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Original article

Rituximab *vs* mycophenolate and *vs* cyclophosphamide pulses for induction therapy of active lupus nephritis: a clinical observational study

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Abstract

Objective. We report the first comparison between rituximab (RTX) and either MMF or CYC pulses in the treatment of active LN.

Methods. Fifty-four patients with active LN received three methylprednisolone pulses for 3 consecutive days followed by oral prednisone and RTX 1 g at days 3 and 18 (17 patients) or MMF 2–2.5 g/day (17 patients) or six CYC pulses (0.5 g every fortnight) (20 patients). At 4 months MMF, AZA or ciclosporin were associated to prednisone as a consolidation/maintenance therapy in all groups. The outcomes of the three groups were compared at 3 and 12 months.

Results. Patients in the RTX group were older, had a longer duration of SLE and LN, had more renal flares, had higher activity and had higher chronicity indexes at renal biopsy than the other two groups. Four patients in each group had acute renal dysfunction and ~50% had nephrotic syndrome. At 3 months, proteinuria was reduced by 50% in 58.8% of patients on RTX, in 64.7% on MMF and in 63.1% on CYC. At 12 months, complete remission was present in 70.6% of patients on RTX, in 52.9% on MMF, and in 65% on CYC. Partial remission was reached in 29.4% on RTX, 41.2% on MMF, and 25% on CYC.

Conclusion. RTX seems to be at least as effective as MMF and CYC pulses in inducing remission. Considering that patients treated with RTX had more negative renal prognostic factors, this drug should be considered a viable alternative for the treatment of active LN.

Key words: systemic lupus erythematosus, lupus nephritis, rituximab therapy, cyclophosphamide pulse therapy, mycophenolate therapy.

CLINICAL SCIENCE

Introduction

LN is one of the major complications of SLE and is associated with a high rate of morbidity and mortality. The current recommended induction treatment for severe forms of LN is a combination of corticosteroids, i.v. methylprednisolone pulses (MPPs) followed by high-dose prednisone, associated with CYC, either administered intravenously or orally, or mycophenolate salts (MMF) [1]. A number of meta-analyses of randomized controlled trials (RCTs) that compared CYC with MMF in patients with biopsy-proven proliferative LN showed that MMF is as effective as CYC in achieving remission. The risk of amenorrhoea, leucopenia and alopecia is lower, while the risk of diarrhoea is higher with MMF than CYC [2–4]. However, few data are available regarding long-term results of MMF therapy [5] and of MMF treatment in severe forms of LN [6, 7]. Rituximab (RTX), an anti-CD20 monoclonal antibody, has emerged as a novel therapeutic alternative for SLE patients. An RCT of RTX [the Lupus Nephritis Assessment with Rituximab (LUNAR) study] [8] failed to show any additional effect of RTX as an

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add-on therapy to the steroid–MMF combination for LN type III/IV in incident patients. Instead, two reviews of non-controlled studies [9, 10] and two large multicentre retrospective studies [11, 12] reported that RTX obtained complete or partial remission at 12 months in 67–74% of patients with diseases refractory to standard therapy or with a renal flare of LN. A renal recurrence occurred in ~30% of patients within 2–24 months after administration of the drug [13]. However, with few exceptions [14–16], in the majority of non-controlled studies RTX was administered together with i.v. CYC [17–20] or while continuing the ongoing immunosuppression [21–23], and for this reason the real efficacy and toxicity of RTX in comparison with conventional therapy is not known.

In this clinical observational study we compared for the first time the efficacy on renal and extra-renal manifestations and the toxicity of induction therapy with RTX alone *vs* CYC pulses and *vs* MMF in patients with active LN.

Patients and methods

From 2005 to January 2011, 54 patients with active LN (47 females, 7 males) followed in two Italian renal units (Ospedale Maggiore Ca' Granda and Ospedale San Carlo Borromeo, Milano) entered this open prospective study. Fifty patients were Caucasian, two were Asian and two were Hispanic. At first presentation, all patients fit the diagnosis of SLE according to the ACR criteria [24]. The mean age at diagnosis of LN was 31.4 years (S.D. 11.3). SLE was diagnosed at 49.6 months (S.D. 64.1) before the diagnosis of LN. Three patients were not submitted to renal biopsy for severe thrombocytopenia, while in the other patients renal biopsy showed class III in 9, class IV in 26, class V in 2, class III + V in 4 and class IV + V in 10 according to the International Society of Nephrology (ISN)/Renal Pathology Society (RPS) classification [25]. Twenty-seven patients entered the study at the diagnosis of LN, the other 22 patients at the diagnosis of a new flare of LN and 5 patients for refractory renal disease. Ten of these patients have previously been reported [16].

Endpoints of the study

Primary endpoints

The primary endpoints were renal response at 3 months and complete renal remission at 12 months.

Secondary endpoints

The secondary endpoints were response of clinical and biochemical extra-renal parameters and side effects.

Definitions

(i) Renal response at 3 months: improvement of serum creatinine if impaired at baseline and reduction of 50% of proteinuria and of microscopic haematuria.
(ii) Complete renal remission at 12 months: serum creatinine <1.2 mg/dl (or return to the baseline value in patients with chronic renal dysfunction) and proteinuria <0.5 g/24 h and <5 urinary erythrocytes (UE)/high power field (HPF).
(iii) Partial renal remission at 12 months: serum creatinine <1.2 mg/dl (or return to the baseline value in patients with chronic renal dysfunction) and proteinuria of 0.5–2 g/day.
(iv) No response: no improvement in proteinuria or in serum creatinine if impaired at baseline.
(v) Acute renal dysfunction: increase of serum creatinine to >1.2 mg/dl and creatinine clearance <75 ml/min.
(vi) Chronic renal insufficiency: doubling of plasma creatinine lasting for at least 6 months with a value of plasma creatinine >2 mg/dl and creatinine clearance <40 ml/min without any improvement over time.

Methods

Global disease activity was evaluated using the SLEDAI [26].

Statistical methods

For comparison of variables at baseline and at follow-up Student's *t*-test was used for normally distributed parameters, the non-parametric Mann–Whitney test was used for non-normally distributed parameters and the chi-square test was used for dichotomized variables.

Therapeutic schedules

At enrolment, all specific therapies were stopped with the exception of HCQ and the patients received one i.v. MPP (0.5 g for body weight <50 kg, 1 g for weight >50 kg) for 3 consecutive days followed by prednisone 0.5–0.75 mg/kg/day for 1 month, then progressively tapered at the discretion of the clinicians; and RTX 1 g i.v. at the end of the third infusion of MPP and at day 18 (patients received standard premedication with antihistamine and paracetamol); or MMF 2–2.5 g/day or six i.v. CYC pulses of 0.5 each, one every fortnight [27].

At the beginning of the fourth month, as maintenance therapy, in addition to prednisone patients received AZA 1–2 mg/kg/day or MMF 1–2 g/day or ciclosporin 1–2 mg/kg/day. The kind of treatment was chosen on the basis of patient choice or the physician's clinical judgment. Written consent according to the Declaration of Helsinki was obtained from all subjects. Approval from the local ethics committee was not sought because therapy regimens were in compliance with standards currently applied in Italy and because of the observational nature of the study.

Results

Characteristics of the three groups of patients at the beginning of the study

Seventeen patients (15 females, 2 males) were treated with RTX, 17 patients (15 females and 2 male) with MMF and 20 patients (17 females and 3 males) with CYC pulses. Three patients on RTX, 10 on MMF, and 14 on CYC pulses entered the study at diagnosis of LN. The duration of SLE before the diagnosis of LN was

Table 1 Comparison between characteristics of patients treated with RTX, MMF and CYC

	MMF ($n = 17$)	RTX ($n = 17$)	CYC ($n = 20$)	*P*-value RTX *vs* MMF	*P*-value RTX *vs* CYC
Sex, F/M	15/2	15/2	17/3	0.6	0.8
Age at diagnosis of LN, mean (S.D.), years	32.35 (17.75)	31.09 (9.48)	31.0 (8.34)	0.8	0.97
Age at the beginning of the study, mean (S.D.), years	34.76 (15.2	38.4 (6.9)	32.05 (8.43)	0.37	0.02
Duration of SLE, mean (S.D.), years	6.96 (5.82)	12.82 (6.3)	5.26 (6.8)	0.008	0.001
Duration of LN, mean (S.D.), years	3.1 (3.73)	7.1 (4.54)	1.62 (4.7)	0.008	0.000
Previous therapy, no therapy/P alone/P + AZA/ P + CYC/P + MMF/P + CsA/P + MTX, *n*	5/4/4/6/0/0/2	1/0/8/10/8/7/2	7/4/5/3/4/1/1	0.01	0.007
Number of flares, mean (S.D.)	0.82 (1.19)	2.5 (1.5)	0.2 (0.41)	0.01	0.000
Therapy at enrolment, no therapy/P alone/P + AZA/ P + MMF/P + CsA, *n*	6/7/3/0/0	1/5/3/5/3	8/7/2/3/0	0.018	0.05
Class III/IV/V/V + III/V + IV, *n*	4/7/1/2/0 (3 NA)	1/11/1/0/4	4/8/0/2/6	0.07	0.2
Activity index, mean (S.D.)	4.4 (3.41)	7.25 (2.7)	6.9 (2.43)	0.037	0.68
Chronicity index, mean (S.D.)	0.9 (0.99)	1.6 (1.7)	0.8 (1.1)	0.15	0.05
Serum creatinine, mean (S.D.), mg/dl	1.2 (0.99)	1.08 (0.8)	0.94 (0.34)	0.7	0.5
GFR, mean (S.D.), ml/min	88.4 (40.5)	89.75 (35.9)	95.25 (38.3)	0.9	0.65
Proteinuria, mean (S.D.), g/day	3.5 (2.9)	4.5 (2.9)	3.28 (2.2)	0.3	0.15
Urinary red blood cells, mean (S.D.)	33 (46.5)	44.7 (44.4)	40.9 (25.6)	0.45	0.7
Albuminaemia, mean (S.D.), g/dl	3 (0.81)	2.85 (0.32)	3.2 (1.05)	0.48	0.19
Haemoglobin, mean (S.D.), g/dl	11.2 (2.0)	11.8 (1.58)	11.0 (2.2)	0.33	0.2
C3, mean (S.D.), mg/dl	63.5 (28.6)	63.3 (28.2)	61.4 (29.6)	0.98	0.8
C4, mean (S.D.), mg/dl	10.3 (6.7)	8.9 (6.4)	8.8 (5.4)	0.5	0.9
Anti-DNA antibodies, mean (S.D.), U/ml	168 (140.1)	216.6 (122.0)	184.5 (134.3)	0.3	0.4
SLEDAI, mean (S.D.)	15.1 (6.45)	14.1 (4.53)	18.35 (4.8)	0.6	0.01
P after MPP, mean (S.D.), mg/day	39.9 (14.6)	26.3 (6.5)	37.7 (12)	0.01	0.001
P after MPP, mean (S.D.), mg/kg/day	0.66 (0.28)	0.42 (0.1)	0.62 (0.21)	0.002	0.001

F/M: females/males; P: prednisone; CsA: ciclosporin A; GFR: glomerular filtration rate; MPP: methylprednisolone pulse.

4.62 years (S.D. 6.51, median 1.52). Eleven patients on RTX, seven on MMF and four in the CYC group entered the study at diagnosis of a new renal flare. Before the renal flare, 4 of the 11 patients of the RTX group were in complete remission, 6 in partial remission and 1 with stable chronic renal insufficiency. Of the seven patients in the MMF group, two were in complete remission, three in partial remission and two had stable chronic renal insufficiency (serum creatinine 2.1 and 2.6 mg/dl and proteinuria 0.23 and 0.36 g/day, respectively). Of the four in the CYC group, three were in complete remission and one in partial remission. Three patients in the RTX group and two in the CYC group entered the study for refractory renal disease. Before entering the study, all these patients were receiving treatment with low-dose steroids associated with AZA in six, MMF in seven, ciclosporin in three and MTX in one.

In comparison to patients treated with MMF, patients who received RTX had a significantly longer duration of SLE [12.82 years (S.D. 6.3) *vs* 6.96 (5.82), $P = 0.008$] and LN [7.1 years (S.D. 4.54) *vs* 3.1 (3.73), $P = 0.008$], had a significantly higher number of renal flares before entering the study [2.5 (S.D. 1.5) *vs* 0.82 (1.19), $P = 0.01$] and had a higher activity index at renal biopsy [7.25 (S.D. 2.7) *vs* 4.4 (3.41), $P = 0.037$] (Table 1).

Compared with patients treated with CYC pulses, patients who received RTX were significantly older when entering this study [38.4 years (S.D. 6.9) *vs* 32.05 (8.43), $P = 0.02$], had significantly longer duration of SLE (12.82 years (S.D. 6.3) *vs* 5.26 (6.8), $P = 0.001$] and LN [7.1 years (S.D. 4.54) *vs* 1.62 (4.7), $P = 0.000$], had a significantly greater number of renal flares before entering the study [2.5 (S.D. 1.5) *vs* 0.2 (0.41), $P = 0.000$] and had a higher chronicity index at renal biopsy [1.6 (S.D. 1.7) *vs* 0.8 (1.1), $P = 0.05$] (Table 1).

At the beginning of the study there were no significant differences in the mean value of serum creatinine, proteinuria, number of red blood cells at urinary sediment score between patients treated with RTX and those treated with MMF and those with CYC pulses, although the mean value of proteinuria was higher and the mean value of serum albumin was lower in the RTX group compared with the other groups. Four patients on RTX (23.5%) had acute renal dysfunction (in one superimposed with pre-existing chronic renal insufficiency) compared with four on MMF (23.5%) (in two superimposed with pre-exsisting chronic renal insufficiency) and three in the CYC group (15%). Nephrotic syndrome was present in 10 patients on RTX (59%) in 8 on MMF (47%) and in 8 in the CYC group (40%) (*P*-value not significant).

There were no significant differences between RTX and MMF and between RTX and the CYC group in the percentage of patients with albumin <3.5 g/dl (93% *vs* 64.7% and *vs* 61.1%, *P*-value not significant), in C3 <90 mg/dl

TABLE 2 Comparison of prednisone dosages in patients treated with RTX, MMF and CYC

Prednisone, mg/day	Baseline	1 month	2 months	3 months	6 months	12 months
RTX	26.3 (6.5)	21.7 (7.7)	20.7 (5.8)	17.9 (6.8)	13.2 (3.7)	8.8 (2.7)
MMF	36.9 (14.6)	27.1 (10.3)	22.2 (10.3)	17.2 (6.4)	12.4 (4.4)	8.01 (4.3)
CYC	37.7 + 12	30.9 + 9.9	26.2 + 10.9	17.5 + 8.45	11.03 + 6.13	8.5 + 7.9
P: (RTX *vs* MMF)	0.01	0.09	0.6	0.7	0.5	0.5
P: (RTX *vs* CYC)	0.001	0.008	0.08	0.8	0.2	0.9

Values are given as mean (S.D.).

(76.4% *vs* 76.4% and *vs* 78.9%, *P*-value not significant), in C4 <15 mg/dl (76.4% *vs* 58.8% and *vs* 78.9%, *P*-value not significant) and in haemoglobin <12 g/dl (47% *vs* 64.7% and *vs* 70.9%, *P*-value not significant).

After MPP the mean basal dosage of prednisone was significantly lower in patients on RTX [26.3 mg/day (S.D. 6.5) and 0.42 mg/kg/day (S.D. 0.1)] than on MMF [39.9 mg/day (S.D. 14.6), $P = 0.01$ and 0.66 mg/kg/day (S.D. 0.2), $P = 0.002$] and in the CYC group [37.7 mg/day (S.D. 120.0), $P = 0.001$ and 0.62 mg/kg/day (S.D. 0.21), $P = 0.001$]. The dosage of prednisone continued to be lower in the RTX group than in the MMF and CYC groups until the third month. From the 3rd month to the 12th month the mean dosage of oral prednisone was not different among the three groups (Table 2).

Outcome of the three groups

At 3 and 12 months significant improvement in almost all the parameters evaluated was observed in all groups (Tables 3 and 4). At 3 months, renal response occurred in 10 patients (58.8%) on RTX, in 11 (64.7%) on MMF and in 12 of 19 (63.1%) in the CYC group (*P*-value not significant).

At the beginning of the fourth month MMF was given to 73.3% of patients in the RTX group and to 53% in the CYC group, AZA was given to 13.3% in the RTX group and to 35% in the CYC group and ciclosporin A was given to 13.3% in the RTX group and 11.8% in the CYC group. All patients in the MMF group continued the same therapy.

At 12 months, 12 patients on RTX (70.6%) were in complete remission, compared with 9 (52.9%) on MMF and 13 (65%) in the CYC group. Partial remission was observed in five patients on RTX (29.4%), seven (41.1%) on MMF and five (25%) in the CYC group. One patient on MMF (5.9%) and two in the CYC group (10%) had no response. At 12 months, serum albumin was <3.5 g/dl in none of the patients on RTX, in three on MMF and in two in the CYC group. C3 was <90 mg/dl and C4 <15 mg/dl, respectively, in 9 and 8 patients on RTX, in 8 and 3 on MMF and in 10 and 12 in the CYC group (*P*-value not significant). Anti-DNA antibodies continued to be positive in nine patients on RTX, four on MMF and eight in the CYC group.

The SLEDAI score progressively decreased in all groups without differences between groups. Table 4 reports the response to therapy of the extra-renal manifestations of SLE in the three groups at baseline, 3 and 12 months. Skin and joint manifestations progressively improved in all groups while haematological manifestations, in particular anaemia, persisted in one third of patients in each group. No patients died or developed renal or extra-renal flares during the observation period.

Immunological parameters and histological classes at renal biopsy as predictors of response/remission in the RTX group

Anti-DNA antibodies were positive at the baseline in 15 of 17 patients treated with RTX. Of these, 60% achieved response at 3 months, compared with neither of the two patients with negative anti-DNA antibodies. At 12 months, 73% of the patients with positive anti-DNA antibodies were in complete remission, compared with 50% of those with negative anti-DNA antibodies at baseline. The differences were not significant; however, the mean values of anti-DNA antibodies at baseline in patients who achieved remission tended to be higher [243.9 (S.D. 125.6)] compared with those of patients who did not achieve remission [125.2 (S.D. 86.1), $P = 0.1$]. Thirteen of 17 patients (76.5%) treated with RTX had low complement fractions (C3 and/or C4) at baseline. Of these, nine achieved response at 3 months (69%), compared with one of the four (25%) patients with normal complement. At 12 months, 10 of the 13 patients (77%) with low complement were in complete remission, compared with two of the four (50%) with normal complement. The differences were not significant.

No significant differences emerged in response/remission to RTX of the different histological classes at renal biopsy. Of the 11 patients in class IV, 72% achieved response at 3 months and 82% achieved complete remission at 12 months, while of the four patients in class IV + V, only one (25%) achieved response at 3 months and two (50%) entered complete remission at 12 months. The only patient in class III did not achieve response at 3 months and achieved partial remission at 12 months. Instead, the patient in class V achieved a response at 3 months and then achieved complete remission at 12 months.

Side effects

In the RTX group, one patient had an allergic reaction at the second infusion, two patients had a mild infection (flu syndrome and gastroenteritis) a few weeks after RTX infusion, one patient had otitis and a gluteal abscess and another patient complained of diffuse pruritus for some

Table 3 Outcome of biochemical, immunological and urinary tests in patients treated with RTX, MMF or CYC

	RTX				MMF				CYC pulses			
	Baseline	3 months	12 months	*P*-value baseline *vs* 12 months	Baseline	3 months	12 months	*P*-value baseline *vs* 12 months	Baseline	3 months	12 months	*P*-value baseline *vs* 12 months
Prednisone, mg/day	26.3 (6.5)	17.9 (6.8)	8.8 (2.7)	0.000	36.9 (14.6)	17.2 (6.4)	8.01 (4.31)	0.000	37.7 (12)	17.5 (8.45)	8.51 (5.2)	0.000
SLEDAI	14.1 (4.53)	7.8 (4.1)	5.3 (4.0)	0.000	15.1 (6.45)	5.7 (5.3)	5.4 (6.46)	0.000	18.3 (4.8)	8.3 (3.2)	7.9 (7.1)	0.000
Serum creatinine, mg/dl	1.08 (0.8)	1.04 (0.7)	0.97 (0.6)	0.6	1.2 (0.99)	0.95 (0.65)	0.97 (0.7)	0.4	0.94 (0.34)	0.9 (0.3)	0.87 (0.3)	0.5
Proteinuria, g/day	4.5 (2.9)	1.9 (1.4)	0.77 (0.8)	0.000	3.5 (2.9)	1.38 (1.1)	0.79 (0.91)	0.000	3.28 (2.2)	1.65 (1.7)	0.72 (0.76)	0.000
UE/number of HPF	44.7 (44.4)	19.5 (33.1)	7.8 (15.9)	0.007	33 (46.5)	25.3 (36.1)	9.43 (15.4)	0.056	40.9 (25.6)	22.8 (24.1)	15.1 (13.8)	0.000
Serum albumin, g/dl	2.85 (0.3)	3.4 (0.4)	3.96 (0.3)	0.000	3.0 (0.81)	3.68 (0.5)	3.96 (0.6)	0.000	3.2 (1.05)	3.6 (0.6)	3.9 (0.4)	0.01
Haemoglobin, g/l	11.8 (1.6)	12.8 (1.5)	12.8 (1.5)	0.7	11.2 (2.0)	12.2 (1.7)	12.4 (1.9)	0.08	11.0 (2.2)	12.5 (1.6)	12.5 (1.6)	0.02
C3, mg/dl	63.3 (28.2)	82 (20)	83.2 (22)	0.03	65.3 (28.6)	86.8 (20.2)	83.9 (21.2)	0.04	61.4 (29.6)	85.1 (24.1)	88 (25.1)	0.005
C4, mg/dl	8.9 (6.4)	13.9 (5.5)	14.2 (6.3)	0.04	10.3 (6.7)	16.1 (5.7)	15.6 (7)	0.01	8.8 (5.4)	13.9 (5.6)	14.6 (6)	0.003
Anti-DNA antibodies, U/ml	217 (122)	71.1 (59.2)	69.3 (45.6)	0.000	168 (140.1)	45.8 (69.8)	57.3 (68)	0.03	184.5 (134)	107.7 (124)	102 (111)	0.07

Values are given as mean (s.d.). *P*-value between baseline and 3 months <0.05 for all parameters with the exception of serum creatinine as well as UE and haemoglobin in the RTX group, UE and haemoglobin in MMF group and serum albumin and anti-DNA antibodies in CYC group. UE: urinary erythrocytes; HPF: high power field.

Table 4 Response of the extra-renal manifestations of SLE in patients treated with RTX, MMF or CYC

	RTX			MMF			CYC		
	Baseline	3 months	12 months	Baseline	3 months	12 months	Baseline	3 months	12 months
Skin involvement	5 (29.4)	3 (17.64)	0	1 (5.88)	1 (5.88)	0	5 (25)	3 (15)	2 (10)
Joint involvement	7 (41.1)	2 (11.76)	2 (11.76)	8 (47)	3 (17.64)	0	4 (20)	0	1 (5)
Neurological manifestations	0	0	0	1 (5.88)	0	1 (5.88)	1 (5)	0	0
Fever	2 (11.76)	0	0	3 (17.64)	1 (5.88)	0	3 (15)	0	0
Serositis	0	0	0	1 (5.88)	0	0	3 (15)	0	0
Anaemia	8 (47)	5 (29.4)	5 (29.4)	11 (64.7)	8 (47)	5 (29.4)	14 (70)	7 (35)	7 (35)
Leucopenia	1 (5.88)	1 (5.88)	0	3 (17.64)	1 (5.88)	0	4 (20)	0	2 (10)
Thrombocytopenia	0	0	0	1 (5.88)	0	0	1 (5)	0	0

Values are given as number of patients (%).

Table 5 Clinical status at the last observation of patients treated with RTX, MMF or CYC

	MMF	RTX	CYC
Follow-up beyond month 12, mean (S.D.)	32.3 (36.1)	30.52 (21.1)	51.8 (30.6)
Serum creatinine, mean (S.D.), mg/dl	0.98 (0.76)	1 (0.8)	0.99 (0.45)
Proteinuria, mean (S.D.), g/day	0.4 (0.31)	0.6 (0.66)	0.8 (0.75)
Proteinuria >0.5 g/day, *n* (%)	2 (11.8)	2 (11.8)	5 (25)
Chronic renal insufficiency, *n* (%)	2 (11.8)[a]	1 (5.9)[a]	1 (5)[b]
End-stage renal disease, *n* (%)	0	0	0
Extra-renal flares, *n* (%)	0	1 (5.9)	4 (20)
Proteinuric flares, *n* (%)	1 (5.9)	1 (5.9)	7 (35)[c]
Nephritic flares, *n* (%)	0	0	1 (5)
Months from the end of the study to flares	4	6	Mean 22 (S.D. 10.2), range 8–36

Follow-up beyond month 12: RTX *vs* MMF, $P = 0.8$; RTX *vs* CYC, $P = 0.03$; MMF *vs* CYC, $P = 0.09$. No other significant differences were found between groups for the other variables of the table. [a]Patients with renal insufficiency at the beginning of the study. [b]Patient who developed renal insufficiency during the study. [c]One flare was due to non-compliance with therapy.

weeks. In the MMF group, three patients developed diarrhoea (in one associated with leucopenia) requiring reduction of the MMF dosage, one patient developed pneumonia, acute rhinitis and diarrhoea, and another patient developed varicella zoster. In the CYC group, two patients developed leucopenia, one patient developed severe anaemia requiring blood transfusion, one patient developed acute gastroenteritis, one patient had two episodes of urinary sepsis and two patients had herpes zoster. Three patients developed amenorrhoea, transient in one patient and persistent in the other two patients.

Outcome of the patients beyond month 12

Follow-up beyond 12 months (Table 5) was shorter in patients who received RTX compared with those treated with CYC (30.5 *vs* 51.8 months, $P = 0.03$), and not different from those of patients treated with MMF (30.5 *vs* 32.3, $P = 0.8$). At last observation there were no significant differences among the three groups in the mean values of serum creatinine and proteinuria. However, the number of patients who developed flares was higher in the CYC group than in the other two groups. No patients entered end-stage renal disease and no patients died.

Discussion

Based on the available data on the treatment of LN, RTX emerged as ineffective in prospective trials [8, 28] but beneficial in clinical practice [10]. In fact, the LUNAR trial [8] failed to demonstrate the efficacy of RTX as an add-on therapy to steroids and MMF in incident LN patients. Instead, a recent pooled analysis [11] of 164 patients with LN who received RTX in European centres for diseases refractory to standard therapy or for renal flares documented complete response in 30% and partial response in 37% of patients at 12 months. A higher rate of response was achieved in patients with class III (81%) and mixed forms (75%) compared with classes IV (63%) and V (65%). Nephrotic syndrome and renal dysfunction have been reported to be predictive of poor response to RTX therapy [11, 22]. Moreover, recent studies suggest that the concomitant use of RTX and CYC may not provide additional benefit to RTX alone [29, 30]. RTX emerged as a steroid sparing agent in two studies [23, 31]. In another prospective cohort of patients who received a regimen based on RTX and MMF without steroids the majority of patients achieved renal remission [32]. Histological improvement at repeated renal biopsy in terms of a significant reduction of the activity index was reported by some authors [30, 33, 34]. However, the efficacy of RTX not associated with other immunosuppressive drugs has never been compared with that of CYC pulses and MMF, drugs that are considered to be the standard of care for the treatment of severe forms of this disease. In this observational study we present the results of the first comparison of RTX *vs* MMF and *vs* CYC pulses in the treatment of active LN. We used the RA regimen for RTX because it seems to be equally effective as the haematological schedule and because it implies only two hospital admissions. All patients received MMP before RTX, MMF and CYC pulses. After 3 months a maintenance therapy was added in all three groups with the aim of consolidating the response and preventing the well-documented recurrences of the disease after RTX therapy [13]. As maintenance treatment we employed MMF or AZA or ciclosporin, as all these drugs appeared to be equally effective in maintenance therapy, at least in European patients [35, 36]. The majority of patients treated with RTX entered the study at the diagnosis of a new renal flare while the majority of patients on MMF and in the CYC group entered the study at the diagnosis of LN. As a consequence, the durations of SLE and LN were longer in the RTX group than in the other groups. In addition, patients treated with RTX had other negative prognostic factors: they were older and had a higher chronicity index than those who received CYC, and had a higher activity index than patients treated with MMF. At the beginning of the study there was a trend of higher proteinuria and lower serum albumin in the RTX group compared with the other groups.

Three months after the beginning of the induction therapy, renal response occurred with the same frequency in all groups. Of note, these results were achieved with a lower dosage of prednisone during the first 3 months in the RTX group compared with those in the CYC and MMF groups. At 12 months, clinical renal remission (complete or partial) was achieved in all patients treated with RTX and in all but one patient in the MMF group and two in the CYC group. However, due to the lack of control renal biopsies, we cannot exclude persistent histological activity in these patients.

In addition, a significant and comparable improvement in all the other clinical and biochemical parameters evaluated was documented in all groups with the exception of haematological abnormalities, which persisted in around one third of patients in each group.

Side effects seem to be more frequent in the CYC group than in the RTX and MMF groups. Patients have been followed 2.5 to 4 years (mean of 40 months) since the end of the study. At last observation, the mean serum creatinine was in the normal range and the mean proteinuria was <1 g/day in all groups. Patients treated with CYC developed more renal and extra-renal flares than the other two groups. This negative result could be due to the longer follow-up of these patients compared with those in the RTX and MMF groups. Due to the small number of patients included in the RTX group, we were unable to demonstrate a different outcome of the histological classes at renal biopsy as well as a different response to therapy of patients with low or normal complement fractions and in those with positive or negative anti-DNA antibodies.

Our study has some limitations, in particular, the sample size was small, it was not a randomized trial and the decision to assign the patient to one or another group was based on clinical judgment, in particular, patients that have received one or more courses of CYC or MMF entered the RTX group. For all these reasons, our results need to be confirmed in larger randomized trials. However, the results are quite encouraging and seem to indicate that RTX is at least as effective as MMF 2–2.5 g/day and 3 g CYC pulses in inducing remission in the majority of patients with active LN. The addition of a maintenance therapy at the beginning of the fourth month consolidated the results and achieved the goal of complete remission in the majority of patients. Similar results have been shown in RCTs in systemic vasculitis [37], where RTX has been shown to be equivalent to or even more effective than CYC, particularly in recurrent forms. This is particularly important in situations where CYC avoidance is desirable, such as in young patients, to preserve fertility, if previously treated with CYC, in patients intolerant to MMF or CYC and in refractory cases.

Rheumatology key messages

- Rituximab (RTX) seems to be as effective as standard treatment in active LN.
- RTX might induce LN remission with a lower dosage of corticosteroids.

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