Title: Therapeutic efficacy of rituximab for the management of adult-onset steroid-dependent nephrotic syndrome: A retrospective study

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Abstract

Background

Recent reports have described the efficacy of rituximab in treating steroid-dependent nephrotic syndrome (SDNS) in pediatric patients. However, few reports describe data regarding adult-onset SDNS. We investigated the efficacy of rituximab for the management of adult-onset SDNS.

Methods

We performed a retrospective cohort study investigating 8 patients with adult-onset SDNS who were treated with rituximab. Clinical data were obtained at the initiation of rituximab therapy. The primary outcomes evaluated were successful suppression of relapses and CD19+ cells after rituximab treatment. The corticosteroid- and immunosuppressant-sparing effect and adverse events were additionally evaluated.

Results

All 8 patients were diagnosed with minimal change nephrotic syndrome and received immunosuppressants in addition to corticosteroid. Total number of relapses was 10.5 times as a median value. Rituximab administration was repeated in 2 patients, whereas 6 received single-dose rituximab. 3 of 8 (37.5%) patients showed relapse after rituximab

therapy. A rituximab-induced depletion in CD19+ cells noted initially was followed by their reappearance in all patients. There were cases with no relapse after the reappearance of CD19+ cells. The median relapse time pre- and post-rituximab therapy showed a decrease from 1 time/year (interquartile range [IQR] 1–3 times/year) to 0 time/year (IQR 0–1 time/year). Rituximab treatment induced a significant reduction in the required doses of corticosteroid and cyclosporine ($P < 0.01$). No serious adverse events were observed.

Conclusion

Rituximab treatment was effective not only in childhood-onset but also in adult-onset SDNS. Further studies are needed to establish optimal treatment regimens.

Introduction

Minimal change nephrotic syndrome (MCNS) shows a high prevalence in pediatric patients; however, most patients show a good response to corticosteroid therapy and > 90% of patients achieve complete remission with initial treatment. MCNS is also a major cause of idiopathic nephrotic syndrome (NS) in adults. Although MCNS patients show a favorable response to steroids, the relapse rate following a reduction of the steroid dose is reportedly as high as 30–70% [1, 2]. In those with steroid-dependent NS (SDNS) and frequently-relapsing NS (FRNS), relapse prevention and reduction of steroids is managed with the addition of immunosuppressants such as cyclosporine (CyA), cyclophosphamide (CPA), mizoribine (MZR), tacrolimus (TAC), and mycophenolate mofetil (MMF). However, serious side effects and increased medical costs associated with long-term administration act as major drawbacks related to the use of these drugs.

Recent reports have described the therapeutic efficacy of rituximab, a monoclonal antibody targeting the CD20 antigen of B lymphocytes, in treating idiopathic NS. Standard treatment methods for membranous nephropathy (MN) remain controversial [3]. Ruggenenti et al. demonstrated the efficacy and safety of rituximab in a large-scale cohort study [4]. The authors also reported the therapeutic effect of

rituximab in Japanese patients with MN [5]. Moreover, rituximab has been reported to significantly reduce the relapse rate in children with SDNS and FRNS [8-16]. However, few reports have described the efficacy of rituximab in adults [17-22]. Additionally, the optimal dosing regimen for rituximab treatment has not been established. We evaluated the efficacy of rituximab in treating adult-onset SDNS.

Materials and methods

Study design and population

We performed a retrospective cohort study at a single hospital. Our study comprised 8 patients with adult-onset SDNS who were administered rituximab at Nagoya University Hospital between May 2014 and March 2017. NS was diagnosed based on the clinical practice guidelines established by the Collaborative Working Group of the Ministry of Health, Labour and Welfare NS Research Group and the Japanese Society of Nephrology [6]. This study protocol was approved by The Ethics Committee for Human Research of Nagoya University Hospital (IRB approval number 2017-0192).

Clinical data collection

Baseline characteristics including clinical findings at the initiation of rituximab therapy and medical history were obtained from patients' medical records. The clinical parameters evaluated were age, gender, body mass index, systolic and diastolic blood pressure, complete blood count, serum total protein and albumin, serum total and low-density lipoprotein cholesterol, serum creatinine, glomerular filtration rate (GFR calculated using the equation for Japanese patients: eGFR [mL/min/1.73 m^2] = $194 \times Scr^{-1.094} \times Age^{-0.287}$ [×0.739 if female]) [7], CD19+ cell levels, and urinary protein-creatinine ratio (uPCR). With regard to medical history, hypertension, diabetes mellitus, total number of relapses prior to initiation of rituximab therapy, previous use of immunosuppressive agents, and the dosage of prednisolone (PSL) at the time of rituximab infusion were recorded.

Renal pathological diagnosis

A renal biopsy was performed in all patients to establish the histopathological diagnosis. The kidney specimens were studied by light microscopy (LM),

immunofluorescence stain (IF), and electron microscopy (EM) using standard techniques. For direct IF stain, 3-μm cryostat sections were stained using polyclonal fluorescein isothiocyanate conjugated antibodies to IgG, IgA, IgM (Medical & Biological Laboratories, Nagoya, Japan), C3, C4, and C1q (Dako, Copenhagen, Denmark). Renal pathological diagnosis was done by two independent pathologists to gain objective assessment.

Rituximab treatment

Screening for infectious diseases was performed in all patients prior to the initiation of rituximab. When necessary, the influenza and pneumococcal vaccines were administered to patients. A single intravenous dose of rituximab was administered to all patients. Only in the first patient, rituximab was infused at a dose of 375 mg/m^2 of body surface area. After the second patient, a single fixed dose of rituximab (500 mg) was administered. In the first 2 patients, rituximab was regularly administered every 6 months, whereas the remaining 6 patients were administered only a single dose of rituximab (500 mg), and thereafter the clinical course was monitored in all patients. In those who

presented with sequential relapses, rituximab was added at a dose of 500 mg after complete remission (CR) was obtained by increasing the steroid dose. Rituximab was reconstituted in saline and instilled at a recommended rate based on the attached document. We administered 100 mg of hydrocortisone sodium succinate, 30 mg of diphenhydramine hydrochloride, and 400 mg of acetaminophen 30 min prior to the rituximab infusion as premedication to minimize infusion reactions. Patients were closely observed, and in those in whom infusion reactions were suspected, we decreased the rate of rituximab administration. The CD19+ cell levels were confirmed immediately prior to the initiation of rituximab and measured each month after the drug was administered. Written informed consent was obtained from all patients prior to the initiation of rituximab therapy.

Outcomes and definitions

The primary endpoint was the occurrence of relapse following rituximab therapy. The reappearance of CD19+ cells was also evaluated. Additionally, the doses of corticosteroid and immunosuppressants required were compared prior to and after

administration of rituximab. CR was defined as a decline in the proteinuria to < 0.3 g/day. Relapse was defined as the condition that urine protein ≧ 1 g/day runs or ≧ (2+) continues 2-3 times in a row after CR. If collection of 24-hour urine was impossible, the uPCR (represented as g/g creatinine, g/gCr) measured in a spot urine sample was utilized for diagnostic and therapeutic evaluation. SDNS was defined as follows based on the therapeutic response—steroid therapy is impossible to discontinue because repeated over 2 times relapses appear after the reduction or discontinuation of steroid. These definitions were based on the clinical practice guidelines established for NS [6].

Statistical analysis

Clinical data have been presented as medians with interquartile ranges (IQR) or as numbers denoting percentages (%). Differences between groups were analyzed using the Wilcoxon rank-sum test (for continuous variables) or the Fisher exact test (for categorical variables). The paired t-test was used to analyze changes in clinical parameters. The Kaplan–Meier method was used to evaluate the relapse and CD19+ cell reappearance after rituximab therapy. Statistical significance was set at a p value of <

0.05. All statistical tests were performed using the Stata software version 14.0 (Stata Corp LLC, College Station, TX, USA).

Results

Patient characteristics

Patients' baseline characteristics have been summarized in Table 1. The median age at the initiation of rituximab therapy was 43 years (IQR 30–49.5 years) with a predominance of women (62.5% of the patients). The median total number of relapses was 10.5 times (IQR 5.5–14.5 times), and the maximum number of relapses was 16. Regarding renal function, the median serum creatinine and eGFR were 0.69 mg/dL (IQR 0.61–0.99 mg/dL) and 76.7 ml/min/1.73m^2 (IQR 68.2–87.8 ml/min/1.73m^2), respectively. The median urinary protein was 0.10 g/gCr (IQR 0.02–0.42 g/gCr), and 6 of 8 patients showed CR. PSL was administered in all patients at the initiation of rituximab therapy at a median dose of 22.5 mg/day (IQR 15–31.3 mg/day). CyA was also administered in all patients prior to the initiation of rituximab therapy. TAC, MZR, MMF, and CPA were the other immunosuppressive drugs that were administered.

Renal pathological findings

LM findings revealed no significant lesions including cellular proliferation and segmental sclerosis. IF showed nonspecific deposition of IgG or IgM in 1 and 3 cases, respectively. On EM, the effacement of foot process was confirmed and electron dense deposits were not observed. Based on these pathological findings, cases presented were finally diagnosed with MCNS by pathologists.

Relapse and reappearance of CD19+ cells after rituximab therapy

The clinical course of all patients with SDNS who were treated with rituximab has been summarized in Table 2. The median observation period was 13.9 months (IQR 11.6–20.0 months). Among 8 patients included in the study, rituximab administration was repeated in 2 (patient # 1, 2). The number of infusions was 7, and 3, respectively, totaling 3100 mg, and 1500 mg of rituximab, respectively. The remaining 6 patients received a single dose of rituximab (500 mg). Two patients (patient # 2, 5) did not demonstrate CR prior to rituximab treatment but achieved and maintained CR after treatment. We observed that 3 of 8 (37.5%) patients showed relapse (patient # 6–8) during the

observation period. The time until the onset of relapse after rituximab was 8.1, 10.0, and 13.6 months, respectively (Fig 1). No relapse was observed in 2 patients in whom rituximab injections were repeated. The relapse rate was 50% at a median observation period of 13.0 months (IQR 10.3–14.9 months) in those who received a single dose of rituximab (500 mg). In 3 patients who relapsed after rituximab therapy, PSL dosage was temporarily increased to 15-30 mg/day. CR was confirmed 7 days (patient # 6), 14 days (patient # 7) and 21 days (patient # 8) after increasing steroid, and subsequently the second rituximab administration was carried out. CD19+ cells rapidly declined after rituximab therapy but reappeared in all patients. The median period until reappearance was 8.9 months (IQR 5.4–15.6 months) (Fig 1). Restricted to the single-dose rituximab group, CD19+ cells reappeared with a median of 6.5 months (IQR 5.3–10.3 months). The reappearance of CD19+ cells was confirmed prior to relapse in 2 of 3 patients who showed relapse. The interval between reappearance and relapse was 3.3 and 4.9 months, respectively in these patients. One patient showed an overlap between the reappearance of CD19+ cells and relapse. No patient demonstrated a relapse while the CD19+ cells were observed to be suppressed. In 5 patients (patient # 1–5), CR was maintained even

after CD19+ cells reappeared. The number of relapses during a year prior to and after rituximab therapy was compared in 6 patients with observation period of more than 1 year. The median relapse times were decreased from 1 time/year (IQR 1–3 times/year) to 0 time/year (IQR 0–1 time/year) (P = 0.08) (Fig 2).

Corticosteroid and immunosuppressive drugs

At the time of last observation, the corticosteroid dose could be reduced in 87.5% of patients. In 50%, the dose of PSL could be decreased to 5 mg or less, and steroid administration was discontinued in 1 patient. The median PSL dosage at the last visit was 5.5 mg/day (IQR 2.5–7 mg/day), which was significantly lower than that required prior to rituximab treatment (P < 0.01). CyA was used in 7 of 8 patients at the time of initiation of rituximab therapy at a median dose of 75 mg/day (IQR 75–150 mg/day). After rituximab administration, CyA could be reduced in all 7 patients. Among these, CyA was discontinued in 3. The dosage of CyA at the final observation time point was 25 mg/day (IQR 0–100 mg/day), which was significantly lower than that required prior to initiation of rituximab therapy (P < 0.01) (Fig 3). Similarly, even in the single-

dose group, a significant reduction in PSL and CyA dosages were observed from 20 mg/day (IQR 10–27.5 mg/day) to 6 mg/day (IQR 5–8 mg/day) (P = 0.03), and 87.5 mg/day (IQR 75–150 mg/day) to 25 mg/day (IQR 0–100 mg/day) (P < 0.01). MZR and MMF were being administered in 1 and 2 patients, respectively, at the time of initiation of rituximab therapy; however both drugs could be discontinued. No patient reported using immunosuppressants other than CyA.

Adverse events

Adverse events noted during the observation period have been summarized in Table 3. Among the 19 rituximab infusions administered in 8 patients, infusion reaction of hypotension was observed in association with only 1 infusion (5.3%). This episode was mild and was managed with a decrease in the drip rate. Adverse events such as infectious diseases, progressive multifocal leukoencephalopathy, bone marrow suppression, mucocutaneous damage, pneumonopathy, liver dysfunction, gastrointestinal disorder, nephrotoxicity, and malignant neoplasm were not observed during the observation period.

Discussion

Rituximab has been recognized as a novel therapeutic agent to treat idiopathic NS. Several reports have demonstrated the efficacy of rituximab in pediatric patients for the management of SDNS or FRNS, which is primarily categorized as MCNS [9-13]. Randomized controlled trials have already been performed involving children with SDNS, and the effectiveness of this drug has been demonstrated [14, 15]. Iijima et al. have reported a multicenter, double-blind, randomized, placebo-controlled trial in Japan [16] demonstrating that among 48 pediatric patients with SDNS or FRNS, relapses were significantly decreased in the rituximab-treated group compared to a placebo group. However, few studies have described adult SDNS [17-22].

In this cohort study, we showed that rituximab reduced the frequency of relapses. The characteristics of this study are that all subjects were limited to adult-onset SDNS, and in most patients, the clinical course was observed after a single-dose infusion of rituximab. Despite the majority of patients were treated with a single dose of rituximab, the relapse rate was 37.5%, which is comparable with previous reports [17, 18, 20, 21]. The treatment of idiopathic NS is associated with various adverse effects secondary to

the long-term use of corticosteroid and immunosuppressive drugs. Notably, rituximab treatment for adult-onset SDNS enables reduction in the required doses of steroid and immunosuppressants.

Usually, relapses are observed to occur after the reappearance of CD19+ cells in the patient's blood; however, previous reports have shown that a few patients did not show relapse even after the reappearance of B cells [17, 18]. In all our patients, CD19+ cells were depleted immediately after rituximab administration. However, even after they reappeared in all patients, the relapse rate remained 37.5%. In 2 out of 3 patients who showed relapse, CD19+ cells had been observed to reappear prior to confirmation of relapse suggesting that the reappearance of B cells can be a predictive marker of relapse. In contrast, similar to previous reports, a few patients did not demonstrate relapse even after the reappearance of B cells. Ruggenenti et al. have recommended that it is important to disrupt the activity of pathogenic autoreactive B lymphocyte clones [18]. Based on these findings, it is necessary to further investigate whether B cell-target therapy would be a valid intervention in this context. The pathomechanism contributing to the action of rituximab on B lymphocytes to effectively treat idiopathic NS remains unclear, although

this is hypothesized to be the primary pathology of T lymphocyte-related disorders and permeability factors. It has been hypothesized that targeting and removing B cells indirectly interferes with the activity of immunoregulatory cells such as regulatory T cells, natural killer cells, and macrophages [23]. Another pathomechanism suggested by Fornoni et al. indicates that rituximab might promote the stabilization of the podocyte cytoskeleton and prevent apoptosis, through interaction with glomerular epithelial cells [24] thus indicating that the role of rituximab in the management of SDNS is not attributable to only the removal of B cells. Therefore, a few patients maintain remission despite the reappearance of CD19+ cells, suggesting individual differences in the effect of rituximab irrespective of B cell depletion.

To date, the optimal dosing regimen for rituximab therapy to treat SDNS has not been established. Munyentwali et al. reported no relationship between the dose of rituximab and relapse [17]. Our results showed that a few patients maintained remission even with a small dose of rituximab. Further studies are needed to determine whether applied dose complying with non-Hodgkin’s lymphoma, re-treatment following the reappearance of B lymphocytes, or prophylactically repeated administration would be

effective treatment methods. Treatment with single-dose rituximab therapy is beneficial in minimizing the risk of infusion reactions and reducing the frequency of hospitalizations and medical expense. Moreover, exposure to small quantities of rituximab reduces the possibility of antibody production and facilitates re-treatment.

It has not been determined whether CR should be achieved at the time of initiation of rituximab infusion to prevent the loss of rituximab in the urine. In our cohort, 2 patients did not achieve CR, but eventually showed a favorable course. Thus, it may not be necessary to achieve CR at the onset of rituximab treatment. The fact that rituximab is effective in treating nephrotic MN supports this conclusion.

Regarding adverse events, although mild infusion reaction was observed, no serious events were noted. High tolerability is considered a major advantage of rituximab treatment.

Limitations of our study: 1) The lack of a control group and the small number of patients studied would count as a limitation. However, adult-onset SDNS is a rare disease entity, and the number of patients affected is not large. In this cohort, all patients with adult-onset SDNS treated with rituximab at our hospital were enrolled in this study,

thereby reducing the selection bias. 2) The observation period was relatively short and long-term observation is necessary to investigate the utility of single-dose rituximab therapy.

In conclusion, we observed that rituximab treatment for SDNS reduced the number of relapses, as well as the corticosteroid and immunosuppressant dosages required in these patients, not only in childhood-onset but also in adult-onset SDNS. Our study showed that in a few patients, relapse was suppressed despite the reappearance of B cells following single-dose rituximab therapy. Considering the safety, medical costs, and anti-drug antibody formation, it would be beneficial to reduce the dosage of rituximab as much as possible. Further investigations are needed to develop an optimal treatment regimen and to determine predictive factors associated with a therapeutic response.

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Compliance with ethical standards

Conflict of interest

The Department of Nephrology and Rheumatology, Aichi Medical University received research promotion grants from Chugai Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Eisai Co., Ltd., Kyowa Hakko Kirin Co., Ltd., Mitsubishi Tanabe Pharma Co., Novartis Pharma K.K., Otsuka Pharmaceutical Co., Ltd., Pfizer Japan Inc., Takeda Pharmaceutical Co., Ltd., Teijin Pharma Limited., and Torii Pharmaceutical Co., Ltd.

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Human and animal rights

All procedures performed in studies involving human participants were in accordance with the ethical standards of The Ethics Committee for Human Research of Nagoya University Hospital at which the studies were conducted (IRB approval number 2017-0192) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent

The Ethical Committee approved this retrospective cohort study without written informed consent from patients; however, informed consent was obtained from most patients at the time of performing a renal biopsy.

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Table 1

Baseline characteristics of patients with adult-onset steroid-dependent nephrotic syndrome treated with rituximab

Values are shown as either medians with interquartile ranges (IQR) or numbers denoted as percentages (%).

Abbreviations: ACE: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, BMI: body mass index, CPA: cyclophosphamide, Cr: creatinine, CyA: cyclosporine, eGFR: estimated glomerular filtration rate, LDL: low-density lipoprotein, MCNS: minimal change nephrotic syndrome, MMF: mycophenolate mofetil, MZR: mizoribine, NS: nephrotic syndrome, PSL: prednisolone, SDNS: steroid-dependent nephrotic syndrome, TAC: tacrolimus, uPCR: urinary protein-creatinine ratio.

Table 2

Clinical course of all patients with adult-onset steroid-dependent nephrotic syndrome treated with rituximab

Abbreviations: CPA: cyclophosphamide, Cr: creatinine, CyA: cyclosporine, eGFR: estimated glomerular filtration rate, F: female, IS: immunosuppressants, M: male, MCNS: minimal change nephrotic syndrome, MMF: mycophenolate mofetil, MZR: mizoribine, NS: nephrotic syndrome, PSL: prednisolone, SDNS: steroid-dependent nephrotic syndrome, TAC: tacrolimus, uPCR: urinary protein-creatinine ratio.

Table 3

Adverse events noted during the observation period

Fig. 1 Relapse and the reappearance of CD19+ cells after rituximab treatment

Kaplan–Meier curves show the cumulative relapse rate and the reappearance of CD19 positive cells after rituximab treatment in all patients with adult-onset SDNS.

Abbreviations: SDNS: steroid-dependent nephrotic syndrome.

Fig. 2 Comparison of the number of relapses in a year prior to and after rituximab therapy

Rituximab therapy reduced the annual relapse rate in adult-onset SDNS patients. The + symbols show the median value.

P = 0.08

Abbreviations: SDNS: steroid-dependent nephrotic syndrome.

Fig. 3 The corticosteroid- and cyclosporine-sparing effects of rituximab therapy

At the time of the last observation, the doses of corticosteroid and CyA required in patients with adult-onset SDNS were significantly decreased following rituximab therapy. The + symbols show the median value.

* $P < 0.01$

Abbreviations: CyA: cyclosporine, PSL: prednisolone, SDNS: steroid-dependent nephrotic syndrome.

Table 1

Baseline characteristics of adult-onset SDNS patients with rituximab therapy

	All cases (n=8) median [IQR], n (%)
Age at NS onset (years)	23 [22-41.5]
Age at rituximab therapy (years)	43 [30-49.5]
Gender, female	5 (62.5)
Renal pathological diagnosis	
MCNS	8 (100.0)
BMI (kg/m^2)	22.3 [19.1-24.7]
Systolic blood pressure (mmHg)	125.5 [114-131]
Diastolic blood pressure (mmHg)	77 [67.5-83]
Hypertension	1 (12.5)
Diabetes mellitus	2 (25.0)
Total number of relapses before rituximab	10.5 [5.5-14.5]
Laboratory parameters	
White blood cell count (/μL)	10050 [9050-14500]
Hemoglobin (g/dL)	12.8 [11.5-13.1]
Platelet count ($x10^4/\mu L$)	29.7 [21.8-31.3]
Serum total protein (g/dL)	6.2 [5.8-6.5]
Serum albumin (g/dL)	3.8 [3.3-4.0]
Total cholesterol (mg/dL)	231.5 [215-251.5]
LDL cholesterol (mg/dL)	121.5 [99-138.5]
Serum creatinine (mg/dL)	0.69 [0.61-0.99]
eGFR ($ml/min/1.73m^2$)	76.7 [68.2-87.8]
uPCR (g/gCr)	0.10 [0.02-0.42]
CD19 positive cell level (%)	14 [8.5-20.5]
Treatment before rituximab	
ARB	2 (25.0)
ACE	0 (0.0)
PSL	8 (100.0)
CyA	8 (100.0)
TAC	1 (12.5)
MZR	3 (37.5)
MMF	2 (25.0)
CPA	1 (12.5)
PSL dose at rituximab infusion (mg/day)	22.5 [15-31.3]

Table 2

Clinical course of all patients with rituximab treatment for adult-onset SDNS (n=8)

	Patients with repeated rituximab therapy		Patients treated with single-dose regimen of rituximab 500 mg					
			No relapse cases after rituximab			Relapse cases after rituximab		
Patient number	1	2	3	4	5	6	7	8
Age at NS onset (years)	22	24	50	44	22	20	39	22
Age at rituximab therapy (years)	41	25	56	52	34	26	45	47
Gender	F	M	M	F	M	F	F	F
Renal pathological diagnosis	MCNS	MCNS	MCNS	MCNS	MCNS	MCNS	MCNS	MCNS
Observation period (months)	40.4	20.3	13.0	10.3	5.6	19.6	14.9	13.0
Total number of relapses before rituximab, n	16	3	7	15	14	4	7	14
All types of IS before rituximab	CyA,TAC, MZR,CPA	CyA, MZR	CyA	CyA, MZR	CyA, MMF	CyA	CyA	CyA, MMF
IS at rituximab therapy	MMF	CyA	CyA	CyA, MZR	CyA, MMF	CyA	CyA	CyA
PSL dose at rituximab therapy (mg/day)	25	50	20	27.5	20	35	7.5	10
CyA dose at rituximab therapy (mg/day)	0	75	50	150	200	75	100	75
Laboratory parameters at rituximab therapy								
Serum albumin (g/dL)	3.4	2.6	3.7	3.9	4.1	3.1	4.2	3.9
Total cholesterol (mg/dL)	254	217	213	316	181	232	249	231
Serum creatinine (mg/dL)	0.69	1.20	1.79	0.61	0.78	0.58	0.68	0.60
eGFR (ml/min/1.73m^2)	74.1	63.1	32.3	79.2	92.5	102.1	73.3	83.0
uPCR (g/gCr)	0.06	1.11	0.13	0.19	0.64	0.03	0.00	0.00
CD19 positive cell level (%)	12	27	3	16	25	10	7	16
Rituximab treatment frequency, n	7	3	1	1	1	2	2	2
Rituximab total dose (mg)	3100	1500	500	500	500	1000	1000	1000
Relapse times per year before rituximab, n	4	3	1	2	1	1	1	1
Relapse times after rituximab, n/year	0/3.4	0/1.7	0/1.1	0/0.9	0/0.5	1/1.6	1/1.2	1/1.1
Time to reappearance of CD19 positive cell after rituximab (months)	36.2	20.3	5.3	7.5	5.6	10.3	10.9	3.2
Time to relapse after rituximab (months)	–	–	–	–	–	13.6	10.9	8.1
Time to re-treatment of rituximab (months)	–	–	–	–	–	14.7	12.6	9.5
Interval between CD19 positive cell reappearance and relapse (months)	–	–	–	–	–	3.3	0	4.9
CD19 positive cell level at relapse after rituximab (%)	–	–	–	–	–	2	1	6
PSL dose at relapse after rituximab (mg/day)	–	–	–	–	–	4	1	1
Increase of PSL dose for relapse after rituximab (mg/day)	–	–	–	–	–	30	30	15
PSL dose at last visit (mg/day)	3	2	0	6	8	5	6	10
CyA dose at relapse after rituximab (mg/day)	–	–	–	–	–	0	25	50
CyA dose at last visit (mg/day)	0	25	0	100	100	0	50	0
Discontinuation of IS after rituximab	MMF	–	CyA	MZR	MMF	CyA	–	CyA

Table 3

Adverse events during observation period (n=8)

	n (%)
Infusion reaction (hypotension)	1 (12.5)
Bacterial infection	0 (0.0)
Viral infection	0 (0.0)
Reactivation of hepatitis B	0 (0.0)
Progressive multifocal leukoencephalopathy	0 (0.0)
Pancytopenia	0 (0.0)
Neutropenia	0 (0.0)
Agranulocytosis	0 (0.0)
Thrombocytopenia	0 (0.0)
Mucocutaneous disorder	0 (0.0)
Respiratory disturbance	0 (0.0)
Liver functional impairment	0 (0.0)
Gastrointestinal symptom	0 (0.0)
Acute kidney injury	0 (0.0)
Malignancy	0 (0.0)

Fig. 1

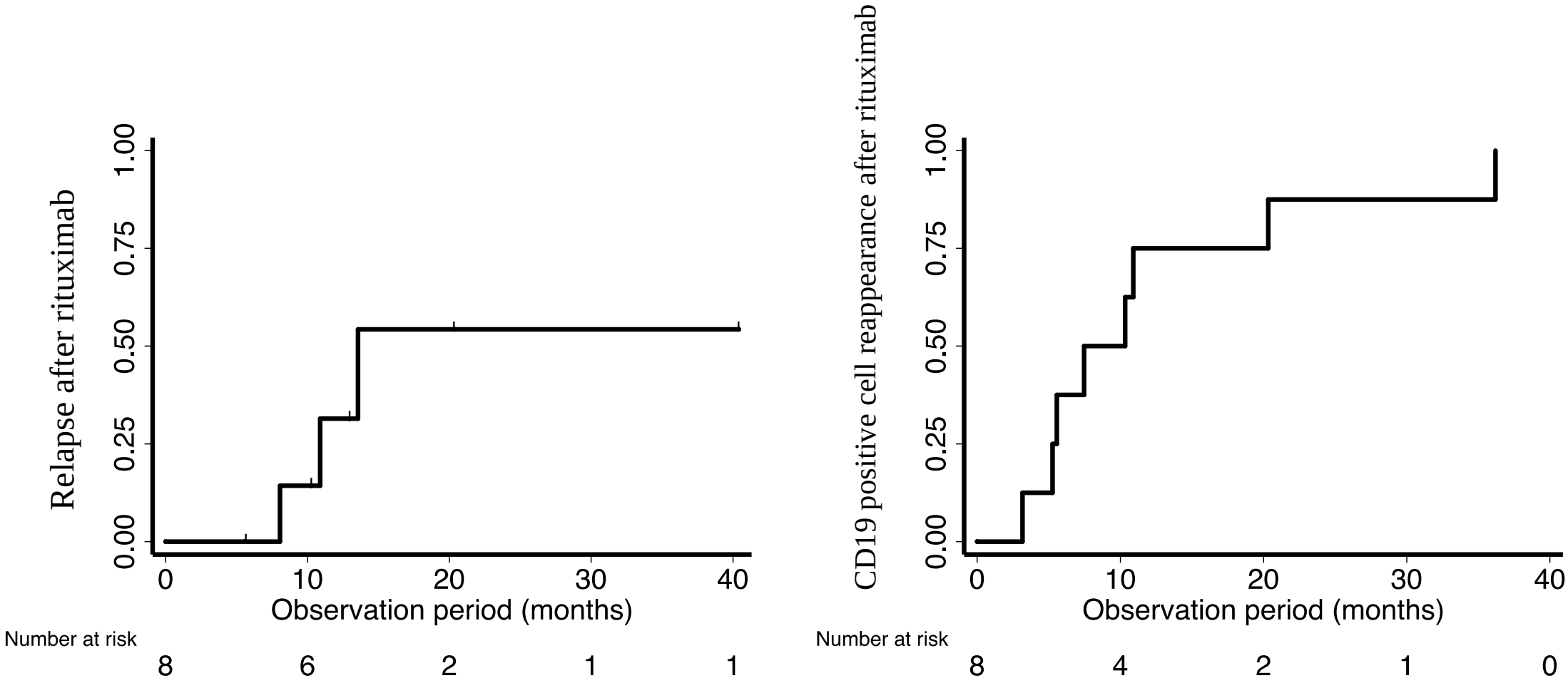

Relapse after rituximab
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
Observation period (months)
Number at risk
8
6
2
1
1
CD19 positive cell reappearance after rituximab
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
Observation period (months)
Number at risk
8
4
2
1
0

Fig. 2

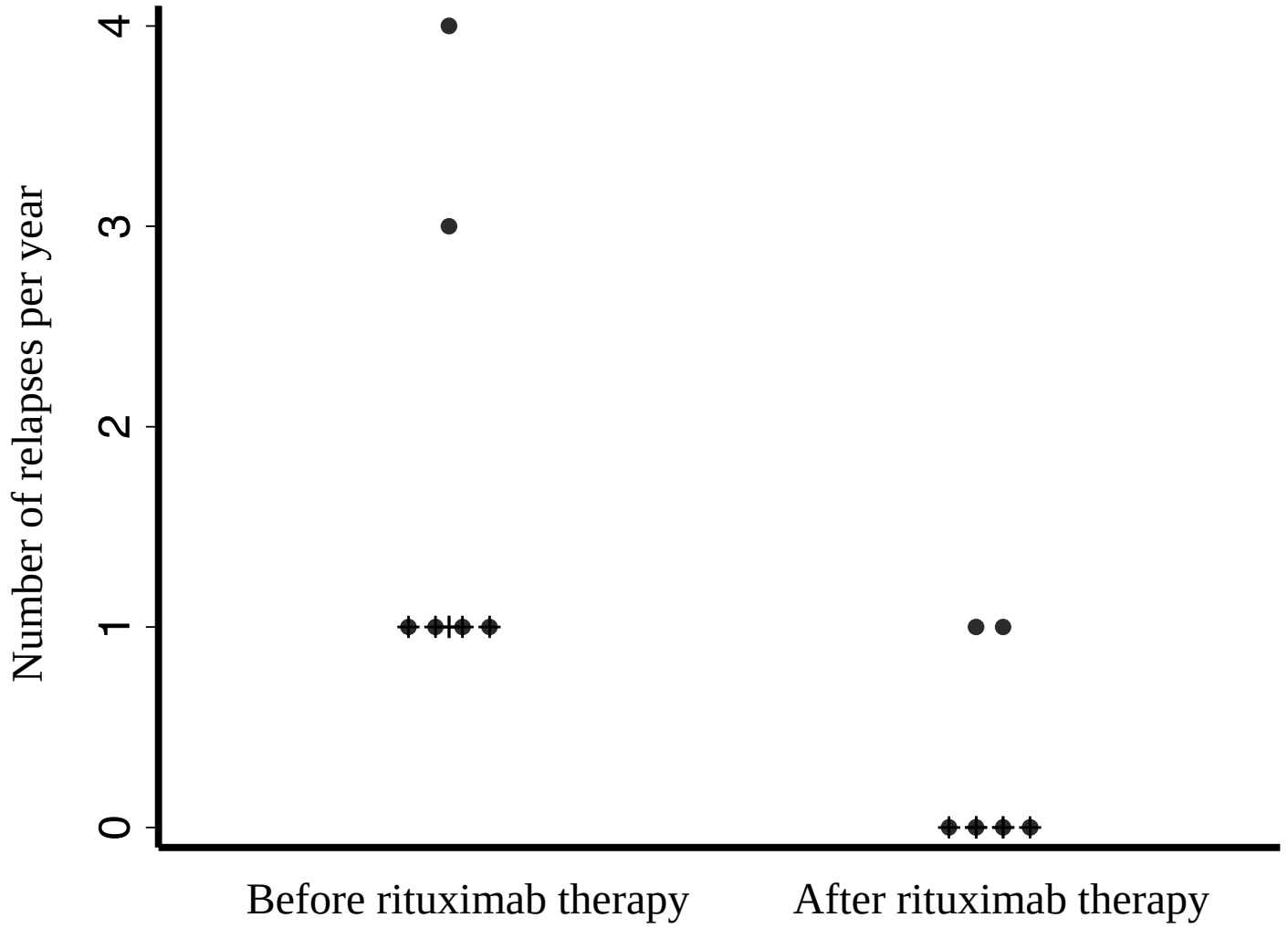
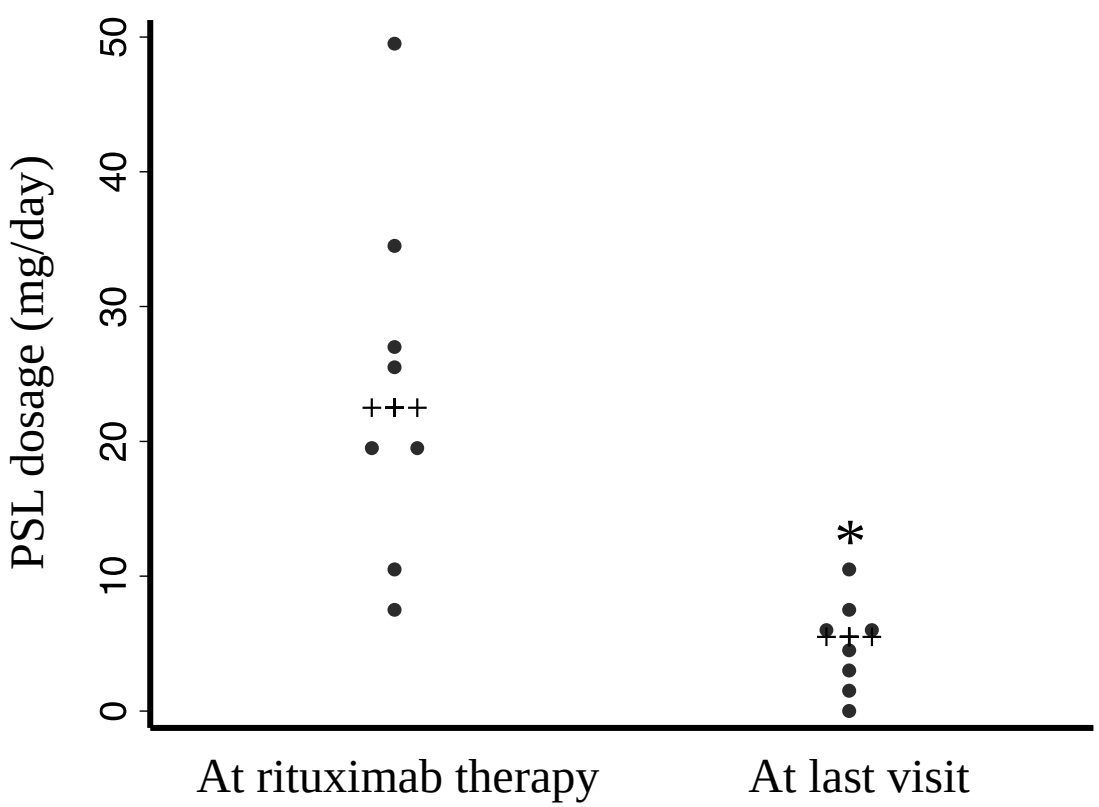
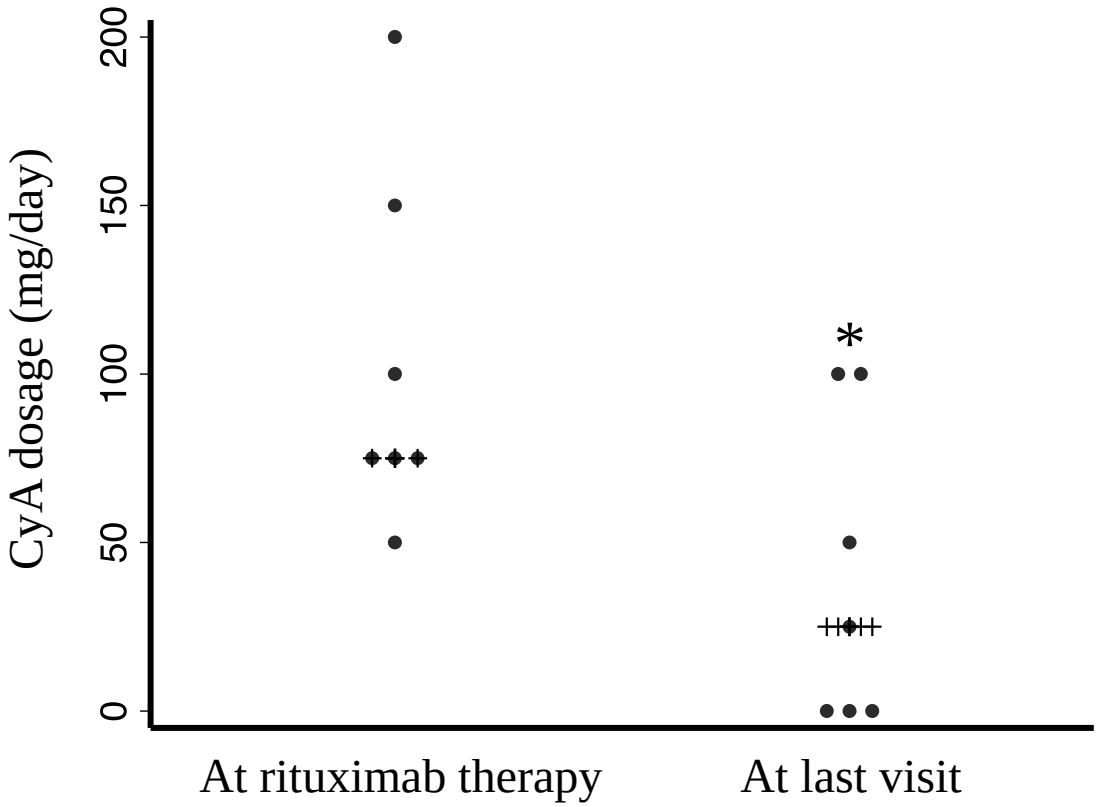

Number of relapses per year
0
1
2
3
4
Before rituximab therapy
After rituximab therapy

Fig. 3


PSL dosage (mg/day)
0
10
20
30
40
50
*
At rituximab therapy
At last visit


CyA dosage (mg/day)
0
50
100
150
200
*
At rituximab therapy
At last visit