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ORIGINAL ARTICLE

Efficacy and safety of rituximab in comparison with common induction therapies in pediatric active lupus nephritis

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Abstract
Background Childhood-onset lupus nephritis (LN) is one of the most severe manifestations of systemic lupus erythematosus (SLE). Despite treatment-related toxicities, cyclophosphamide (CYC) and glucocorticoid-based treatment protocols are still considered standard therapy in managing this multisystem disorder. An effective and safe alternative induction regimen is needed.
Methods Forty-four pediatric patients with active LN aged 3.5–13.8 (median 8.4) years, of whom 32 entered the study at diagnosis of SLE, were followed over 36 months. Induction therapy consisted of methylprednisolone pulses followed by either rituximab (RTX) ($n = 17$), mycophenolate mofetil (MMF) ($n = 12$) or pulse-CYC ($n = 15$), with tapering dose of prednisolone orally. MMF was added as maintenance immunosuppressant (800 mg/m^2 daily) in all children from the third month onward.
Results Flare-free survival was significantly higher at 36 months with RTX compared with MMF and CYC (100% for RTX vs. 83% for MMF. and 53% for CYC, $p = 0{\cdot}006$). Twelve patients (76.5%) achieved complete remission with RTX compared with five (41.7%) and seven (46.7%) with MMF and CYC, respectively, at last follow-up. Requirement of mean daily dosage of prednisone was significantly lower in RTX group [$p = 0.005$ (RTX vs MMF); 0.0001 (RTX vs CYC) at 36 months] compared with other groups after the 3-month follow-up. In comparison with few minor adverse events in the other two cohorts, several serious adverse events occurred in the CYC group.
Conclusions Efficacy and medium-term safety of RTX induction followed by MMF maintenance therapy in inducing and maintaining remission among children with LN were evident in this study.

Keywords Pediatric SLE · Lupus nephritis · Rituximab · Mycophenolate mofetil · Cyclophosphamide

Introduction

Lupus nephritis (LN) is one of the most severe manifestations of systemic lupus erythematosus (SLE) and is associated with a high rate of morbidity and mortality. Approximately 50–60% of adult patients with SLE develop LN [1, 2]. Compared with adult-onset disease, LN in children is more severe, with increased damage accrual. Hence, managing childhood LN is challenging, and therapeutic regimens are mostly derived from adult protocols. The current recommended induction treatment for severe forms of LN includes corticosteroids in conjunction with cyclophosphamide (CYC) or mycophenolate mofetil (MMF) [3, 4]. However, response with CYC is often slow and associated with increased risks for adverse effects, including gonadal toxicity [5, 6]. MMF, a less toxic alternative, was at least as effective as CYC in induction treatment in various trials [7–13]. Although the renal response rates among patients receiving CYC or MMF treatment reach 50–80%, many of these responses are partial [14].

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In SLE, B cells contribute to disease pathology by facilitating antigen presentation and autoantibody production, together with permitting the secretion of cytokines and the costimulation of T cells. Rituximab (RTX), as a B-cell-depleting agent, offers an alternative or adjunctive therapeutic option for patients with SLE. RTX has produced conflicting results regarding its efficacy across various studies on adult populations [15–19]. Therefore, the search for an effective and less toxic therapeutic option for children is essential.

Patients and methods

Study population

We retrospectively reviewed the medical records of all children (<14 years) diagnosed with active LN at NRS Medical College, Kolkata, India, between February 2008 and January 2016. The diagnosis of SLE was made according to American College of Rheumatology (ACR) criteria. LN was classified as per International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification of kidney biopsy [20]. Patients with class IIIA or IIIA/C (±V); class IVA or IVA/C (±V) LN, and pure class V nephritis with nephrotic-range proteinuria were classified as active LN and included in the study. Patients were excluded when any of the following criteria were met: treatment with RTX or CYC within the previous year, and patients already under chronic renal replacement therapy (RRT) at study entry.

Baseline parameters and follow-up data

Patient demographics and clinical courses were obtained from hospital case records. For each patient, the following data were collected: gender, age, age at presentation, clinical manifestations, treatment received, duration of follow-up, any flare, and final outcome. Clinical manifestations included symptoms, signs, and organ involvement at presentation. Results of biochemical, immunological, and histological investigations were also collected from hospital records. The study was approved by our Institutional Review Board.

Treatment protocol

Induction therapy

Induction therapy consisted of methylprednisolone pulses IV (15 mg/kg daily for 3 days) and dialysis if indicated. This was followed by two RTX infusions (375 mg/mt^2 weekly) or MMF 1200 mg/mt^2 daily or six CYC pulses IV of 500 mg/mt^2 once every fortnight; along with oral prednisolone (2 mg/kg daily) for 1 month, then progressively tapered at the discretion of the clinicians. Before January 2010, we mostly used CYC or MMF as the induction agent, but we later preferred either MMF or RTX induction for better efficacy–toxicity ratio of RTX when compared with CYC. Selection of the induction agent was individualized by the pediatric nephrologist team based on the patient's specific clinical condition. In general, we preferred MMF for newly diagnosed cases and CYC (before 2010) or RTX (after 2010) for older cases. However, specific choice of drug was confirmed following detailed discussion with parents regarding existing data of efficacy and safety of a particular drug.

Circulating B cells were measured 24 h after the second RTX administration. If more than five B-cells/mm^3 were observed, they were measured again 1 week later. If the count was still five B-cells/mm^3, third and fourth doses of RTX were administered. Cotrimoxazole (20 mg/kg; three times a week) was systematically given to all RTX recipients during the period of B-cell depletion for pneumocystis prophylaxis.

Maintenance therapy

Maintenance therapy consisted of tapering doses of daily prednisolone orally and MMF 800 mg/m^2 every day in two divided doses from the third month onward. Patients received maintenance MMF therapy for 2–3 years depending on further flare and disease activity.

Flare management

Any flare was treated with reinstitution of induction therapy with either MMF or RTX, followed by maintenance therapy. No patient was treated with more than two courses of RTX.

Definitions

Estimated glomerular filtration rate (eGFR) was calculated using the modified Schwartz formula [21]. Hypertension was described as systolic/diastolic blood pressure ≥95th percentile for sex, age, and height [22]. Proteinuria was classified as subnephrotic [urine protein–creatinine ratio (Up/Uc) between 0.2 and 2] or nephrotic (Up/Uc >2). Other defined terminologies were hematuria [≥5 red blood cells/high-power field (HPF) in centrifuged specimen]; anaemia (hemoglobin <11 g/dl); thrombocytosis (platelet >450 × 1000 cells/mm^3), and deranged liver function tests [aspartate aminotransferase (AST) or alanine aminotransferase (ALT) to >50 IU/L]. Global disease activity was evaluated using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) [23].

Treatment response was defined as complete renal remission if there was improvement in kidney function as determined by eGFR (>90 ml/min/1.73m^2) or return to the baseline in patients with chronic renal dysfunction), proteinuria (≤0.5 g/24 h), and inactive urinary sediment (≤5 white blood cells HPF and ≤5 red blood cells per HPF); complete remission was defined as if there was attenuation of clinical manifestations of SLE flare

along with complete renal remission; partial renal remission was defined as partial improvement of renal function (≤25% decrease in baseline eGFR, ≥50% decrease in baseline proteinuria, or proteinuria <1 g/24 h, but not fulfilling criteria of complete renal remission); partial remission was partial attenuation of clinical manifestations of SLE flare along with partial renal remission; and treatment failure by no improvement or a deterioration of clinical symptoms and renal function. We diagnosed LN flare if there was reappearance or deterioration of clinical manifestations of LN and renal biochemical parameters (≥25% decrease in baseline eGFR or proteinuria ≥1 g/24 h), along with rising titers of immunological parameters after initial postinduction stabilization or improvement.

Primary and secondary outcomes

The primary outcome was flare-free survival. Secondary outcomes were overall patient survival, renal survival, time to first flare after induction, number of flares, and drug-related adverse reactions. The end-point for renal survival analysis was commencement of long-term RRT, while that for patient survival was death due to any cause.

Statistical analysis

Considering the limited sample population, we performed nonparametric tests for all statistical analyses. Continuous data were analyzed using Mann–Whitney *U* test and Wilcoxon signed-rank test; nominal data were examined using Fisher's exact test. Throughout the text, data are expressed as mean [standard deviation (SD)] and percentages, as appropriate, and $p<0.05$ was considered statistically significant. SPSS for Windows version 16 software (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

Baseline demographics

Baseline patient characteristics of the three treatment cohorts are summarized in Table 1. Seventeen patients were treated with RTX, 12 with MMF, and 15 with CYC. Thirty-two patients entered the study upon SLE diagnosis, and 12 (RTX 6, MMF 2, CYC 4) were previously known cases of SLE. Before the new renal flare at study entry, two of the six patients in the RTX group, both patients in the MMF group, and three of the four patients in the CYC group were in complete remission. All other patients were in partial remission. Before entering the study, all patients except one from the RTX group were receiving maintenance treatment with low-dose steroids and azathioprine (AZA). The children selected for MMF were mostly (83.3%) new patients with shorter SLE duration.

Baseline disease characteristics

Table 2 summarizes clinical manifestations and biochemical and immunological parameters at the time of study entry. Fever, headache, hypertension, and renal involvement with active urinary sediments were the most common manifestations of all children. Thirty-six (81.8%) presented with uremic symptoms necessitating dialysis for a variable period. Serositis was recognized among 14 (31.8%) children, and two of whom had pericardial effusion. Four (9%) children had neurological manifestations and two (4.5%) had melena. Most patients had a varying degree of anemia, leucopenia, or thrombocytopenia at the time of presentation. Deranged liver enzymes were found in two (4.5%) patients. All patients were positive for antinuclear antibody (ANA), and 39 (88.6%) of them were anti-double-stranded (anti-dsDNA) positive. Forty patients (90.9%) had decreased complement 3 (C3) levels, whereas 37 (84%) had decreased complement 4 (C4) levels. We documented increased erythrocyte sedimentation rates (ESR) and elevated serum C-reactive protein (sCRP) levels in all patients. No one was positive for perinuclear antineutrophil cytoplasmic autoantibodies (p-ANCA), cytoplasmic ANCA (cANCA), hepatitis B surface antigen (HBsAg), or anti-hepatitis C virus (anti-HCV). Renal histologies of all patients are summarized in Table 1. A varying degree of interstitial fibrosis was found in 11 (25%) patients. Children in the MMF arm had better clinical and renal parameters than those started on RTX or CYC (mean SLEDAI score 17.5, 13.2, and 15.4, respectively; $p = 0.03$ (RTX vs MMF).

Outcome after induction therapy (at 3-month follow-up)

After completion of the induction therapy as per protocol, almost all (97.7%) children showed significant improvement in clinical and renal parameters (Table 2). Excluding one child from the CYC cohort, all others became independent of dialysis at the 3-month follow-up. Mean eGFR was significantly improved in the RTX cohort in comparison with MMF and CYC cohorts (95.4 vs 71.6 and 78.6 ml/min/1.73m^2 respectively; $p = 0.02$ (RTX vs. MMF); $p = 0.4$ (RTX vs. CYC). Twelve patients (76.5%) achieved complete remission among the RTX cohort in comparison with five (41.7%) in the MMF group (RTX vs. MMF; $p = 0.14$) and seven (46.7%) in the CYC group (RTX vs. CYC; $p = 0.28$). Requirement of mean daily dosage of prednisone was also significantly lower in patients with RTX when compared with MMF and CYC groups (Table 3).

Outcome at 36-month follow-up

Detailed treatment outcome at 36 months is summarized in Table 2. The flare-free survival rate was similar during the

Table 1 Patient baseline characteristics

	RTX ($n = 17$)	MMF ($n = 12$)	CYC ($n = 15$)
Gender *n*(%)			
Male	8 (47.1%)	5(41.7%)	8 (53.3%)
Female	9 (52.9%)	7 (58.3%)	7(46.7%)
Age at study entry *(year); mean (SD)*	8.4(4.6)	8.1(3.2)	8.7(4.1)
Diagnosis of SLE at study entry *n*(%)			
New patients	11(64.7%)	10(83.3%)	11(73.3%)
Old patients	6(35.3%)	2(16.7%)	4(26.7%)
Duration of SLE *(months); mean (SD)*	11.6(6.4)	7	13.4(4.3)
Previous flares *n*(%)			
Patients with previous renal flare	4(23.5%)	1(8.3%)	3(20%)
Patients with previous extra renal flare	2(11.8%)	0	1(6.7%)
Total numbers of flare *n*	6	1	4
Previous therapy *n*(%)			
no therapy	11(64.7%)	10(83.3%)	11(73.3%)
P alone	0	1(8.3%)	1(6.7%)
P + AZA	1(5.9%)	0	1(6.7%)
P + CYC	2(11.8%)	0	0
P + MMF	3(17.6%)	0	2(13.3%)
P + RTX	0	0	0
Therapy at enrolment *n*(%)			
No therapy	11(64.7%)	10(83.3%)	11(73.3%)
P alone	1(5.9%)	1(8.3%)	2(13.3%)
P + AZA	4(23.5%)	0	2(13.3%)
P + MMF	1(5.9%)	0	0
Renal biopsy class *n*(%)			
III(A)	4(23.5%)	6(50%)	5(33.3%)
IV	11(64.7%)	3(25%)	9(60%)
IV-G(A)	3(17.6%)	2(16.6%)	2(13.3%)
IV-G(A/C)	1(5.8%)	0	1(6.6%)
IV-S(A)	6(35.2%)	1(8.3%)	6(40%)
IV-S(A/C)	1(5.8%)	0	0
V	2(11.8%)	3(25%)	1(6.7%)
Presence of Interstitial fibrosis	9(52.9%)	3(25%)	6(40%)

P prednisolone, *AZA* azathioprine, *RTX* rituximab, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil, *A* active lesions, *A/C* active and chronic lesions, *G* global, *S* segmental, *SLE* systemic lupus erythematosus

first 2 years but subsequently diverged between treatment arms, resulting in a significantly higher 36-month flare-free survival with RTX as compared with MMF and CYC (100% vs. 83% and 53%, respectively; $p = 0.006$) (Fig. 1). There was no further renal or extrarenal flare among the RTX cohort during the follow-up period. All flares in CYC and MMF arms were in previous cases of SLE; there were no new flares in any SLE newly-diagnosed cases at study onset. One child in the MMF cohort developed both renal and extrarenal flares at 23 months and was successfully treated with RTX. Another child from the MMF cohort showed features of a new renal flare at 25 months and was treated with MMF induction. Two children from the CYC cohort developed both renal and extrarenal flares at 23 and 28 months of follow-up and was treated with RTX induction. One of these patients died at 28 months following two consecutive renal and neurological flares. There were another four children with new renal flares in the CYC arm; three were treated with RTX and the other with MMF induction. Kidney function was well recovered in all patients in the RTX and MMF groups, and there were no patients with eGFR <30 ml/min/1.73m^2. One child continued chronic RRT since first induction into the CYC group. The dosage of prednisone continued to be lower in the RTX than the MMF and CYC groups after the 3-month follow-up (Table 3).

Table 2 Clinical, biochemical, immunological, and overall disease outcome according to treatment cohorts over the study period

	Baseline			3 months			36 months		
	RTX ($n = 17$)	MMF ($n = 12$)	CYC ($n = 15$)	RTX ($n = 17$)	MMF ($n = 12$)	CYC ($n = 15$)	RTX ($n = 17$)	MMF ($n = 12$)	CYC ($n = 14$)
Clinical manifestations									
Fever *n*(%)	15(88.2%)	9(75%)	15(100%)	0	0	0	0	0	0
Weight loss *n*(%)	4(23.5%)	1(8.3%)	2(13.3%)	0	0	0	0	0	0
Skin rash/ulcers *n*(%)	11(64.7%)	7(58.3%)	9(60%)	1(5.9%)	1(8.3%)	2(13.3%)	1(5.9%)	1(8.3%)	2(14.3%)
Arthralgia/arthritis *n*(%)	17(100%)	11(91.7%)	13(86.7%)	0	0	1(6.7%)	0	0	0
Neurological involvement (CNS lupus) *n*(%)	3(17.6%)	0	1(6.7%)	1(5.9%)	0	1(6.7%)	1(5.9%)	0	1(7.1%)
Serositis *n*(%)	7(41.2%)	3(25%)	4(26.7%)	0	0	0	0	0	0
Hypertension *n*(%)	17(100%)	12(100%)	15(100%)	7(41.2%)	9(75%)	9(60%)	4(23.5%)	5(41.7%)	8(57.1%)
Oliguria *n*(%)	17(100%)	10(83.3%)	15(100%)	1(5.9%)	2(16.7%)	3(20%)	0	1(8.3%)	2(14.3%)
Gross hematuria *n*(%)	4(23.5%)	0	1(6.7%)	0	0	0	0	0	0
Uremia *n*(%)	17(100%)	6(50%)	13(86.7%)	0	0	1(6.7%)	0	0	0
Biochemical and hematological parameters									
Range of eGFR *(ml/min/1.73mt^2); n*(%)									
15	7(41.2%)	0	6(40%)	0	0	1(6.7%)	0	0	1(7.1%)
>15–30	8(47.1%)	4(33.3%)	6(40%)	1(5.9%)	2(16.7%)	2(13.3%)	0	0	2(14.3%)
>30–60	1(5.9%)	2(16.7%)	1(6.7%)	2(11.8%)	3(25%)	2(13.3%)	1(5.9%)	1(8.3%)	4(28.6%)
>60–90	0	0	0	1(5.9%)	2(16.7%)	3(20%)	1(5.9%)	0	0
>90	0	0	0	13(76.5%)	5(41.7%)	7(46.7%)	15(88.2%)	11(91.7%)	8(57.1%)
Range of urine protein creatinine ratio (mg/mg) *n*(%)									
0.2	1(5.9%)	2(16.7%)	2(13.3%)	15(88.2%)	9(75%)	13(86.7%)	14(82.3%)	12(100%)	9(64.3%)
>0.2–2	14(82.3%)	7(58.3%)	12(80%)	2(11.8%)	3(25%)	2(13.3%)	1(5.9%)	0(0%)	4(28.6%)
>2	2(11.8%)	3(25%)	1(6.7%)	0	0	0	0(0%)	0(0%)	1(7.1%)
Range of hematuria *(RBC/HPF); n*(%)									
5	0	0	0	13(76.5%)	5(41.7%)	7(46.7%)	17(100%)	11(91.7%)	8(57.1%)
>5	1(5.9%)	1(8.3%)	0	3(17.6%)	5(41.7%)	5(33.3%)	0	1(8.3%)	4(28.6%)
plenty	16(94.1%)	11(91.7%)	15(100%)	1(5.9%)	2(16.7%)	3(20%)	0	0	2(14.3%)
Hematological paramaters *n*(%)									
Anaemia	17(100%)	12(100%)	15(100%)	4(23.5%)	7(58.3%)	8(53.3%)	3(17.6%)	3(25%)	6(42.9%)
Leucopenia	14(82.3%)	8(66.7%)	11(73.3%)	1(5.9%)	2(16.7%)	4(26.7%)	0	0	0
Thrombocytopenia	17(100%)	10(83.3%)	14(93.3%)	2(11.8%)	3(25%)	5(33.3%)	0	0	1(7.1%)
Immunological parameters									
Range of anti-dsDNA antibody titer *(IU/ml); n*(%)									
<30 (negative)	4(23.5%)	0	1(6.7%)	13(76.5%)	5(41.7%)	7(46.7%)	15(88.2%)	11(91.7%)	8(57.1%)
30 to 60 (low positive)	1(5.9%)	0	1(6.7%)	4(23.5%)	7(58.3%)	8(53.3%)	2(11.8%)	1(8.3%)	4(28.6%)
>60 to 200 (positive)	3(17.6%)	2(16.7%)	5(33.3%)	0	0	1(6.7%)	0	0	2(14.3%)
>200 (strong positive)	9(52.9%)	10(83.3%)	8(53.3%)	0	0	0	0	0	0
Range of C3 concentration *(g/L); n*(%)									
>1.8	0	0	0	0	0	0	0	0	0
0.9 to 1.8 (normal)	3(17.6%)	1(8.3%)	0	14(82.3%)	5(41.7%)	7(46.7%)	16(94.1%)	12(100%)	10(71.4%)
<0.9 (low)	14(82.3%)	11(91.7%)	15(100%)	3(17.6%)	7(58.3%)	8(53.3%)	1(5.9%)	0	4(28.6%)
Range of C4 concentration *(g/L); n*(%)									
>0.47	1(5.9%)	0	0	1(5.9%)	0	0	0	0	0
0.16 to 0.47 (normal)	1(5.9%)	2(16.7%)	3(20%)	16(94.1%)	7(58.3%)	9(60%)	17(100%)	12(100%)	12(85.7%)
<0.16	15(88.2%)	10(83.3%)	12(80%)	1(5.9%)	5(41.7%)	6(40%)	0	0	2(14.3%)
SLEDAI score *mean (SD)*	17.5(5.6)	13.2(4.5)	15.4(4.2)	7.6(4.1)	9.8(3.4)	10.3(3.7)	5.1(2.3)	7.2(2.5)	8.7(3.1)
Overall outcome									
Died *n*(%)				0	0	0	0	0	1(7.1%)
Complete remission *n*(%)				12(70.6%)	5(41.7%)	7(46.7%)	14(82.3%)	9(75%)	8(57.1%)
New SLE cases				11(64.7%)	5(41.6%)	7(46.6%)	11(64.7%)	9(75%)	8(57.1%)
Old SLE cases				1(5.8%)	0	0	3(17.6%)	0	0
Partial remission *n*(%)				5(29.4%)	7(58.3%)	7(46.7%)	3(17.6%)	3(25%)	5(35.7%)
No response *n*(%)				0	0	1(6.7%)	0	0	1(7.1%)
Complete renal remission *n*(%)				13(76.5%)	5(41.7%)	7(46.7%)	15(88.2%)	11(91.7%)	9(64.3%)
Partial renal remission *n*(%)				4(23.5%)	7(58.3%)	7(46.7%)	2(11.8%)	1(8.3%)	4(28.6%)
End stage renal disease *n*(%)				0	0	1(6.7%)	0	0	1(7.1%)
Patients with no further flare *n*(%)				17(100%)	12(100%)	15(100%)	17(100%)	9(75%)	9(64.3%)
Patients with further renal flare *n*(%)				0	0	0	0	2(16.7%)	6(42.9%)
1 flare				0	0	0	0	2(16.7%)	5(35.7%)
2 flare				0	0	0	0	0	1(7.1%)
Patients with extrarenal flare *n*(%)				0	0	0	0	1(8.3%)	2(14.3%)

RTX rituximab, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil, *eGFR* estimated glomerular filtration rate, *dsDNA* double-stranded DNA, *CNS* central nervous system, *SLE* systemic lupus erythematosus, SLEDAI Systemic Lupus Erythematosus Disease Activity Index

Table 3 Requirement of prednisone according to study arms

	RTX (n = 17)	MMF (n = 12)	CYC (n = 15)	RTX vs MMF (p)	RTX vs CYC (p)
Prednisolone dose *(mg/kg/day), mean (SD)*					
Baseline[a]	1.3(0.7)	0.9(0.4)	0.9(0.8)	0.08	0.14
3 month	0.9(0.7)	1.4(0.5)	1.3(0.3)	0.04	0.04
12 month	0.6(0.5)	0.8(0.2)	1.1(0.5)	0.20	0.0008
24 month	0.5(0.3)	0.8(0.6)	1.2(0.7)	0.08	0.0007
36 month	0.3(0.2)	0.7(0.5)	0.9(0.5)[b]	0.005	0.0001
No. of patients off steroid, *n*(%)					
12 month	7(41%)	4(33%)	3(20%)	0.71	0.26
24 month	14(82%)	8(67%)	7(47%)	0.13	0.01
36 month	14(82%)	9(75%)	9(64%)[b]	0.29	0.08

RTX rituximab, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil

[a] Before starting induction therapy

[b] At 36 -months; *n* = 14 in CYC arm

Drug-related side effects

Adverse events were reported in five (29.4%) patients in the RTX group compared with seven (58.3%) in MMF and 15 (100%) in CYC groups (Table 4). No serious adverse events occurred after RTX or MMF therapy. Four patients from the RTX cohort had urticarial rashes soon after infusion, but no further complications developed. One patient developed varicella zoster infection, but that did not require hospitalization. Acute gastroenteritis was the most frequent adverse event among the MMF cohort, necessitating temporary dose reduction in three patients. Temporary stoppage of MMF was also required in two patients with deranged liver function tests. Although it is difficult to distinguish drug-related adverse effects from manifestations of SLE itself, there were 29 adverse events in the CYC cohort, two of which were serious requiring in-patient care. All serious adverse events with CYC were documented during the induction period.

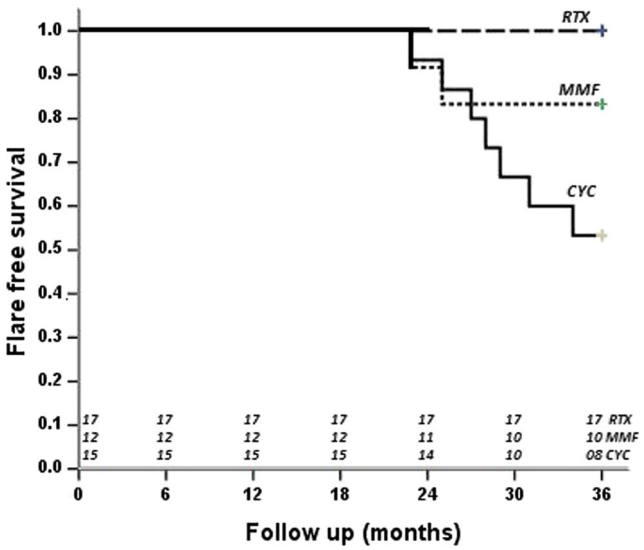

Fig 1 Flare-free survival of patients treated with rituximab (RTX) vs. cyclophosphamide (CYC) vs. mycophenolate mofetil (MMF) (Log-rank $p = 0.006$)

Discussion

Ultimate goals of treatment in SLE are long-term preservation of renal function, flare prevention, avoiding treatment-related harm, and improved quality of life and survival [2]. Treatment of pediatric LN is more challenging than in adults, and

Table 4 Adverse events during observation period

	RTX (n = 17)	MMF (n = 12)	CYC (n = 15)
Deaths	0	0	1
No. of patients with at least one adverse events	5	7	15
Total numbers of adverse events	8	10	29
Adverse events			
Infusion related urticarial rash	4	0	1
Flu syndrome	1	0	3
Acute gastroenteritis	2	5	0
Otitis media	0	0	2
Pneumonia	0	0	3(1SAE)
Abscess	1	0	3
Nasopharyngitis	0	1	1
Urinary tract infection	0	0	3
Varicella zoster	1	0	2
Meningitis	0	0	1(SAE)
Nausea/vomiting	0	0	7
Alopecia	0	0	3
Deranged liver function test	0	2	0

SAE serious adverse event, *RTX* rituximab, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil

therapeutic options are limited. Moreover, the accepted pediatric treatment regimens are mostly derived from those developed for adults. Although steroids and CYC still constitute the crux for effective induction therapy of pediatric LN, SLE patients always remain prone to further flares. Aside from this, treatment toxicity is a major cause of chronic morbidity and early mortality in pediatric SLE, and a focus of clinical research has been optimizing CYC dosing and evaluation of alternative immunosuppressives, both for remission induction and flare prevention.

In this retrospective cohort study of pediatric LN patients, we show improved flare-free patient and renal survival with RTX in comparison with MMF and CYC—drugs considered to be the standard of care for treating severe forms of this disease. Flare-free survival in this study was almost equal during the first 2 years in all treatment arms. New flares in the MMF and CYC arms during the second half of the study period were probably due to gradual weaning of immunosuppression produced by the induction regimens. In contrast, there were no further new flares in the RTX arm during the study period. The point of interest in this study is that the RTX group demonstrated better long-term treatment outcomes despite the presence of poorer baseline disease characteristics. RTX has been reported to be a promising treatment option in several case series and off-label studies in patients with SLE [18, 19, 24]. Prospective data from the French Registry also revealed better safety and clinical efficacy of RTX among patients with refractory SLE [19]. In addition, patients with LN in the European cohorts demonstrated complete response in 30% and partial response in 37% of patients at 12 months after RTX therapy [18]. Our study also revealed similar findings in line with these studies of RTX in SLE. Besides this, 82% of our patients required dialysis at presentation, which suggests a more severe spectrum of disease activity. However, in two recent randomized placebo-controlled trials with adult SLE patients, RTX failed to achieve the primary end points [15, 25]. The LUNAR trial also failed to demonstrate the efficacy of RTX as an add-on therapy to steroids and MMF in incident LN patients [15]. However, the LUNAR trial was not targeted at LN but SLE, and RTX was added to the treatment regimen of SLE patients who were heavily treated, so improvement of outcome may not have been shown for this reason. The varying efficacy of RTX across different studies is possibly due to the fact that RTX was administered in some studies as a last therapeutic option in patients who failed other steroid-sparing therapies.

Meta-analyses of smaller studies have suggested that more patients respond to MMF than to CYC [11–13]. The ALMS trial in adult LN showed comparable response rates between MMF and CYC [26]. However, due to the ease of administration and the more favorable toxicity profile of MMF, EULAR recommends it as the favored option to treat most cases of class III–IV LN [2]. EULAR also recommends low-dose CYC over high-dose CYC as initial treatment for class III–IV (±V) LN, especially in Caucasian adults, based on a better efficacy–toxicity ratio [2]. Some studies also demonstrate that the efficacy of CYC varies between racial and ethnic groups [27]. In our study also, we detected better efficacy of MMF in compared with CYC induction.

Although there have been increasing reports of the excellent immunosuppressive effect of RTX against childhood LN, most patients are likely to develop further flares following recovery of B cells [18, 19, 24]. To consolidate the response of induction therapy and prevent further new flares, we added MMF maintenance therapy in all three groups after 3 months. Although MMF, AZA, or calcineurin inhibitors all appeared to be equally effective in maintenance therapy, at least in adult European patients, we preferred MMF due to a better efficacy–toxicity ratio [28, 29]. Most of our older SLE patients were already on AZA before entering the study, and calcineurin inhibitors have various known drug-related toxicities. Besides this, a larger randomized clinical trial suggested a difference between the two drugs in favor of MMF after initial response to either MMF or CYC [30]. Notably, long-term follow-up data of the MAINTAIN nephritis trial do not indicate that MMF is superior to AZA as maintenance therapy in a Caucasian adult population with proliferative LN [31]. In our study, maintenance therapy with MMF after RTX induction in children with LN significantly improved patient outcome in maintaining remission and preventing further flares. We speculate that immune modulation by MMF has an additive impact in maintaining remission, even after B-cell recovery.

Treatment choices in this condition are not only driven by efficacy but also by drug tolerability and safety considerations. CYC is associated with significant gonadotoxicity and may increase long-term cancer risk [2, 6]. We detected various adverse events, including two serious, in children in the CYC cohort. In contrast, RTX and MMF were relatively safe, at least over the medium-term follow-up of this study. Moreover, the relatively stronger steroid-sparing effect of RTX decreased cumulative steroid load and associated steroid toxicities on a long-term basis for children in the RTX cohort. There were minor transfusion-related reactions in five (29%) patients and infectious complications among four (23%) patients with RTX. This is in line with a large study that reported transfusion reaction, albeit relatively infrequently severe, is the major adverse event with RTX, occurring in 17% of patients [32]. However, a Spanish study revealed infection as the major complication with RTX [33].

We recognize several limitations to our study. The patient number was small, and therefore statistical analysis may not be conclusive. Our findings were restricted to children who had eGFR <60 ml/min/1.73m^2. We did not directly compare the efficacy of RTX to that of MMF and CYC among children with active LN. There were possibilities of selection biases due to the nonrandomized selection of induction agents for

each patient following discussion of their efficacy and safety with parents.

Our three treatment cohorts are actually rituximab–MMF, MMF–MMF, and CYC–MMF groups, as they all received MMF treatment 3 months after respective induction therapy. As the rituximab–MMF cohort received more intensive treatment, we speculate that more intensive, early treatment may result in better outcome and fewer flares in these patients.

Although our study is retrospective, we conclude that RTX induction followed by MMF maintenance therapy may be an ideal and safe regimen to consider for inducing and maintaining remission among children with LN. Further randomized clinical trials are needed among such children to establish these findings. RITUXILUP, a clinical trial, is currently underway, which includes children using RTX as an induction agent [34].

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Compliance with ethical standards

Ethics The study was approved by the Institutional Review Board of our institute.

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