

Rituximab treatment for steroid dependent or steroid resistant nephrotic syndrome in adult patients

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Abstract

Background: Minimal change disease (MCD) and focal segmental glomerular sclerosis (FSGS) are major causes of nephrotic syndrome (NS). The patients of steroid-dependent (SD) or steroid-resistant (SR) NS are exposed to high doses of steroid, more adverse effects, and worse outcomes. This study applied B cell-oriented rituximab therapy in MCD or FSGS adult patients with SD or SR, to investigate its efficiency and safety.

Methods: Eight patients with steroid-dependent and frequent-relapsing (SD/FR) NS and six patients with steroid-resistant (SR) NS were enrolled. B-cell-oriented (<5 cells/mm³) rituximab administration was used with single dose of 375 mg/m² adjusted according to eGFR.

Results: During the follow-up period of 15.0 (8.8-18.0) months, B-cell depletion was achieved and maintained in all the 14 patients. Four, two, seven, one patients received two, three, four, five infusions of rituximab respectively. No adverse event was observed. All the eight SD/FR patients maintained complete remission without relapse. Six of them stopped steroid in 10 (2.3-12.3) months and four of them further stopped immunosuppressants. All of them maintained stable kidney function (eGFR 107.4 ± 27.4 vs. 111.0 ± 31.5 mL/min/1.73m², $P=0.600$). All the six SR patients showed no response and presented with severe nephrotic syndrome. Five of them presented with kidney function deterioration (eGFR 49.6 ± 35.7 vs. 15.9 ± 11.5 mL/min/1.73m², $P=0.047$) and three of them went into ESRD.

Conclusion: B cell depletion-oriented regimen of rituximab was effective and safe for MCD or FSGS adult patients with SD/FR nephrotic syndrome, which could reduce drug doses and adverse events. This regimen showed no therapeutic effect for SR patients.

Background

Minimal change disease (MCD) and focal segmental glomerular sclerosis (FSGS) are major causes of nephrotic syndrome. MCD accounts for approximately 70%-90% of pediatric and 10%-15% of adult patients with nephrotic syndrome[1, 2]. FSGS accounts for ~ 15% of end-stage renal disease (ESRD) with the annual incidence rate ranging from 0.2 to 1.8/100,000 population per year around the world[3].

The patients with MCD often respond well to steroids and achieve complete remission. However, nearly 50% of the patients in remission will develop to steroid-dependent (SD) and frequent-relapsing (FR) nephrotic syndrome[4]. According to KDIGO guidelines, these patients would receive immunosuppressants including cyclophosphamide, calcineurin inhibitors such as cyclosporine or tacrolimus, or mycophenolate mofetil[5]. The patients with FSGS often appear as steroid-resistant (SR) nephrotic syndrome. Some patients may require immunosuppressants, such as calcineurin inhibitors and mycophenolate mofetil. These intensive regimens present with various responses in achieving and maintaining remission, but are associated with drug-related adverse effects, such as growth retardation, infections, diabetes, osteoporosis, hypertension and renal toxicity[6].

Rituximab is a chimeric monoclonal antibody against CD20 and has been successfully used in patients with B-cell non-Hodgkin's lymphoma, autoimmune diseases including idiopathic thrombocytopenic purpura, rheumatoid arthritis, ANCA-associated vasculitis, and membranous nephropathy[7–10]. From 2004[7], rituximab was reported being effective in the treatment of refractive nephrotic syndrome in children, more successful induction of remission in SD/FR patients than in SR patients[11–14]. Most patients received the standard four-dose protocol with rituximab 375 mg/m²/week for four weeks, which may be associated with more side effects and costs. Since Ruggenenti et al. reported that B cell-oriented rituximab treatment effectively and safely prevented the relapse of nephrotic syndrome and reduced the need for immunosuppressants in SD or FR patients[13], we performed B-cell-oriented rituximab treatment for adult patients with MCD or FSGS who presented with SD/FR or SR nephrotic syndrome.

In this study, we reported the therapeutic effects and safety of B-cell-oriented regimen of rituximab in the management of adult patients with SD/FR or SR nephrotic syndrome due to MCD or FSGS.

Methods

Patients

From 2013 to 2018, biopsy-proven adult patients who were diagnosed as MCD or primary FSGS and presented with refractory nephrotic syndrome as SD/FR or SR were enrolled in this study. The patients in combination with hepatitis B virus or hepatitis C virus infection, tuberculosis, pregnancy, diabetes mellitus, or malignancy, were excluded. Clinical and pathological data were collected from medical records. All patients were followed up every one to three months as routine clinical practice, and clinical data were collected on every visit.

The research was in accordance with the Declaration of Helsinki and was approved by the ethics committee of Peking University First Hospital. All patients signed fully informed written consent. For patients below 18 years old, their parents/legal guardians signed fully informed written consent.

Rituximab treatment

Rituximab was administered with a single dose of 375 mg/m², adjusted according to estimated glomerular filtration rate (eGFR). Circulating B cells were measured 24 hours after rituximab infusion and on every visit. B-cell depletion was defined as < 5 B cells/mm³ in the circulation. All patients received B-cell-oriented regimen that rituximab would be reused when B-cell was > 5 cells/mm³, regardless of the urinary protein levels.

Treatment responses

SD was defined as two consecutive relapses during steroid therapy or within 2 weeks of ceasing therapy. FR was defined as two or more relapses within 6 months of initial response or four or more relapses in any 12-month period. SR was defined as failure to achieve complete remission after 8 weeks of steroid therapy.

For evaluation of therapeutic responses, complete remission was defined as urinary protein excretion < 0.3 g/d with normal kidney function. Partial remission was defined as urinary protein excretion < 3.5 g/d and 50% or greater reduction from peak values with stable serum creatinine. No remission was defined as failure to reduce urinary protein excretion by 50% from baseline. Relapse was defined as recurrence of urinary protein excretion > 3.5 g/d after a period of remission.

For evaluation of kidney outcomes, ESRD was defined as eGFR < 15 ml/min/1.73 m² or initiation of renal replacement therapy. Kidney dysfunction was defined as a decline of eGFR more than 50% of baseline with eGFR being < 60 ml/min/1.73 m². eGFR was estimated according to the CKD-EPI equation[15].

Statistical analysis

Statistical analysis was performed using the SPSS statistical software package (17.0, SPSS, Chicago, IL, USA). Parametric data were presented as means ± standard deviation (SD). Non-parametric data were presented as median [interquartile range (IQR)]. Differences in quantitative parameters between groups were tested using Mann-Whitney U-test. Differences of qualitative data were assessed using chi-square test or Fisher's exact test. A two tailed p-value < 0.05 was considered statistically significant.

Results

Patients

There were 14 patients enrolled in the present study, including eight patients of SD/FR and six patients of SRNS. The demographic and clinical data of them are shown in Table 1.

All patients were over 15 years old with an average age of 31.1 ± 17.6 years. Before enrollment they had undergone the disease for 5 (2.0-11.0) years. Six patients with MCD received repeat kidney biopsy before rituximab administration, with three patients changed the diagnosis into primary FSGS and three patients remained the initial diagnoses. Thus, of the eight SD/FR patients, six patients were MCD and two patients were primary FSGS, with one of not otherwise specified (NOS) variant and one of tip variant. All the six SR patients were diagnosed as primary FSGS NOS variant.

Previous treatments

All patients received previous steroids and immunosuppressive therapies, including the combination of steroids and tacrolimus in ten patients, cyclosporine in eight patients, cyclophosphamide in six patients, mycophenolate mofetil in eight patients, leflunomide in ten patients and azathioprine in three patients.

All the eight SD/FR patients underwent more than three times of relapse with the average of 9 (6-12) times. They all achieved complete remission by steroids and immunosuppressive drugs at the time of rituximab infusion, with six patients receiving prednisolone alone (32.5 ± 14.4 mg/d; range 15-55 mg/d), one patient receiving cyclosporin, and one patient receiving prednisolone combined with tacrolimus.

Before rituximab treatment, they had received steroids for 10.5 (4.5-11.0) years with a cumulative dose of 49.1 ± 20.6 g.

All the six SR patients never responded to any regimens and presented with nephrotic syndrome at the time of rituximab infusion. By the time of enrollment, the average level of urinary protein was 15.7 ± 6.0 g/d, serum albumin was 17.8 ± 1.4 g/L, serum creatinine was 196.1 ± 86.4 $\mu\text{mol/L}$ and eGFR was 45.8 ± 33.3 ml/min/1.73m². The immunosuppressive drugs were stopped at least three months before rituximab administration, while prednisolone was continued (21.7 ± 15.1 mg/d; range 5-50 mg/day). Before rituximab treatment, they had received steroids of 2.0 (1.0-6.5) years and 25.3 ± 29.6 g.

Rituximab regimen

All the 14 patients received B-cell-oriented rituximab administration, with each single dose of 375 mg/m² adjusted according to eGFR. The amount of B cells was examined every one month. If it is over 5 cells/mm³, rituximab was prescribed with one single dose of 375 mg/m². During the follow-up period of 15.0 (8.8-18.0) months, four patients received two infusions with the total dose of 975 ± 263 mg, two patients received three infusions with the total dose of 1600 ± 141 mg, seven patients received four infusions with the total dose of 2229 ± 605 mg, and one patient received five infusions with the total dose of 2900 mg. The total dose of rituximab was comparable between the SD/FR patients and the SR patients (1812.5 ± 780.9 vs. 1850.0 ± 828.9 mg, P=0.845). (Table 2).

Treatment responses

All the eight SD/FR patients maintained complete remission. No relapse was observed in the follow-up period of 15.0 (8.8-18.0) months. Among them, six patients (5 MCD, 1 FSGS NOS) gradually withdrew and stopped steroid from 25.0 (11.3-36.3) mg/d in 10 (2.3-12.3) months. Four of them stopped both steroids and immunosuppressants treatments, one patient continued cyclosporin treatment and one patient continued tacrolimus treatment. The other two patients (1 MCD, 1 FSGS Tip) maintained steroid treatment, while the dose was decreased from 20 mg/d and 55 mg/d to 10 mg/d and 20 mg/d, respectively.

The six SR patients all showed no response to rituximab treatment and presented with severe nephrotic syndrome during the whole period of follow-up. Only one patient showed improvement of proteinuria, with the level of urinary protein decreased from 11.6 g/d to 6.1 g/d and the serum albumin increased from 17.6 g/L to 25.5 g/L, not achieving partial remission.

Kidney outcomes

During the follow-up period of 15.0 (8.8-18.0) months, all the eight SD/FR patients remained stable kidney function with eGFR 107.4 ± 27.4 ml/min/1.73m² at the time of rituximab treatment and eGFR 111.0 ± 31.5 ml/min/1.73m² at the end of follow-up (P=0.600).

Of the six SR patients, one patient who got improvement of proteinuria also got improvement of kidney function, with the serum creatinine decreased from 212 $\mu\text{mol/L}$ (eGFR 26.6 ml/min/1.73m²) to 123 $\mu\text{mol/L}$ (eGFR 51.2 ml/min/1.73m²). The other five patients showed kidney function deterioration with the serum creatinine increased from 192.8 \pm 96.1 $\mu\text{mol/L}$ to 515.8 \pm 303.1 $\mu\text{mol/L}$ (P=0.047) and eGFR decreased from 49.6 \pm 35.7 ml/min/1.73m² to 15.9 \pm 11.5 ml/min/1.73m² (P=0.047). Three of them went into ESRD.

Adverse effects

Rituximab was well tolerated and no adverse event was observed at the time of infusion. During follow-up, there was one SR patient once suffering from pneumonia and acute kidney injury at two months after the second dose of rituximab. At that time, the amounts of B cells and CD4+ T cells were 4.1/mm³ and 623.4/mm³, respectively. Her serum creatinine increased from 212 $\mu\text{mol/L}$ to 390 $\mu\text{mol/L}$. All the etiological examinations were negative. The infection was successfully and empirically treated with moxifloxacin, sulperazone, oseltamivir, SMZCo and meropenem. Then the serum creatinine decreased to 123 $\mu\text{mol/L}$. No other severe adverse event was observed in other patients. No patient developed neutropenia, pneumocystis infection or cardiac arrhythm.

Discussion

In this study, 14 patients with refractory nephrotic syndrome received B cell depletion-oriented rituximab treatment in 15.0 months. Eight patients with SD/FR all maintained complete remission and six of them stopped steroids. However, six patients with SR and primary FSGS NOS variant all failed to respond. We found that the B cell depletion-oriented rituximab treatment could successfully maintain remission and reduce relapse in SD/FR patients, but failed to induce remission in SR patients.

In the current study, all SD/FR patients had good responses to rituximab treatment. In other studies, 47%-100% of complete remission rate was reported [11, 4, 12–14]. Several reasons might explain the difference in remission rate. First, the patients in this study were all adults, who might be less likely carrying the genetic mutation of pediatric patients in previous studies[12, 13], thus being more sensitive to immunosuppressive treatment. Second, in this study, complete remission was achieved before rituximab infusion. Rituximab may be lost via urinary excretion and the level of proteinuria may affect its pharmacokinetics[16]. The efficacy of rituximab was higher when the urinary protein was negative[11, 14]. However, Guitard et al. found that the remission of nephrotic syndrome was not a prerequisite before rituximab therapy and they suggested that rituximab alone might be sufficient to induce remission in adulthood[17]. In our study, complete remission was obtained in all SD/FR patients and they responded well to the rituximab treatment without any relapse. Our findings suggested that achieving complete remission in SD/FR patients before rituximab infusion might be beneficial to better pharmacokinetics and higher efficacy of this treatment. However, no conclusion could be made based on this retrospective study with small sample size.

The six SR patients in this study failed to response to rituximab and no remission was induced. Three of them further progressed into ESRD. All the six SR patients are FSGS, which is less sensitive to steroids and immunosuppressive drugs and is more likely to progress to ESRD, compared to the patients with MCD. A study included five SR (2 MCD, 3 FSGS) patients treated with rituximab and reported four patients achieving complete remission and one patient achieving partial remission[18]. Another study showed two SR patients with FSGS achieved partial remission by one single dose of rituximab[19]. However, Fernandez-Fresnedo et al. found that none of eight SR adult patients with FSGS achieved complete or partial remission after four doses of rituximab[20]. An open-label, randomized, controlled trial in 31 children with idiopathic nephrotic syndrome unresponsive to prednisone and calcineurin inhibitor showed all patients failed to respond to two doses of rituximab treatment[21]. Since at least 38 genes have been identified associated with FSGS, genetic variants may partly explain why some FSGS patients are resistant to the treatments. Considering the inconsistent results from different studies, further large-scale randomized controlled trials (RCT) and genetic screening at enrollment are needed in the future.

Different regimens of rituximab were used to achieve or maintain remission in patients with SD/FR or SR in different clinical studies[18, 11, 19, 20, 12, 21, 17, 13, 14]. Ruggenti et al. reported that remission was achieved by one single dose of 375 mg/m² in 28 of 30 patients with idiopathic nephrotic syndrome[13]. However, Kemper et al. found that the remission duration in patients received one or two doses of rituximab was obviously shorter than that in those received three or four doses[22]. Repeated or prolonged exposure to rituximab might induce the production of anti-rituximab antibodies or human anti-chimeric antibodies, which may be associated with treatment failure or severe adverse effects[23]. Most patients relapsed after B-cell recovery[24]. Therefore, in the current study we took the B cell depletion-oriented one single dose of rituximab regimen, which might have the lowest drug exposure. The quite good results on the eight SD/FR patients for maintaining complete remission and withdrawing steroids and immunosuppressants supported us to recommend this regimen to SD/FR patients for maintaining remission. Large-scale RCTs with long terms of follow-up are needed to make more definitive conclusion for this regimen on SD/FR patients. However, this regimen may be not suitable for SR patients. More intensive usage of rituximab or other new therapeutic projects are needed for SR patients.

As a chimeric monoclonal antibody, rituximab targets CD20 + B cells by apoptosis, antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity[25]. The potential mechanisms of rituximab to MCD and FSGS have been explored. First, it could decrease the numbers of T cells by targeting a small subset of T cells that express CD20. In patients with rheumatoid arthritis, a small subset of T cells expressing few amounts of CD20 were depleted by rituximab[26]. In patients with Sjogren's syndrome, CD20 was co-expressed on IL-17-producing T cells and was depleted by rituximab[27]. Second, rituximab may influence T cells functions by interfering the interaction between B cells and T cells. B-cell depletion may increase Treg cells. Rocatello et al. found that the number of Treg cells increased remarkably in MN patients treated by rituximab[28]. B cells could regulate T cell responses via co-stimulatory molecules. Tokunaga et al. reported that rituximab decreased the CD40 and CD80 expressing cells and downregulate CD40L and CD69 on CD4 + cells in patients with systemic lupus erythematosus[29]. Increased expression

of CD80 interferes the integrity of podocytes cytoskeleton and slit diaphragm[30]. Third, rituximab could influence the secretion of some cytokines. In patients with atopic eczema, rituximab treatment was associated with the decrease of IL-13 and Th17-related cytokines [31, 32]. Forth, rituximab could directly remodel the podocyte cytoskeleton by targeting sphingomyelin-phosphodiesterase-acid-like 3b (SMPDL-3b)[33].

Conclusions

In conclusion, rituximab is an effective and safe treatment for MCD or FSGS adult patients with SD/FR nephrotic syndrome, but has poor therapeutic effect for FSGS patients with SR. The B cell depletion-oriented single dose regimen after patients achieving complete remission is beneficial for lower drug exposure and higher drug efficacy. RCTs of large cohort and long term of follow-up are needed to decide an appropriate regimen for rituximab treatment in SD/FR patients.

Abbreviations

MCD: Minimal change disease; **FSGS**: Focal segmental glomerular sclerosis; **NS**: nephrotic syndrome; **SD**: Steroid-dependent; **SR**: Steroid-resistant; **ESRD**: End-stage renal disease; **FR**: Frequent-relapsing; **eGFR**: Estimated glomerular filtration rate; **NOS**: Not otherwise specified; **RCT**: Randomized controlled trials; **SMPDL-3b**: Sphingomyelin-phosphodiesterase-acid-like 3b.

Declarations

Funding

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Availability of data and materials

The present study was derived from a developing clinical cohort. Data from the cohort will not be shared currently since the whole project is not finished and the privacy of patients should be protected. If there are any requests about details of the project, please contact with the corresponding author Zhao Cui.

Authors' contributions

JH, PW and ZC formed the study concept, conducted the study, analyzed the data, interpreted the results, and drafted the manuscript. YMZ, FW, LQM, XYC, GL and FDZ made substantial contributions to the acquisition of data, and the analysis and interpretation of data. MHZ is the major sponsor, and he raised

lots of important and helpful suggestions in manuscript writing and revision. Each author contributed important intellectual content during manuscript drafting or revision and agreed to be accountable for all aspects of the work. The authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The research was in compliance of the Declaration of Helsinki and approved by the ethics committee of Peking University First Hospital. All participants gave written contents for data collection.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Tables

Table 1. Baseline characteristics of the patients receiving rituximab treatment.

Patients (n)	Total (n=14)	SD/FR (n=8)	SR (n=6)
Male/Female (n)	11/3	7/1	4/2
Age of onset (years)	23.2 ± 20.3	18.9 ± 20.4	29.0 ± 20.5
Age of rituximab treatment (years)	31.1 ± 17.6	29.1 ± 18.7	33.7 ± 17.6
History before rituximab treatment (years) [median (IQR)]	5.0 (2.0-11.0)	10.5 (4.5-11.0)	2.0 (1.0-6.5)
Urinary protein (g/24h)	7.3 ± 8.9	0.1 ± 0.1	15.7 ± 6.0*
Serum albumin (g/L)	30.6 ± 12.0	40.1 ± 4.8	17.8 ± 1.4*
Serum creatine (µmol/L)	129.3 ± 81.1	79.2 ± 14.4	196.1 ± 86.4*
eGFR (ml/min/1.73m ²)	81.0 ± 42.8	107.4 ± 27.4	45.8 ± 33.3*
CKD-2, n (%)	9 (64.3%)	7 (87.5%)	2 (33.3%)
CKD-3, n (%)	2 (14.3%)	1 (12.5%)	1 (16.7%)
CKD-4, n (%)	3 (21.4%)	0 (0%)	3 (50%)
Kidney pathology			
MCD, n (%)	6 (42.9%)	6 (75.0%)	0 (0%)
FSGS, n (%)	8 (57.1%)	2 (25.0%)	6 (100%)
Tip variant, n (%)	1 (7.1%)	1 (12.5%)	0 (0%)
NOS variant, n (%)	7 (50.0%)	1 (12.5%)	6 (100%)
Cell variant, n (%)	0 (0%)	0 (0%)	0 (0%)
Collapsing variant, n (%)	0 (0%)	0 (0%)	0 (0%)
Previous therapies			
Prednisone, n (%)	14 (100%)	8 (100%)	6 (100%)
Cyclosporine, n (%)	8 (57.1%)	5 (62.5%)	3 (50.0%)
Tacrolimus, n (%)	10 (71.4%)	5 (62.5%)	5 (83.3%)
Cyclophosphamide, n (%)	6 (42.9%)	2 (25.0%)	4 (66.7%)
Mycophenolate mofetil, n (%)	8 (57.1%)	5 (62.5%)	3 (50.0%)
Leflunomide, n (%)	10 (71.4%)	6 (75.0%)	4 (66.7%)
Azathioprine, n (%)	3 (21.4%)	3 (37.5%)	0 (0%)
Times of relapse before rituximab treatment [median (IQR)]	-	9 (6-12)	-

*P < 0.05 between the patients with SDNS/FR and those with SRNS. SD: steroid-dependent; FR:

frequent-relapsing; SR: steroid-resistant; MCD: minimal change disease; FSGS: focal segmental

glomerular sclerosis; NOS: not otherwise specified.

Table 2. Clinical characteristics of patients after rituximab treatment

Patients (n)	Total (n=14)	SD/FR (n=8)	SR (n=6)
Follow-up time (months) [median (IQR)]	15.0 (8.8-18.0)	15.0 (9.0-18.8)	16.0 (9.8-17.8)
Completion rate n (%)	8 (66.7%)	8 (100%)	0 (0%)
Average dose of rituximab	1828.6 ± 770.0	1812.5 ± 780.9	1850.0 ± 828.9
Average times of rituximab	3.4 ± 1.0	3.0 ± 0.9	3.8 ± 1.0
Number of rituximab in every patient (2/3/4/5 courses), n	4/2/7/1	3/2/3/0	1/0/4/1
Urea nitrogen (g/24h)	6.4 ± 9.4	0.2 ± 0.3	13.7 ± 9.5*
Urea albumin (g/L)	34.5 ± 11.7	44.2 ± 3.8	23.1 ± 5.2*
Urea creatinine (µmol/L)	237.9 ± 273.4	78.6 ± 15.3	450.4 ± 315.0*
CRF (ml/min/1.73m ²)	72.8 ± 52.5	111.0 ± 31.5	21.8 ± 17.7*
CKD-2, n (%)	7 (50.0%)	7 (87.5%)	0 (0%)
CKD-3, n (%)	2 (14.3%)	1 (12.5%)	1 (16.7%)
CKD-4, n (%)	2 (14.3%)	0 (0%)	2 (33.3%)
CKD-5, n (%)	3 (21.4%)	0 (0%)	3 (50.0%)
Glucocorticoids and immunosuppressive therapies			
All stopped, n (%)	6 (42.9%)	4 (50.0%)	2 (33.3%, dialysis)
Prednisone, n (%)	6 (42.9%)	2 (25.0%)	4 (66.7%)
Cyclosporin, n (%)	1 (7.1%)	1 (12.5%)	0 (0%)
Tacrolimus, n (%)	2 (14.3%)	1 (12.5%)	1 (16.7%)

*P < 0.05 between SD/FR and SR patients.