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The effectiveness and safety of rituximab in juvenile idiopathic arthritis: Hints from the ITHACA monocentric registry

Maria Rosa Pellico [a,b,c,†], Marco Pandolfi [a,b,c,†], Andrea Amati [a,b,c], Claudia Iannone [a,b,c], Sabino Germinario [a,b,c], Stefania Costi [b], Francesco Baldo [b], Maurizio Gattinara [b], Elisabetta Miserocchi [d,e], Maria Gerosa [a,c], Achille Marino [b], Roberto Caporali [a,b,c], Cecilia Beatrice Chighizola [a,b,*]

[a] Department of Clinical Sciences and Community Health, Dipartimento di Eccellenza 2023-2027, University of Milan, Milan, Italy
[b] Unit of Pediatric Clinical Rheumatology, ASST G. Pini – CTO, Milan, Italy
[c] Unit of Clinical Rheumatology, ASST Gaetano Pini – CTO, Milan, Italy
[d] Department of Ophtalmology, IRCCS San Raffaele Scientific Institute, Milan, Italy
[e] Faculty of Medicine, Vita-Salute San Raffaele University, Milan, Italy

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ABSTRACT

B cells contribute to the pathogenesis of juvenile idiopathic arthritis (JIA), suggesting a therapeutic potential for B cell depleting agent rituximab (RTX).

This retrospective study describes the effectiveness and safety of RTX in a monocentric cohort of JIA patients. Disease activity was assessed using DAS28-CRP at baseline and at each RTX infusion.

Thirty-seven JIA patients (56.8 % with polyarticular JIA) received RTX between 2008 and 2023, at a median age of 23.5 years. Most patients had a refractory disease: 45.9 % of the cohort received >2 prior biologics. The median exposure time to RTX was 2.5 years, with a median number of 5 cycles per patient and a median follow-up from first infusion of 7.44 years. At 6 months, 73 % of patients responded to RTX, and 48.6 % achieved remission. At 12 months, the trend in reducing DAS28-CRP levels persisted. ACPA positivity improved remission rates although not significantly; in most cases, uveitis did not respond to RTX. Six patients (16.2 %) discontinued RTX thanks to a prolonged remission, none requiring further biologics at a follow-up of 1.4 years. Both hypogammaglobulinemia and clinically relevant infections occurred in 27 % of the cohort. Receiving >4 RTX cycles predicted the development of hypogammaglobulinemia and/or infections (sensitivity 71.9 %, specificity 60.0 %).

Although the optimal patient selection strategy remains unclear, RTX might be regarded as an effective treatment for refractory cases, particularly in oligo/polyarticular JIA, with a manageable safety profile when exposure is limited to 4 cycles.

Introduction

Rituximab (RTX), a chimeric monoclonal antibody targeting human CD20 on B cells, has demonstrated significant efficacy and safety in the treatment of several immune-mediated disorders thanks to its depleting effects on pathogenic B lymphocytes [1,2]. Juvenile idiopathic arthritis (JIA), the most common rheumatologic condition with pediatric onset, is classically regarded as a T cell driven condition, but several lines of evidence support a contribution of B cells to disease pathogenesis, in particular in oligoarticular (oligoJIA) and polyarticular JIA (polyJIA). Firstly, JIA patients present abnormalities in subpopulations of circulating B cells, mainly in terms of decreased regulatory B cells [3]. B cells from peripheral blood and synovial fluid of JIA patients display atypical B cell regulation, altered maturation and increased activation [3]. B cell activation is supported by the increased levels of B cell activating factor (BAFF), A proliferation-inducing ligand (APRIL), interleukin (IL) −6 and 21 registered in the peripheral blood and synovial fluid from JIA patients [3]. Consistently, patients with early-onset disease have been

* Corresponding author: Unit of Pediatric Clinical Rheumatology, ASST G. Pini – CTO, Department of Clinical Sciences and Community Health, University of Milan, Piazza Cardinal Ferrari 1, 20122 Milan, Italy.
E-mail address: cecilia.chighizola@unimi.it (C.B. Chighizola).
† These two authors contributed equally

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shown to display an expression profile in peripheral blood mononuclear cells enriched in genes related to B lymphocytes but not cells of the myeloid lineage [4]. In addition, circulating autoantibodies suggest a breach in self-tolerance leading to the proliferation of autoreactive B cells: anti-nuclear antibodies (ANA) are the most prevalent in JIA, with approximately half of patients across different disease categories being ANA positive. Even though its contribution to JIA etiopathogenesis remains unclear, ANA positivity was shown to be related to the development of lymphoid structures in synovial tissue and to the rate of infiltrating plasma cells [5]. Rheumatoid factor (RF) and antibodies against citrullinated peptides (ACPA) are detected in a minority of JIA subjects, mainly in the polyarticular subset, with an overall prevalence around 5 % [7]. RF and ACPA have been identified as poor prognostic factors, both conveying a higher risk of aggressive disease course with bone erosions [6]. RF and ACPA exert a deleterious role in JIA joints by precipitating as immune complexes, activating complement cascade and macrophages via Fcγ receptor thus inducing the release of proinflammatory cytokines [8,9]. In JIA B cells and plasma cells infiltrate the synovial tissues, where they worsen the articular proinflammatory milieu by synthetizing key pathogenic mediators as IL-6 and tumour necrosis factor (TNF) α [3]. In addition, in situ B cells drive the activation and polarization of T cells via the upregulation of costimulatory molecules. Consistently, a higher rate of CD20+ *B* cells in the synovial tissue has been related to a more severe disease course, regardless of the subtype of JIA and previous treatment exposure [10]. The relevance of B cells in JIA pathogenesis suggests that B cell depletion might be a promising treatment strategy, in particular in case of seropositive JIA or a severe and recalcitrant disease. Given the paucity of available data, the aim of the present study consists in retrospectively exploring the effectiveness and safety of RTX in a monocentric cohort of JIA patients.

Patients and methods

Patients regularly attending the outpatient transition clinic in the rheumatology department of ASST G. Pini – CTO in Milan, Italy were consecutively included in this retrospective cohort study in case of: i) JIA diagnosis formulated according to ILAR criteria [11] and ii) at least one cycle of treatment with RTX. Patients were further classified in disease categories according to ILAR criteria: oligoJIA, polyJIA, and enthesitis-related arthritis (ERA) [11]. RTX treatment consisted of two intravenous infusions of 1 gr separated by a 2-week interval, preceded by premedication with methylprednisolone 100 mg to prevent infusion reactions. Subsequent courses of RTX could be repeated every six months or with an "on demand" strategy. Clinical data were retrospectively extracted from the monocentric ITHACA registry of JIA patients (study approval 4085_S_P, obtained on December 13th, 2023). Patients were subgrouped upon the age at RTX start as follows: <14 years, 14–18 years and >18 years. Treatment persistence was calculated as the time between the first and the last RTX infusion whereas follow-up was defined as the time between the first RTX infusion to the latest rheumatological assessment at our institution. Disease activity was assessed using DAS28-CRP at RTX initiation, then at 6 and 12 months and/or at treatment discontinuation. Disease activity status was defined upon DAS28-CRP scores as follows: remission (DAS28-CRP<2.6), low (2.6≤DAS28-CRP<3.2), moderate (3.2≥DAS28-CRP≤5.1) and high disease activity (DAS28-CRP>5.1). Response to RTX was defined as a DAS28-CRP decrease of at least 0.6. Disease flares were defined as an increase in DAS28-CRP above 1.2 [12]. Potential side effects due to CD20 depletion were investigated at each RTX infusion and at the latest follow-up visit. In particular, infections were defined as clinically relevant when hospitalization and/or treatment discontinuation were deemed necessary or in case of recurrent infections despite adequate antibiotic treatment. Hypogammaglobulinemia was defined in case of gamma globulins <10 % of total proteins at serum electrophoresis.

Statistical analysis

Continuous data were expressed as median (interquartile range [IQR]) and categorical data as percentages. The association between categorical variables was assessed by chi-squared or Fisher exact tests as appropriate; Mann-Whitney test was applied to compare continuous variables between subgroups of patients, as appropriate. Wilcoxon signed rank test was applied to evaluate clinical variables before and after RTX treatment. DAS28-CRP scores over follow-up were compared within the same subject by Friedman's test. ROC curves were drawn to identify the baseline DAS28-CRP value with the highest predictive role for response to RTX at 6 months and the number of RTX cycles predictive of hypogammaglobulinemia and clinically relevant infections. Treatment persistence was assessed by Kaplan-Meier method, where RTX discontinuation represented the event. Difference between the curves was assessed by log-rank method. P-values <0.05 were regarded as significant. Statistical analysis was performed using SPSS v29.

Results

From June 2008 to December 2023, 37 patients fulfilling inclusion criteria were started on RTX and thus included in this study (Table 1). Most patients had polyJIA (56.8 %) with only 4 subjects (10.8 %) presenting ERA. OligoJIA represented the second most prevalent disease subset (32.4 %); in 4 cases (33.3 %) the disease was classified as oligo-extended JIA. Fourteen subjects (37.8 %), all with oligoJIA, also developed JIA-uveitis. All patients had longstanding JIA, with a median disease duration of 13.9 years. The median age at first RTX infusion was 23.5 years, with 7 patients (18.9 %) receiving RTX in pediatric age: 3 (8.1 %) were aged <14 years (10, 11 and 13 years), while 4 subjects (10.8 %) started RTX between 15 and 17 years of age. Most patients had failed at least one anti-TNFα agent; 2 patients (5.4 %) received RTX as first-line biological disease modifying anti-rheumatic drug (bDMARD) due to comorbidities: one subject had a concomitant autoimmune thrombocytopenia and another a primary myelofibrosis with CALR somatic mutation. Most patients (25, 69.4 %) had been treated with at least 2 different bDMARDs, and 12 patients (32.4 %) had failed at least two mechanisms of action before RTX. As detailed in Table 2, 17 patients (45.9 %) received RTX in association with a conventional disease modifying anti-rheumatic drugs (csDMARD). Out of 37 patients, 2 (5.4 %) received treatment "on demand" in case of active articular disease. All other patients received RTX infusions every 6 months until discontinuation. The median number of cycles per patient was 5 (IQR 9, range 1–26), with a median persistence time in treatment with RTX of 2.5 years (IQR 6.77 years, range 6–192 months). Patients were followed-up for a median of 7.44 years (IQR 7.26, range 1–16 years) from the first RTX infusion.

The introduction of RTX did not allow dose tapering in any of the 4 patients (10.8 %) on chronic oral medium-high glucocorticoids: in 2 cases, prednisone was maintained due to active refractory arthritis [12.5 mg daily], in one due to active treatment-resistant uveitis [15 mg daily] and in another due to severe steroid-dependent neutrophilic skin lesions [17.5 mg daily]. In 12 patients (32.4 %), chronic treatment with low dose prednisone was continued after RTX initiation (10 patients on 5 mg daily and 2 on 2.5 mg daily). Six patients (16.2 %) were prescribed with oral glucocorticoids in order to control articular disease activity before receiving RTX, then discontinuing the treatment [prednisone 25 mg in 2 cases and 5 mg in 3 cases). Seven patients (18.9 %) required intra-articular knee injection of corticosteroids over RTX treatment due to persistently active arthritis. At 6 months, a response to RTX was registered in 27 patients (73 %): 9 subjects (24.3 %) reached a low disease activity and 18 patients (48.6 %) achieved remission; disease flared in a single patient. DAS28-CRP scores at 6 months from RTX initiation were lower compared to those registered at baseline (median at baseline 3.91 [IQR 3.42–4.54], after 6 months of RTX treatment 2.73 [IQR 3.59–4.54]; $p < 0.01$, median change −1.13 [IQR −2.8–0.5], Fig. 1A). Patients who

Table 1
Demographics and disease features of patients stratified according to response and remission at 6 months of treatment with rituximab.

	Whole cohort ($n = 37$)	Response at 6 months ($n = 27$)	No response at 6 months ($n = 10$)	p-value*	Remission at 6 months ($n = 18$)	No remission at 6 months ($n = 19$)	p-value**
Age at JIA diagnosis, median (IQR)	10.5 (12.9)	11.46 (12.03)	6.72 (12.1)	0.171	10.54 (12.91)	11.59 (13.37)	0.271
Age at starting treatment with RTX, median (IQR)	23.5 (10.4)	24.41 (10.34)	22.87 (9.62)	0.121	23.47 (10.28)	24.41 (10.02)	0.125
<14 years at RTX start, n (%)	3 (8.1)	2 (7.4)	1 (10)	0.965	2 (11)	1 (5)	0.874
14–18 years at RTX start, n (%)	4 (10.8)	3 (11.1)	1 (10)		2 (11)	3 (15.7)	
>18 years at RTX start, n (%)	30 (81.1)	22 (81.4)	8 (8)		14 (77)	15 (78.95)	
Female gender, n (%)	31 (83)	23 (85)	8 (80)	0.310	16 (88)	15 (78.95)	0.412
Disease subset, n (%)							0.915
oligoJIA	12 (32.4)	7 (26)	5 (50)	0.933	5 (27.8)	7 (36.8)	
polyJIA	21 (56.76)	17 (63)	4 (40)		11 (61.1)	10 (52.6)	
ERA	4 (10.81)	3 (11)	1 (10)		2 (11.1)	2 (10.6)	
RF positivity, n (%)	14 (37.84)	11 (40.7)	3 (30)	0.829	8 (44)	6 (31.58)	0.494
ACPA positivity, n (%)	11 (29.71)	9 (33.3)	2 (20)	0.702	7 (38)	4 (21)	0.278
ANA positivity, n (%)	16 (43)	11 (40.7)	5 (50)	0.102	8 (44)	8 (41)	0.738
Uveitis, n (%)	14 (37)	10 (37)	4 (40)	0.205	7 (38)	7 (36.84)	0.612
Comorbidities, n (%)	16 (43)	11 (40.74)	6 (60)	0.551	8 (38)	9 (47.37)	0.191

oligoJIA: Oligoarticular Juvenile Idiopathic Arthritis; polyJIA: Polyarticular Juvenile Idiopathic Arthritis; ERA: Enthesitis-Related Arthritis; RTX: Rituximab; IQR: Interquartile Range; RF: Rheumatoid Factor; ACPA: Anti-Citrullinated Peptides Antibodies; ANA: Anti-Nuclear Antibodies.
* p-value refers to the comparison between patients obtaining response to rituximab at 6 months versus those non obtaining response.
** p-value refers to the comparison between patients on remission after 6 months of treatment with rituximab versus those non in remission.

Table 2
Baseline clinical and therapeutical features of patients stratified according to response and remission at 6 months of treatment with rituximab.

	Whole cohort ($n = 37$)	Response at 6 months ($n = 27$)	No response at 6 months ($n = 10$)	p-value*	Remission at 6 months ($n = 18$)	No remission at 6 months ($n = 19$)	p-value**
Disease duration at RTX start in years, median (IQR)	13.9 (9.0)	14.1 (7.2)	16.1 (13.9)	0.089	14.0 (10.2)	14.3 (10.2)	0.442
DAS28-CRP at starting treatment with RTX, median (IQR)	3.9 (1.1)	3.97 (1.70)	3.6 1.0)	0.881	3.91 (1.1)	3.9 (1.2)	0.467
DAS28-CRP at after 6 months of treatment with RTX, median (IQR)	2.6 (1.5)	2.51 (1.19)	4.3 (1.1)	0.910	2.735 (1.54)	2.8 (1.4)	0.330
DAS28-CRP at last follow-up with RTX, median (IQR)	2.3 (1.2)	2.3 (1.0)	3.6 (1.4)	0.561	2.6 (1.3)	2.2 (1.5)	0.320
Pre-RTX bDMARDs, median (IQR)	2 (2)	2 (2)	2 (1.7)	0.874	2 (2)	2 (2)	0.773
>2 previous bDMARDs, n (%)	17 (45.9)	13 (48.2)	4 (40.0)	0.944	7 (38.9)	10 (52.6)	0.781
MTX during RTX treatment, n (%)	16 (43.2)	9 (33.3)	7 (70)	0.046	10 (55.5)	6 (31.6)	0.143
LEF during RTX treatment, n (%)	1 (2.7)	1 (3.7)	0 (0)	0.991	0 (0)	1 (5.3)	0.891
IACI during RTX treatment, n (%)	7 (18.9)	4 (14.8)	3 (30)	0.321	2 (11.1)	5 (26.3)	0.735
Clinically relevant infections, n (%)	10 (27)	6 (22.2)	4 (40)	0.615	5 (27.8)	5 (26.3)	1.000
Hypogammaglobulinemia, n (%)	10 (30.3)	7 (25.9)	3 (30)	0.122	5 (27.8)	5 (26.3)	0.631

RTX: Rituximab; IQR: Interquartile Range; DAS28-CRP: Disease Activity Score in 28 Joints using C-Reactive Protein; bDMARDs: Biologic Disease Modifying Anti-Rheumatic Drugs; MTX: methotrexate (Methotrexate); LEF: Leflunomide; IACI: intra-articular corticosteroid injection.
* p-value refers to the comparison between patients obtaining response to rituximab at 6 months versus those non obtaining response.
** p-value refers to the comparison between patients on remission after 6 months of treatment with rituximab versus those non in remission.

responded to RTX at 6 months had higher -although not significantly- DAS28-CRP at baseline (4.25 vs 3.53; $p = 0.09$); in particular, subjects with a baseline DAS28-CRP >3.68 had the highest chance of responding to RTX at 6 months (area under the curve [AUC] 0.724, sensitivity 66.7 %, specificity 56.7 %). The response rate to RTX was similar across the different JIA subsets (oligoJIA 58.3 %, polyJIA 80.9 %; ERA 75 %; $p = 0.370$) even though DAS28-CRP values decreased significantly overtime in polyJIA and oligoJIA but not in ERA ($p = 0.0126$, $p = 0.049$ and $p = 0.281$ respectively; Fig. 1B). Conversely, the remission rate at 6 months was higher, although not significantly, in the polyarticular subset compared to the oligoJIA (52.4 % vs 41.7 %, $p = 0.75$). Disease duration and age at RTX start -even when stratified in 3 age categories (Fig. 1C)- did not impact response nor remission rates at 6 months.

No difference in both the response and remission rates at 6 months from first RTX cycle emerged for patients subgrouped upon ANA (response: 68.8 % in ANA+ vs 76.2 %, $p = 0.613$ and remission: in ANA+ 50 % vs 37 %, $p = 0.404$) and RF status (response: 78.6 % in RF+ vs 69.6 %, $p = 0.549$, and remission: 57.1 % in RF+ vs 43.5 %, $p = 0.419$). The response and remission outcomes were better in case of positive ACPA (response: 81.8 % in ACPA+ vs 69.2 %, $p = 0.430$ and remission: 63.6 % in ACPA+ vs 42.3 %, $p = 0.235$) and double RF/ACPA seropositivity (response: 66.7 % in RF/ACPA double positive vs 50 %, $p = 0.382$ and remission: 55.5 % vs 46.4 %, $p = 0.633$), without reaching conventional statistical significance.

Combination therapy with MTX (pursued in 43.2 % of cases) conferred a lower rate of response to RTX (56.3 % vs 85.7 %, $p = 0.046$) but did not affect the remission rate (55.5 % vs 42.1 %, $p = 0.413$) at 6 months. The remission rate was similar between patients who had been treated with 2 or more bDMARDs before RTX start and those who had received only one bDMARD before RTX (76.4 % vs 70 %, $p = 0.658$).

At RTX start, uveitis was active in 4 cases (28.5 %); patients with active uveitis were invariably treated with low dose topical corticosteroids during the first 6 months of treatment with RTX. After 6 months of treatment with RTX, 3 of the 4 patients (75 %) with active uveitis at RTX

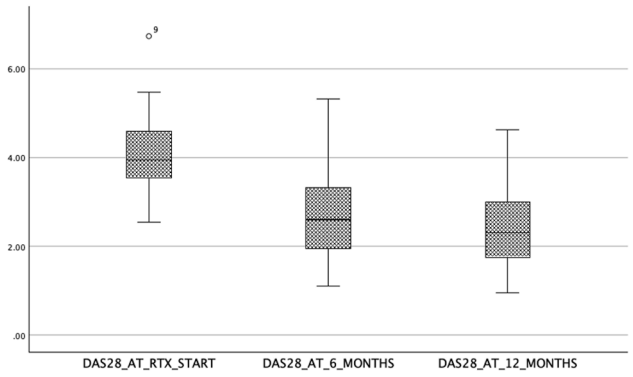

Fig. 1A. Fluctuation of DAS28-CRP in patients with JIA receiving rituximab at baseline and at each semester of treatment.
Median DAS28-CRP at baseline 3.92 [IQR 1.12], at 6 months 2.74 [IQR 1.55], at 12 months 2.31 [IQR 1.21], $p < 0.001$.

start still presented active ocular disease and discontinued RTX after 1 or 2 cycles (2 and 1 subject respectively). Out of the 14 with uveitis, 2 patients (14.3 %) experienced uveitis relapses over 6 months of follow-up, necessitating initiation of topical corticosteroid therapy. RTX was discontinued in one case (50 %), with a switch to infliximab while the other patient continued RTX achieving uveitis remission at 12 months. No new-onset ocular complications were observed during the treatment period.

Response to rituximab beyond 6 months of treatment

At 12 months, 32 patients were still under RTX treatment as 5 subjects (13.5 %) had discontinued treatment: 3 patients (60 %) due to primary non-response (active arthritis/enthesitis), 2 (40 %) because of clinically relevant infections. Although the most marked improvement was registered between baseline and 6 month, DAS28-CRP maintained the reduction trend even at 12 months (Friedman statistics=27.44; $p < 0.001$, Fig. 1A); a response to RTX was registered in 3 additional patients (8.1 %), with 15 further patients (40.5 %) achieving disease remission. No new disease flares were registered at 12 months, and no other subjects achieved disease remission during treatment course with RTX.

At the latest follow-up, RTX was discontinued in 23 additional patients (62.2 %, Fig. 2). In particular, 4 patients (10.8 %) discontinued due to secondary non-response (active arthritis/enthesitis), one subject (2.7 %) because of the desire of a pregnancy, 7 patients (18.9 %) due to side effects; 6 patients (16.2 %) stopped RTX after achieving prolonged remission (median number of cycles 13, IQR 11.5–20.5). At a median time of 1.45 years [IQR 0.13–2.81] from the last RTX administration, all these 6 subjects were still in remission (median DAS28-PCR 1.82 [IQR 1.46–2.50]). One subject continued MTX; none of these patients required to resume RTX or a new bDMARD due to poor disease control. At the latest follow-up, 5 patients were still receiving RTX (median number of RTX cycles 11, IQR 6–13; median persistence time in RTX 6.57 years, IQR 5.67) with satisfactory disease control and no side effects.

Safety profile

The 7 patients (18.9 %) who discontinued RTX due to side-effects experienced infusion-related adverse events ($n = 5$) or post-infusive cutaneous manifestations ($n = 2$).

Ten patients (27 %) manifested hypogammaglobulinemia. Patients who developed hypogammaglobulinemia had received a higher number of RTX cycles, a difference which almost attained statistical significance (10.5 vs 4 cycles, U statistic 164.5, $p = 0.053$).

Ten patients (27 %) experienced clinically relevant infections during RTX treatment. Hospitalization was required in 4 cases (40 %): 3 for pneumonia and 1 for osteomastoiditis; all other patients received domiciliary therapies. Three patients (30 %) experienced recurrent infections during treatment with RTX: 2 patients (20 %) had recurrent urinary tract infections, while the other (10 %) presented recurrent sinusitis. In 3 other cases (30 %), infections required RTX discontinuation. Patients with clinically relevant infections were exposed to a similar number of RTX administration than subjects who did not develop such complication ($p = 0.163$); consistently, no significant correlation emerged between the number of RTX administrations and clinically relevant infections ($p = 0.507$). Hypogammaglobulinemia did not convey a higher risk of infections ($r = 0.283$, $p = 0.111$), whereas a moderate significant negative correlation was observed between the number of severe infections and the number of treatments with other

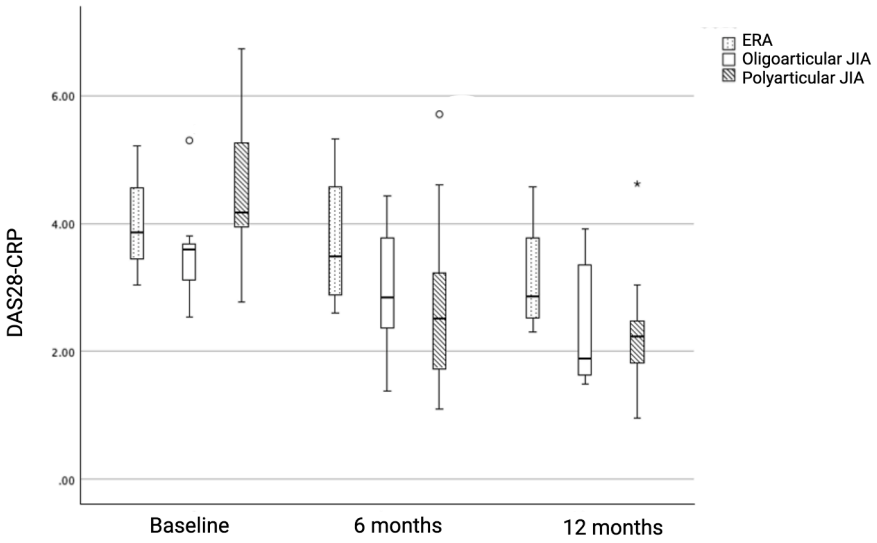

Fig. 1B. Fluctuation of DAS28-CRP in patients with JIA subgrouped upon disease category receiving rituximab at baseline and after 6 and 12 months of treatment.
ERA: enthesitis-related arthritis; JIA: juvenile idiopathic arthritis.
Oligoarticular JIA: Median DAS28-CRP at baseline 3.6 [IQR 0.6], at 6 months 2.73 [IQR 1.4], at 12 months 2.29 [IQR 0.47], $p = 0.0126$.
Polyarticular JIA: Median DAS28-CRP at baseline 4.18 [IQR 1.4], at 6 months 2.73 [IQR 1.8], at 12 months 2.19 [IQR 1.34], $p = 0.049$.
ERA: Median DAS28-CRP at baseline 3.86 [IQR 0.6], at 6 months 2.62 [IQR 1.2], at 12 months 2.88 [IQR 0.79], $p = 0.281$.

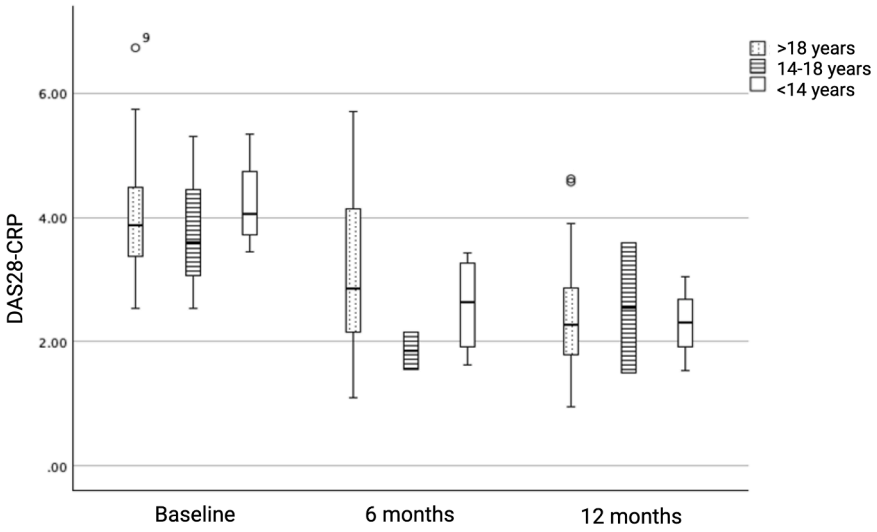

Fig. 1C. Fluctuation of DAS28-CRP in patients with JIA subgrouped upon age at first infusion receiving rituximab at baseline and after 3 and 6 months of treatment.
<14 years: Median DAS28-CRP at baseline 3.6 [IQR 1.38], at 6 months 2.85 [IQR 0.44], at 12 months 1.91 [IQR 0.29], $p = 0.038$.
14–18 years: Median DAS28-CRP at baseline 4.06 [IQR 0.56], at 6 months 2.85 [IQR 0.95], at 12 months 2.06 [IQR 0.69], $p = 0.173$.
>18 years: Median DAS28-CRP at baseline 3.91 [IQR 1.12], at 6 months 2.73 [IQR 1.54], at 12 months 2.29 [IQR 1.23], $p = 0.008$.

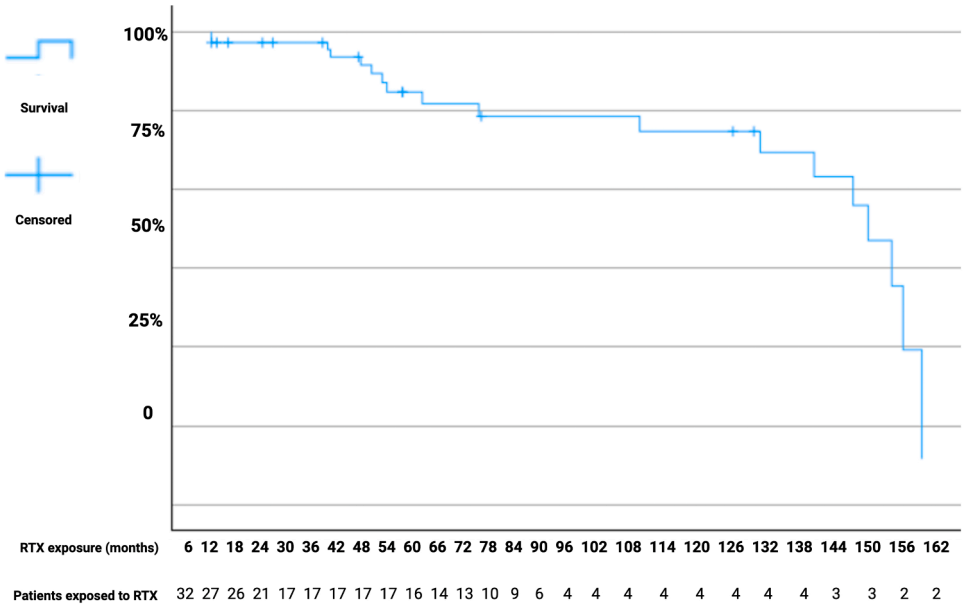

Fig. 2. Persistence in treatment with rituximab of patients with JIA over follow-up.
Patients still receiving rituximab were censored at latest follow-up.

bDMARDs before RTX ($r = -0.40$; $p = 0.014$). Patients receiving RTX on the top of MTX treatment did not display a higher risk of clinically relevant infections, hypogammaglobulinemia or both compared to those on RTX monotherapy (25 % vs 28.5 %, $p = 0.808$, 25 % vs 28.5 %, $p = 0.808$, and 37.5 % and 42.8 %, $p = 0.742$ respectively).

Patients with hypogammaglobulinemia and/or clinically relevant infection had a similar persistence in treatment with RTX compared to subjects who did not experience these complications ($p = 0.7$, log-rank 0.111). Receiving >4 cycles of RTX predicted the development of hypogammaglobulinemia and/or clinically relevant infections (AUC 0.62, sensitivity 71.9 %, specificity 60.0 %).

Response to biologics after rituximab

After RTX discontinuation, 16 patients (43 %) started another bDMARD with a different mechanism of action. Seven patients (43 %) received an anti-TNFα, while 5 patients (31.5 %) were started on treatment with abatacept. Two patients (12.5 %) were started on anti-IL1 (anakinra), a single patient (6.2 %) on anti-IL6 (tocilizumab) and another (6.2 %) on JAK-inhibitor (tofacitinib). Six months after starting a new bDMARD following the discontinuation of RTX, 11 patients (68.75 %, 6 patients on anti-TNFα therapy, 4 patients on abatacept, and 1 patient on tofacitinib) achieved a DAS28-CRP score below 3.2, indicating improved disease control. In case of failure of the first post-RTX bDMARD, 7 patients (43 %) were switched to a second biologic tool (5 [71.4 %] with anti-TNFα, 1 [14.3 %] with tofacitinib, and 1 [14.3 %] with tocilizumab); in this instance, 5 patients (71.4 %) reached a DAS28-CRP score below 3.2 at 6 months.

Discussion

The data from the monocentric ITHACA registry support a potential therapeutic niche for RTX in the pharmacological approach to recalcitrant JIA. At 6 months, a response to RTX was registered in approximately three-quarters of our study cohort, with nearly half of the 37 subjects achieving disease remission. Given that RTX has yet to find its therapeutic role in the pharmacological management of non-systemic JIA, the description of our clinical experience with CD20 depletion -the second largest available in literature to date and the one with the longest follow-up [13,14]- acquires particular importance. Indeed, the 2019 ACR guidelines for the treatment of JIA favour TNFα inhibitors, abatacept or tocilizumab over RTX for patients with moderate/high active polyarthritis refractory to at least 2 bDMARDs [15]. Such recommendation stems from the paucity of data available for the CD20 targeting agent in this scenario, with no randomized clinical trial establishing RTX efficacy in JIA [15]. Despite the lack of any evidence, guidelines suggest considering earlier RTX in case of RF positive polyarthritis, translating data from adult rheumatoid arthritis [15]. Interestingly enough, in the ITHACA cohort nor ANA nor RF did significantly predict a better response to RTX, with a trend towards better outcome in case of ACPA or RF/ACPA double positivity. Consistently, we observed a higher, although not significantly, remission rate at 6 months in the polyarticular subset, with a significant decrease of DAS28-CRP value in both oligoJIA and polyJIA. This observation finds support in synovial pathobiology, given the relevance of B cell activity even in the extended oligoarticular subtype [10].

Our data suggest that continuing MTX while instituting a CD20 depletion regimen is not beneficial: the combo of RTX with MTX even led to a lower response -but not remission- rate at 6 months, possibly unveiling a selection bias. Indeed, in our experience patients with longstanding JIA usually display very poor tolerance to MTX, possibly because of the enduring exposure to high dose of this anti-folate agent during early stages of disease. Therefore, we tend to reserve MTX combination treatment with bDMARDs only to subjects with more severe and recalcitrant disease presentation.

In the present study, despite a substantial reduction in disease activity, RTX did not enable glucocorticoid dose tapering, and some patients still required intra-articular steroid injections. To note, the remission rate was lower –although far from statistical significance- among patients who had been treated with 2 or more bDMARDs before RTX start. This finding might be biased by the composition of our cohort, which was enriched in patients with longstanding and refractory disease while other case series or case reports described RTX effectiveness at the failure of a first TNF inhibitor [13,14,16,17]. The ITHACA experience mirrors the figures extrapolated from an UK registry: due to RTX failure, 43 % of our patients were prescribed with another bDMARD, as compared to 41 % in the Biologics for Children with Rheumatic Diseases (BCRD) study [13]. The rate of response to the new therapeutic tool was satisfying, in agreement with the intriguing hypothesis that RTX might affect the response to subsequent treatment strategies [17].

Data extrapolated from the ITHACA registry on JIA-uveitis add on the vibrating debate about RTX efficacy in controlling non-infectious uveitis, with few yet contrasting results [18–20]. In our cohort, 3 of 4 patients with active uveitis at RTX initiation experienced persistently active ocular disease despite treatment, challenging the enlisting of RTX among the therapeutic options to be reserved to patients with JIA uveitis.

The present study offers the longest duration of RTX exposure in JIA patients to date available, allowing to dissect the long-term safety of such agent. In the ITHACA registry, severe infections, including cases requiring hospitalization, were recorded in 8.1 % of cases, consistently with previous reports [13]. Interestingly, severe infections occurred more commonly in case of previous exposure to multiple bDMARD treatments while no correlation emerged between the number of RTX cycles and severe infections. These lines of evidence suggest that the infection risk may not directly escalate with prolonged RTX use, but rather be underpinned by the interplay between disease status and prior immune modulation.

Hypogammaglobulinemia presented in 27 % of our patients, a slightly higher figure than the 20 % rate previously reported in JIA [14]. Nevertheless, the rate of serious infections in patients with hypogammaglobulinemia was not significantly different than in those with normal levels, indicating variability in clinical impact [21]. Importantly, our clinical experience suggests that exposing JIA patients to a maximum of 4 RTX cycles ensures a good safety profile, minimizing the rate of infections and hypogammaglobulinemia. Despite a theoretical risk of an increased rate of complications, adding RTX on the top of ongoing MTX treatment did not impact the risk of developing infections and/or hypogammablulinemia in our cohort.

It should be acknowledged that the present study has limitations, including its retrospective nature and the limited sample size. The monocentric nature of the study, with patients mostly of Caucasian origin, prevents the generalizability of findings. Being a tertiary referral center for pediatric and adult rheumatology, the present cohort could also be affected by a selection bias being not representative of the whole spectrum of JIA but only of severe disease. Surely, the heterogeneous composition of the study cohort impinges the drawing of definite conclusions about the fine profiling of patients most likely to respond to RTX (e. g., JIA subset, ANA positivity, uveitis, age at disease onset, previous treatments). Even the optimal dosing strategy to be reserved to JIA patients needs to be further elucidated, as only 2 subjects received RTX on demand. Disease activity was evaluated by the means of DAS28-CRP only, which has however been recently identified as the most reliable clinimetric score among adults with JIA [22]. Unfortunately, monitoring of peripheral blood lymphocyte subsets was inconsistently available for included patients, thus $CD19^{+}$ *B* cell depletion and/or repopulation were not taken into account in the evaluation of clinical response to RTX. Lastly, it should be mentioned that the management modalities and therapeutic approaches to JIA might have progressively evolved over the 15-year study period.

In conclusion, RTX might be regarded as a therapeutic option for refractory JIA, particularly in the poly/oligoarticular courses with positive ACPA, when other bDMARDs have failed. In 16.2 % of treated JIA patients, RTX allowed to obtain long-term disease remission without requiring further treatments. While showing promise in the management of refractory JIA, RTX also raises safety concerns: limiting RTX exposure to 4 cycles might allow to reduce the rate of side effects. While waiting for further research insights into the clinical as well as synovial profile of patients most likely to benefit from RTX, careful patient selection and vigilant monitoring are essential to optimize outcomes and minimize risks.

Ethical approval

The project has been approved by the local ethical committee (study approval 4085_S_P, obtained on December 13th, 2023; ITHACA study).

All recruited subjects provided written informed consent.

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Data sharing statement

Data generated by the present research study can be made available upon request.

CRediT authorship contribution statement

Maria Rosa Pellico: Writing – original draft, Formal analysis, Data curation. **Marco Pandolfi:** Writing – original draft, Formal analysis,

Data curation. **Andrea Amati:** Writing – review & editing, Data curation. **Claudia Iannone:** Writing – review & editing, Investigation. **Sabino Germinario:** Writing – review & editing, Investigation, Data curation. **Stefania Costi:** Writing – review & editing, Supervision, Methodology. **Francesco Baldo:** Writing – review & editing, Resources. **Maurizio Gattinara:** Writing – review & editing, Supervision. **Elisabetta Miserocchi:** Writing – review & editing, Formal analysis. **Maria Gerosa:** Writing – review & editing, Supervision, Conceptualization. **Achille Marino:** Writing – review & editing, Supervision. **Roberto Caporali:** Writing – review & editing, Supervision, Conceptualization. **Cecilia Beatrice Chighizola:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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