Anti CD20 (Rituximab) therapy in refractory pediatric rheumatic diseases

Reis J[1], Aguiar F[2], Brito I[3]

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ABSTRACT

Objectives: We aim to report the efficacy and safety of rituximab (RTX) in patients diagnosed with juvenile systemic lupus erythematosus (JSLE) or juvenile idiopathic arthritis (JIA) refractory to conventional treatment.

Methods: A retrospective review was made of all medical records of patients with JSLE or JIA treated with RTX between January 2009 and January 2015 in the Pediatric Rheumatology Unit of a central hospital.

Results: Five patients, 4 with JSLE and 1 with extended oligoarticular JIA, received 10 cycles of RTX (23 infusions). The scheme of RTX frequently used was 750 mg/m^2 two weeks apart. The median follow-up time after receiving the first cycle of RTX was 24 months (12 – 70). The four patients with JSLE were female (three caucasian and one black). The patient with JIA was a caucasian male. The median age at diagnosis was 10 years (16 months – 17years). The median evolution time until receiving RTX was 6 years (5 months – 15 years). Refractory class IV lupus nephritis was the most common indication for receiving RTX. Previous treatment to RTX included nonsteroidal anti-inflammatory drugs, disease-modifying anti-rheumatic drugs, immunosuppressive drugs and corticosteroids in all patients and anti-TNFα (etanercept) in the patient with JIA. It was possible to reduce the mean oral corticosteroid dose after RTX, ranging from 23 mg/day (20--25mg/day) before RTX to 11 mg/day (0–20 mg/day) at the last evaluation. Disease activity also improved when comparing the period before RTX with the last evaluation. The SLEDAI score, for JSLE, decreased from a median of 15, 5 (11–18) to 3 (0–6), and the JADAS-27 score, for JIA, also diminished from 40.4 to 3.5. Adverse events occurred in 2 patients, including delayed second dose after the diagnosis of cryptococcosis and respiratory tract infection with concomitant hypogammaglobulinemia needing of immunoglobulin replacement and antibiotic therapy.

Conclusions: Rituximab might have a role in the treatment of JSLE and JIA. However controlled studies and long term follow-up are needed to evaluate its safety and efficacy.

Keywords: JSLE; JIA; Rituximab, Pediatric Rheumatology

1- Faculdade de Medicina da Universidade do Porto
2. Reumatologia, Centro Hospitalar S. João
3. Unidade Reumatologia Pediátrica, Centro Hospitalar S. João

INTRODUCTION

Juvenile idiopathic arthritis (JIA) and juvenile systemic lupus erythematosus (JSLE) are probably the most clinical relevant chronic rheumatic disorders of pediatric age where autoimmunity and inflammation are crucial for the development of the disease[1, 2].

JIA represents a heterogeneous group of arthritis of unknown etiology presenting before 16 years and lasting at least 6 weeks and it is subdivided into 7 distinct subtypes[3]. There is evident heterogeneity among JIA subtypes with respect to clinical, demographic, genetic and pathophysiological features, translating into distinct responses to therapies currently available[4,5]. The discovery of new immunological markers is expected to improve diagnosis and treatment[6].

The incidence of JIA ranges between 1 per 100,000 in Japan to more than 20 per 100,000 children/year in northern Europe[1]. Prevalence rates varies between 10 and 150 per 100000 children[7, 8], but is probably underdiagnosed[9].

Unlike JIA, JSLE is substantially the same disease as in adults[10]. JSLE represents about 10% to 20% of the total cases of systemic lupus erythematosus (SLE)[2]. Most studies use either 16 years or 18 years as the upper cutoff age for JSLE[2]. The incidence is between 0,36 and

0,9 per 100000 children/year in North American and European studies[11] and the prevalence ranges from 3,3 to 8,8 per 100000 children[2].

Despite the similarities, JSLE patients have more active disease at presentation and over time[12]. JSLE carries a higher risk of developing lupus nephritis, malar rash, anti-DsDNA antibody positivity and hemolytic anemia[13]. Usually JSLE patients also receive more intensive drug therapy and accumulate more related damage[12].

Recent discoveries regarding the inflammation process and autoimmunity allowed the specific management of the aberrant responses present in both diseases. These discoveries resulted mainly in major improvements in short and medium-term outcomes and better control of acute-life threatening events[14].

These patients are usually treated with a combination of nonsteroidal anti-inflammatory drugs (NSAID), corticosteroids, disease-modifying anti-rheumatic drugs (DMARDs) such as methotrexate (MTX), sulfasalazine or leflunomide (LEF), and immunosuppressive agents such as azathioprine (AZT), cyclophosphamide (CYC) or mycophenolate mofetil (MMF).

However, not all patients respond to these treatments and they are sometimes associated with toxicities that limit long-term use or diminish compliance[15]. Such patients are therefore candidates for treatment with biologic agents.

In recent years, several non-controlled studies have demonstrated that rituximab (RTX) might be effective in JSLE and JIA[16-23] in accordance with the important role that B cells might have in autoimmune diseases through three different ways: producing antibodies, presenting antigens to T-cells and producing cytokines[24, 25]. However large clinical trials in adults evaluating RTX for renal[26] and non-renal lupus[27] did not prove its efficacy, contrary to rheumatoid arthritis[28], but the failure might be more related with the study design than with RTX itself[29].

RTX is a chimeric monoclonal antibody (mAb) initially approved for the treatment of B cell malignancies[30]. Currently it is also approved for rheumatoid arthritis, granulomatosis with polyangiitis and microscopic polyangiitis in adults[31], but its use in JIA and JSLE is still off-label. RTX binds with high affinity to cells that express CD20 antigen in their surface. This antigen is only present on B cells (malignant or not)[32]. CD20 antigen is not expressed in lymphoid progenitors in bone marrow allowing repopulation after stopping the treatment[24]. Plasma cells do not express CD20 antigen either and they are not directly depleted by RTX. Consequently not all antibodies decrease after treatment[24].

RTX causes peripheral B cell depletion by two different mechanisms: antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC)[33]. For ADCC occur it is necessary that mAb Fc interacts with Fc receptors for IgG (Fc Rs) on the surface of effector cells, localized in the reticuloendothelial system (RES), especially in the liver[34]. Fc RIIA and Fc RIIIA are two of the most important Fc R in human, and RTX s efficacy increases if mAb Fc affinity to Fc RIIIA is improved [24]. Circulating B cells are rapidly cleared at RES while B cells residing in lymphoid tissues are slowly eliminated by this mechanism as they need to have access to the circulation. The time needed to eliminate cells that does not circulate is even longer as they depend solely on the CDC[34].

Binding of RTX to podocytes membranes has also been reported, inducing remodeling of glomeruli that might explain the early decrease on proteinuria in lupus nephritis even before immune changes occur[35].

Therefore, we aim to report our single center experience with the use of RTX in patients diagnosed with JSLE and JIA resistant to other treatments in terms of safety and efficacy.

MATERIALS AND METHODS

We made a retrospective review of all medical records of patients with JSLE or JIA treated with RTX between January 2009 and January 2015 in the Pediatric Rheumatology Unit of a central hospital. This study was approved by the ethics committee along with all the treatments made.

The patients with JSLE included fulfilled the American College of Rheumatology criteria for the diagnosis of SLE[36]. The patients with JIA were classified according to the criteria of the International League of Associations for Rheumatology[3].

The medical records were reviewed for patients' demographic characteristics, age at diagnosis, baseline rheumatic disease, previous and current medical treatments. Indication for RTX, age at the time of the first treatment with RTX, protocol used and follow-up time were also collected.

The overall disease activity in JSLE and JIA was evaluated using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)[37] and the 27-joint Ju-

venile Arthritis Disease Activity Score (JADAS-27)[38], respectively. We also collected laboratory measures for JSLE (hemoglobin, platelets, erythrocyte sedimentation rate, complement C3 and C4, anti-double-stranded DNA antibodies, serum immunoglobulins, plasma creatinine, urine protein, and urinary sediment). Brain natriuretic peptide was also collected for one patient with JSLE. For the patient with JIA we collected the laboratory measures as in JSLE, but included the number of active and restrictive joints affected, and excluded the complement levels and auto-antibodies.

All serious adverse events, medically important infections and infusion reactions were also evaluated.

RESULTS

Five patients were included (Table I). Four had JSLE (all female, 3 caucasian and 1 black) and 1 had extended oligoarticular JIA (caucasian male). The median age at diagnosis was 10 years (16 months – 17years) and the median evolution time until receiving RTX was 6 years (5 months – 15 years). All of them have received previous treatments that included high-dose prednisolone (n=5), methylprednisolone pulses (n=5), cyclophosphamide pulses (n=3), methotrexate (n=2), hydroxychloroquine (n=3), mycophenolate mofetil (n=3), azathioprine (n=2), leflunomide (n=1) and etanercept (n=1).

RTX was indicated for refractory class IV lupus nephritis (in three patients), JSLE with refractory multisystem involvement (in one patient), and severe polyarthritis (in a patient with JIA who had anti-TNFα-induced lupus-like nephritis).

Ten cycles of RTX were performed, in a total of 23 infusions (Table II). The most common dosage used was 750 mg/m^2 (maximum of 1 g) administered 2 weeks apart. One patient had also administration of CYC during the first RTX cycle. Three patients needed more than one cycle of RTX. The median time for a new cycle of RTX was 8 months (6–10 months).

The median follow-up time after receiving the first cycle of RTX was 24 months (12–70 months).

It was possible to reduce the dose of oral corticosteroids (CS) in all patients, including discontinuation of oral CS in the patient with JIA. The mean daily oral CS dose before RTX was 23 mg/day (20-25mg/day). At the last evaluation it was 11 mg/day (0–20 mg/day). During and/or after RTX patients with JSLE continued therapy with MMF.

The scores of disease activity also improved. The SLEDAI scores decreased from a median of 15, 5 (11-18) before RTX, to 3 (0 – 6) at the last evaluation. The JADAS-27 score also diminished from 40.4 before RTX to 3.5 at the last evaluation.

It was recorded one case of respiratory tract infection 3 months after RTX with concomitant hypogammaglobulinemia (IgG of 423 mg/dL) and one case of in-

TABLE I. DEMOGRAPHIC, THERAPEUTIC AND CLINICAL CHARACTERISTICS OF PATIENTS

Patients	1	2	3	4	5
Gender	F	F	F	F	M
Race	Caucasian	Caucasian	Caucasian	Black	Caucasian
Disease	JSLE	JSLE	JSLE	JSLE	Extended oJIA
Age at diagnosis	10y	10y	10y	17y	1y
Disease duration before RTX	15y	6y	7y	5m	5y
Age at first administration of RTX	25y	16y	17y	17y	6y
Medication before RTX	MP, CYC, PDN, AZA, MMF, HCQ	MP, CYC, PDN, MMF	MP, CYC, PDN, MMF, AZA, HCQ	MP, PDN, MTX, HCQ	MP, PDN, MTX, LEF, Etanercept
RTX indication	Anemia of CD+ refractory class IV lupus nephritis	Refractory class IV lupus nephritis	Refractory class IV lupus nephritis	Refractory multisystem involvement	Severe polyarthritis (previous anti-TNFα induced lupus-like nephritis)

AZA=azathioprine; CYC = cyclophosphamide; F= Female; M= Male; y= years; m = months; oJIA = oligoarticular Juvenile idiopathic arthritis; JSLE = juvenile systemic lupus erythematosus; HCQ = hydroxychloroquine; LEF = leflunomide; MMF = mycophenolate mofetil; MP= methylprednisolone pulses; MTX = methotrexate; PDN = prednisolone ; RTX = Rituximab; CD = Chronic disease

TABLE II. TREATMENT CHARACTERISTICS AND OUTCOME OF THE PATIENTS

Patients	1	2	3	4	5
Follow-up time after first RTX treatment	12 months	19 months	70 months	24 months	28 months
No. of doses	4	4	3	2	8
Regimen of RTX used* (days of administration)	1st 750 (0, 15 d) 2nd750 (0, 15 d)	1st 750 (0, 15 d) + CYC 375 (1d) 2nd 750 (0,15 d)	750 (0, 15, 30d)	750 (0, 50 d)	1st375 (0, 15d) 2nd750 (0, 15 d) 3rd750 (0, 15 d) 4th750 (0, 15 d)
Time to retreatment	10 months	8 months	None	None	7 months, 9 months and 8 months
Adverse events	None	None	Respiratory tract infection and transient hypogammaglobulinemia	Cryptococcosis	None
Outcome	Hematologic response. Renal improvement, but persistent proteinuria	Initial response. Retreated later with renal improvement	Global improvement, but persistent proteinuria	Improvement. Deterioration after stopping MMF In another hospital	Initial complete response with multiple flare. Complete remission nowadays
Drug dosage previous to RTX	MMF: 2,5 g/d PDN: 25 mg/d	MMF: 2g/d PDN: 20 mg/d	MMF: 2g/d PDN:25 mg/d	MMF: 2g/d PDN:20mg/d	PDN: 20 mg/d
Drug Dosage at last evaluation	MMF: = PDN:↓5mg/d	MMF: = PDN:↓10mg/d	MMF:= PDN:↓15mg/d	MMF:↓0,5g/d PDN:↓5mg/d	PDN: ↓20 mg/d (stopped)

*The doses are in mg/m². CYC = Cyclophosphamide; MMF = mycophenolate mofetil; PDN = prednisolone; RTX = rituximab; "↓" = decreased;"= " = maintained; "d" = day.

fection by Cryptococcus diagnosed a month after the first RTX infusion.

Next we present the clinical particularities for each patient (Table III):

Patient 1: Caucasian female diagnosed with SLE at 10 years old (1999) initially with mucocutaneous, articular, hematologic involvement and positivity to antinuclear antibodies (ANAs) and anti-DsDNA antibodies. At 14 years old (2003) developed nephrotic proteinuria and cylindruria with complement consumption. Kidney biopsy revealed class IV lupus nephritis. She was treated with methylprednisolone pulses (MP) and CYC and later with AZT and prednisolone (PDN). Due to leucopenia AZT was switched to MMF. She kept stable with PDN and MMF until March 2013. She developed asthenia, peripheral edema, increasing blood pressure, anemia of chronic disease, complement consumption, and worsening of renal function. MMF was increased to 2,5 g/day, but maintained disease activity during the next months. The kidney biopsy showed class IV lupus nephritis with great activity and mild chronicity. There was no significant response to the treatment with MP pulses. For these reason she received the first RTX cycle. There was hematological response and renal improvement but maintained persistent proteinuria. The dose of MMF and PDN was reduced. Six months later she developed again edema and nephrotic proteinuria with complement consumption and active sediment. MMF was increased to 2,5 g/day, along with losartan and furosemide. The sediment became inactive and proteinuria turned subnephrotic. Another cycle of RTX was made and at the last evaluation the patient presented with no edema, inactive sediment, normal blood pressure, but maintaining proteinuria.

Patient 2: Caucasian female diagnosed with SLE at 10 years old (2006) presenting with renal involvement. The biopsy showed class IV lupus nephritis. Clinically stable after induction treatment with CYC and maintenance treatment with MMF. She stopped hydroxychloroquine due to dermatologic toxicity. In 2012 developed progressive proteinuria with active sediment that did not respond to treatment optimization. The kidney biopsy revealed class IV lupus nephritis. She was treated with RTX plus CYC. Five months later the proteinuria had significantly decreased together with inactive sediment. The platelet values and hemoglobin values also improved. Because proteinuria worsened again it was decided to do another cycle of RTX, eight months after the first one. At the last evaluation the laboratory results showed improvement in all parameters evaluated.

Patient 3: Caucasian female diagnosed with SLE at 10 years old (2002) with hemolytic anemia and severe thrombocytopenia together with lupus nephritis class IV. Patient achieved hematologic response but kept lupus nephritis refractory to induction treatment with CYC and maintenance with PDN and MMF. Since 2009 she developed severe nephrotic proteinuria and hypertension without complement consumption. A new biopsy confirmed the previous result. She made pulses of MP, followed by PDN with unsatisfactory response and was decided to start RTX. There was a significant decrease in proteinuria and normalization of anti-DsDNA antibodies. Three months later she was hospitalized due to lower respiratory tract infection that was treated successfully with antibiotic therapy. The laboratory results showed hypogammaglobulinemia [IgG 423 mg/dL (N 650 – 1500]) and a B cell count of 1,46 cel/mm^3. Intravenous immunoglobulin (IvIg) was administered. Since then she is clinically stable without hypertension and reduced proteinuria.

Patient 4: Black female hospitalized at another hospital in 2012 with 17 years old due to prolonged febrile syndrome, lymphadenopathies, fatigue, and weight loss. She was then transferred to our hospital already with oral ulcers, arthritis and malar rash. Laboratory exams showed positive ANAs, anti-DsDNA, anti-ribonucleoproteins antibodies and normochromic, normocytic anemia with positive Coombs. She was diagnosed with JSLE and was treated with PDN and hydroxychloroquine (HCQ). After discharge she started MTX and reduced PDN (20 mg/day).

She was hospitalized again 3 months later with spiking fever (40º C), painful hepatomegaly with increased liver enzymes, increased PCR, anemia and thrombocytopenia, together with leukopenia that appeared later. Macrophage syndrome was excluded after bone marrow biopsy. MTX and HCQ were suspended for suspected toxic hepatitis. The suspected lupus flare was unresponsive to the pulses of MP. She maintained elevated hepatic enzymes and pancytopenia. She was treated with one pulse of RTX and initiated MMF (2 g/day). After a week there was an improvement of pancytopenia and hepatic enzymes. Due to maintained tachycardia, she was evaluated by cardiology that diagnosed a heart failure with severe systolic dysfunction (ejection fraction 24%) with magnetic resonance images compatible with autoimmune myocarditis. She was treated with IvIg, PDN (50 mg/day) and increased MMF's dosage. A month after first RTX

TABLE III. EVOLUTION AFTER RTX TREATMENT

1	1st cycle RTX 750 mg/m² (0 d; 15 d)	4 months	6 months	8 months-2nd cycle RTX 750 mg/m² (0d ;15 d)	12 months (last evaluation)
	Hb:8,9	Hb: 11,3	Hb:12,4	Hb: 12,1	Hb:13,7
	Plat: 297	Plat: 311	Plat:236	Plat: 284	Plat.: 318
	ESR: 93	ESR:45	ESR: 29	ESR: 24	ESR: 46
	Creat: 1.13	Creat: 0.86	Creat 0.83	Creat : 0,93	Creat: 0,91
	P:cr = 0.88	24 h P: 2,44	24h P: 3.36	24h P: 1,44	24h P: 2,5
	Linf CD19+ : 118	Linf CD19+: NA	Linf CD19+: 1,27	Linf CD19+: 2,8	Linf CD19+: NA
	IgG: 842; IgM: 48	IgG: 752; IgM: 32	IgG 641; IgM: 37	IgG 662; IgM: 56	IgG:814; IgM 41
	C3: 74; C4: 17	C3: 80; C4: 17	C3: 67; C4: 13;	C3: 66; C4: 14	C3:109; C4: 27
	Anti-DsDNA: 237	Anti-DsDNA: 194.4	Anti-DsDNA: 239	Anti-DsDNA: 196.8	Anti-DsDNA:178,4
	Active sediment	Inactive sediment	Active sediment	Inactive sediment	Inactive sediment
	SLEDAI: 18	SLEDAI:8	SLEDAI:16	SLEDAI:8	SLEDAI:62
2	1st cycle RTX 750 mg/m² (0d;15 d) + CYC 375 mg/m² (1d)	5 months	8 months-2nd cycle RTX 750 mg/m² (0d ;15 d)	15 months	19 months (last evaluation)
	Hb: 10,5	Hb: 11,8	Hb: 11,3	Hb: 11,4	Hb: 12,8
	Plat: 71	Plat: 499	Plat: 341	Plat: 296	Plat: 254
	ESR: 71	ESR: 69	ESR: 43	ESR: 29	ESR: 44
	Creat: 0,58	Creat: 0.83	Creat: 1,6	Creat: 0.56	Creat: 0.75
	24h P: 3.31	24h P: 0,36	24h P: 1,55	24 h P :0.62	24h P: 0.24
	IgG: 742; IgM: 108	IgG:385	IgG: 504; IgM: 95	Inactive sediment	IgG: 550
	C3: 100; C4: 18	C3:103; C4: 50	C3: 137; C4: 31	SLEDAI:0	C3:109; C4: 28
	Anti-DsDNA:190,4	Anti-DsDNA: NA	Anti-DsDNA: 84,2		Anti-DsDNA: <10
	Active sediment	Inactive sediment	Inactive sediment		Inactive sediment
	SLEDAI:15	SLEDAI:0	SLEDAI:6		SLEDAI:0

3	1st cycle RTX 750 mg/m² (0d ;15d;30 d)	3 months	12 months	20 months	48 months	58 months	70 months (last evaluation)
	Hb: 12,3	Hb: 13,8	Hb: 11,2;	Hb: 11.1;	Hb: 12,3;	Hb: 12,8	Hb:12.5
	Plat: 313	Plat: 290	Plat: 348	Plat: 265	Plat: 280	Plat: 311	Plat: 283
	ESR: 46	ESR: 19	ESR: 24	ESR:10	ESR: 19	ESR: 20	ESR:19
	Creat: 0,72	Creat: 0,83	Creat: 0,77	Creat: 0.70	Creat: 0.59	Creat: 0,69	Creat: 0,65
	P:Cr: 5,15	IgG :423; IgM: 61	P:Cr: 0.43	24h :0.32	24h P: 1,43	24h P: 0.4	24h P: 1.42
	Linf CD19+: NA	C3:106; C4:29;	Linf CD19+: 1,5	C3: 101; C4:21	C3: 111; C4: 24	IgG: 921; IgM: 66	IgG: 723; IgM: 47
	IgG: 407; IgM: 78	Anti-DsDNA: 19,5	IgG 881; IgM 61	Anti-DsDNA: 9.0	Anti-DsDNA: 39,7	C3: 105; C4: 27	C3:106; C4: 29
	C3: 91; C4: 15;	Inactive sediment	C3: 117;C4: 19	Active sediment	Inactive sediment	Anti-DsDNA: 20,7	Anti-DsDNA: <10

(continues on the next page)

TABLE III. CONTINUATION

	1st cycle RTX 750 mg/m² (0d ;15d;30 d)	3 months	12 months	20 months	48 months	58 months	70 months (last evaluation)
	Anti-DsDNA: 25.8	SLEDAI:0	Anti-DsDNA: 18,9	SLEDAI:8	SLEDAI:4	Inactive sediment	Inactive sediment
	Active sediment		Inactive sediment			SLEDAI:0	SLEDAI:4
	SLEDAI:16		SLEDAI:0				

4	1st cycle RTX 750 mg/m² (0d;50 d)	7 months	Emigrated to France Follow-up on local hospital	12 months	24 months (last evaluation)
	Constitutional symptoms	No constitutional symptoms.	Suspended mycophenolate	Constitutional symptoms	No constitutional symptoms
	Polyarthritis	Oligoarthritis	mofetil and augmented	Polyarthritis	No polyarthritis
	Hb: 6.1 ; WBC: 3.20 ; Plat: 64	Hb: 13.4; WBC: 3.0 ;Plat: 308	prednisolone	Hb: 12.4; WBC 3.55 ; Plat:305	Hb 11,2; WBC: 3,45 ; Plat 352
	ESR: 103	ESR: 31		ESR: 36	ESR: 49
	Creat:0,42	Creat:0.56		Creat: 0.54	Creat: 0,52
	24h P: 0.4	C3: 132; C4: 44		C3: 132 ; C4: 44	C3:102; C4: 24
	C3: 98; C4: 19	BNP: 57.4		BNP: 70.5	BNP: 59.1
	BNP: 2100	Anti-DsDNA: >800		Anti-DsDNA: >800	Anti-DsDNA: >800
	Anti-DsDNA: >800	No proteinuria and inactive		No proteinuria and inactive	No proteinuria and inactive
	Inactive sediment	sediment		sediment	sediment
	Heart failure with myocarditis	NYHA I		SLEDAI:6	SLEDAI:2
	Systolic dysfunction of left ventricle (EF 24%)	SLEDAI:2			
	SLEDAI:11				

5	1st cycle RTX 375 mg/m² (0d;15 d)	7 months-2nd cycle RTX 750 mg/m² (0d ;15 d)	12 months	16 months-3rd cycle RTX 750 mg/m² (0d;15 d)	22 months	24 months-4th cycle RTX 750mg/m2 (0d ;15)	28 months (last evaluation)
	A. joints: 12	A. joints: 4	A. joints: 0	A. joints: 11	A. joints: 1	Worsening of polyarthritis	A. joints:2
	R. joints: 12	R. joints: 3	R. joints: 2	R. joints: 12	R. joints: 3	Hb:12.2	R. joints: 0
	Hb: 12,0	Hb:11.8	Hb: 13.0	Hb:12.2	Hb: 13.4	Plat: 445	Hb:13.1
	Plat: 558	Plat: 484	Plat: 447	Plat: 482	Plat: 519	ESR: 24	Plat: 322
	ESR: 24	ESR: 13	ESR: 2	ESR: 47	ESR: 7	Creat: 0.29	ESR: 8
	Creat: 0,30	Creat: 0.29	Creat: 0.34	Creat: 0.29	Creat:0.30	P:Cr:NA	Creat: 0.28
	P:Cr: 0,087	P:Cr: 0.098	P:Cr: 0,094	P:Cr:NA	P:Cr:NA	Linf CD19+: 358	P:Cr: 0,095
	IgG:1220; IgM: 106	Linf CD19+: 298	Linf CD19+: 1.0	Linf CD19+: 260	Linf CD19+:4.7	Inactive sediment	Linf CD19+: 0,0
	Inactive sediment	IgG: 1030;IgM: 32	IgG: 879; IgM: 19	IgG: 914;IgM: 30	IgG: 659; IgM:7	JADAS 27: 39.4	since last RTX cycle
	JADAS 27: 40.4	Inactive sediment	Inactive sediment	Inactive sediment	Inactive sediment		Inactive sediment
		JADAS 27: 14	JADAS 27: 3	JADAS 27: 39.4	JADAS 27:8		JADAS 27: 5

Hb = haemoglobin concentration (g/dL); **Plat** = platelet count (x 10^9/L) ; **ESR** = Erythrocyte sedimentation rate (mm/h) ; **Creat** = Creatinine serum concentration (mg/dL) ; **P:cr** = protein –creatinine ratio on urine sample; **24P** = 24-hour urine protein (grams) ; **Ig** = Immunoglobulin (mg/dL); **Anti-DsDNA** = Anti-double-stranded DNA antibody (UI/ml); **WBC**=White blood cell count (x 10^9/L); **Linf CD19+** = lymphocytes CD19+ (per mm^3); **NYHA** = New York Heart association functional classification of heart failure; **C3** and **C4** = complement (mg/dL); **EF** = systolic ejection fraction; **RCP** = reactive C-protein (mg/mL); **BNP** = B-type natriuretic peptide (ng/mL); **A.joints** = active joints; **R.joints** = restrictive joints; **RTX** = Rituximab; **CYC** = Cyclophosphamide; **NA** = Not available; **SLEDAI** = Systemic Lupus Erythematosus Disease Activity Index; JADAS-27: 27-joint Juvenile Arthritis Disease Activity Score. Abnormal values or important information are in **Bold**

pulse, Cryptococcus was detected in blood cultures. She was treated with amphotericin B and fluconazole. Fifty days later, after an improvement of systolic function and laboratory analyses she made a second pulse of RTX and reduced PDN to 30 mg/day. After discharge she initiated again HCQ (400 mg/day) and reduced PDN to 15 mg/day, while maintaining 1,5g/day of MMF. She maintained clinically stable with no signs of heart failure. She immigrated to France. When she returned MMF had been suspended, the PDN augmented to 20 mg/day and she referred febrile spikes and polyarthritis. She resumed therapy with MMF (1,5 g/day) and PDN (15 mg/day) and at the last evaluation she was asymptomatic but slightly anemic.

Patient 5: Caucasian male, 8 years old, diagnosed with extended oligoarticular JIA since 16 months of age, with uveitis and clinical evolution dependent of CS and refractory to treatment with MTX and LEF. He started etanercept (0,4 mg/Kg/dose) in September of 2010 resulting in complete clinical response and suspension of CS. In December of 2011 he was hospitalized due to a nephrotic syndrome together with acute kidney lesion, hypertension with complement consumption and positive ANAs. The biopsy revealed membranoproliferative glomerulonephritis, interpreted as possible glomerulonephritis lupus–like induced by anti-TNFα. It was decided to stop etanercept and administrate PDN and MTX, with clinical and renal improvement. However arthritis recurred even with optimized dosage of CS and MTX. Because Abatacept was not available, he made one cycle of RTX, and a second cycle eight months later, with clinical and analytical improvement. PDN was reduced to 2,5 mg/day. In January 2014 he presented again with polyarthritis. PDN was augmented to 5 mg/day and made one more cycle of RTX obtaining clinical remission again. In October of 2014 the polyarthritis worsened again and another cycle was administrated. Since that time B cells have been completely depleted and achieved clinical remission. No signs of renal lesion were observed after stopping etanercept.

DISCUSSION

The American College of Rheumatology published recently recommendations for the Treatment of JIA[39] with an update on systemic JIA released in 2013[40]. There are no approved drugs for treatment of juvenile lupus nephritis and the treatment relies on off-label use of medications approved for any other reason, as is the case of RTX[41]. Frequently the therapeutic strategies used are similar to what it is done in adult age. However, various adverse events limit their use compared to adults[10,12,42] and the optimal dosage and safety is not determined[41].

Besides the side effects, the conventional therapies also have limitations in their efficacy. The induction treatment of proliferative lupus nephritis with conventional first-line treatments as CYC or MMF has reported failure rates ranging from 10% to 43%[43]. In JIA rates of non-response to therapy with NSAIDs, CS or DMARDs vary from 5% to 30 %[44].

As mentioned before, an increasing number of open-label, retrospective studies and case reports have demonstrated that RTX might be effective in JSLE and JIA[16-23]. However assessing the efficacy after RTX is complicated by the fact that it takes a variable amount of time to respond and in the meanwhile patients might need adjustments in conventional therapy to control the clinical situation before obtain a response[45], as in our case.

Three of the patients received RTX due to refractory lupus nephritis. All of them had lupus nephritis classified as class IV according to World Health Organization. Class IV represents 40 to 60% of all cases as described in literature and has the worst prognosis [10]. Kidney involvement has a great impact in morbidity and mortality, and it has a major impact in the choice of immunosuppressive therapy[14], being usually the main indication for RTX treatment[20], as in our case.

Generally, for all the patients with JSLE, there was an improvement in disease activity scores, clinical and laboratory parameters after each RTX pulse. The median SLEDAI score diminished from 15,5 (11 – 18) in the beginning, to 3 (0 – 6) at last evaluation, representing an important decrease in disease activity, especially because values over 5 are associated with initiating or changing therapy in more than 50%[46]. Two of them obtained values below five, suggesting a mild activity disease, and one achieved 0 points, suggesting no active disease[46]. A significant improvement in SLEDAI score was also seen in all the first evaluations after RTX. There was also an improvement in the values of erythrocyte sedimentation rate, serum creatinine, complement, anti-DsDNA antibodies and improvement in hematuria, casts and leucocytes in urinalysis. There was also a reduction in proteinuria. A decrease in anti-Ds -DNA antibodies and an increase in C3 have been associated with a good response to RTX[29]. This is clearly

seen in patient 3 that maintained stable remission during 70 months. However, in patient 1 and 2, the response was not permanent as shown by the increase of SLEDAI scores by more than 3 points, suggesting a moderate flare[46], and the increase of proteinuria and serum creatinine, requiring an adjustment of the angiotensin-converting-enzyme inhibitor and conventional therapy followed by another cycle of RTX. This is in accordance with literature where is reported that 30% needed more than one cycle and account for 58% of the cycles[20]. Although the degree of B cell depletion usually correlates with better responses[29], relapses may occur even with a low B cell level as observed in patient 1, probably reflecting RTX's mechanism of action.

In the case of patient 4, after RTX treatment used to control refractory multisystem involvement, SLEDAI score fell significantly; there was an improvement in hemoglobin concentration, platelets and leukocytes, along with decrease in liver enzymes. With respect to the heart failure, the findings from magnetic resonance were indicative of myocarditis, which was treated as possible autoimmune manifestation. Clinically significant myocarditis in JSLE is quite uncommon, however it might be the initial manifestation[14]. The improvement might also be attributed to the previous use of RTX. In the only case report founded, RTX was used with success to treat myocarditis in childhood lupus with improvement after a week[47]. However, it was not possible to rule out cryptococcosis as a cause.

Overall, patients showed a good hematological response, even if that was not the reason to administrate RTX. There was an improvement in hemoglobin concentration and platelet's count, as seen in other larger cohorts[20]. Evidence also suggests that depletion of B cell can be efficacious and safe for treatment of autoimmune thrombocytopenia and/or autoimmune hemolytic anemia in JSLE refractory to first line treatment[47].

Patient 5, diagnosed with AIJ, required a total of four cycles. The worsening of polyarthritis, with concomitant increase of JADAS-27 score, was the main reason for repeating RTX [18] and was always preceded by an increase of $CD19^+$ B cell count[48]. Nevertheless, some studies find no correlation between B cell count and symptoms in JIA[18]. At least for JIA the evidence suggests that 75% of patients required repeat cycles of RTX to suppress disease activity, with remission rates improving over time, as shown by improvement in JADAS-27 score. This suggests that patients with highly refractory JIA can achieve high levels of improvement by continued treatment with RTX[18]. Patient 5 also has a past history of "lupus-like" nephritis after using etanercept. A growing body of evidence suggests that TNFα antagonists can induce lupus like nephritis especially with etanercept[49,50].

The dosage of RTX typically used in our study was 750 mg/m^2, with exception of the first cycle in patient 5 where we used 375 mg/m^2, taking into account his age. Whenever possible the second dose was administered 14 days apart. We only included CYC (375mg/m^2) in patient 2 because the other patients have reached the maximum cumulative dose. The dosage of RTX we used was in accordance with the published literature[16,19,20,22], although there are reports of lower doses (375 mg/m^2) used weekly during 4 weeks[18,21,23] or with two weeks apart[17]. These schemes usually combine RTX with CYC in the treatment of SLE in the majority of the patients, but not in the case of JIA[18].

In our opinion it was important to spare the use of CYC in JSLE patients since it is known for its capacity to induce irreversible infertility and increase the risk of malignancy[42].

Furthermore, we managed to decrease the mean dose of CS between the evaluation previously to RTX and the last evaluation, consistent with the published literature[16-23]. All the patients with JSLE maintained the therapy with MMF. Data from other larger cohorts indicate that in the majority of the patients at least another immunosuppressive drug is maintained along with PDN[21,22]. This reduction in CS is especially important since children have more damages related to their use, including cataracts and avascular necrosis. 50.9% of the damage observed in JSLE appears to be related to the use of CS compared to 29.3% in adults[12]. The abnormal growth, delayed puberty and alterations in body image can have a major impact in adolescents' identity and relationships contributing to a worst compliance with treatments[10].

In the case of lupus nephritis it was demonstrated to be possible to induce remission with rituximab while keeping maintenance therapy with MMF, allowing reduction or total withdraw of corticosteroids[51,52].

However several studies showed that JSLE is more severe, frequently needing higher doses of oral CS and immunosuppressive therapies and having more admissions in intensive care units compared to adults[53-55]. So the complete withdrawal of CS might not be possible.

In our cohort RTX was usually safe. In a total of 23 infusions, there are no reports of adverse events rela-

ted to the infusion. As mentioned before, we report one respiratory tract infection with concomitant hypogammaglobulinemia and one Cryptococcus' infection. In literature concerning pediatric age, the most common adverse events reported are mild infusion reactions and infections [16-23],which is similar to what is reported in adults[26,27]. The reported incidence of infections requiring hospital admission is of 86,9/1000 person-year[22],and alarge cohort showed that only 2% of JSLE treated with RTX needed IvIg replacement[20]. Although in adults IgG levels are usually unchanged after RTX, because CD20 antigen is not present in plasma cells[45], prolonged deficiency of immunoglobulin or $CD20^+$ B cell was documented in some pediatric cases even after 12 months[21]. Although very rare the European Medicines Agency recently actualized RTX product information to include the occurrence of progressive multifocal leukoencephalopathy and reactivation of hepatitis B virus[31].

CONCLUSION

The overall response to RTX was favorable in our case series, since it was possible to reduce the use of corticosteroids without compromising disease control. It was also possible to induce remission of lupus nephritis with RTX without CYC while maintaining therapy with MMF and PDN. Some patients needed more than one cycle with a mean time of 8.4 months. This is probably related to the RTX's mechanism of action and with the degree of depletion of B cells, especially in the youngest patients. The drug was globally safe. Limitations to this study include its retrospective nature and the small sample size.

Controlled studies are needed to demonstrate the efficacy and the safety at long term. Rituximab might be able to spare children from deleterious effects of corticosteroids and other immunosuppressive drugs. However, we cannot rule out RTX interference with the immune system and future consequences upon chronic usage.

CORRESPONDENCE TO
Brito I
Al. Prof. Hernâni Monteiro,
4200-319 Porto, PORTUGAL
E-mail: mimed09070@med.up.pt

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