Efficacy and safety of off-label use of rituximab in refractory lupus: data from the Italian Multicentre Registry

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Abstract

Objective

To evaluate the efficacy and safety of rituximab (RTX) in patients with systemic lupus erythematosus (SLE) refractory to standard therapy in the clinical practice setting.

Methods

145 SLE patients (ACR criteria) were treated with RTX in 11 Italian Centres: 118 with two infusions (1 g), two weeks apart; 27 with 4 infusions (375 mg/m^2), one week apart, followed in 10 cases by two further doses, after 1 and 2 months. Systemic complete response (CR) was defined as European Consensus Lupus Activity Measurement (ECLAM) score ≤1 and partial response (PR) as 1< ECLAM ≤3. Renal CR (RCR) and renal PR (RPR) were defined according to EULAR recommendations for management of lupus nephritis.

Results

Data from 134 (92.4%) patients were available. The mean±SD follow-up was 27.3±18.5 months. After the first course of RTX, CR or PR were observed in 85.8% and CR in 45.5% of cases; RCR or RPR in 94.1% and RCR in 30.9% of patients after 12-month follow-up. Disease flares occurred in 35.1% and renal flares in 31.2% of patients during observational period. Among patients retreated, CR or PR were observed in 84.4% and CR in 57.8% of cases.

Adverse events, infections, and infusion reactions occurred after first RTX course in 23.8%, 16.4%, and 3.8% of patients and after retreatment in 33.3%, 22.2% and 11.1%, respectively. No severe infusion reactions or deaths occurred.

Conclusion

Data from Italian multicentre RTX Registry confirmed the efficacy and safety of RTX in SLE patients refractory to standard treatment in clinical practice setting.

Key words

systemic lupus erythematosus, rituximab, Italian registry, lupus activity, complete response, lupus nephritis

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disorder associated with a wide spectrum of clinical features, such as skin rash, arthritis, serositis, nephritis, seizures, psychosis, haemolytic anaemia and cytopenia (1, 2). The number of randomised controlled trials (RCTs) in SLE is limited, thus, therapeutic recommendations are mainly based on uncontrolled studies and include corticosteroids and hydroxychloroquine for mild-to-moderate disease and immunosuppressants as adjunctive therapy for severe disease (3, 4). Unfortunately, long-term use of corticosteroids and immunosuppresants is burdened by several side effects which increase the morbidity and mortality of SLE patients, especially in those with major organ involvement (5, 6).

B cells play a pivotal role in the pathogenesis of SLE (7), since they act as antigen-presenting cells and produce autoantibodies, cytokines, and chemokines. In the last few years, anti-B cell therapy has largely been used for the treatment of SLE, particularly rituximab (RTX), a chimeric anti-CD20 monoclonal antibody which induces B-cell depletion, and belimumab, a fully-human monoclonal antibody directed against BLyS (B-lymphocyte stimulator) (8). The efficacy of RTX in SLE was suggested by several open-label and retrospective studies which reported the achievement of clinical response in a high percentage of patients, confirming a corticosteroid-sparing effect and a good safety profile (9-15). Unfortunately, two phase III RCTs testing RTX in SLE patients with glomerulonephritis (the Lupus Nephritis Assessment with Rituximab [LUNAR] study) (16) and without glomerulonephritis (the Exploratory Phase II/III SLE Evaluation of Rituximab [EXPLORER] trial) (17) failed to meet their primary endpoints. Despite that, the *off-label* use of RTX is considered a valid treatment option, especially in patients with some refractory manifestations of the disease.

In the present study, the efficacy and safety of RTX were analysed in a nationwide multicentre cohort of Italian SLE patients.

Patients and methods

This study is based on a multicentre and observational data collection of adult patients with SLE, refractory to standard therapy, treated with at least one course of RTX.

All Italian tertiary centres for SLE management were invited to take part in this National Registry. The inclusion of patients in the Registry did not interfere with current clinical practice. From May 2003 until August 2012 data on 145 SLE patients from 11 Italian Centres were collected.

All patients were classified according to the 1982 American College of Rheumatology (ACR) revised criteria for SLE (18) and the definition of refractory clinical manifestations of SLE was based on physician's judgment, like in other European registries (11, 12). However, RTX was used after the failure of at least one immunosuppressant, and was added to or replaced previous immunosuppressant. Three RTX regimens were used: two infusions (1 g), two weeks apart (scheme A), 4 infusions (375 mg/m^2) one week apart (scheme B) or 4 infusions (375 mg/m^2), one week apart followed by two more doses administered 1 and 2 months after the last weekly infusion (scheme C). Patients treated with scheme C concomitantly received two pulses of cyclophosphamide, 750 mg, (Days 4 and 17) and three pulses of methylprednisolone, 15 mg/kg, (Days 1, 4 and 8). All patients received standard premedication with antihistamine, acetaminophen, and intravenous 40-100 mg methylprednisolone (depending on patients' weight and allergic diathesis), 30 minutes before RTX infusion.

Data on clinical manifestations, serologic abnormalities and previous immunosuppressive treatment were collected on all patients prior to the first RTX infusion. Disease activity was assessed by the European Consensus Lupus Activity Measurement (ECLAM) score (19). ECLAM score and response to treatment were analysed at baseline, 3, 6, and 12 months after RTX infusion. Mild-to-moderate SLE activity was defined as ECLAM score ≤5 and high disease activity as ECLAM score >5. For the evaluation of the response

Table I. Demographic characteristics, clinical and autoantibodies features of 145 SLE patients enrolled in the RTX Italian Registry.

Patients	145
– Female	130 (89.7%)
– Male	15 (10.3%)
Age at diagnosis (mean±SD, years)	27.8 ± 11.2
Age at the first treatment (mean±SD, years)	37.3 ± 12.4
Disease duration (mean±SD, years)	9.3 ± 7.3
Clinical manifestations at baseline	
– Renal	68 (50.7%)
– Musculoskeletal	35 (26.1%)
– Haematologic	25 (18.6%)
– Cutaneous	11 (8.2%)
– Neurologic	9 (6.7%)
– Serositic	5 (3.7%)
– Visceral vasculitis	1 (0.7%)
Autoantibodies at baseline	
– ANA	145 (100%)
– Anti-dsDNA	105 (72.4%)
– Anti-Sm	24 (16.5%)
– Anti-U1RNP	30 (20.7%)
– Anti-SSA	50 (34.5%)
– Anti-SSB	15 (10.3%)
– Anti-cardiolipin	47 (32.4%)
– Anti-β2GPI	19 (13.1%)
– Lupus anticoagulant	43 (29.7%)
Past treatment	
– Oral corticosteroids	129 (89.0%)
– Azathioprine	75 (51.7%)
– Cyclophosphamide	64 (44.1%)
– Cyclosporine A	72 (49.6%)
– Mycophenolate mofetil	66 (45.5%)
– Methotrexate	43 (29.7%)
– Antimalarials	72 (49.6%)
– IV immunoglobulins	20 (13.8%)
– Leflunomide	6 (4.1%)
Concomitant treatment	
– Oral corticosteroids	130 (89.7%)
– Mycophenolate mofetil	40 (27.6%)
– Antimalarials	34 (23.4%)
– Cyclophosphamide	20 (13.8%)
– Methotrexate	19 (13.1%)
– Azathioprine	15 (10.3%)
– Cyclosporine A	13 (9.0%)
– IV corticosteroids	11 (7.6%)
– Leflunomide	1 (0.7%)

ANA: anti-nuclear antibody; dsDNA: double stranded DNA; IV: intravenous; SD: standard deviation.

to treatment, complete response (CR) was defined as ECLAM ≤1 and partial response (PR) as 1<ECLAM≤3. For the evaluation of the organ specific response to the treatment, in patients with lupus nephritis, renal CR (RCR) and renal PR (RPR) were defined according to the European League Against Rheumatism (EULAR) recommendations for management of lupus nephritis [20] and were assessed at 3, 6 and 12-month follow-up and, for the evaluation of organ specific extra-renal (*i.e* joint, skin, vasculitis, etc.) response to treatment, CR and PR were based on physician's judgment and were assessed at 3, 6 and 12-month follow-up. Finally, anti-double stranded (ds) DNA antibody levels were measured by Enzyme Linked Immunosorbent Assay (ELISA), and in patients with lupus nephritis, serum creatinine and 24h-proteinuria were assessed at baseline, 3, 6, and 12-month follow-up.

All data were collected and managed by the Rheumatology Unit, University of Padova, and reported in an *ad hoc* excel file (.xlsx). Missing data were minimised by asking physician from each Centre to fulfill missing data in the *ad hoc* excel file whenever requested. Patients with less than 6-month follow-up were excluded from the efficacy and safety analysis.

Physicians were allowed to retreat patients after 6 or 12-month follow-up (retreatment schedule) or in case of disease flare. Disease flares were defined according to physician judgment.

Adverse events (AEs) were carefully recorded at every clinical evaluation for all patients during the follow-up (first treatment or retreatment). AEs were defined as severe when hospitalisation was required and/or death and/or life-threatening manifestations occurred. Infections were defined as severe if hospitalisation and/or intravenous antibiotics were required, and/or death had occurred. Infusion reactions were considered severe when intensive care unit support was required for treatment.

Written informed consent was obtained according to Helsinki Declaration and the study was carried out according to standards currently applied in Italy.

Statistical analysis

Data were analysed using the SPSS 20.0 software. The frequency of categorical variables were compared among groups by Pearson's chi-square test. For continuous variables we used paired or unpaired Student's *t*-test, when appropriate. *p*-values below 0.05 were considered statistically significant.

Results

Patients' characteristics

Past clinical manifestations were musculoskeletal in 108 patients (74.5%), haematologic in 106 (73.1%), glomerulonephritis in 86 (59.3%), skin rash in 89 (61.4%), photosensitivity in 79 (54.5%), serositis in 43 (29.7%), oral ulceration in 21 (14.5%), neurologic in 13 (9.0%) and discoid lupus in 8 (5.5%). Demographic characteristics, clinical and serological features of patients treated with RTX are summarised in Table I.

ECLAM score (mean±SD) was 4.11±1.73 (range 3–9) before the first RTX infusion; a high disease activity score (ECLAM≥5) was observed in 34

patients (25.4%). Among 68 patients with glomerulonephritis, the mean±SD proteinuria and creatinine serum levels were 4.04±2.91 g/day and 1.09±0.63 mg/dl, respectively.

Of 145 SLE patients included in the Registry, 118 (81.4%) were treated with scheme A, 17 (11.7%) with scheme B, and 10 (6.9%) with scheme C. Fifty-nine patients were treated with a second course and 18 with a third course of RTX. The mean±SD follow-up period encompassing all treatment courses was 27.3±18.5 months (range 6–84).

As detailed in Table I, RTX was added to background immunosuppressant in 110 cases (75.9%). Antimalarials were concomitantly administered in 34 (23.4%), oral corticosteroids in 130 (89.7%), intravenous (IV) pulse methylprednisolone (500, 750 or 1000 mg) in 13 (8.9%) patients.

Efficacy

• *First course of RTX*

Data for analysis of efficacy and safety after at least the 6-month follow-up were available in 134 patients (92.4%). Cumulative CR and PR were observed in 119 patients (88.8%) at the 6-month follow-up and 115 patients (85.8%) at the 12-month follow-up and CR in 61 patients (45.5%) after 6 and 12 months of follow-up. Refractory manifestations requiring RTX and data on efficacy of RTX are detailed in Table II and III. The efficacy of RTX in patients treated with scheme C has already been published (21).

The ECLAM score (mean±SD) at baseline, 3,- 6-, and 12-month follow-up was 4.11±1.73, 2.17±1.46, 1.77±1.39, and 1.84±1.67, respectively (Fig. 1). In Figure 2 the proportion of patients stratified according to ECLAM score at baseline, 3-, 6-, and 12-month follow-up is reported. Among 68 patients with glomerulonephritis, RCR and RPR were observed in 64 patients (94.1%) and RCR in 21 patients (30.9%) at the 12-month follow-up. Mean±SD 24h proteinuria levels (g/day) at baseline and at 3-, 6- and 12-month follow-up were 4.1±2.9, 1.9±1.9, 1.3±1.5, and 1.1±1.9, respectively (Fig. 3). No differences in creatinine serum levels were found during the follow-up.

Table II. Cumulative rates of complete and partial organ specific response to the first RTX course in 134 patients with SLE by 12-month follow-up.

Manifestations	Patients n.	CR n. (%)	PR n. (%)	CR+PR n. (%)
Renal	68	21 (30.9)	43 (63.2)	64 (94.1)
Musculoskeletal	35	18 (51.4)	13 (37.2)	31 (88.6)
Haematologic	25	14 (56.0)	10 (40.0)	24 (96.0)
Cutaneous	11	8 (72.7)	2 (18.2)	10 (90.9)
Neurologic	9	5 (55.5)	3 (33.3)	8 (88.8)
Serositic	5	4 (80.0)	1 (20.0)	5 (100.0)
Visceral vasculitis	1	1 (100.0)	–	1 (100.0)

CR: complete response; PR: partial response; RTX: rituximab; SLE: systemic lupus erythematosus. Renal PR and CR were defined according to the European League Against Rheumatism (EULAR) recommendations for management of lupus nephritis (20). CR or PR regarding non-renal organ specific involvement was based on physician judgment.

Table III. Response rate after first course of RTX in 11 patients with cutaneous and 9 with neuropsychiatric manifestations.

Manifestations	Patients n.	CR n. (%)	PR n. (%)	NR n. (%)
Cutaneous				
Acute	3	2 (66)	1 (34)	–
Subacute	4	3 (75)	1 (25)	–
Chronic	1	1 (100)	–	–
Vasculitic	2	1 (50)	–	1 (50)
Urticaria	1	1 (100)	–	–
Neuropsychiatric				
Cerebral vasculitis	4	2 (50)	2 (50)	–
Seizure	2	2 (100)	–	–
Lupus headache	1	-	–	1 (100)
Psycosis	1	-	1 (100)	–
Organic brain syndrome	1	1 (100)	–	–

CR: complete response; PR: partial response; NR: non response; RTX: rituximab.

No differences in the proportion of patients who achieved PR and CR were observed according to corticosteroids (yes/no) or immunosuppressant (yes/no) intake. However, the rate of non-responders tended to be higher in patients who did not take immunosuppressants compared with those who did (11.6% *vs*. 2.9%; p=0.059) and patients who were treated with cyclophosphamide achieved RCR and RPR more frequently than those who were cyclophosphamide free (p=0.032).

Mean±SD anti-dsDNA antibody levels (KIU/L) were 152.1±169.4 at baseline and 88.9±80.8 (p<0.001), 80.7±85.3 (p<0.001) and 85.8±89.3 (p<0.001) at 3-, 6- and 12-month follow-up, respectively.

• *Flares after the first course of RTX*

After the first course of RTX a disease relapse (renal and extrarenal) occurred in 47 out of 119 responders (39.5%), after a mean±SD observational period of 16.9±18.8 months (range 6–84) from the last RTX infusion, without any difference between patients who achieved PR and CR or between patients treated with corticosteroids and those who were corticosteroid free. Patients treated with mycophenolate mofetil had a lower flare rate than those not treated with this drugs (14.8% *vs*. 34.9%; p=0.011); on the other hand patients treated with methotrexate had a higher flare rate than those not treated with this drug (23.4% *vs*. 7.2% p=0.01).

Renal flares occurred in 20 (31.2%) out of 64 patients who achieved a renal response, either RCR or RPR, after a mean±SD follow-up period of 19.6±21.7 months (range 6–73) from the last RTX infusion. Extra-renal disease relapses requiring RTX retreatment were haematologic (10 patients),

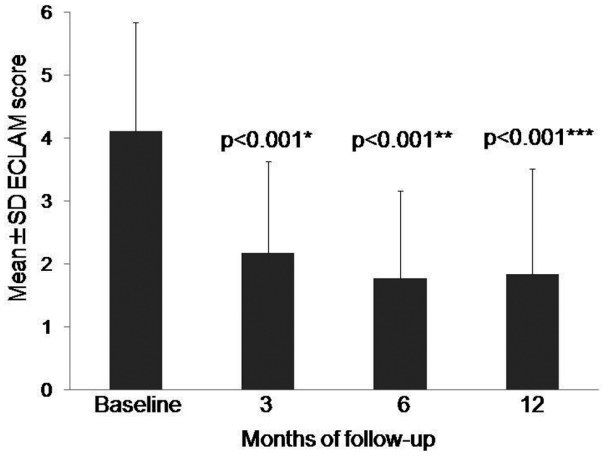

Fig. 1. Mean±SD ECLAM score at baseline and at 3-, 6- and 12-month follow-up in 134 SLE patients treated with RTX (exact values are reported in the text)
*baseline *vs*. 3 months; **baseline *vs*. 6 months; ***baseline *vs*. 12 months

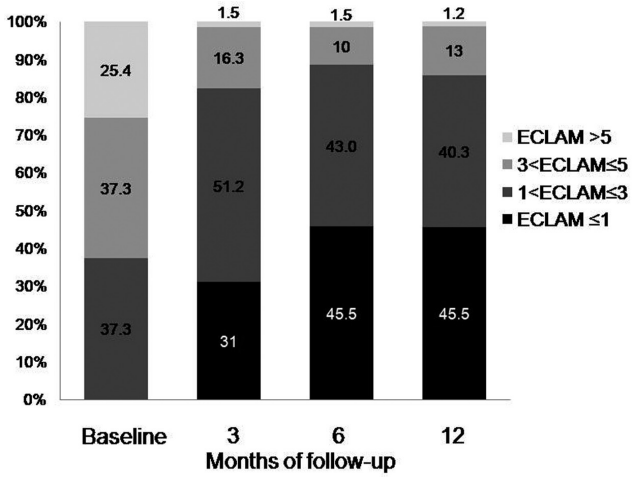

Fig. 2. Proportion of patients stratified according to ECLAM score at baseline, 3-, 6-, and 12-month follow-up.

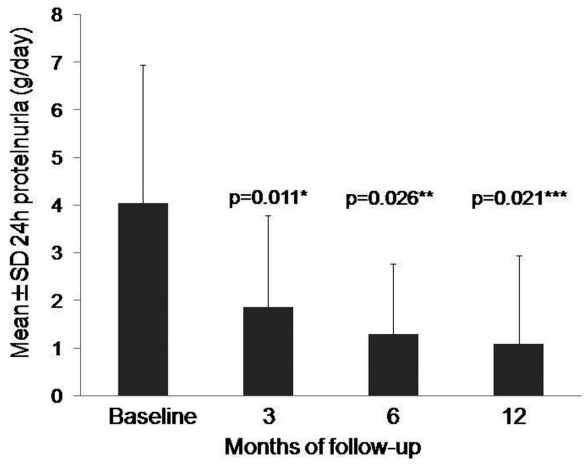

Fig. 3. Mean 24 hours proteinuria at baseline and at 3-, 6- and 12-month follow-up in 68 patients with lupus nephritis treated with RTX (exact values are reported in the text)
*baseline *vs*. 3 months; **baseline *vs*. 6 months; ***baseline *vs*. 12 months.

musculoskeletal (10 patients), neurologic (3 patients), cutaneous (3 patients), and vasculitic (1 patient).

– Second course of RTX

Fifty-nine (40.7%) patients were treated with a second course of RTX. RTX was added to immunosuppressive therapy in 37 out of 59 (62.7%) retreated patients: 16 patients were taking mycophenolate mofetil (27.1%), 9 methotrexate (15.2%), 7 azathioprine (11.9%) and 5 cyclosporine A (8.5%). Antimalarials were used in 19 cases (32.2%). Data on efficacy and safety were available in 45 patients who had at least 6-month follow-up. Mean±SD lag time between the first and second course was 21.3±17.6 months (range 6-84).

CR or PR was observed in 38 patients (84.4%) and CR in 26 patients (57.8%). The mean±SD ECLAM score was 2.68±1.49 at baseline, 1.47±1.33 after 3 months (p<0.001), 1.47±1.38 after 6 months (p<0.001) and 1.39±0.96 after 12 months (p<0.001). Among 20 patients retreated due to renal flare, RCR and RPR was observed in 18 patients (90%) and RCR in 12 patients (60%). The mean±SD 24h-proteinuria was 1.61±1.63 g at baseline, 0.81±0.59 g after 3 months (p=0.011), 0.72±0.41 g after 6 months (p=0.026), and 0.57±0.51 g after 12 months (p=0.021).

• *Flares after the second course of RTX*

After the second RTX course, disease relapse occurred in 12 (31.6%) out of 38 patients who achieved CR or PR, after a mean±SD observational period of 21.8±9.8 months (range 6–36).

• *Third course of RTX*

Eighteen patients (12.4%) were treated with a third course of RTX. Data on efficacy and safety were available in 13 patients who had at least a 6-month follow-up. Mean±SD lag time between second and third course was 16.9±10.8 months (range 6–33). PR and CR was achieved in 11 patients (84.6%) and CR in 9 patients (69.2%).

Safety

AEs were observed in 32 patients (23.9%), who had undergone the first RTX course, including infections in 22 (16.4%) and infusion reactions in 5 patients (3.7%). A severe infection was observed in 8 patients (6%): sepsis in 4, intestinal infection in 2, and pulmonary infection in 2. No differences were found in the frequency of AEs between patients treated or not with immunosuppressant or corticosteroid. However, patients concomitantly treated with intravenous pulse methylprednisolone had more frequently infections (13.6% *vs* 0%; p=0.004) and patients concomitantly treated with cyclosporine A had more frequently sepsis (50% *vs*. 7.9%; p=0.042) than patients who were not treated with these drugs.

AEs were observed in 15 patients (33.3%) after the second RTX course and in 5 (38.5%) after the third course.

Table IV. Safety profile of RTX after first, second, and third course of treatment with RTX.

	First course (%) (134 pts)	Second course (%) (45 pts)	Third course (%) (13 pts)
Adverse events	**32 (23.9)**	**15 (33.3)**	**5 (38.5)**
Severe adverse events	12 (8.9)	3 (6.7)	0
Infusion reaction	**5 (3.7)**	**5 (11.1)**	**2 (15.3)**
Severe infusion reactions	0	0	0
Infections	**22 (16.4)**	**10 (22.2)**	**3 (23.1)**
Urinay tract	4 (3.0)	6 (13.3)	0
Respiratory	6 (4.5)	4 (8.9)	3 (23.1)
Intestinal	3 (2.2)	0 0	
Herpes reactivation	3 (2.2)	1 (2.2)	0
Sepsis	4 (3.0)	0	0
Cutaneous	4 (3.0)	0	0
Cerebral	0	1 (2.2)	0
Severe infections	8 (6.0)	3 (6.7)	0

After the second course, 10 patients (22.2%) had infections and 3 (6,7%) had severe infections, 2 involving high respiratory tract and one the central nervous system. After the third course three patients had mild infections (23.1%); no severe infections were observed. Infusion reactions occurred in 11.1% and 15.3% of patients during the second and third RTX course, respectively.

No severe infusion reactions and no deaths were observed during the three RTX courses. Detailed data on safety are summarised in Table IV.

Discussion

B cell depleting therapy, such as RTX, is currently used in the treatment of a number of systemic autoimmune diseases (8, 21-24). Despite the failure of two RCTs (16, 17), RTX is currently considered a valid therapeutic option for SLE patients, especially in those with refractory or life-threatening manifestations, as suggested by a number of open label studies and registries (9-15, 25-27). Its use in refractory class III/IV glomerulonephritis was also suggested by the European and American experts who elaborated the recommendations for the management of lupus nephritis (20, 28). Although the major goal of registries is to establish drug safety in clinical practice, in the case of RTX, registries give also valuable information on drug efficacy and are the main source of information to support the *off-label* use of RTX in SLE. Our study is based on data collected from the Italian Registry of RTX in SLE, in which safety and efficacy have been evaluated. The registry reflects the therapeutic attitude of Italian physicians involved in SLE management in their daily clinical practice. In Italian Registry only 23% of patients were concomitantly treated with antimalarials; this low rate could be due to the inclusion in the Registry of 68 (50.7%) patients with glomerulonephritis mostly managed in the two Nephrologic Centres. However, the antimalarial use was similar to that reported in the German Registry (12) (Table V).

Disease activity was assessed by ECLAM score, which is a validated and simple tool to use in everyday clinical practice (29). The results of the Italian Registry confirm the good clinical efficacy of RTX in patients with SLE, with and without glomerulonephritis, even in those treated with a second and third course. Notably, in our cohort the concomitant use of mycophenolate mofetil was be associated with a lower risk of disease flare and the concomitant use of cyclophosphamide with a better renal outcome. However, it has to be pointed out that 10 of the 20 patients receiving cyclophosphamide were also treated with an intensive RTX scheme (21). Conversely, the use of methotrexate resulted not to be protective against disease flare in our patients.

The good efficacy of RTX in patients with SLE has been demonstrated in other European observational Registries (11, 12, 14, 15). The French Autoimmunity and Rituximab (AIR) Registry (11) investigated the efficacy of RTX in 113 SLE patients, 40 with biopsy-proven lupus nephritis, with a clinical response observed in 71% of patients. The Spanish Registry (14) evaluated RTX treatment in 107 SLE patients, of whom 77% achieved a clinical response. Likewise, the German Registry for Autoimmune Diseases (GRAID) (12) evaluated 85 SLE patients treated with RTX, reporting a clinical response in 81% of cases. Baseline characteristics of patients in these cohorts were similar. Altogether, data from registries showed a good efficacy and safety profile of RTX in patients with renal and extra-renal SLE manifestations although they are not directly comparable due to differences in disease activity scores used, clinical response criteria, and length of follow-up (Table V).

Notably, in our registry, data on efficacy were assessed after 12 months, as in the Spanish Registry (14), whereas efficacy was evaluated after 6±3 months in the AIR (11) and after a mean of 9.6±7.4 months in the GRAID Registry (12). We also reported ECLAM score, anti-dsDNA titer, 24 h-proteinuria and creatinine serum levels at baseline, and at the 3-, 6-, and 12-month follow-up. Unlike other registries, in the Italian Registry, data on 2nd and 3rd course of RTX were collected and analysed.

Patients achieved a clinical and serological response within three months after RTX infusion and clinical response remained stable during the following months, suggesting a long-term efficacy of RTX. Moreover, the rate of patients achieving CR tended to be higher after the second and third course of RTX suggesting the usefulness of re-treatment in case of disease flare.

Interestingly, one of the confounding factors which mostly contributed to the failure of the two RCTs carried out using RTX in patients with renal (LUNAR) and non-renal (EXPLORER) lupus (16, 17) was the concomitant aggressive immunosuppressive therapy, especially high-dose corticosteroids, which may have masked the efficacy of RTX (8, 30). Long-term use of corticosteroids leads to several complications and increases damage accrual which can, in turn, affect long-term survival in patients with SLE (5, 6, 31-33). In European registries concomi-

Table V. Results of four European Registries on the use of rituximab in patients with refractory lupus.

		Italian Registry	French Registry	German Registry	Spanish Registry
Author, year and reference		Present study	Terrier *et al*., 2010 (11)	Witt *et al*., 2013 (12)	Ramos-Casals *et al*., 2010 (14)
Patients n.		134	113	85	107
Patients with glomerulonephritis n. (%)		66 (50.7)	42 (37.2)	31 (36.5)	49 (45.8)
Disease activity score		ECLAM	SELENA-SLEDAI	SELENA-SLEDAI	–
Age at first infusion (mean± SD, years)		37.3±12.4	39.1±14.4	36.6*	35.9±1.15
Disease duration (mean± SD, years)		9.3±7.3	8.9±6.7	9.8±8.0	–
Response criteria		ECLAM	SELENA-SLEDAI	Physicians' judgement	EULAR/ACR
Follow-up period (months)		12	6±3	9.6±7.4 (mean follow-up)	12
RTX administration				**	
1 mg x 2 infusions		81.4%	60%	–	15%
375 mg x 4 infusions		18.6%	36%	–	85%
Concomitant therapy					
Prednisone		89.7%	92%	92.4%	100%
Immunosuppressant		75.9%	52%	59.5%	60%
Antimalarials		23.4%	53%	29.1%	–
Efficacy response					
Disease activity	complete	45.5%	71%***	46.8%	45%
	partial	40.3%		34.2%	32%
Articular	complete	51.4%	52%	–	78%
	partial	37.2%	20%	–	–
Cutaneous	complete	72.7%	48%	–	33%
	partial	18.2%	23%	–	–
Renal	complete	30.9%	45%	–	79%
	partial	63.2%	29%	–	–
Safety					
Adverse events		23.9%	–	–	17%
Severe adverse events		8.9%	12%	–	–
Infusion reactions		3.7%	11%	21.2%	–
Severe infusion reaction		0%	1.5%	–	2%
Infections		16.4%	–	14.1%	11%
Severe infections		6.0%	9%	4.7%	–
Death		0%	4%	0%	5%

Standard deviation (SD) is not reported; ** The Authors reported the mean dosage of RTX per treatment course (1887±670 mg, median 2000 mg); *** The Authors did not differentiate the rate of complete and partial response.
NA: not available; RTX: Rituximab; ECLAM: European Consensus Lupus Activity Measurement; SELENA-SLEDAI: Safety of Estrogens in Lupus Erythematosus: National Assessment (SELENA) version of the SLE Disease Activity Index (SLEDAI); EULAR: European League against Rheumatism; ACR: American College of Rheumatology.

tant corticosteroids were administered in 92–100% of patients (11, 12, 14). In our cohort, 10.3% of patients were corticosteroids free and few patients received IV pulse corticosteroids, without differences in terms of efficacy between patients who did and did not take corticosteroid therapy. The chance of avoiding the use of corticosteroids has also been suggested by the pioneering study performed by Condon *et al*. (13) on the efficacy of RTX in 50 patients with lupus nephritis treated with RTX and mycophenolate mofetil without oral corticosteroids for maintenance.

The strength of observational studies is the monitoring of the safety in a real-life context, which is in contrast with RCTs where patients are highly selected and cases with concomitant medications or affected with comorbidities are often excluded. This is of particular value for the safety assessment of a drug, especially in an *off-label* setting.

A good safety profile of RTX was found in the Italian Registry, which is similar to that reported in other registries. The rate of infections was 11% in the Spanish Registry (14), 14.1% in the GRAID Registry (12) and 16.4% in Italian Registry. Notably, the lowest rate of infusion reactions was reported in our cohort (Table V), where no severe infusion reactions were observed; two severe infusion reactions occurred in the AIR (11) and in the Spanish Registry (14), and one severe reaction was observed in the GRAID Registry (12). In addition, the Spanish Registry (14) reported five deaths, one due to pneumonia and four due to disease progression. Five deaths were reported in the AIR as well (11): three due to endocarditis, septicaemia, and cholangitis, and two due to disease related causes. No deaths were observed in our and in the GRAID Registry (12).

The percentage of patients who experienced AEs, especially infusion reactions and infections, tended to be higher after the second and third course of RTX than after the first course. The higher rate of infusion reactions was probably due to sensitisation to the drug, similarly to what observed for other biologics. Moreover, the small increase in the risk of infections after RTX retreatment suggests an appropriate tight follow-up in these patients. Notably, in our cohort the use of cyclosporine A, and intravenous pulse methylprednisolone seems to be associated with a higher risk of infections.

In conclusion, in keeping with the results from other European registries, the Italian RTX Registry showed a good efficacy of RTX in the treatment of active, refractory renal and extra-renal SLE. Safety profile was also good; however, in case of retreatment, a higher incidence of AEs, especially infusion reactions and infections should be expected.

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References

1. ZEN M, BASSI N, NALOTTO L *et al*.: Disease activity patterns in a monocentric cohort of SLE patients: a seven-year follow-up study. *Clin Exp Rheumatol* 2012; 30: 856-63.
2. RAHMAN A, ISENBERG DA: Systemic lupus erythematosus. *N Engl J Med* 2008; 358: 929-39.
3. MOSCA M, TANI C, ARINGER M *et al*.: European League Against Rheumatism recommendations for monitoring patients with systemic lupus erythematosus in clinical practice and in observational studies. *Ann Rheum Dis* 2010; 69: 1269-74.
4. BERTSIAS G, IOANNIDIS JP, BOLETIS J *et al*.: Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. EULAR recommendations for the management of systemic lupus erythematosus. Report of a Task Force of the EULAR Standing Committee for International Clinical Studies Including Therapeutics. *Ann Rheum Dis* 2008; 67: 195-205.
5. DORIA A, IACCARINO L, GHIRARDELLO A *et al*.: Long-term prognosis and causes of death in systemic lupus erythematosus. *Am J Med* 2006; 119: 700-6.
6. DORIA A, GATTO M, ZEN M, IACCARINO L, PUNZI L: Optimizing outcome in SLE: treating-to-target and definition of treatment goals. *Autoimmun Rev* 2014; 13: 770-7.
7. GATTO M, ZEN M, GHIRARDELLO A *et al*.: Emerging and critical issues in the pathogenesis of lupus. *Autoimmun Rev* 2013; 12: 523-6.
8. GATTO M, KISS E, NAPARSTEK Y, DORIA A: In-/off-label use of biologic therapy in systemic lupus erythematosus. *BMC Med* 2014 published on 17 February 2014. doi: 10.1186/1741-7015-12-30.
9. LU TY, NG KP, CAMBRIDGE G *et al*.: A retrospective seven-year analysis of the use of B cell depletion therapy in systemic lupus erythematosus at University College London Hospital: the first fifty patients. *Arthritis Rheum* 2009; 61: 482-7.
10. RAMOS-CASALS M, SOTO MJ, CUADRADO MJ, KHAMASHTA MA: Rituximab in systemic lupus erythematosus: a systematic review of off-label use in 188 cases. *Lupus* 2009; 18: 767-76.
11. TERRIER B, AMOURA Z, RAVAUD P *et al*.: Safety and efficacy of rituximab in systemic lupus erythematosus. Results from 136 patients from the French autoimmunity and rituximab registry. *Arthritis Rheum* 2010; 62: 2458-66.
12. WITT M, GRUNKE M, PROFT F *et al*.: German Registry of Autoimmune Diseases (GRAID) Investigators. Clinical outcomes and safety of rituximab treatment for patients with systemic lupus erythematosus (SLE) - results from a nationwide cohort in Germany (GRAID). *Lupus* 2013; 22: 1142-9.
13. CONDON MB, ASHBY D, PEPPER RJ *et al*.: Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids. *Ann Rheum Dis* 2013; 72: 1280-6.
14. RAMOS-CASALS M, GARCÍA-HERNÁNDEZ FJ, DE RAMÓN E *et al*.: BIOGEAS Study Group. Off-label use of rituximab in 196 patients with severe, refractory systemic autoimmune diseases. *Clin Exp Rheumatol* 2010; 28: 468-76.
15. DÍAZ-LAGARES C, CROCA S, SANGLE S *et al*.: UK-BIOGEAS Registry. Efficacy of rituximab in 164 patients with biopsy-proven lupus nephritis: pooled data from European cohorts. *Autoimmun Rev* 2012; 11: 357-64.
16. ROVIN BH, FURIE R, LATINIS K *et al*.: LUNAR Investigator Group. Efficacy and safety of rituximab in patients with active proliferative lupus nephritis: the Lupus Nephritis Assessment with Rituximab study. *Arthritis Rheum* 2012; 64: 1215-26.
17. MERRILL JT, NEUWELT CM, WALLACE DJ *et al*.: Efficacy and safety of rituximab in moderately-to severely active systemic lupus erythematosus: the randomized, double-blind, phase II/III systemic lupus erythematosus evaluation of rituximab trial. *Arthritis Rheum* 2010; 62: 222-33.
18. TAN EM, COHEN AS, FRIES JF *et al*.: The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982; 25: 1271-7.
19. VITALI C, BENCIVELLI W, ISENBERG DA *et al*.: Disease activity in systemic lupus erythematosus: report of the Consensus Study Group of the European Workshop for Rheumatology Research. *Clin Exp Rheumatol* 1992; 10: 541-7.
20. BERTSIAS GK, TEKTONIDOU M, AMOURA Z *et al*.: European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association. Joint European League Against Rheumatism and European Renal Association-European Dialysis and Transplant Association (EULAR/ERA-EDTA) recommendations for the management of adult and paediatric lupus nephritis. *Ann Rheum Dis* 2012; 71: 1771-82.
21. ROCCATELLO D, SCIASCIA S, ROSSI D *et al*.: Intensive short-term treatment with rituximab, cyclophosphamide and methylprednisolone pulses induces remission in severe cases of SLE with nephritis and avoids further immunosuppressive maintenance therapy. *Nephrol Dial Transplant* 2011; 26: 3987-92.
22. ENGEL P, GÓMEZ-PUERTA JA, RAMOS-CASALS M, LOZANO F, BOSCH X: Therapeutic targeting of B cells for rheumatic autoimmune diseases. *Pharmacol Rev* 2011; 63: 127-56.
23. FERRI C, CACOUB P, MAZZARO C *et al*.: Treatment with rituximab in patients with mixed cryoglobulinemia syndrome: Results of multicenter cohort study and review of the literature. *Autoimmun Rev* 2011; 11: 48-55.
24. NALOTTO L, IACCARINO L, ZEN M *et al*.: Rituximab in refractory idiopathic inflammatory myopathies and antisynthetase syndrome: personal experience and review of the literature. *Immunol Res* 2013; 56: 362-70.
25. WEIDENBUSCH M, ROMMELE C, SCHROTTLE A, ANDERS HJ: Beyond the LUNAR trial. Efficacy of rituximab in refractory lupus nephritis. *Nephrol Dial Transplant* 2013; 28: 106-11.
26. REDDY V, JAYNE D, CLOSE D, ISENBERG D: B-cell depletion in SLE: clinical and trial experience with rituximab and ocrelizumab and implications for study design. *Arthritis Res Ther* 2013; 15 (Suppl. 1): S2.
27. FERNANDEZ-NEBRO A, DE LA FUENTE JM, CARRENO L *et al*.: Multicenter longitudinal study of B-lymphocyte depletion in refractory systemic lupus erythematosus: The LES-IMAB study. *Lupus* 2011; 21: 1063-76.
28. HAHN BH, MCMAHON MA, WILKINSON A *et al*.: American College of Rheumatology guidelines for screening, treatment, and management of lupus nephritis. *Arthritis Care Res* 2012; 64: 797-808.
29. ROMERO-DIAZ J, ISENBERG D, RAMSEY-GOLDMAN R: Measures of adult systemic lupus erythematosus. *Arthritis Care Res* 2011; 63: S37-S46.
30. ISENBERG DA: Rituximab – it was the best of times, it was the worst of times. *Autoimmun Rev* 2012; 11: 790-1.
31. SEGURO LPC, ROSARIO C, SHOENFELD Y: Long-term complications of past glucocorticoid use. *Autoimmun Rev* 2013; 12: 629-32.
32. LUIJTEN RKMAC, FRITSCH-STORK RD, BIJLSMA RWJ, DERKSEN RHWM: The use of glucocorticoids in Systemic Lupus Erythematosus. After 60 years still more an art than science. *Autoimmun Rev* 2013; 12: 617-28.
33. ZEN M, CANOVA M, CAMPANA C *et al*.: The kaleidoscope of glucorticoid effects on immune system. *Autoimmun Rev* 2011; 10: 305-10.