Mycophenolate Mofetil Following Rituximab in Children With Steroid-Resistant Nephrotic Syndrome

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BACKGROUND: Rituximab is being increasingly used in children with idiopathic nephrotic syndrome resistant to standard treatments. In spite of good initial response, rituximab responders always remain prone to further relapse, necessitating either repeat course of rituximab or addition of another steroid-sparing immunosuppressant.

METHODS: A retrospective analysis of baseline clinico-pathologic presentation and treatment outcome (complete remission, partial remission, or no response) was performed among 24 children with refractory-idiopathic SRNS over a follow-up period of 24 months. Children received 2 to 4 rituximab infusions (375 mg/m² weekly) depending on circulating B-cell level. At 3-month follow-up, a second course of rituximab was administered (if >5 B cells/mm³) along with MMF (1200 mg/m² per day) maintenance therapy.

RESULTS: Of 24 patients, 54% (13/24) and 46% (11/24) had minimal change disease and focal segmental glomerulosclerosis, respectively, on renal histopathology. After the first course of rituximab, 21% (5/24) of children achieved complete remission; however, most (4/5) of them relapsed again at a median interval of 53 (interquartile range 46–72) days. Depending on response to the first course of rituximab, MMF was started on 15 children at 3 months. After 6 months, 67% (10/15) of children on MMF achieved complete remission and 33% (5/15) remained at partial remission. At 24 months overall, 25% (6/24) and 42% (10/24) of children were in complete remission and partial remission, respectively; 33% (5/15) of children continued sustained complete remission after postrituximab-MMF maintenance therapy in comparison with no sustained complete remission with rituximab alone at 24 months (P < .001).

CONCLUSIONS: MMF may be an effective and safe maintenance therapy to consider as an additive immunosuppressant after induction with rituximab in maintaining remission among children with refractory SRNS.

WHAT’S KNOWN ON THIS SUBJECT: Treatment of idiopathic steroid-resistant nephrotic syndrome is challenging, and therapeutic options are limited. In spite of good initial response with rituximab, responders always remain prone to further relapse, necessitating either repeat course of rituximab or addition of another steroid-sparing immunosuppressant.

WHAT THIS STUDY ADDS: Mycophenolate mofetil may be an effective maintenance therapy to consider as an additive immunosuppressant after induction with rituximab in maintaining remission among children with refractory steroid-resistant nephrotic syndrome.
Idiopathic nephrotic syndrome affects 1 to 3 per 100,000 children <16 years of age. Whereas most of them respond well to corticosteroid treatment, as many as 20% experience a complicated course with steroid resistance (steroid-resistant nephrotic syndrome [SRNS]) associated with poor renal survival. The most commonly used steroid-sparing protocols in SRNS are intravenous cyclophosphamide courses and maintenance calcineurin-inhibitor (CNI) therapy. But treating patients with SRNS who fail to respond to CNI, mycophenolate mofetil (MMF), alkylating agents, or various combinations of these steroid-sparing agents with or without steroid, is challenging, and therapeutic options are limited if patients do not respond even after maintaining adequate drug trough levels. In 3 randomized clinical trials (RCTs), cyclosporin resulted in complete remission in 31% and partial remission in 38% during 6 months of therapy. MMF revealed 33% complete or partial remission in 1 RCT. Two RCTs comprising 84 children with SRNS demonstrated no significant differences in the number achieving remission with cyclophosphamide and prednisone compared with prednisone alone. Complete remission was significantly higher with tacrolimus (52.4%) than with cyclophosphamide (14.8%) in 1 RCT. To overcome this therapeutic problem and long-term adverse complications of these alternative medications, rituximab, a recently introduced alternative steroid-sparing therapy, is being increasingly used in subjects who failed other steroid-sparing therapies. Rituximab, an anti-CD20 monoclonal antibody, mainly acts by rapidly depleting B lymphocytes in children with nephrotic syndrome. There are several reports about the use of rituximab in children with complicated nephrotic syndrome, but it seems that the effects of rituximab may result in a higher percentage of remission and cumulative sustained remission in steroid-dependent (SDNS) and frequent-relapse nephrotic syndrome rather than in SRNS. In spite of good initial response, rituximab responders always remain prone to further relapse with regeneration of B lymphocytes, necessitating either repeat course of rituximab or addition of another steroid-sparing immunosuppressant. Reports suggest efficacy of rituximab may vary depending on disease pathology, clinical course, and simultaneous use of other immunosuppressants. In this retrospective study, we analyzed the efficacy of MMF maintenance therapy after rituximab in children with refractory-idiopathic SRNS. All children were treated with a first course of rituximab induction followed by a second course of rituximab reinfusion (if needed) as per center practice to achieve adequate B-lymphocyte suppression and daily MMF therapy to maintain the immunosuppressive effect induced by rituximab.

METHODS

Study Design and Setting

We retrospectively reviewed the medical records of 24 children with idiopathic SRNS and postrituximab follow-up of at least 24 months at a tertiary-care medical college between September 2011 and May 2014. The study was approved by the institutional review board of our institute.

Inclusion and Exclusion Criteria

All patients included in this retrospective analysis met the following criteria at study entry: (1) age 2 to 16 years, (2) with idiopathic nephrotic syndrome, (3) no response despite at least 6 months of continuous therapy with CNIs (with adequate drug trough level of 50–150 ng/mL for cyclosporin and 5–10 ng/mL for tacrolimus) or presence of drug-related toxicities (eg, convulsion, hyperglycemia, progressive deterioration of estimated glomerular filtration rate [eGFR], hirsutism), (4) eGFR >60 mL/min per 1.73 m², and (5) either minimal change disease (MCD) or focal segmental glomerulosclerosis (FSGS) on renal histopathology. Exclusion criteria were congenital nephrotic syndrome, secondary causes of nephrotic syndrome (known etiology; eg, lupus erythematosus, immunoglobulin A nephropathy, amyloidosis; known chronic infection like tuberculosis, HIV, hepatitis B or C; and known malignancy), considerable glomerular sclerosis (>50% sclerosis) on histopathology, and previous history of rituximab treatment.

Definitions

Standard definitions were used for nephrotic syndrome, remission, and relapses. Remission was defined as complete (urine protein to creatinine ratio [Up/Uc] <0.2 mg/mg or <20 mg/mmol) or partial (Up/Uc between 0.2 and 2 mg/mg, or 20–200 mg/mmol; serum albumin >2.5 g/dL or >3.62 µmol/L; and no edema). No response (nonresponder) was defined as the presence of nephrotic range proteinuria (Up/Uc >2 mg/mg or >200 mg/mmol), serum albumin <2.5 g/dL (<3.62µmol/L), or edema. “Initial resistance” was defined by the lack of remission at the first episode of nephrotic syndrome, and “late resistance” was considered in patients who were steroid sensitive initially, but showed steroid resistance during a subsequent relapse.

Rituximab Therapy

Rituximab was administered to patients with SRNS if there was lack of remission despite at least 6 months of continuous therapy with CNI or presence of drug-related toxicities. We excluded Epstein-Barr virus, cytomegalovirus, HIV, tuberculosis, hepatitis B, and hepatitis C, along with any pyogenic focus in all children before giving the first dose of
rituximab. After premedication with steroids and antihistamine, 2 rituximab infusions were administered once every week at standard dose (intravenous infusion of rituximab 375 mg/m²). We directly measured circulating B-lymphocyte counts (measured with flow cytometry) 24 hours after rituximab administration to monitor adequacy of rituximab, instead of measuring it indirectly with CD19 or CD20. If >5 B lymphocytes per mm³ were observed, they were again measured 1 week later. If counts were still >5 B lymphocytes per mm³, third and fourth doses of rituximab were administered.

Concomitant Therapy
Oral immunosuppressive drugs were tapered down or discontinued gradually for all patients. MMF and CNI were discontinued immediately and prednisone was tapered off over 1 month after B-lymphocyte (<5 B cells per mm³) depletion. In those children who did not show any sign of improvement (nonresponder), immunosuppressive agents were continued along with the lowest possible alternate-day steroid and other experimental drugs as per center practice. Enalapril was given at 0.5 mg/kg per day in all patients with hypertension. Furosemide was used for control of edema, if indicated. Cotrimoxazole was systematically given to all patients during the period of B-lymphocyte depletion for Pneumocystis prophylaxis.

Intervention at 3-Month Follow-up
At 3-month follow-up, a second course of rituximab infusion (if >5 circulating B lymphocytes per mm³) was administered and MMF was started (1200 mg/m² per day in 2 divided doses) to all patients with partial remission and in patients with subsequent relapse after complete remission with the first course of rituximab. To determine the number of rituximab doses, a similar strategy was followed depending on circulating B lymphocytes as in the first course of rituximab therapy. No patient was treated with more than 2 courses of rituximab.

Clinical and Biochemical Assessment
Clinical histories with relapses were recorded through standardized screening of the patients’ hospital records. At every 3 months during the 24-month follow-up period, we reviewed number of relapses, side effects, proportion of patients with sustained remission, and time to first relapse after rituximab infusion and after adding of MMF. Proteinuria was assessed by measurement of a 24-hour urine sample or by assessing Up/Uc. In addition, blood count, serum creatinine, serum albumin, and serum cholesterol values were obtained at study entry and every 3 months. GFR was estimated at baseline and then every 6 months by using the modified Schwartz formula. All patients performed regular proteinuria dipstick at home and contacted the treatment center when 3 to 4+ proteinuria occurred for more than 2 consecutive days.

Statistical Analysis
Continuous data between subgroups were analyzed by using Mann-Whitney U test, Wilcoxon signed rank test, Student’s t test, or analysis of variance, as appropriate, and nominal data were examined using Fisher’s exact test. Kaplan-Meier survival analysis was performed separately during the first 3 months after the first course of rituximab therapy (n = 24) and thereafter up to 24 months after addition of MMF therapy (n = 15). Throughout the text, data are expressed as means ± SDs, medians (interquartile range [IQR]), and percentages, as appropriate, and P ≤ .05 was considered statistically significant. The SPSS for Windows version 16 software (IBM SPSS Statistics, IBM Corporation, Chicago, IL) was used for all statistical analyses.

RESULTS
Baseline characteristics of 24 children with SRNS (15 children had no response with 6 months of CNI therapy and CNI was stopped in 9 children due to drug-related toxicities) who were evaluated by retrospective analysis are summarized in Table 1. Fourteen patients were initial SRNS and 10 patients were late SRNS. Of 24 patients treated with rituximab, 13 (54%) had MCD and 11 (46%) had FSGS on histopathology of kidney; 38% (5/13) of children with MCD and 64% (7/11) of children with FSGS required a total of 4 doses of rituximab initially for adequate B-lymphocyte suppression (<5 B cells per mm³). All patients had various steroid toxicities, such as obesity, cushingoid features, and/or poor height velocity. Five children had early posterior subcapsular cataracts.

Outcome at 3 Months (After Completion of First Rituximab Course) and Retreatment
Five (MCD 4; FSGS 1) children of the total of 24 (21%) achieved complete remission within 2 weeks of completion of initial rituximab course (Fig 1). The median time to response was 12 days (IQR 8–14 days) after the last dose of rituximab; however, 4 (80%; 4/5) of them who responded completely, relapsed again within 90 days of the last rituximab infusion at a median interval of 53 days (IQR 46–72 days). Of these 4 children, 2 relapsed in spite of continued adequate B-lymphocyte suppression and were not considered for rituximab retreatment. MMF was started in all 4 of these children. Partial remission was achieved in 46% (11/24) of children and no response was documented among 33% (8/24) of children. MMF was started in all 11 children who achieved partial remission. Children
who did not respond at all were treated with other experimental drugs as per center practice. All children with partial remission or no remission achieved adequate B-lymphocyte depletion after rituximab treatment.

### Outcome at 6 Months

One child continued sustained complete remission without any steroid or immunosuppressant after the first course of rituximab. Ten (67%) of a total of 15 children who were on MMF therapy achieved complete remission and 5 (33%) remained in partial remission. At 6 months, 7 (50%) of 14 patients with initial SRNS and 9 (90%) of 10 patients with late SRNS were in complete or partial remission. Complete or partial remission was seen in 85% (11/13) of patients with MCD and 45% (5/11) of patients with FSGS. Complete response in children with MCD was almost double in comparison with FSGS. Only 55% (6/11) of children among partial responders (with first course of rituximab) in comparison with all (4/4) children among initial complete responders (with first course of rituximab) achieved complete remission after the second course of rituximab and addition of MMF therapy.

### Outcome at 12 Months

Of 10 children who achieved complete remission at 6 months after MMF therapy, 60% (6/10) sustained complete remission, but 40% (4/10) relapsed. Trough levels were obtained in those patients who failed MMF treatment, and adequate concentrations of >2.5 µg/mL were observed. All (4/4) children with initial complete response (with first course of rituximab) continued complete remission. One child remained in sustained remission throughout this study period after initial rituximab course.

### Outcome at 24 Months

At 24 months of follow-up, overall 25% (6/24) and 42% (10/24) of children were in complete and partial remission, respectively. Interestingly, all children with complete remission were MCD. Five (33%) of the total 15 children who failed to continue sustained complete response with the first course of rituximab, attained sustained complete remission after MMF maintenance therapy in comparison with no relapse-free survival with rituximab alone at 24 months (Figs 1 and 2).

### Predictive Factors for Favorable Clinical Response

Kaplan-Maier curve (Fig 2) depicts relative efficacy of MMF maintenance therapy after rituximab in comparison with rituximab alone. Interestingly, all children with sustained complete remission at 24-month follow-up were MCD ($P < .001$) and late steroid resistant ($P < .001$). So, these factors might be associated with favorable treatment response.

### Adverse Effects of Drugs

No serious adverse events occurred after rituximab therapy. One patient had dizziness and mild dyspnea soon after infusion, but no further complications. Two other children developed respiratory tract infections that did not require hospitalization. No patient developed neutropenia (<500 per mm$^3$). During MMF therapy, 1 child had diarrhea, which settled with symptomatic treatment.

### DISCUSSION

Our study reveals a high rate of cumulative sustained remission among pediatric patients with idiopathic SRNS treated with rituximab followed by MMF therapy. The point of interest regarding this report is a homogeneous rituximab infusion protocol with adequate B-cell depletion and a systematic rituximab reinfusion to maintain adequate B-lymphocyte suppression followed by daily MMF therapy to maintain the immunosuppressive effect induced by rituximab.

Although there have been many reports of the excellent steroid-sparing effect of rituximab against
FIGURE 1
Long-term outcome of children with SRNS (n = 24).
idiopathic complicated childhood nephrotic syndrome, most patients were likely to relapse after recovery of B lymphocytes. As a single infusion or a single course, rituximab can deplete B lymphocytes for a limited time only; the efficacy of additional rituximab administrations just after the reemergence of B lymphocytes has been reported in children with SDNS. In our report, 67% (16/24) of children achieved complete or partial remission at a median interval of 17 days after the first course of rituximab therapy. All children revealed adequate B-lymphocyte suppression, yet 33% (8/24) revealed no sign of improvement. Four of 5 children who responded completely relapsed again within 90 days of the last rituximab infusion. Of these 4 children, 2 relapsed in spite of continued adequate B-lymphocyte suppression and they were not considered for rituximab retreatment. Interestingly, all 5 children continued sustained complete remission at 24-month follow-up after the second course of rituximab and MMF treatment, even after recovery of >5 B lymphocytes. Therefore, it may be concluded that B-lymphocyte depletion or B-lymphocyte recovery is not the only criterion for maintaining remission or relapse. But of course immunomodulation of MMF had an additive impact in maintaining remission even after B-lymphocyte recovery. In this study, the mean time interval from B-lymphocyte recovery to nephrotic syndrome relapse after the first course of rituximab therapy was 1.8 months, but the interval significantly increased to 11.2 months after addition of MMF.

The single child who maintained complete sustained remission after the first course of rituximab therapy, and even after regeneration of adequate B lymphocytes at 5 months, ultimately relapsed after 17 months. We had not treated this child with a second course of rituximab, as the parents of the child did not agree for further rituximab and we started MMF. This case suggests that a relapse may occur even after a long interval of B-lymphocyte recovery. A child may relapse or may not respond at all with no detectable circulating B lymphocytes in the peripheral blood, a finding that might be related to the escape of some intraorgan B cells to rituximab-induced apoptosis, and the subsequent persistence of B lymphocytes in specific body compartments. So, the repeated prophylactic use of rituximab after B-lymphocyte recovery is not always justifiable and must be individualized. We did not treat any child with more than 2 courses of rituximab infusion because it may not be cost-effective and may produce severe immunsuppression with a threat for serious infections. Whether prophylactic periodic maintenance reintjection of rituximab depending on B-lymphocyte regeneration should be proposed to patients with a complete clinical response remains a matter of debate. Moreover, response of rituximab in SRNS also depends on multiple factors, such as degree of glomerulosclerosis or presence of podocytopathy. Advanced glomerular fibrosis, as well as low eGFR, may adversely affect the treatment outcome even after adequate immunsuppression, depending on the numbers of actively working glomeruli. A more important point is to administer rituximab during remission or at least in partial remission. It is believed that the effect of rituximab is lower during the nephrotic range proteinuria phase, as a significant amount of rituximab is lost in urine, resulting in lower serum rituximab levels and faster recovery of B lymphocytes.

In this study, maintenance therapy with MMF after rituximab in children with SRNS significantly improved the patient outcome in maintaining remission without repeated exposure of rituximab (Fig 2). We chose MMF for maintenance therapy after rituximab, as most of the children were also suffering from various stages of CNI nephrotoxicity and interestingly this nephrotoxicity gradually reversed to various extents over time during rituximab and MMF therapy after withdrawal of CNI. Severe adverse events are rarely experienced with MMF treatment, and MMF treatment can be endured long-term. Similar to our study, the editorial commentary of Filler et al regarding management of SDNS also stated that the addition of MMF to the therapeutic regimen after rituximab may provide an effective tool to prevent further relapse once the CD19/CD20 count returns to normal. Ito et al performed a cohort study on SDNS and followed 9 patients who...
received MMF after rituximab infusion and 7 patients as the control group without MMF administration after rituximab. They found that the number of relapses were significantly lower in patients who were treated with the combination of rituximab and MMF within 1 year of treatment. Sharma and Filler reported that children with FSGS could successfully maintain long-term remission with MMF after rituximab, and described a case treated with rituximab and MMF; relapse did not occur in this patient despite an increase in CD19 cells. Fujinaga et al. reported the value and efficacy of maintenance therapy with ciclosporin after a single dose of rituximab, even for patients with previously ciclosporin-refractory SDNS and emphasized the improvement in drug sensitivity to ciclosporin after rituximab treatment and noted that ciclosporin treatment could prevent relapse after rituximab. However, the long-term use of ciclosporin increases the risk of nephrotoxicity. MMF may eliminate the need to repeat rituximab infusions, and consequently reduces the side effects of its repeated doses. Our report suggests that the efficacy of combined rituximab and MMF is better among children with renal histology of MCD or with late steroid resistance.

We recognize several limitations to our study. The patient number in each subgroup was small and, therefore, statistical analysis may not be conclusive. We did not directly compare the efficacy of rituximab versus rituximab-MMF among children with SRNS. Also, we did not routinely perform therapeutic drug monitoring for MMF, the cost of which exceeds available resources in most developing countries. A recent report suggested that individualized dosing of MMF according to pharmacokinetic profiling may markedly augment its efficacy in nephrotic syndrome. Finally, routine evaluation for genetic mutations was not done in our patients because of the variability in availability, significant cost, low to absent prevalence observed in Indian populations, and current uncertainty about the individual response to therapy and prognosis when a mutation is identified.

**CONCLUSIONS**

Although our study is a retrospective study, we conclude that MMF may be an ideal and safe maintenance therapy to consider as an additive immunosuppressant after induction with 1 or 2 courses of rituximab in maintaining remission among children with SRNS. However, an RCT is needed among these children to further establish the fact.

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**ABBREVIATIONS**

CNI: calcineurin inhibitor
eGFR: estimated glomerular filtration rate
FSGS: focal segmental glomerulosclerosis
IQR: interquartile range
MMF: mycophenolate mofetil
RCT: randomized clinical trials
SDNS: steroid-dependent nephrotic syndrome
SRNS: steroid-resistant nephrotic syndrome
Up/Uc: urine protein to creatinine ratio

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