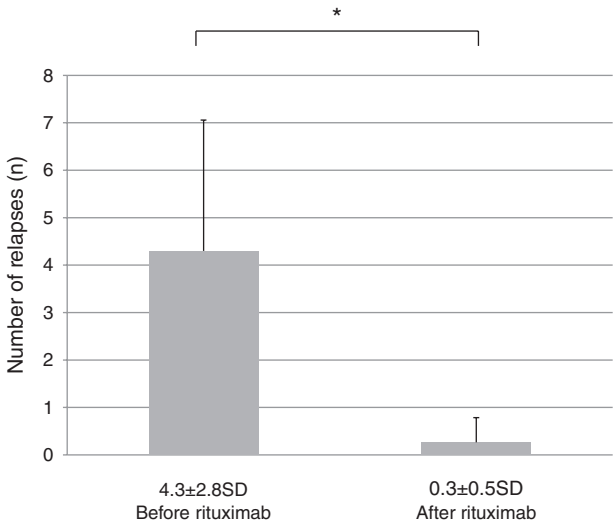

FIGURE 2. Number of relapses during the 24-month period before and 24-month period after the 1st rituximab administration. Results are expressed as means ± S.D. $*P < 0.05$.

were also significantly lower (CyA, 20 vs. 5; $P < 0.001$; MZ, 6 vs. 0; $P < 0.001$). TAC could be discontinued by 24 months after the first rituximab administration in all patients. To assess the improvement in the adverse effects of PRED, we measured the bone density at the baseline and at 24 months after the first rituximab administration. The BMD (g/m^2) and Z scores were significantly higher at 24 months as compared with the values recorded at the baseline (0.84 ± 0.2 vs. 0.95 ± 0.10; $P < 0.05$, -1.7 ± 1.5 vs. -0.7 ± 1.0; $P < 0.01$).

Long-Term Outcomes

Complete remission was maintained in all patients at the end of 24 months after the first infusion of rituximab (Table 2). Of the 20 patients who continued to receive the 6-monthly rituximab infusions after 24 months (treatment continuation group), 4 discontinued the rituximab treatment after the fifth infusion and 2 discontinued the treatment after the sixth infusion of their own will; however, complete remission was maintained in all the 20 patients from 36 to 54 months after the first rituximab infusion. In the treatment discontinuation group, 1 of the 5 patients developed relapse with B-cell repletion at 8 months after the last rituximab infusion, and the rituximab treatment was resumed.

B-Cell Depletion

The peripheral blood B-cell count increased significantly by 6 months after the first infusion in 18 of the 25 patients (Figure 3). Furthermore, 6 of these 18 patients developed relapse by around 6 months after the first rituximab infusion. The B cell count increased again by 6 months after the 2nd rituximab infusion in 7 of the 25 patients; 2 of these 7 patients developed relapse by around 6 months after the 2nd rituximab infusion. Thereafter, complete B-cell depletion was achieved again in all the 25 patients after the 3rd and after the 4th rituximab infusions, and significant increase of the peripheral blood B cell count occurred in 3 patients by 6 months after the 3rd rituximab infusion and in 4 patients by 6 months after the 4th infusion. However, none of these patients developed relapse by 18 or 24 months after the first rituximab infusion.

In 3 of the 20 patients of the treatment continuation group, the B-cell counts increased at 30 months after the first rituximab infusion, however, none of these patients developed relapse. Furthermore, 2 of the 16 patients who continued the treatment after the 5th rituximab infusion showed an increase of the B-cell count at 36 months after the first rituximab infusion, although neither of these patients developed relapse either relapse. In addition, 1 of the 14 patients who received the 7th rituximab infusion showed increase of the B-cell count at 42 months after the first rituximab infusion, and this patient did not show relapse either. In all of the 5 patients of the treatment discontinuation group (treatment discontinued after the 4th rituximab infusion), the B-cell count increased from 6 to 10 months after the 4th rituximab infusion; 1 (patient No. 2) of these patients was increased B-cell count by around 10 months after the last rituximab infusion, and the rituximab treatment was resumed in consideration of relapse at 13 months after the last rituximab infusion. In the remaining 10 patients with B-cell repletion (including 4 from the treatment discontinuation group and 6 from the treatment continuation group) complete remission was maintained despite the B-cell repletion.

Adverse Events

Of the 133 infusions in 25 patients, 13 infusions (9.8%) in 9 patients (36%) were associated with adverse events, including mild infusion reactions such as cough and hiccups. One patient developed exanthema almost immediately after the start of administration of rituximab, which was observed as a fixed drug eruption on the trunk and improved following treatment with betamethasone. One patient developed leukopenia (white blood cell count less than $3000/mm^3$) at 9 months from the baseline, and the white blood cell count improved to $5000/mm^3$ by 12 months. There were no severe adverse events, such as infectious episodes or hematological toxicities during the study period. Caution is needed in respect of the risk of late complications such as multifocal progressive leukoencephalopathy.

DISCUSSION

Rituximab which is known to cause B-cell depletion has been shown to have significant efficacy against immunological disorders caused by autoantibodies.[15] Several reports have suggested the possible efficacy of rituximab in pediatric patients with nephrotic syndrome.[4–13] In our previous study, we demonstrated that rituximab exerted almost the same efficacy in adult patients with steroid-dependent MCNS.[14] More recently published studies have also reported the efficacy of this drug in adult patients with MCNS.[16–18] In one reported study, there was no difference in the efficacy of rituximab between patients with adult and childhood onset of the disease.[18]

The rituximab doses used vary greatly among reports. In most studies, rituximab was given at the dose of $375\,mg/m^2$ BSA once a week for 4 weeks.[6,19] In most cases of relapse, the relapse developed simultaneously with the recovery of the B-cell count.[5] The time to recovery of the B-cell counts ranged from 5 to 10 months (average 7.5 months). Therefore we administered single-dose infusions of rituximab at intervals of 6 months and demonstrated the efficacy of two doses of rituximab.[14]

We observed significant increases of the B-cell counts by 6 months after the 1st rituximab infusion in 18 patients, by 6 months after the 2nd rituximab infusion in 7 patients, by 6 months after the 3rd infusion in 7 patients, and by 6 months after the 4th rituximab infusions in 7 patients (Figure 3). Six of

TABLE 2. Clinical Courses of all the 25 Patients With Steroid-Dependent MCNS. (Classification by Colors [deep Gray, Bright Gray and White] Links With Figure 3)

No	Onset (Age)	Start of RTX (Age)	Sex	Relapse Before RTX (/2 year)	UP at Start of RTX (g/d)	PRED Dose and Immunosuppressants at Start of RTX	Relapse After RTX (/2 year)	RTX Number (Every 6 mo + Addition)	Follow Up After 4th RTX (Standard Protocol) (mo)	Follow Up After Last RTX (mo)	Relapse After 4th RTX	Current PRED Dose and Immunosuppressants	
1	8	24	M	10	0	PRED5 mg + CyA75 mg + MZ150 mg	0	4 + 4	27	1	1	(-)	Relapse at 8 months after 4th RTX and add 4th RTX
2	21	26	M	3	16.9	PRED5 mg	1	4 + 2	41	2	0	PRED5 mg + MMF500 mg	B cell recovery at 8 months after 4th RTX. Additional RTX at 13 months and 25 month.
3	14	23	M	2	0	PRED30 mg + CyA140 mg	0	4	27	27	0	PRED2.5 mg + CyA100 mg	
4	39	51	M	3	0	PRED20 mg + CyA150 mg	0	4	25	25	0	(−)	
5	56	59	F	2	0.84	PRED25 mg + CyA100 mg	0	4	18	18	0	(−)	
6	3	25	M	3	0.12	PRED25 mg + CyA150 mg	0	5	19	13	0	(−)	At 13 months after 5th RTX, dropout of outpatients
7	16	29	M	3	0	PRED17.5 mg + CyA100	0	5	15	10	0	(−)	At 10 months after 5th RTX, dropout of outpatients
8	10	41	F	3	0.13	PRED25 mg + MMF1250 mg	0	5	39	30	0	(−)	
9	17	20	M	5	0.15	PRED55 mg + CyA150 mg	0	5	12	12	0	CyA 25 mg	
10	5	19	M	2	4.86	PRED30 mg + CyA100 mg	0	6	23	13	0	(−)	
11	30	35	F	2	3.53	CyA25 mg	0	6	23	13	0		
12	2	18	M	3	0	PRED15 mg + CyA200 mg + MZ300 mg	1	13	54	5	0	(−)	
13	8	23	M	8	4.45	PRED25 mg + CyA75 mg	1	10	41	5	0	(−)	
14	10	41	M	2	1.71	PRED30 mg + CyA100 mg	0	9	30	1	0	PRED 2 mg	PRED5 mg administration owing to steroid withdrawal symptom
15	8	23	M	3	0	PRED10 mg + CyA100 mg+	1	9	30	1	0	(−)	
16	29	34	M	11	0	PRED14 mg + CyA200 mg + MZ150 mg	0	8	28	3	0	(−)	
17	33	45	M	2	17.25	PRED15 mg + CyA100 mg	1	8	24	0	0	(−)	
18	4	20	M	3	3.60	PRED40 mg + CyA75 mg	0	7	24	4	0	(−)	
19	10	19	M	4	0	PRED30 mg + CyA100 mg + 150 mg	0	7	23	4	0	(−)	
20	16	19	M	5	0	PRED40 mg + TAC3 mg	0	7	22	3	0		
21	41	52	F	10	8.57	PRED30 mg + CyA75 mg	2	7	23	3	0	PRED 5 mg	PRED5 mg administration owing to steroid withdrawal symptom
22	2	28	M	6	0	PRED25 mg	0	7	22	3	0	(−)	
23	7	22	M	2	0	PRED30 mg + CyA150 mg	0	7	21	2	0	CyA 25 mg	Current gradually decrease of CyA
24	17	36	M	4	0	PRED15 mg + MZ150 mg	0	7	19	0	0	(−)	
25	12	21	M	7	0.65	PRED30 mg + CyA80 mg	1	7	18	1	0	PRED 0.33 mg	PRED5 mg administration owing to steroid withdrawal symptom

CyA = cyclosporine, d = day, F = female, M = male, mo = month, MZ = mizoribine, PRED = predmisolone, RTX = rituximab, TAC; tacrolimus, UP = urinary protein, yr = year.

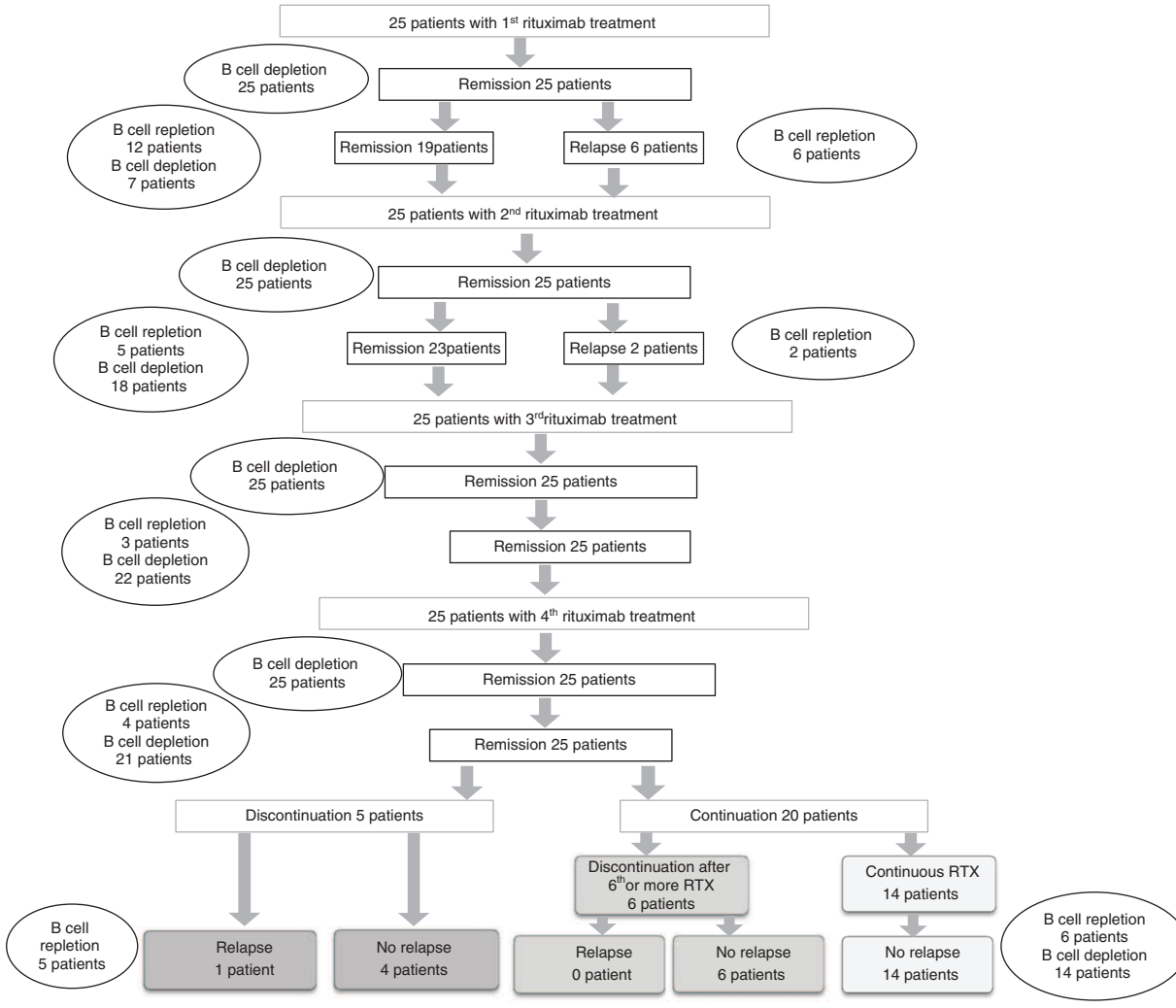

FIGURE 3. Study flow chart.

the 18 patients who showed an increase of the B-cell count by 6 months after the 1st rituximab infusion and 2 of the 7 patients who showed an increase of the B-cell count by 6 months after the 2nd rituximab infusion developed relapse. In all of the 5 patients of the treatment discontinuation group who discontinued rituximab after the 4th rituximab infusion, the B-cell count increased again by 6 months after the 4th rituximab infusion, and 1 of these 5 patients developed relapse. On the other hand, remission was maintained in all of the patients in whom B-cell depletion was maintained. Interestingly, complete remission was also maintained in the 10 patients who showed B-cell repletion after discontinuation of the rituximab therapy. There is a possibility that depletion of B cells for 24 months influenced the improvement of the morbidity of steroid-dependent MCNS, suggesting that B-cell restitution is always associated with relapse.

Rituximab therapy could facilitate reduction of the steroid and immunosuppressant drug doses. The reduced exposure to steroid and CyA most likely accounted for the progressive reduction of the blood pressure and amelioration of dyslipidemia observed during the observation period in our study. Rituximab significantly blunted the progressively increasing bone density deficit observed following long-term steroid exposure, with normalization of the bone density by the end of the 2-year observation period. The risk of infections was probably reduced because of the increased IgG and CD4/8 ratio. There were no cases of severe infection during the observation period. The therapy for steroid-dependent MCNS was recommended using the CYC or CyA.[20] However rituximab therapy should be preferentially used for patients with a risk of adverse effect of immunosuppressive medications such as PRED, CYC, and CyA.

B-lymphocytes may play an important regulatory and potentially pathogenic role in MCNS through antibody-independent mechanisms such as antigen presentation, T-lymphocyte activation, and production of cytokines. Imbalance between T-helper 1 (Th1) and 2 (Th2) lymphocytes (Th1/Th2 imbalance) has been implicated in the development of MCNS, and a Th2 shift caused by increased IL-4 has been observed.[21] In this study, while a tendency towards a Th2 shift caused by increased IL-4 was observed, pathogenesis remained unclear and further research is required. There are detailed reports on the rate and phenotype of B-lymphocyte subpopulation recovery after rituximab treatment in patients with

rheumatoid arthritis: after B-cell depletion, immature B lymphocytes reappear first, followed by naïve B lymphocytes; CD27+ memory B lymphocytes may remain depleted for up to 2 years after a single dose of rituximab.[22] It has also been reported that rituximab may have the effect of stabilizing the podocyte cytoskeleton and decreasing proteinuria through this mechanism,[23] however the pathophysiological mechanism underlying the efficacy of rituximab against MCNS remains unclear.

In our trial, rituximab therapy was associated with maintenance of complete remission. The limitations of this study were that it was not a randomized controlled trial and the number of patients enrolled was small. Complete remission was maintained in all the patients showing B-cell depletion. Complete remission was also maintained in most of the patients with B-cell repletion following discontinuation of rituximab therapy after B-cell depletion had been maintained for at least 12 months. Thus, rituximab treatment may be considered as a radical therapeutic agent for patients with steroid-dependent MCNS.

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