

Efficacy of rituximab therapy in children with refractory nephrotic syndrome: a prospective observational study in Shanghai

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Background: Idiopathic nephrotic syndrome is the most common glomerular disease in children. This study was undertaken to observe the efficacy and side-effects of rituximab (RTX) in treating children with different types of refractory primary nephrotic syndrome.

Methods: Twelve patients with steroid dependent nephrotic syndrome (SDNS), frequently relapsing nephritic syndrome (FRNS), and steroid resistant nephrotic syndrome (SRNS) were enrolled in our study. There were obvious drug side-effects, and proteinuria remained difficult to control. RTX was administered at a dose of 375 mg/m² body surface area, once or twice weekly.

Results: The male to female ratio was 3:1, and the onset age was 1.6-8.9 years. There were 9 patients with steroid sensitive nephrotic syndrome (SDNS or FRNS), and 3 patients with SRNS. There were 7 patients with minimal change disease (MCD), 3 patients with focal segmental glomerular sclerosis (FSGS), 1 with focal proliferative glomerulonephritis, and 1 without renal biopsy. The total effective treatment rate of RTX was 91.67%, and for 77.78% of the patients, steroid dosage could be reduced. Six months before and after RTX infusion, the mean steroid dosage was significantly decreased ($P=0.014$) and the recurrence number was significantly reduced ($P<0.001$). The results were better in MCD patients than in FSGS patients ($P=0.045$). There was no significant difference between FRNS/SDNS and SRNS patients ($P=0.175$). During RTX administration, 3 patients developed skin rashes, 1 developed hypotension,

and 1 developed a fever. One patient experienced a persistent decrease in serum immunoglobulin level but without serious infection.

Conclusion: RTX was effective in the treatment of refractory nephrotic syndrome, and it could significantly reduce the use of steroid and immunosuppressants.

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Key words: refractory nephritic syndrome; rituximab

Introduction

Idiopathic nephrotic syndrome is the most common glomerular disease in children. Its main pathological type is minimal change disease (MCD). Since MCD often responds to steroids, such patients are recognized as steroid-sensitive nephrotic syndrome (SSNS). However, nearly 50% of the patients will develop to frequent-relapsing nephrotic syndrome (FRNS) and/or steroid-dependent nephrotic syndrome (SDNS). Focal segmental glomerular sclerosis (FSGS) often appears as steroid-resistant nephrotic syndrome (SRNS). Patients with FRNS, SDNS, and SRNS who are difficult to be controlled by variable immunosuppressants are thought to have refractory nephrotic syndrome.

Patients with refractory nephrotic syndrome^[1] usually have to take various immunosuppressant (IS) agents, including cyclophosphamide (CYC), cyclosporine A (CyA), tacrolimus (FK506), and mycophenolate mofetil (MMF). More than 25% of SSNS children still require steroids and/or IS drugs in their adulthood.^[2] Treatment of SRNS is difficult. Some patients may require multiple target therapy, combined with steroids, mycophenolate mofetil and calcineurin inhibitors. A related issue for these patients is severe drug-related toxicity, which can lead to growth retardation, cataract, osteoporosis, hypertension, and renal toxicity. Therefore, the treatment of FRNS/SDNS and SRNS has long been the focus of pediatric nephrologists.

A study^[3] hypothesized that B cells may be

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involved in the pathogenesis of nephrotic syndromes. Since 2004, many studies using rituximab (RTX) to treat pediatric SDNS, MCD, SRNS, and FSGS, and even recurrent FSGS after renal transplantation have shown satisfactory results.^[4-10] But there is lack of strong data on the optimal number of RTX infusions per treatment course and a suitable maintenance therapy after remission.^[11] There are few reports^[12,13] on RTX treatment in China. The present study was undertaken to examine the response of RTX treatment and its side-effects in Chinese children with refractory idiopathic nephrotic syndrome.

Methods

Patients

Patients who were enrolled in this study met the following criteria: (1) they were younger than 16 years; (2) they had idiopathic nephrotic syndrome; (3) the disease was characterized by SDNS, FRNS, and/or SRNS^[14,15]; (4) IS agents were taken such as CYC, CyA, FK506, or MMF, with obvious side-effects; (5) they had no history of RTX treatment; and (6) genetic test (including *NPHS1*, *NPHS2*, *WT1*, *PLCE1*, *CD2AP*, *TRPC6*, *APOL1*, *INF2*, *MYO1E*, and *MYH9*) was carried out to exclude genetic mutation in children with SRNS. This study was approved by the ethical committee of the hospital. All patients signed the consent form.

Treatment protocol

Routine examination before RTX treatment included: (1) complete blood count, liver function, serum lipids, plasma protein, and immune function assessment; (2) electrocardiography and cardiac ultrasound examination; (3) blood pressure measurement; (4) detection of hepatitis B infection, hepatitis C infection, tuberculosis infection and human immunodeficiency virus infection; and (5) none of other active infections.

The dosage of RTX was 375 mg/m² body surface area, with a maximum of 500 mg. The treatment regimens were different, according to whether the patients were in remission again. For patients with remission, due to the use of prednisolone or other IS agents, RTX was administered only once. For patients without remission, RTX was administered once a week for a maximum of 2 weeks. After remission, the dose of IS agents was gradually reduced for 3-6 months. This study was ended on February 29, 2012.

We checked serum immunoglobulin (Ig) levels and CD19+ B-cell counts by fluorescence activating cell sorting (FACS) until complete B-cell depletion, and then we monitored once a month. B-cell depletion was defined as peripheral CD19+ cell less than 1%

and 5 cells/mm³, and B-cell recovery was defined as peripheral CD19+ cell more than 3% and 15 cells/mm³.

Effectiveness of RTX

The evaluation of RTX effectiveness was to withdraw one or more IS agents with a reduced proteinuria relapse frequency. Complete effect: there was a decreased proteinuria relapse frequency while reducing IS agent dosage by more than 50%; partial effect: there was a reduced proteinuria relapse frequency while reducing IS dosage by less than 50%; and ineffective treatment: IS dosage could not be reduced, but proteinuria relapse frequency increased or renal functional deterioration occurred.

Remission of nephrotic syndrome

Estimated glomerular filtration rate (eGFR) was calculated with the Schwartz formula. Complete remission was defined as negative proteinuria, proteinuria-to-creatinine ratio <0.2, 24-hour proteinuria level <150 mg/kg per day, negative microalbuminuria, and normal renal function and blood pressure. Partial remission was defined as proteinuria level reduced by 50%, proteinuria-to-creatinine ratio <2.0, 24-hour proteinuria level <50 mg/kg per day, and stable renal function and blood pressure. Absence of remission was defined as positive proteinuria, proteinuria-to-creatinine ratio >2.0, 24-hour proteinuria level reach the nephrotic range, and deterioration of renal function and blood pressure.

Maintenance therapy program

According to B cell recovery and/or relapse of nephrotic syndrome, we suggested another dose of RTX. For patients with negative proteinuria but B cell recovery who wouldn't use 2nd dose RTX, we suggested a maintenance therapy with MMF. Patients with positive proteinuria, regardless of B cell status, should receive another dose of RTX or be given a different IS agent according to their willingness.

Statistical analysis

SPSS 11.5 software was used for statistical analyses in this study. Paired *t* test was used to compare the frequency of relapse and the dosage of prednisolone before and after RTX infusion. The Chi-square test was to compare the RTX efficacy, in different steroid responses and in different pathological types. A *P* value less than 0.05 was considered statistically significant.

Results

Twelve patients were enrolled in this clinical trial

Table 1. Patients' characteristics

Patient No.	Gender	Age at onset (y)	NS duration (mon)	Treatment response	Renal histology	IS used before RTX	Infusion number of RTX
1	F	1.6	6	SRNS	FSGS	CyA, MMF, FK506	2 [†]
2	M	3.0	66	SSNS (SDNS)	ND	CYC, MMF	1
3	M	4.0	11	SSNS (FRNS/SDNS)	MCD	MMF, FK506	2 [†]
4	F	2.3	35	SRNS (CyA dependent)	MCD	CyA	2 [†]
5	M	2.0	169	SSNS (FRNS/SDNS)	FSGS	CYC, CyA, MMF	2 [‡]
6	M	8.9	26	SSNS (CyA dependent)	FPGN	CyA	1
7	M	5.1	92	SSNS (SDNS)	FSGS	MMF	1
8	M	2.3	98	SSNS (FRNS/SDNS)	MCD	CYC, CyA, MMF	1
9	F	3.5	33	SRNS (CyA dependent)	MCD	CyA	1
10	M	5.9	125	SSNS (SDNS/CyA dependent)	MCD	CYC, CyA	1
11	M	4.0	13	SSNS (FRNS/SDNS)	MCD	FK506	2 [‡]
12	M	2.5	21	SSNS (SDNS)	MCD	CYC	1

NS: nephrotic syndrome; IS: immunosuppressant; M: male; F: female; SSNS: steroid sensitive nephrotic syndrome; SRNS: steroid resistant nephrotic syndrome; SDNS: steroid dependent nephrotic syndrome; FRNS: frequently relapsing nephrotic syndrome; MCD: minimal change disease; FSGS: focal segmental glomerular sclerosis; FPGN: focal segmental glomerulonephritis; ND: not done; CYC: cyclophosphamide; MMF: mycophenolate mofetil; CyA: cyclosporine; FK506: tacrolimus; RTX: rituximab. *: receiving 2 continuous doses of rituximab; †: B-cell recovery without proteinuria relapse; ‡: proteinuria relapse without B-cell recovery.

from October 2010 to October 2011. Their clinical characteristics are shown in Table 1. Three SRNS patients underwent genetic test, and no mutation was found. The patients were followed up for 4-16 months (average: 8±4 months).

Efficacy of RTX

In this series, 10 patients showed complete remission of nephrotic syndrome, 1 had partial remission, and 1 had no remission at all. The total remission rate in this series was 91.67%. RTX was completely effective in 9 of the 12 patients, partially effective in 2, and ineffective in 1. The total effective rate of RTX was 91.67%.

Treatment with steroids was discontinued in 5 of the 9 SSNS patients. The dosage of steroid was reduced by more than 50% in 2 patients and by less than 50% in 2. The rate of steroid reduction was 77.78%. The average dosage of steroid was significantly decreased after the treatment with RTX ($\chi^2=162.44$ vs. $\chi^2=82.92$, $P=0.014$; Fig.). All the patients stopped the use of IS agents at 6 months. At the end of observation, 3 patients had discontinued all drugs, 4 continued only MMF after B-cell recovery, 3 continued with a single steroid at a reduced dosage, and 1 continued steroid use and MMF after B-cell recovery with relapse. Efficacy in MCD patients was better than that in FSGS patients ($P=0.045$) (Table 2). At six months after RTX infusion, we compared the relapse frequency and found that it decreased significantly after treatment ($\chi^2=1.45$ vs. $\chi^2=0.18$, $P<0.001$; Table 3).

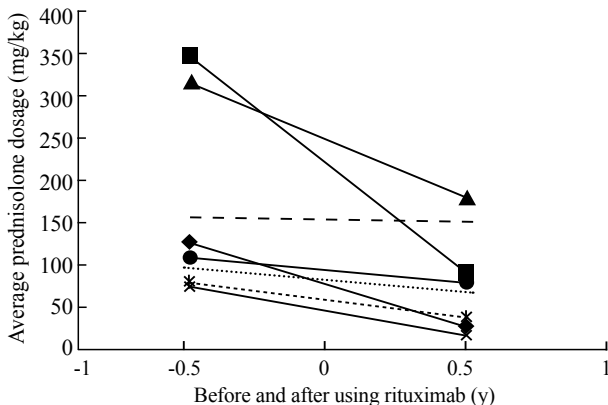


Fig. The average prednisolone dosage of 9 patients with steroid-sensitive nephrotic syndrome before and after 6 months of rituximab infusion (mg/kg).

Table 2. Rituximab effectiveness for different steroid responses and pathological types

Variables	FRNS/SDNS (n=9)	SRNS (n=3)	MCD (n=7)	FSGS (n=3)
Complete remission (%)	8	2	7	1
Partial remission (%)	1	0	0	1
Absent remission (%)	0	1	0	1
P value	0.175		0.045	

FRNS: frequently relapsing nephrotic syndrome; SDNS: steroid dependent nephrotic syndrome; SRNS: steroid-resistant nephrotic syndrome; MCD: minimal change disease; FSGS: focal segmental glomerulosclerosis.

Change in CD series and plasma immunoglobulin

All patients' peripheral B-cell counts reached depletion within 2 weeks. For 11 patients, B-cell count increased at an average of 4.38 months. For one patient who received 2 continuous doses, B-cell recovery occurred at 5.5 months. Two patients recovered at 11 months after administration of a second dose of RTX due to B-cell recovery without relapse. The other 2 patients maintained depleted B-cells levels by receiving a second dose (due to proteinuria relapse without B-cell recovery) at the end

Table 3. Comparison of the relapse frequency 6 months before and after rituximab treatment in the 11 treatment-effective patients

Patient No.	Number of relapse	
	During the 6 months before RTX	During the 6 months after RTX
2	1	0
3	2	0
4	1	0
5	1	1
6	1	0
7	1	0
8	2	0
9	1	0
10	2	0
11	2	1
12	2	0
Mean	1.45*	0.18*
SD	0.52	0.40

*: $P < 0.001$. SD: standard deviation; RTX: rituximab.

of observation (4 and 9 months).

At the end of observation, there were 6 patients with B-cell recovery without relapse. One patient had B-cell recovery with relapse. Two patients relapsed without B-cell recovery. Two patients remained in remission without B-cell recovery.

Two patients maintained reduced serum Ig levels. One patient never achieved negative proteinuria. The other patient had low Ig levels, though negative proteinuria continued for a while. His bone marrow CD classification also showed a significantly decreased CD19 cell level.

Adverse side effects

During treatment of RTX, 3 patients developed rashes, 1 developed hypotension, and 1 developed a fever. After reducing drip-speed or administering steroids, these symptoms disappeared quickly. No patients were given prophylactic antibiotics, and no severe infection occurred. However, one patient had persistent low serum Ig. During follow-up, this patient had fever 3 times, upper respiratory infection twice, and pneumonia once. All his infections could be effectively controlled. At present, he is under close observation.

Discussion

In the past few years, the successful results of anti-CD20 monoclonal antibody (RTX) in treating FRNS/SDNS and SRNS have attracted much attention from researchers. Our data confirmed that RTX allows a reduction in the use of steroid and other IS agents, which is beneficial for avoiding their side-effects and reducing relapse frequency.

Our data showed that the remission rate for FRNS/SDNS patients (100%) was higher than that for SRNS

patients (66.7%). The complete remission rate and total remission rate were better in MCD patients than in FSGS patients. This finding was consistent with the literature.^[16,17] Therefore, when RTX is used, both steroid response and pathological type should be considered. Are SRNS patients with FSGS not suitable for receiving rituximab treatment? A previous case report described 2 SRNS patients with FSGS who achieved partial remission after the administration of a single agent, rituximab.^[18]

Except for one patient with persistent proteinuria, all other patients in this study used either steroids or CyA to successfully reduce proteinuria before RTX treatment. Studies^[9,19] showed that when proteinuria was negative, RTX efficacy was high. Some researchers supposed that the pharmacokinetics of RTX may be affected by the nephrotic syndrome remission state, and that despite depletion of circulating B-cells, noncirculating B-cells may reach only partial clearance. Therefore, proteinuria remission is an important factor for efficacy of RTX.

Until now, no uniform protocol of RTX has been used for the treatment of idiopathic nephrotic syndrome.^[4-10] Some authors used a single dose, some used it once a week for 2-4 weeks, and others used one or more doses according to B-cell recovery and/or proteinuria relapse. In our study, 4 patients received a second dose of RTX. The B-cell depletion duration for these 4 patients was significantly longer than that for the patient who received 2 continuous doses. A recent study^[20] found that the B-cell depletion duration for children receiving 1 or 2 doses ($n=5$, mean: 7.1 months) was similar to that for those receiving 3 or 4 doses ($n=17$, mean: 8.2 months). Whereas, 19 children received repeated RTX treatment, extended the B-cell depletion duration of at least 15 months. Therefore, a multicenter randomized controlled trial should be performed to determine how to cost-effectively use RTX.

B-cell recovery has always been used to predict whether nephrotic syndrome will relapse. A study^[20] has shown that most patients, but not all, relapse immediately after B-cell recovery. B-cell recovery does not necessarily mean a relapse. In our group, if B-cell count recovered, we gave an additional dose of RTX or used MMF to maintain persistent B-cell depletion, to reduce recovery, and to prevent relapse.

Because the therapeutic effect of RTX is temporary, some patients may need to use it more than once. Because long-term side-effects are a concern, there will be a limit to the increase in frequency of use. In order to maintain remission and minimize side-effects, it is important to choose proper maintenance phase medication. It has been reported that RTX can

increase patient sensitivity to IS drugs. Long-term side-effects are the most important consideration for any drug in a long-term maintenance therapy. Therefore, MMF has more advantages than CyA. In addition, the effectiveness of MMF as a drug for maintenance therapy has been confirmed.^[11] In our study, 5 patients used MMF in sustained remission.

Primary side-effects occurred during the infusion period, and after timely treatment, symptoms disappeared rapidly. No serious adverse side-effects were observed. In 1 patient, we observed sustained low serum Ig levels that did not match the proteinuria level. Therefore, during therapy, in addition to monitoring CD classification, it is also prudent to monitor serum Ig levels. None of the patients in our study used prophylactic antibiotics, and no fatal infection was observed. However, we also should be aware of some rare but serious side-effects.

In short, RTX is a drug for rescue therapy for pediatric refractory nephrotic syndrome. It can reduce side-effects and allow some patients to discontinue administration of all medications. Because of the limited number of patients and short follow-up period of our study, we recommend a large-scale, long-term, randomized double-blind study of RTX treatment for pediatric refractory nephrotic syndrome in the future.

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Competing interest: None.

Contributors: Sun L wrote the main body of the article under the supervision of Xu H. All authors contributed to the design and interpretation of the study and to further drafts.

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