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ARAŞTIRMA / RESEARCH

Rituximab treatment in children with difficult-to-treat nephrotic syndrome

Tedavisi zor nefrotik sendromlu çocuklarda rituksimab tedavisi

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Abstract

Purpose: Rituximab (RTX) has been offered as rescue therapy for patients with difficult-to-treat nephrotic syndrome (frequent relapsing, steroid-dependent and steroid resistant). We aimed to assess the efficacy and long-term outcomes of RTX treatment in children with difficult nephrotic syndrome and shared our experiences

Materials and Methods: Medical records of children with difficult nephrotic syndrome who were treated with RTX were retrospectively evaluated. The relapse-free survival rate at 12 month and monitoring of B-cell depletion were assessed.

Results: In the study included 20 children of which 8 had steroid-dependent (SDNS), 6 had frequent relapsing (FRNS), and 6 had steroid-resistant nephrotic syndrome (SRNS). The median number of relapses at 1 year before and after treatment in FRNS/SDNS patients receiving RTX treatment were compared. The median number of relapses decreased from 2 (1-4) to 0 (0-1) times/year. The mean duration of the follow-up period after RTX treatment was 23 (12-59) months, and 8 patients developed relapse. Repeated doses of RTX were administered to 5 patients who relapsed after RTX treatment. In these patients, $CD19^+B$ cells re-emerged during remission, while depletion of memory B-cells remained.

Conclusion: The RTX treatment prolonged the remission time in FRNS/SDNS patients, but it was ineffective in SRNS patients. It was determined that the RTX doses can be repeated to maintain remission in these patients, and the best memory B-cell counts can help in timing the repeat doses.

Keywords:. Rituximab, nephrotic syndrome, memory b-cell count, children

Öz

Amaç: Rituksimab (RTX), tedavisi zor nefrotik sendromlu (sık tekrarlayan, steroide bağımlı ve steroide dirençli) hastalar için kurtarma tedavisi olarak önerilmektedir. Amacımız zor nefrotik sendromlu çocuklarda RTX tedavisinin etkinliğini ve uzun dönem sonuçlarını değerlendirmek ve deneyimlerimizi paylaşmaktır.

Gereç ve Yöntem: RTX ile tedavi edilen zor nefrotik sendromlu çocukların tıbbi kayıtları geriye dönük olarak değerlendirildi. Oniki ayda nükssüz sağkalım oranı ve B hücre deplesyonun izlemi değerlendirildi.

Bulgular: Çalışmaya 8'i steroide bağımlı (SBNS), 6'sı sık tekrarlayan (STNS) ve 6'sı steroide dirençli nefrotik sendromlu (SDNS) 20 çocuk dahil edildi. RTX tedavisi alan STNS/SBNS hastalarının tedavi öncesi ve sonrası 1 yıllık ortalama nüks sayısı karşılaştırıldı. Ortalama nüks sayısı 2 (1-4)'den 0 (0-1) kez/yıla geriledi. RTX tedavisi sonrası ortalama takip süresi 23 ay (12-59) ve 8 hastada nüks gelişti. RTX tedavisi sonrası nüks eden 5 hastaya, tekrarlayan dozlarda RTX uygulandı. Bu hastalarda, $CD19^+B$ hücreleri remisyon sırasında yeniden ortaya çıkarken, hafıza B hücrelerinin deplesyonu devam etti.

Sonuç: RTX tedavisi, STNS/SBNS hastalarında remisyon süresini uzattı, ancak SDNS hastalarında etkisiz kaldı. Bu hastalarda remisyonun idamesi için RTX dozunun tekrarlanabileceği ve tekrarlama dozlarının zamanlamasında en iyi hafıza B hücre sayısının yardımcı olabileceği saptandı.

Anahtar kelimeler: Rituksimab, nefrotik sendrom, hafıza b hücre sayısı, çocuk

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INTRODUCTION

Idiopathic nephrotic syndrome (INS) is the most common glomerular disease in children and is clinically challenging, with a worldwide incidence and prevalence in children of 2-7/100,000 and 16/100,000, respectively[1]. Corticosteroids, which are cornerstones in the treatment of INS, are not able to provide complete remission in some patients in 4 weeks. INS have classified according to the International Study of Kidney Disease in Children (ISKDC) as a steroid-sensitive nephrotic syndrome (SSNS) and steroid-resistant nephrotic syndrome (SRNS)[2]; however, ≥50% of SSNS patients develop frequent relapsing nephrotic syndrome (FRNS) and, among children with FRNS, 50-60% meet the definition of steroid-dependent nephrotic syndrome (SDNS). Also, 10-20% of patients with INS have SRNS, and 36-50% of children with SRNS progress to end-stage renal disease (ESRD) within 10 years[3].

Although the pathogenesis of INS is not fully understood, an underlying immunological defect have suspected, and the standard treatment is immunosuppressive agents, including levamisole, cyclophosphamide, calcineurin inhibitors (CNIs) (cyclosporine [CsA] and tacrolimus), and mycophenolate mofetil (MMF)[4]. These treatments are generally successful in most patients; however, relapses may persist in approximately 20% of INS patients. This condition is defined as complicated FRNS/SDNS. Additionally, approximately 1-3% of INS patients exhibit resistance to steroids and immunosuppressive agents, placing them defined as refractory SRNS[5]. The failure of the currently available therapies suggests that there is a need for novel agents to be used to treat complicated FRNS/SDNS and refractory SRNS.

Rituximab (RTX) is a chimeric anti-CD20 monoclonal human antibody that is produced via genetic engineering. The mechanism of action of RTX involves temporary complete depletion of the peripheral pool of CD20 B-lymphocytes via complement-dependent lysis or apoptosis of these cells[6]. RTX is used as a 'rescue medication' in multi-drug-resistant patients with idiopathic nephrotic syndrome[7]. While RTX shows promising efficacy for the prevention of relapses in children with FRNS/SDNS, its effect remains unknown in children with SRNS. There is no consensus regarding the use of RTX in the treatment of INS, such as initial treatment doses, the number of infusions, the necessity of additional doses, and dose intervals, especially the duration of B cell depletion. In fact, RTX efficiency is related to B cell depletion.

Our hypothesis is that the efficacy of RTX treatment in difficult INS patients can be maintained with additional doses, and pre-emptive RTX treatment can be administered by measuring $CD19^+$B cell counts and memory B-cell counts, the duration of remission can be prolonged. There is no information in the literature about the biomarker that can help in the timing additional doses and repeated use of RTX treatment in INS patients. The aim of this study was to evaluate the long-term outcomes of a maintenance therapy based on $CD19^+$B cell and memory B-cell detection in children with difficult-to-treat INS that were treated with RTX and to share our experiences.

MATERIALS AND METHODS

Study patients and definitions

The study was approved by the institutional ethics committee (Başkent University Institutional Review Board and Ethics Committee (Date: 23 June 2020, Project no: KA20/269), and informed consent was obtained from each patient.

This retrospective study consisted of 394 pediatric patients who were followed up with a diagnosis of idiopathic nephrotic syndrome was conducted in the department of pediatric nephrology between January 2009 and December 2019. The study was performed by the department of Physiology, Therapeutic Apheresis Unit. The patients' data were retrieved from the hospital's electronic patient registry (Nucleus Automation System) and patient files. In total of 20 patients diagnosed with difficult-to-treat INS were included in the study. Patients who were included in this study met the following criteria: (1) onset of INS at age >1 year; (2) initiation of RTX treatment at age younger than 18 years; (3) multi-drug-resistant INS (complicated FRNS/SDNS and refractory SRNS); (4) followed-up for ≥12 months. The exclusion criteria were congenital nephrotic syndrome, secondary causes of nephrotic syndrome (systemic lupus erythematosus, IgA nephropathy), estimated glomerular filtration rate (GFR) <60 mL/min/1.73m^2, known active chronic infections (tuberculosis, HBV, HCV), uncontrolled hypertension, neutropenia or thrombocytopenia, live-attenuated vaccination within last 1 month, and patients with known genetic causes such as NPHS1, NPHS2, WT1, or LAMB2.

The definitions and criteria for NS, remission, relapse, frequent relapse, steroid dependency, and steroid resistance were those of the International Study of Kidney Disease in Children[2]. Before RTX treatment, baseline clinical and laboratory parameters were recorded. The estimated glomerular filtration rate (eGFR) was calculated using the updated Schwartz formula and the constants k=0.413[8].

Therapeutic protocol

RTX was administered at the dose of 375 mg/m^2 (max 500 mg) once a week for 4 weeks (4 doses) in patients with SRNS and biweekly for 8 weeks (4 doses) in patients with FRNS/SDNS. Prednisolone, acetaminophen, and diphenhydramine were administered 30 minutes premedication before every dose. As RTX was administered, the patient's general condition and vital signs were monitored. Following RTX treatment, preventive trimethoprim-sulfamethoxazole was given to all patients.

Cell isolation and flow cytometry

The B-cell depletion was defined as a CD19$^+$ cell count <1% of total lymphocytes, based on flow cytometry[9]. Flow cytometric studies were performed using a Navios 3L10C device (Beckman Coulter, San Jose, USA). For flow cytometric studies cells were separated from peripheral blood samples and CD19 PC.5, CD27 PE, CD38 APC A750, and CD45 KRO monoclonal antibodies were used. All monoclonal antibodies and reagents were obtained from Beckman Coulter.

Lymphocytes were gated on the CD45/SS graph and CD19$^+$B lymphocytes were selected over the lymphocytes. Cells selected as CD27$^+$/CD38$^-$ on B lymphocytes were evaluated as memory B cells. Data obtained were reported as % values. After 1 January 2019, detection of memory B cells in peripheral blood mononuclear cells (PBMCs) was performed and re-emergence was defined as a PBMC concentration >0.05%[10]. Analyzes were made in 1,000,000 cells. Data were analyzed using Kaluza Analysis v.2.1.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows v.23.0 (IBM Corp., Armonk, NY, USA). Continuous variables normally distributed are presented as mean ± SD (P >0.05 based on the Kolmogorov-Smirnov test or Shapiro-Wilk test [n<30]), whereas continuous variables not normally distributed are shown as median. Comparisons between groups were made using Student's t test. Between-group comparison of normally distributed data was performed via Student's t test, versus the Mann-Whitney U test for data not normally distributed.

Baseline-post RTX treatment data were analyzed using the Friedman test and Wilcoxon test, and comparison of within group time-dependent measurements was performed via repeated measure analysis. Between-group comparison of categorical variables was performed using the chi-square test or Fisher's exact test. A survival curve was plotted using the standard Kaplan-Meier methodology. The level of statistical significance was set at $p< 0.05$.

RESULTS

The study included 20 patients (12 male) with difficult-to-treat INS that received RTX. Among the patients, 8 had SDNS, 6 had FRNS, and 6 had SRNS. At the time of RTX treatment, SDNS and FRNS patients were in remission. The median age at disease onset was 3.9 (1-12.5) years. The median age at the start of RTX treatment was 9.9 (3.8-17) years. The median disease duration before RTX treatment was 52 (4-184) months. The median duration of follow-up after RTX treatment was 19 (12-59) months. Renal biopsy was performed in 13 patients, showed focal and segmental glomerulosclerosis (FSGS) in 12 and *mesangioproliferative glomerulonephritis* (MesPGN) in 1 of the patients. During the administration of RTX treatment, all patients continued with concomitant oral immunosuppressive therapy. Patient characteristics at baseline are presented in Table 1.

None of the patients are shown any infusion reactions, neutropenia, hypogammaglobulinemia, serious infections, or any adverse events during the treatment period. The median disease duration of 14 FRNS/SDNS patients before RTX treatment was 73 (19-183) months. The median number of relapses during the first 1 year after RTX was 0 (0-1), as compared to 2 (1-4) during the 1 year before RTX ($p=0.001$). The median duration of the follow-up period after RTX treatment was 23 (12-59) months, and 8 patients developed relapse. The median time to relapse development of 8 patients after treatment was 13 (8-40) months (Figure). It was calculated by Kaplan-Meier analysis that FRNS/SDNS patients had 64% the relapse-free probability in the first year after RTX treatment and the estimated relapse time

was 31±9 (95% CI: 12-49) months. During the follow-up, 5 patients who relapsed after the first course of RTX treatment (4 RTX infusions) were received additional dose RTX infusions. SRNS patients did not respond to RTX treatment that there was no significant difference in eGFR, serum albumin, creatinine, and proteinuria levels (P=0.22, P=0.59, P=0.14, and P=0.46, respectively) (Table 2). In the first year of RTX treatment, the eGFR of 2 patients declined less than 60 ml/min/1.73m^2.

Table 1. Baseline characteristics of the patients

Characteristics (n=20)	**Data**
Female/Male (n)	8/12
Type of Disease	
SDNS, n	8
FRNS, n	6
SRNS,	6
Age at disease onset, years	3.9 (1-12.5)
Age at the start of the RTX treatment, years	9.9 (3.8-17)
Disease duration before RTX treatment, months	52 (4-184)
Duration of follow-up after RTX treatment, months	19 (12-59)
Biopsy result	
FSGS, n	12
MesPGN, n	1
No biopsy performed, n	7
RTX doses and dosage interval	
4 doses, biweekly (SDNS/FRNS)	14
4 doses, once a week (SRNS)	6
Immunosuppressive agents before RTX treatment	
Prednisone (PRD), n (%)	20 (100)
Calcineurin inhibitors (CNIs), n (%)	20 (100)
Cyclophosphamide, n (%)	14 (70)
MMF, n (%)	8 (40)
Levamisole, n (%)	2 (14)
Immunosuppressive agents after RTX treatment	
PRD, n	4
PRD+CNIs, n	13
PRD+CNIs+MMF, n	3

Data are presented as the median or n (%). SDNS, steroid-dependent nephrotic syndrome; FRNS, frequent relapsing nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; RTX, Rituximab; FSGS, focal and segmental glomerulosclerosis; MesPGN, mesangioproliferative glomerulonephritis; PRD, Prednisone; CNIs, calcineurin inhibitors; MMF, Mycophenolate mofetil

Table 2. Response of the SRNS patients to RTX treatment

Patients no	**Serum creatinine (mg/dL)**		**eGFR (ml/min/1.73m^2)**		**Serum albumin (gr/dL)**		**Proteinuria (mg/m^2/h)**	
	Baseline	At 12-month follow-up	Baseline	At 12-month follow-up	Baseline	At 12-month follow-up	Baseline	At 12-month follow-up
1	0.7	0.7	80	78	2.3	2.6	152	162
2	1.0	4.8	72	14	2.1	1.5	174	268
3	0.3	0.4	136	110	2.4	2.2	59	74
4	0.3	0.3	138	136	2.8	2.1	95	110
5	0.4	4.4	104	9	2.6	1.5	250	385
6	0.5	0.4	125	128	2.5	2.4	62	58

eGFR, estimated glomerular filtration rate

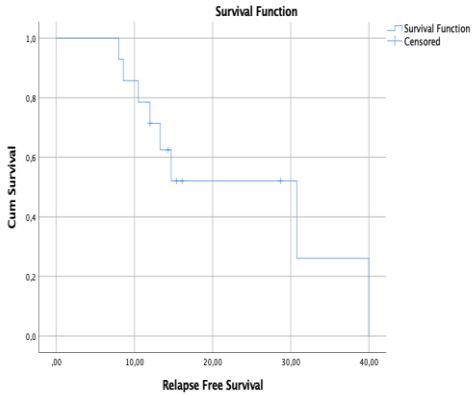

Figure 1. Kaplan-Meier survival curves for relapse-free survival of FRNS/SDNS patients

The CD19⁺B-cell counts were evaluated at baseline and after 12 months of RTX treatment only in FRNS/SDNS patients. None of the SRNS patients achieved remission after RTX treatment; therefore, the CD19⁺B-cell counts were evaluated only at baseline. In patients with FRNS/SDNS, the mean baseline CD19⁺B-cell counts were 11.2% (3.1-16%) before RTX treatment, versus 2.9% (0.3-6.6%) after 12 months of RTX treatment and, the difference was significant (P=0.028); on the other hand, since irregular monitoring of CD19⁺B-cell counts, the median duration of B-cell depletion was unknown.

After 1 January 2019, follow-up of FRNS/SDNS patients, memory B-cell counts were also evaluated as well as CD19⁺B-cell counts. The CD19⁺B-cell and memory B-cell counts were measured both in remission and at relapse time, especially after RTX. The CD19⁺B-cell and memory B-cell counts of 5 patients who relapsed after receiving RTX treatment were compared with the values of post-RTX remission and post-RTX relapse, and both values were significantly lower during remission (P =0.04 and P=0.04, respectively) (Table 3); however, the correlation coefficient was not calculated due to the small number of patients. Whereas CD19⁺B cells re-emergence in these patients during remission, memory B-cells depletion remained. Relapse occurred at a mean of 7.8 (4-15) months after CD19⁺B cells re-emergence.

Table 3. The CD19⁺B cell and memory B-cell counts in remission and relapse after RTX of 5 patients (FRNS/SDNS) who relapsed after receiving RTX treatment

Patients no	Remission after RTX treatment			Relapse after RTX treatment		
	Time of assessment (months)	CD19⁺B-cell counts (%)	Memory B-cell counts (%)	Time to relapse (months)	CD19⁺B-cell counts (%)	Memory B-cell counts (%)
1	8	4.58	0.05	14	28.37	0.71**
2	3	1.0*	0.004	12	4.3	0.05**
3	7	2.2	0.05	12	37.9	0.36**
4	36	10.9	0.45**	40	13.4	0.5**
5	9	7.04	0.05	24	11.4	0.39**

*CD19⁺ cell depletion was considered <1% of total lymphocytes
**Memory B-cell counts were considered as re-emergence >0.05% in peripheral blood mononuclear cells

DISCUSSION

The present retrospective study analyzed RTX treatment in complicated FRNS/SDNS and refractory SRNS patients. While the RTX treatment had positive therapeutic effects in patients with FRNS/SDNS, it was ineffective in patients with SRNS. Following RTX treatment, the relapse rate decreased, and the relapse-free period was prolonged in patients with FRNS/SDNS. A multicenter, double-blind, randomized, placebo-controlled study by Iijima et al. reported that RTX treatment resulted in longer relapse-free periods, and fewer relapses, which suggests that RTX is a promising treatment for FRNS/SDNS patients[11]. Yet, there is no consensus concerning the optimal RTX dose and dose interval. In some studies, it has been shown that single or less dose RTX treatment has a minimal effect on the relapse-free period[12]. Maxted et al. observed that patients treated with single RTX dose of 375 mg/m² had more frequent relapses than the patients treated with RTX 750 mg/m² as 2 doses[13]. Kimata et al. reported that 4 dosages of RTX 375 mg/m² administered at 3-month intervals resulted in a 1-year relapse-free period and fewer side effects[14]. In our study, 4 doses of RTX 375 mg/m² were administered at 2-week intervals, so as to both reduce side effects and to prolong the relapse-free period. In the period

after RTX treatment, our patients had the estimated relapse time was 31±9 (95% CI: 12-49) months and in about half of the patients did not develop relapse. While building the ideal treatment regimen for FRNS/SDNS patients, the efficacy, safety, and cost-effectiveness of RTX treatment should be considered.

Another aim of RTX treatment is to decrease the dose of or completely discontinue prednisolone and CNIs, thereby preventing their side-effects. In the literature, there are several approaches about continuing concomitant immunosuppressive drugs for patients receiving RTX treatment. Some researchers recommend discontinuing immunosuppressive after RTX treatment, whereas others have suggested employing as adjunct therapy consisting of CNIs or MMF to maintain the remission provided by RTX treatment[15-17]. We continued concomitant immunosuppressive treatment, our patients used prednisolone 10 mg/m^2, on alternate days and use of CNIs was followed-up via monitoring serum levels. We observed that continuing concomitant immunosuppressive drugs supported the maintenance of the prolonged relapse-free period.

It has been recently suggested that repeated and long-term use of RTX treatment has safe and effective. Kim et al. reported 18 children with SDNS who received repeated RTX infusions by a mean of 5.2 ± 2.3 cycles, and that of the 71% cycles were administered due to re-emergence of B-cells without relapse[18]. In our study, RTX treatment was re-administered in 5 patients due to relapse. It is not known the time of CD19$^+$B cells re-emergence onset, as CD19$^+$B cell counts were not regularly measured. However, despite the re-emergence of CD19$^+$B cells, remission has been continued. Systematically monitoring B cells re-emergence can help organize the recurrent doses of RTX treatment, thereby reducing both the cost and side effects of the treatment.

B-lymphocytes are composed of subgroups with multiple phenotypes and functions. Some studies investigated the subgroups of B-lymphocytes in SSNS patients[19,20]. In the literature, monitoring of memory B cell counts is recommended in the follow-up of neurological and rheumatic diseases treated with RTX[21-23.] Colucci et al. observed that SSNS patients in relapse had significantly more memory B cells than other B cell subpopulations[24]. Memory B cells play a role in antigen supplementation and immunoglobulin release, proliferation, and activation, thereby functioning as highly specialized B-cells. In our study, CD19$^+$B cell and memory B-cell counts of 5 patients who received recurrent RTX treatment were measured during remission and relapse after RTX. Despite re-emergence of CD19$^+$B cells during post-RTX remission in these patients, memory B cell count was still found to be delayed reconstitution; therefore, the pre-emptive additional RTX injections can be administered by evaluating the memory B cell count instead of the CD19$^+$B cell count. Bhatia et al. compared CD19$^+$B cell count and memory B-cell count after RTX treatment in SDNS patients, and B cells were re-emergence after a median time of 183 (180.5-190) days, the memory B cells were similar between 6 and 12 months in patients who maintained sustained remission. Therefore, they reported that the CD19$^+$B cell count recovered after 12 months while the memory B cell count did not recover[20]. Colucci et al. showed that total memory B cells started to re-emergence approximately 6 months after RTX infusion in INS patients, however, although total CD19$^+$B cells reached baseline levels at 12 months, the re-emergence of memory B cells still stayed significantly delayed at this time point. Due to the physiologic B cell ontogeny, memory B cells are the last cells to emerge from the B cell subset[24]. Therefore, instead of the CD19$^+$B cell count, memory cell count monitoring can be used as a reliable biomarker both in reducing the number of additional RTX doses and predicting relapse.

Refractory SRNS is an extremely challenging clinical entity and renal outcomes are especially poor. Data on the efficacy of RTX treatment in patients with refractory SRNS are limited. In the literature, there are studies with positive outcomes with remission rates varying between 18% and 80%, as well as negative outcomes reporting that there is no change in proteinuria and no remission is achieved[25-28]. Steroid resistance (according to type) and renal pathology findings are significant factors associated with the response to RTX treatment. Patients with early steroid resistance and FSGS disease had remission rates of 44% and 42%, respectively[29]. In our study, RTX treatment did not have a positive effect on eGFR or proteinuria rate in patients with SRNS, and 2 patients progressed to ESRD during follow-up and initiated hemodialysis. Although serum creatinine levels of these patients were normal, proteinuria levels were very high, and had both early steroid resistance and FSGS findings. Therefore,

steroid resistance type and biopsy findings should be considered in patient choice for treatment in patients with SRNS who are planned to receive RTX treatment.

In this study has some limitations. First, it was a retrospective and a small population study. Second, the time of $CD19^+$B cells re-emergence onset is unknown, as the $CD19^+$B cell counts were not measured regularly. Third, the pure effect of RTX treatment might not have been fully shown since concomitant immunosuppressive drugs continued to be given to the patients as well.

The response to RTX treatment is variable in patients with difficult-to-treat INS. While RTX treatment prolonged the relapse-free period in FRNS/SDNS patients, it could not provide remission in SRNS patients; therefore, it is ineffective in SRNS patients. Patients planned to be received RTX treatment should be selected with great care. Monitoring B-cell count is important in the follow-up of FRNS/SDNS patients; however, monitoring memory B-cell count can be more helpful than monitoring $CD19^+$B-cell count for determining the timing of pre-emptive RTX injections. Thus, both costs of healthcare and long-term side-effects of RTX can be reduced with monitoring of memory B-cell count. The memory B-cell count can be considered a sensitive novel biomarker for follow-up of patients receiving RTX treatment. Multicenter prospective studies based on long-term observation are needed to clarify these findings.

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