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ORIGINAL ARTICLE

Efficacy and safety of long-term repeated use of rituximab in pediatric patients with nephrotic syndrome

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Abstract

Background We aimed to investigate the efficacy and safety of repeated use of rituximab (RTX) in pediatric patients with nephrotic syndrome (NS).

Methods Retrospective review of 50 patients with steroid-dependent NS (SDNS) who had received more than three cycles of RTX was conducted; each consisted of one to four infusions until B lymphocytes were depleted.

Results The median age of starting the first RTX cycle was 12.4 years (interquartile ranges (IQR) 10.2–14.6). During a median follow-up period of 6.3 (IQR 3.6–8.6) years, patients received a median of 5.0 RTX cycles (IQR 4.0–7.3). The number of relapses decreased from a median of 2.0 relapses per year (IQR 1.0–3.0) to 0.2 relapses per year (IQR 0.0–0.5) after long-term RTX treatments ($P < 0.001$). Longer relapse-free periods were associated with more than four RTX cycles, longer B-cell depletion, older age at each RTX treatment, and lower cholesterol levels. B lymphocytes recovered to 1% at a median of 5.9 months (95% confidence interval 5.7–6.1) after RTX administration. Factors related to a longer period of B-cell depletion included more than five RTX cycles, a higher dose of RTX, older age at treatment, and concurrent use of antimetabolites. During repeated RTX treatments, 8.0%, 6.0%, and 2.0% of patients developed hypogammaglobulinemia, severe infection, and severe neutropenia, respectively.

Conclusions Long-term repeated use of RTX may be effective and safe in pediatric NS patients. Furthermore, the redosing of RTX could be chosen by considering predictive factors for relapse-free and B-cell depletion periods.

Keywords Hypogammaglobulinemia · Mycophenolate mofetil · Nephrotic syndrome · Neutropenia · Rituximab

Introduction

Idiopathic nephrotic syndrome (NS) is characterized by proteinuria, hypoalbuminemia, and edema, with an incidence of 1.15 to 16.9 per 100,000 persons [1]. Despite the good response to steroid therapy in most NS patients, relapse is common, with about 50% of patients developing steroid-dependent NS (SDNS) or frequently relapsing NS (FRNS) [2, 3]. Conventional treatments for SDNS often involve immunosuppressive agents, including cyclophosphamide, calcineurin inhibitors (CNI), and mycophenolate mofetil (MMF), to reduce steroid use and prevent relapse [4, 5]. However, these steroid-sparing agents have adverse effects such as nephrotoxicity, gonadotoxicity, abdominal discomfort, alopecia, hirsutism, and hyperglycemia.

Rituximab (RTX) is a monoclonal anti-CD20 antibody targeting B lymphocytes and is known to be efficacious and safe through short-term studies of complicated SDNS or FRNS for maintaining remission and withdrawal of other immunosuppressants [6–9]. The Kidney Disease: Improving Global Outcomes guidelines recommended RTX as a preferred steroid-sparing therapy, especially for children with SDNS or FRNS [5]. Due to its relative effectiveness compared to other steroid-sparing agents, RTX is widely used

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in clinical practice, and the number of patients receiving repeated administration is increasing. Previous studies have shown that repeated RTX treatment improves clinical outcomes, and adverse events such as hypogammaglobulinemia, agranulocytosis, and infections were tolerable in children with SDNS [10–14]. However, there is a lack of evidence for the efficacy and safety of long-term repeated use of RTX in pediatric NS patients. Furthermore, the optimal approach for repeated RTX treatment has not been established.

This study aimed to evaluate the efficacy and safety of long-term repeated use of RTX in pediatric NS patients who received more than three cycles of RTX.

Materials and methods

Participants and RTX treatment

Fifty pediatric patients with SDNS treated with repeated RTX between 2006 and 2022 were retrospectively analyzed. The patients, aged 1 to 18 years at the onset of SDNS, received at least three cycles of RTX consisting of one to four infusions of 375 mg/m^2 per dose (maximum 500 mg) until B lymphocytes were depleted. Subsequent cycles of RTX were given after B-cell recovery or NS relapse. CD19-positive cell count was measured using flow cytometry prior to RTX treatment, with a subsequent measurement conducted 1–2 weeks after RTX administration. Following this, CD19-positive cell count was monitored every 1–2 months until recovery. Patients with congenital or infantile NS, multidrug refractory NS, and secondary NS were excluded. This study was approved by the Seoul National University Hospital Institutional Review Board (IRB no. 2106–195-1231).

Definitions

B-cell depletion was defined as a CD19-positive cell count of less than 1% of the total lymphocytes. Hypogammaglobulinemia was defined based on laboratory reference values for each age, except for cases with nephrotic-range proteinuria [15]. Neutropenia and severe neutropenia were defined as an absolute neutrophil count of less than 1500 and 500 per microliter, respectively. SDNS was defined as two consecutive relapses during steroid therapy or within 2 weeks of steroid withdrawal. FRNS was defined as four or more NS relapses within 12 months. Steroid-resistant NS (SRNS) was defined as the absence of remission after 4 weeks of 60 mg/m^2/day oral prednisolone. Relapse was defined according to the Kidney Disease: Improving Global Outcomes guidelines as a urine protein to creatine ratio $\geq$ 2.0 mg/mg or $\geq$ 3 + protein on a urine dipstick for 3 consecutive days [5]. Steroids and CNIs were tapered off after each cycle of RTX, with no defined protocol for discontinuation. Concurrent immunosuppressants such as steroids, CNI, and antimetabolites were defined as administration for more than 1 month during the B-cell depletion period after RTX treatment.

Statistical analyses

Data are expressed as a number (percentage) for categorical variables and as a median with an interquartile range (IQR) for continuous variables. Differences in clinical outcomes before and after repeated RTX treatments were analyzed using the paired *t*-test or Wilcoxon signed-rank sum test for continuous variables and the McNemar test for categorical variables. We used a generalized linear mixed model for non-normal distribution and a linear mixed model for normal distribution, with a random effect for time effects associated with longitudinal measurements, to analyze the difference in relapse rate and growth with increasing RTX cycles. The Kaplan–Meier survival curve was used to analyze the time to relapse and B-cell recovery by factors such as repeated cycles, the dose of RTX, sex, onset age, age at each RTX treatment, concomitant medications, and laboratory values at the time of RTX administration. Cox regression mixed-effect models were used for the survival outcome of repeated measures on participants to analyze the predictive factors for relapse-free survival and B-cell depletion duration. If continuous variables, including age and laboratory findings, had a *P*-value of less than 0.100 in the univariate Cox regression mixed-effect model, these were categorized according to the optimal cutoff points derived from continuous values using maximally selected log-rank statistics. The Pearson chi-square test, Fisher's exact test, independent-sample *t*-test, or Mann–Whitney *U* test was used to compare the clinical characteristics between patients with and without adverse effects. Statistical analysis was performed using R version 4.1.2. A *P*-value of < 0.05 was considered statistically significant for all tests.

Results

Patient characteristics

The characteristics of 50 pediatric patients with NS who received a total of 297 cycles of RTX are described in Table 1. The number of patients, doses, and redosing indications per rituximab cycle are presented in Supplementary Table S1. The patient population was predominantly male, with a median onset age of 4.52 years (IQR 2.73–6.75). The median age at the initiation of the first cycle of RTX was 12.40 (IQR 10.16–14.63) years, and the minimum age was 5.53 years old. Kidney biopsies were performed on 29 patients, with minimal change disease observed in 20 (40.0%) patients. Before the first RTX treatment, all patients

Table 1 Clinical characteristics of patients with nephrotic syndrome treated with long-term rituximab treatment

Characteristics	$n=50$
Sex (male: female)	37:13
Age at the onset of NS (years)	4.52 (2.73–6.75)
Age at the first RTX treatment (years)	12.40 (10.16–14.63)
Period from the onset of NS to the first RTX treatment (years)	6.63 (4.58–10.05)
Age at the last follow-up (years)	19.08 (15.29–21.80)
Kidney biopsy	
Minimal change disease	20 (40)
Focal segmental glomerulosclerosis	8 (16)
C1q nephropathy	1 (2)
Not done	21 (42)
Past SRNS	
No	27 (54)
SRNS (initial non-responder)	5 (10)
SRNS (late non-responder)	18 (36)
Frequent relapse	22 (44)
Previous immunosuppressants before RTX treatment	
Steroids	50 (100)
Cyclosporine	49 (98)
Tacrolimus	25 (50)
Cyclophosphamide	38 (76)
Mycophenolate mofetil	20 (40)
Mizoribine	24 (48)
Levamisole	10 (20)
Immunosuppressant at the first RTX treatment	
Cyclosporine	30 (60)
Tacrolimus	15 (30)
Mycophenolate mofetil	2 (4)
Mizoribine	2 (4)
Total cycles of RTX treatment	5.00 (4.00–7.25)

Values are expressed as numbers (%) and median (IQR)

NS nephrotic syndrome, *RTX* rituximab, *SDNS* steroid dependent NS, *SRNS* steroid resistant NS

received oral steroids, and 49 of them received cyclosporine therapy. The most common concurrent immunosuppressants used with RTX were cyclosporine and tacrolimus.

Efficacy of repeated RTX treatment

The median number of RTX cycles was 5.00 (IQR 4.00–7.25) during a median follow-up period of 6.3 (IQR 3.6–8.6) years. Relapse significantly decreased from a median of 2.00 (IQR 1.00–3.00) times per year to 0.22 (IQR 0.00–0.50) times per year after long-term RTX treatments ($P<0.001$) (Table 2). Height growth and hypertension improved significantly, and the estimated glomerular filtration rate remained stable after RTX therapy. There were no differences in body mass index, cataracts, osteoporosis, and diabetes between pre- and post-RTX treatments. Our analysis using the generalized linear and linear mixed models revealed that with an increasing number of repeated cycles, the relapse rate, weight *Z* score, and body mass index *Z* score decreased while the height *Z* score increased (Fig. 1). At the time of the last follow-up, 17 (34.0%) patients remained relapse-free for more than 2 years without requiring additional RTX treatments or other immunosuppressants.

Factors associated with relapse-free period

If the patient did not develop relapse before RTX redosing or after the last treatment of RTX, relapse was defined as the censored time point of the additional cycle or last follow-up. The median relapse-free period was 13.9 months (95% confidence interval (CI) 10.6–16.5) during repeated RTX treatments, which was estimated by Kaplan–Meier analysis (Fig. 2), and the median relapse-free period increased with repeated administration of RTX, ranging from 6.7 months (95% CI, 6.3–8.9) with the first cycle to 62.1 months (95% CI, 17.0–not available) with the sixth cycle. However, the seventh or higher cycles of RTX did not prolong the

Table 2 Efficacy before and after repeated rituximab treatment

	Before rituximab	After rituximab	*P*-value
Relapse/year	2.00 (1.00–3.00)	0.22 (0.00–0.50)	< 0.001
Height *Z*-score	− 1.14 (− 1.79 to − 0.33)	− 0.67 (− 1.49–0.07)	0.002
Body weight *Z*-score	− 0.15 (− 1.07–0.74)	− 0.18 (− 1.23–0.85)	0.682
BMI *Z*-score	0.56 (− 0.66–1.44)	0.10 (− 0.95–1.09)	0.224
eGFR (mL/min/1.73m^2)	111.17 (90.41–133.57)	125.10 (107.77–131.20)	0.454
Hypertension	24 (48)	13 (26)	0.013
Cataract	15 (30)	17 (34)	0.687
Osteoporosis	7 (14)	6 (12)	0.219
Diabetes	2 (4)	3 (6)	1.000

Values are expressed as numbers (%) and median (IQR)

BMI body mass index, *eGFR* estimated glomerular filtration rate

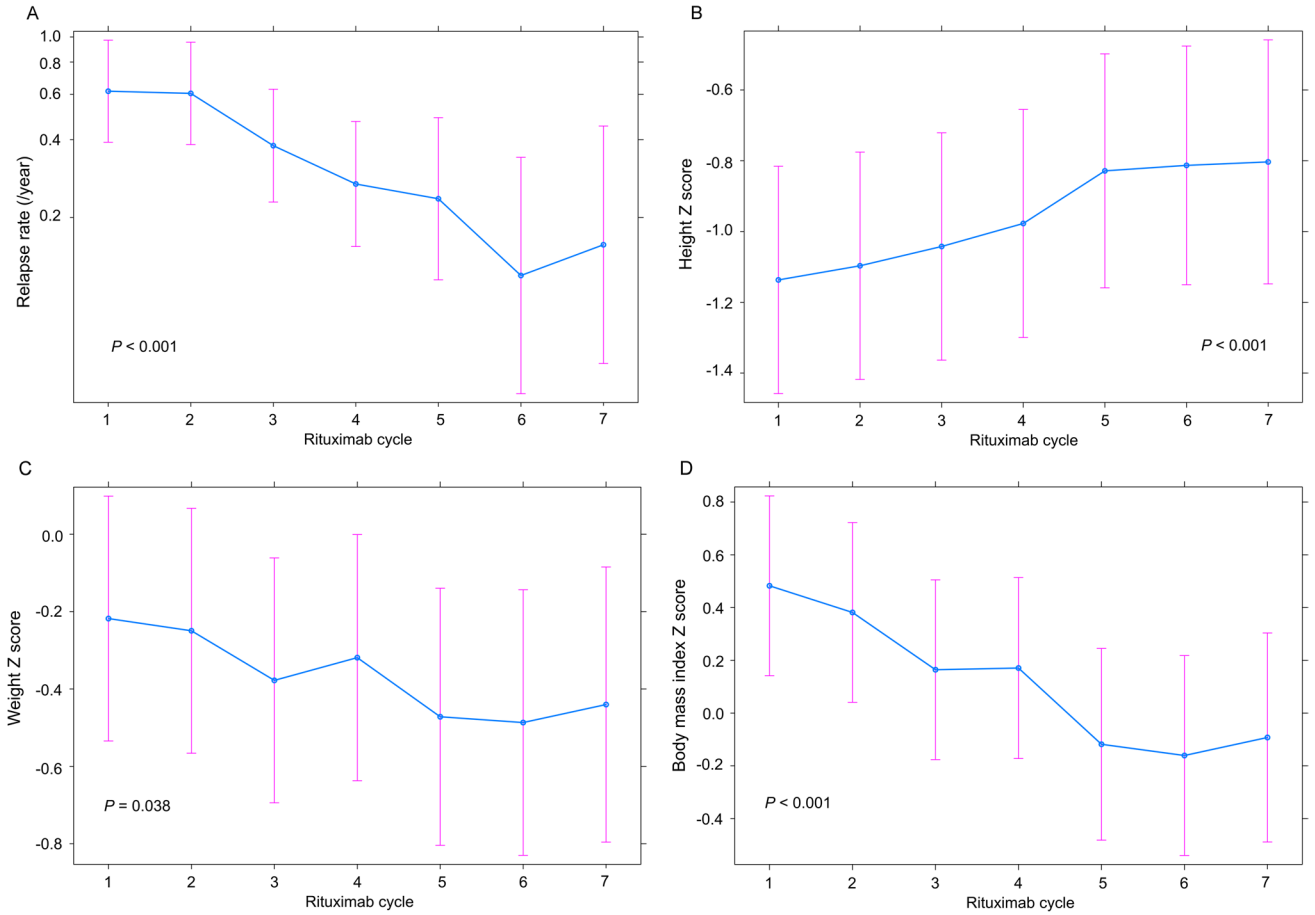

Fig. 1 The relapse rate (**A**), height *Z* score (**B**), weight *Z* score (**C**), and body mass index *Z* score (**D**) over time are based on the results of the generalized linear and linear mixed model analyses. The estimated marginal means for each cycle point are presented, and the vertical bars represent the 95% confidence interval

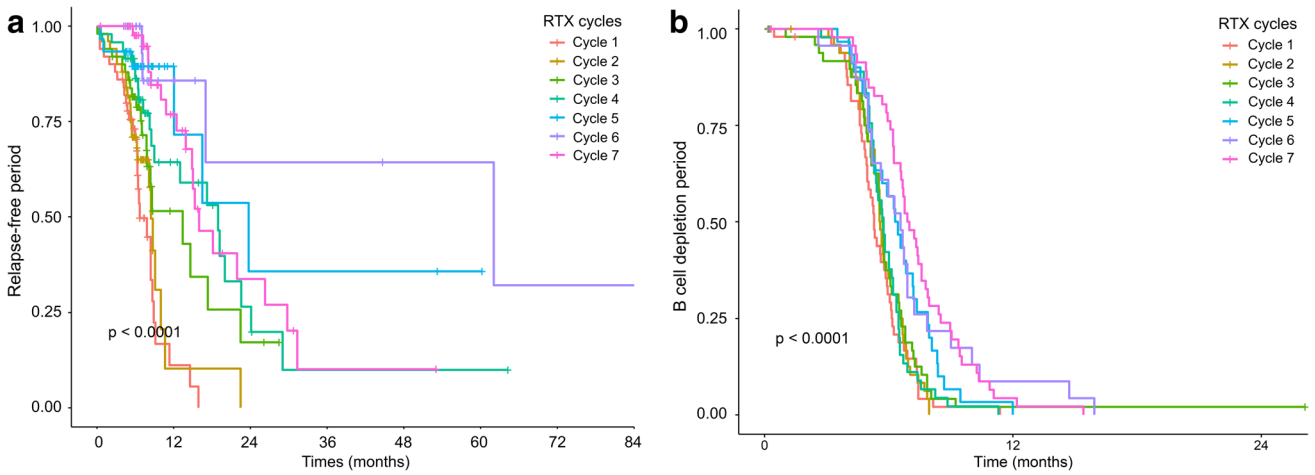

Fig. 2 Kaplan–Meier curve for the relapse-free survival (**a**) and the B-cell depletion period (**b**)

relapse-free period in 18 (36.0%) patients who received more than seven cycles.

Multivariate analysis showed that more than three cycles of RTX, a B-cell depletion period of more than 6.04 months, female sex, age at each RTX treatment of more than 13.37 years, and serum cholesterol level equal to or less than 208 mg/dL were associated with a longer relapse-free period compared with the first cycle of RTX (Table 3).

Factors associated with B-cell depletion period

The B lymphocytes recovered to 1% after a median of 5.85 months (95% CI, 5.68–6.11) post-completion of RTX administration (Fig. 2). The B-cell depletion period sustained within the fourth cycle of RTX ranged from 5.29 to 5.75 months but increased to 6.37–6.95 months after more than four cycles (Table 4). Multivariate analysis demonstrated that more than four cycles of RTX and more than one dose of RTX per cycle were associated with a more extended period of B-cell depletion. In addition, the age at each RTX treatment of more than 12.71 years and concomitant use of antimetabolites were related to a longer B-cell depletion period.

Adverse effects of RTX treatment

The adverse effects of RTX treatment are summarized in Table 5. Acute side effects after infusion were observed in 20 (40.0%) patients, with chest discomfort and urticaria/rash being the most common. However, no patients experienced severe infusion reactions or serum sickness disease. Anti-RTX antibodies were detected in one patient and were associated with failure to achieve B-cell depletion after the third cycle of RTX. Complete blood cell counts were monitored regularly after all RTX treatments. Neutropenia was observed in 19/297 (6.4%) episodes among 13/50 (26.0%) patients after a median of 4.4 months (IQR 2.5–5.6) of treatment with RTX. Two episodes of severe neutropenia occurred in one (2.0%) patient, at 3.1 and 3.4 months after RTX treatment, and spontaneously resolved without intervention. Immunoglobulin G levels were measured in 94 (31.6%) of 297 RTX treatments. Hypogammaglobulinemia was detected in four (8.0%) patients after a median of 1.1 years and 2.5 cycles of RTX treatment. None of the patients required immunoglobulin replacement therapy or discontinuation of RTX. Severe infections requiring hospitalization or intravenous antibiotics were observed in three (6.0%) patients, but no life-threatening infections were identified. There were no statistical differences in sex, age of NS onset, age of the first RTX treatment, history of SRNS, and FRNS between patients with and without long-term side effects, including hypogammaglobulinemia, severe neutropenia, and severe infections (Supplementary Table S2). No secondary neoplasms or opportunistic infections occurred during the study period.

Discussion

In this study, we showed that repeated and long-term usage of RTX in pediatric patients with SDNS is effective and relatively safe. The long-term clinical course after RTX treatment improved, especially in cases of relapse events and height growth, consistent with previous studies [10, 13, 16, 17]. Several predictive factors for relapse-free and B-cell depletion periods were identified in SDNS children receiving repeated RTX treatment.

After randomized controlled trials of RTX demonstrated clinical improvement in children with SDNS [6–8], observational studies have shown that additional RTX treatment after B-cell reconstitution effectively reduces relapse and discontinues the use of steroids and CNIs [13, 16–18]. The optimal number of cycles and indication for retreatment with RTX remain uncertain. Although a Japanese study showed that preemptive repeated RTX treatments to maintain B-cell depletion could be effective for maintaining long-term remission in SDNS patients with a history of SRNS [12], most studies had a strategy to retreat with RTX after relapse or B-cell recovery, similar to our study. Our study analyzed data from patients who received a median of five cycles of RTX over a median follow-up period of 6.3 years and demonstrated that with increasing repeated cycles, clinical outcomes, including relapse rate, height, and body mass index, improved. Additionally, relapse-free survival was extended after repeated cycles of RTX, consistent with a previous study [10]. These findings suggest that repeated cycles of RTX could be increasingly effective in improving long-term clinical outcomes in patients with SDNS.

This study found that a more extended relapse-free period was associated with a longer duration of B-cell depletion, older age at each RTX treatment, female sex, and lower cholesterol levels. RTX has previously been shown to maintain remission in NS patients during B-cell depletion effectively [11, 19–21]. However, this study is the first to identify the B-cell depletion period as a predictive factor for the relapse-free period in patients treated repeatedly with RTX. Previous studies have reported that older age at RTX treatment is related to better outcomes, and our data support this observation [10, 16, 20]. The International Pediatric Nephrology Association practice guidelines recommend that RTX is preferable, both in terms of safety and effectiveness, for children from 7 to 9 years of age [4]. Although there have been a few reports on relapse risk based on sex differences [22, 23], female sex was associated with prolonged remission during repeated RTX treatments in this study. In addition, lower cholesterol concentration at the time of RTX

Table 3 Predictive factors for a relapse-free period

			Mixed effect Cox regression analysis			
	n	Median (95% CI)[a]	HR (95% CI)	*P*-value	Adjusted HR (95% CI)	*P*-value
Rituximab cycle						
1	50	6.7 (6.3–8.9)	Reference		Reference	
2	50	8.4 (8.2–NA)	0.71 (0.40–1.27)	0.250	0.86 (0.46–1.60)	0.631
3	50	13.4 (7.8–NA)	0.38 (0.20–0.71)	0.002	0.56 (0.29–1.11)	0.098
4	47	19.0 (9.0–NA)	0.22 (0.12–0.43)	< 0.001	0.29 (0.13–0.64)	0.002
5	30	23.7 (12.1–NA)	0.09 (0.03–0.26)	< 0.001	0.28 (0.10–0.84)	0.022
6	23	62.1 (17.0–NA)	0.03 (0.01–0.12)	< 0.001	0.10 (0.03–0.38)	< 0.001
≥ 7	47	16.0 (15.0–NA)	0.13 (0.06–0.27)	< 0.001	0.46 (0.19–1.10)	0.081
Rituximab dose per cycle						
1 dose	277	14.9 (12.1–17.3)	Reference		Reference	
2 doses	17	8.7 (8.0–NA)	1.81 (0.86–3.84)	0.120	0.99 (0.45–2.15)	0.973
4 doses	3	11.3 (8.4–NA)	3.07 (0.83–11.31)	0.092	0.91 (0.24–3.49)	0.889
B cell depletion period	288	NA	0.78 (0.69–0.88)	< 0.001		
≤ 6.04 months	158	8.3 (7.0–9.1)	Reference		Reference	
> 6.04 months	130	20.0 (15.0–31.3)	0.28 (0.17–0.45)	< 0.001	0.36 (0.22–0.61)	< 0.001
Sex						
Male	227	10.8 (8.9–15.0)	Reference		Reference	
Female	70	22.5 (15.9–NA)	0.55 (0.28–1.07)	0.080	0.52 (0.28–0.95)	0.034
Age at the onset of NS	297	NA	1.02 (0.92–1.12)	0.717		
History of steroid-resistant NS						
No	143	14.6 (10.0–20.0)	Reference		Reference	
Yes	154	13.9 (9.1–17.2)	1.24 (0.71–2.18)	0.451	0.84 (0.53–1.34)	0.465
Frequent relapsing NS						
No	161	15.9 (13.9–22.5)	Reference		Reference	
Yes	136	9.0 (8.0–15.0)	1.62 (0.95–2.79)	0.079	1.36 (0.87–2.14)	0.177
Age at each rituximab treatment	297	NA	0.86 (0.8–0.92)	< 0.001		
≤ 13.37 years	110	8.4 (7.0–11.3)	Reference		Reference	
> 13.37 years	187	15.3 (13.4–21.9)	0.34 (0.21–0.55)	< 0.001	0.46 (0.29–0.75)	0.002
Cholesterol	297	NA	1.01 (1.00–1.01)	< 0.001		
≤ 208 mg/dL	194	17.2 (15.0–23.7)	Reference		Reference	
> 208 mg/dL	103	7.8 (6.7–8.8)	3.74 (2.44–5.74)	< 0.001	1.89 (1.04–3.42)	0.036
Albumin	297	NA	0.46 (0.33–0.64)	< 0.001		
≤ 3.9 mg/dL	84	7.8 (6.5–14.6)	Reference		Reference	
> 3.9 mg/dL	213	15.9 (13.0–20.0)	0.43 (0.29–0.64)	< 0.001	0.92 (0.54–1.58)	0.774
Urine protein/creatinine	297	NA	1.27 (0.75–2.15)	0.381		
Concomitant medications						
Steroids						
No	161	19.0 (15.3–31.3)	Reference		Reference	
Yes	136	8.4 (7.8–9.1)	2.97 (1.98–4.46)	< 0.001	1.31 (0.74–2.32)	0.353
Calcineurin inhibitors						
No	126	19.0 (15.3–29.0)	Reference		Reference	
Yes	171	8.9 (8.4–13.0)	2.26 (1.42–3.59)	< 0.001	1.05 (0.63–1.74)	0.853
Antimetabolites						
No	242	12.1 (9.1–15.3)	Reference		Reference	
Yes	55	16.5 (13.0–NA)	0.78 (0.44–1.37)	0.390	1.06 (0.58–1.94)	0.858

HR hazard ratio, *CI* confidence interval, *NA* not available, *NS* nephrotic syndrome

[a]Estimated by Kaplan–Meier analysis

Table 4 Predictive factors for B-cell depletion period

			Mixed effect Cox regression analysis			
	n	Median (95% CI)[a]	HR (95% CI)	*P*-value	Adjusted HR (95% CI)	*P*-value
Rituximab cycle						
1	50	5.29 (4.96–5.91)	Reference		Reference	
2	50	5.57 (5.45–6.08)	1.11 (0.72–1.71)	0.634	0.81 (0.51–1.28)	0.359
3	50	5.70 (5.52–6.08)	0.90 (0.58–1.41)	0.656	0.79 (0.48–1.29)	0.348
4	46	5.75 (5.36–6.21)	1.01 (0.65–1.58)	0.950	0.86 (0.52–1.43)	0.560
5	30	6.37 (5.29–7.36)	0.52 (0.31–0.86)	0.011	0.37 (0.20–0.67)	< 0.001
6	23	6.57 (5.19–7.85)	0.44 (0.25–0.76)	0.003	0.31 (0.16–0.59)	< 0.001
≥ 7	47	6.95 (6.60–7.79)	0.23 (0.14–0.38)	< 0.001	0.16 (0.09–0.30)	< 0.001
Rituximab dose per cycle						
1 dose	276	5.75 (5.59–6.08)	Reference		Reference	
2 doses	17	6.14 (5.95–7.43)	0.53 (0.31–0.91)	0.021	0.42 (0.24–0.76)	0.004
4 doses	3	8.15 (6.74–NA)	0.30 (0.09–1.04)	0.058	0.08 (0.02–0.30)	< 0.001
Sex						
Male	226	5.85 (5.68–6.14)	Reference		Reference	
Female	70	5.75 (5.55–6.31)	1.28 (0.65–2.52)	0.482	0.82 (0.43–1.58)	0.559
Age at onset of NS	296	NA	0.95 (0.86–1.05)	0.308		
History of steroid-resistant NS						
No	143	5.91 (5.59–6.44)	Reference		Reference	
Yes	153	5.78 (5.55–6.08)	1.35 (0.75–2.42)	0.319	1.17 (0.64–2.12)	0.616
Frequent relapsing NS						
No	160	6.05 (5.75–6.24)	Reference		Reference	
Yes	136	5.67 (5.29–6.08)	1.29 (0.72–2.33)	0.393	1.35 (0.76–2.39)	0.305
Age at each rituximab treatment	296	NA	0.84 (0.79–0.89)	< 0.001		
≤ 12.71 years	92	5.45 (5.19–5.68)	Reference		Reference	
> 12.71 years	204	6.31 (5.91–6.57)	0.41 (0.27–0.61)	< 0.001	0.44 (0.27–0.72)	0.001
Cholesterol	296	NA	1.00 (1.00–1.00)	0.002		
≤ 253 mg/dL	233	6.01 (5.75–6.24)	Reference		Reference	
> 253 mg/dL	63	5.49 (4.96–5.88)	1.81 (1.27–2.58)	< 0.001	1.16 (0.77–1.77)	0.478
Albumin	296	NA	0.68 (0.54–0.85)	< 0.001		
≤ 4.1 mg/dL	129	5.55 (5.22–5.78)	Reference		Reference	
> 4.1 mg/dL	167	6.21 (5.91–6.57)	0.70 (0.53–0.93)	0.015	0.87 (0.61–1.25)	0.457
Urine protein/creatinine	296	NA	1.31 (0.96–1.80)	0.087		
≤ 0.06 mg/mg	112	6.44 (5.85–6.80)	Reference		Reference	
> 0.06 mg/mg	184	5.65 (5.49–5.95)	1.66 (1.22–2.27)	0.001	1.28 (0.92–1.77)	0.138
Concomitant medications						
Steroids						
No	161	6.08 (5.78–6.47)	Reference		Reference	
Yes	135	5.65 (5.42–5.88)	1.37 (1.02–1.83)	0.034	0.85 (0.56–1.27)	0.420
Calcineurin inhibitors						
No	126	6.21 (5.88–6.51)	Reference		Reference	
Yes	170	5.65 (5.36–5.91)	1.52 (1.10–2.09)	0.011	0.89 (0.61–1.30)	0.545
Antimetabolites						
No	242	5.72 (5.52–6.01)	Reference			
Yes	54	6.52 (5.88–7.33)	0.83 (0.56–1.25)	0.381	0.51 (0.33–0.79)	0.003

HR hazard ratio, *CI* confidence interval, *NA* not available, *NS* nephrotic syndrome

[a]Estimated by Kaplan–Meier analysis

Table 5 Adverse events during long-term rituximab treatment

	Numbers of patients (%)
Acute side effects	
Urticaria/rash	7 (14)
Throat pain/cough/dyspnea	4 (8)
Chest discomfort	8 (16)
Headache/dizziness	5 (10)
Bradycardia/tachycardia	2 (4)
Nausea/vomiting/abdominal pain	5 (10)
Long-term events	
Neutropenia	13 (26)
Severe neutropenia	1 (2)
Infectious history	5 (10)
Cellulitis	1 (2)
Chickenpox	1 (2)
Pneumonia	1 (2)
Minor (gastroenteritis, otitis externa)	2 (4)
Hypogammaglobulinemia	4 (8)
Cancer	0 (0)

administration was associated with a longer relapse-free period. Administering RTX after inducing remission is recommended to maximize efficacy [4, 5], as the serum half-life of RTX is known to be extremely short due to urinary loss in patients with proteinuria [24]. Cholesterol levels may reflect the degree of control of NS since patients with NS have elevated cholesterol levels immediately after remission of proteinuria. Delaying RTX redosing until cholesterol levels decrease after a relapse may be beneficial in extending the relapse-free period.

Similar to our findings, the duration of B-cell depletion after RTX injection was between 5.1 and 6.1 months in the previous studies evaluating repeated RTX treatment [10, 11, 25]. Chan et al. showed that the B-cell depletion period remained consistent after redosing in children with SDNS who received two or more courses of RTX [10]. However, our study observed that a long duration was required for B-cell recovery in patients who received equal to or more than five cycles of RTX. This result discrepancy could be due to the difference in the number of repeated RTX administrations. Our study enrolled patients who received more than three cycles of RTX, with 94% and 64% of the patients having received four and five retreatments, respectively, which is higher than the 34% and 20% reported in a previous study [10]. As RTX treatments increase, clinicians must be aware of the potential prolongation of impaired B-cell immunity. Previous studies have shown that repeated RTX treatment courses increased the incidence of hypogammaglobulinemia [14, 26]. In addition, we found that the duration of B-cell recovery is associated with other factors, including the dose of RTX, age at each RTX treatment, and concurrent use of antimetabolites such as MMF or mizoribine. Previous studies have demonstrated that RTX dose and older age were strongly associated with time to B-cell reconstitution [21, 27].

Concomitant use of other immunosuppressants during RTX treatment has reduced the risk of relapse. However, the optimal immunosuppressive therapy for remission after RTX treatment remains controversial [28–31]. While previous studies have demonstrated that MMF is effective for maintaining remission after RTX treatment [28–30], Fujinaga et al. [31] reported that CNI is superior to MMF after the first RTX treatment. Moreover, long-term follow-up data showed that MMF therapy as a maintenance immunosuppressant after RTX was a predicting factor for early relapse [20]. In our study, the use of antimetabolites was associated with a longer period of B-cell depletion but not a more extended relapse-free period. There is no evidence of an association between the B-cell depletion period and impaired immune systems, including hypogammaglobulinemia and neutropenia. However, prolonged B-cell depletion may cause adverse effects such as infection. Previous studies showed that a history of SRNS, low immunoglobulin G levels at RTX treatment, and younger age were risk factors for developing hypogammaglobulinemia in children receiving RTX [14, 32]. Therefore, clinicians should carefully monitor patients with risk factors for hypogammaglobulinemia when using antimetabolites with repeated RTX treatment. Future studies must identify better immunosuppressive therapy for remission during repeated RTX treatment.

Chronic adverse events, including hypogammaglobulinemia, infection requiring hospitalization, and severe neutropenia, were noted in a relatively low percentage of our patients: 8%, 6%, and 2%, respectively. Furthermore, there were no cases of RTX discontinuation due to these adverse events. While hypogammaglobulinemia events were less frequent in our study, previous studies have shown that hypogammaglobulinemia is a common complication of RTX treatment in children with SDNS [10, 14, 33]. This event might have been underestimated in our study, as immunoglobulin levels were not routinely checked and were measured in only 31.6% of RTX treatments. In a European survey, 65% and 59% of centers reported regularly monitoring immunoglobulin G levels before and after RTX administration [33]. While a retrospective study showed no significant association between hypogammaglobulinemia severity and infection [15], another study reported that 80% of patients with severe infections had hypogammaglobulinemia [33]. Clinicians need to monitor impaired immune function following RTX treatment, particularly in younger patients at higher risk for hypogammaglobulinemia, as reported in previous studies [10, 34]. Additionally, patients on repeated RTX may

be at increased risk of infections due to prolonged use of multiple immunosuppressive agents before and after RTX treatments. Further studies are required to identify chronic side events and their risk factors.

This study had several limitations. First, it was a retrospective observational study, which did not include standardized protocols for indications of repeated RTX, concurrent use of immunosuppressants, tapering schedule of steroid and CNI, and immune system monitoring. Second, despite adjusting for confounding factors, selection bias was potential. Lastly, it was a relatively small study, which may have included patients in whom repeated RTX treatment was relatively effective in controlling NS. Consequently, these limitations present challenges in assessing and drawing definitive conclusions on the efficacy and safety of long-term repeated treatment with RTX and warrant further research. Nevertheless, we identified several factors related to relapse-free and B-cell depletion periods in children receiving repeated RTX treatments during long-term follow-up. Our results could contribute to establishing an appropriate approach to repeated RTX treatment based on the patient's characteristics and laboratory findings.

In conclusion, this study found that long-term repeated treatment with RTX effectively improves the long-term clinical course, particularly in reducing the frequency of relapse events and promoting height growth in pediatric patients with NS. Additionally, we identified several predictive factors for relapse-free and B-cell depletion periods. Based on our findings, it is expected that the redosing of RTX could be chosen by considering predictive factors for relapse-free and B-cell depletion periods.

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Data availability The data underlying this article will be shared upon reasonable request to the corresponding author.

Declarations

Ethics approval This study was approved by the Seoul National University Hospital Institutional Review Board (IRB no. 2106–195-1231).

Conflict of interest The authors declared no competing interests.

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