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Multicenter longitudinal study of B-lymphocyte depletion in refractory systemic lupus erythematosus: the LESIMAB study

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Keyword:	Systemic Lupus Erythematosus, Rituximab, Safety
Abstract:	Objective This study aimed to investigate the effectiveness and safety of single and repeated courses of rituximab in patients with refractory lupus. Methods LESIMAB is a multicenter, retrospective, longitudinal study of lupus patients who have failed to standard therapy and have been treated with rituximab. Response rates at 6 months and at follow-up were defined as efficacy outcomes. Complete response was defined as a SELENA-SLEDAI score of 0 and a SELENA-SLEDAI Flare Index of 0. Partial response was defined as a reduction in the SELENA-SLEDAI score of ≥4 points with no new or worsening of symptoms. The incidence and severity of adverse events were collected. Results Seventy-three (62.9%) of 116 patients achieved a response at 6 months (complete in 10 and partial in 63). Ninety-seven (77.6%) of 128 patients achieved a response after a mean follow-up of 20.0 ± 15.2 months (complete in 35 and partial in 62). High baseline SLEDAI score, previous treatment with ≥100 mg/day prednisone, and no history of severe hematologic flare were associated with response after the first treatment course. The median time to response was 6.5 months (95% CI, 5.0–8.0). Thirty-seven patients (38.1%) relapsed after the first infusion. The flare was severe in 7 cases and mild to moderate in 29 cases. Serious infection rate was 12.6/100 patient-years. Six patients died; 2 of infection and 4 of lupus complications. Conclusion Rituximab can be an effective treatment option for refractory lupus with severe or life-threatening disease with acceptable tolerance profile.

TITLE

Multicenter longitudinal study of B-lymphocyte depletion in refractory systemic lupus erythematosus: the LESIMAB study

RUNNING FOOTLINE:

LESIMAB Study

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ABSTRACT

Objective: This study aimed to investigate the effectiveness and safety of single and repeated courses of rituximab in patients with refractory lupus.

Methods: LESIMAB is a multicenter, retrospective, longitudinal study of lupus patients who have not responded to standard therapy and have been treated with rituximab. Response rates at 6 months and at follow-up were defined as efficacy outcomes. Complete response was defined as a SELENA-SLEDAI score ≤2 and a SELENA-SLEDAI Flare Index of 0. Partial response was defined as a reduction in the SELENA-SLEDAI score of ≥4 points with no new or worsening of symptoms. Adverse events were collected.

Results: Seventy-three (62.9%) of 116 patients achieved a response at 6 months (complete in 22 and partial in 51). Ninety-seven (77.6%) of 128 patients achieved a response after a mean follow-up of 20.0 ± 15.2 months (complete in 50 and partial in 47). High baseline SLEDAI score, previous treatment with ≥100 mg/day prednisone, and no history of severe hematologic flare were associated with response after the first

treatment course. The median time to response was 6.5 months (95% CI, 5.0–8.0). Thirty-seven patients (38.1%) relapsed after the first infusion. The flare was severe in 7 cases and mild to moderate in 29 cases. Serious infection rate was 12.6/100 patient-years. Four weekly doses schedule was associated with more serious infections. Six patients died; 2 of infection and 4 of lupus complications.

Conclusion: Rituximab can be an effective treatment option for refractory lupus with severe or life-threatening disease with acceptable tolerance profile.

KEYWORDS

Systematic Lupus Erythematosus, Rituximab, Effectiveness treatment, Safety, Biological therapy, Infection.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, multisystem inflammatory disease with a wide range of clinical manifestations and characterized by the presence of auto-antibodies directed against a number of self-antigens.

In recent years, the 10-year survival rate for SLE patients has significantly improved (1), but the long-term prognosis remains poor in patients with severe SLE because they tend to suffer more complications and are exposed to more intensive immunosuppression (2). Corticosteroids and immunosuppressant drugs have traditionally been used to treat moderate to severe cases of SLE, but a substantial subset of patients either fail to achieve optimal disease control or develop severe toxicity.

Rationale for T and B cells as therapeutic targets in SLE is supported by a well documented pathogenic role of autoreactive T cells and polyclonally activated B cells in lupus manifestations (3). Rituximab is a chimeric monoclonal antibody that selectively binds the CD20 receptor and induces B-cell depletion (4). Only two controlled randomized trials of rituximab in SLE have been published (5-7). These studies detected positive signals for this treatment, but failed to detect significant differences in the primary outcomes between the active and control arms as groups. Since these results were published, many questions have been raised about the methods employed in these studies, especially regarding the bias of exclusion of severe organ manifestations. Although previous observational studies have investigated the effects of rituximab on SLE, the analysis of sizable cohorts of patients treated for clinical need is still important to improve our understanding of the potential benefit of Rituximab in SLE as used in clinical practice. The aim of this study was to investigate the effectiveness and safety of

single and repeated courses of rituximab in patients with severe refractory SLE in the clinical setting.

PATIENTS AND METHODS

Study design

LESIMAB is a multicenter, retrospective, longitudinal, observational study of SLE patients who have failed to respond to conventional therapy and have been treated with rituximab. This study was designed by the Systemic Autoimmune Diseases Working Group of the Spanish Society of Rheumatology (SADWG-SSR). Nineteen rheumatology units at Spanish university centers with substantial experience in the management of SLE agreed to participate in this study.

Patients

Patients were diagnosed with SLE according to the revised American College of Rheumatology (ACR) criteria (8), who had active disease despite ongoing corticosteroid and/or immunosuppressive treatment, and completed at least 2 evaluations related to anti-CD20 infusions were eligible for the study. The Ethics and Clinical Research Committee of the Carlos Haya University Hospital approved the study as a central approval requirement, and all participants provided local Committee agreement and written informed consent from recruited patients.

Variables and Effectiveness criteria

The primary outcome for effectiveness was the rate of either complete or partial response at 6 ± 3 months after the first course of rituximab. A course was defined as a

complete set of infusions (2 doses of 1,000 mg rituximab given 14 days apart or 4 weekly doses of 375 mg/m^2 rituximab) administered in each cycle of treatment.

The secondary outcomes included the response rate at the end of follow-up (1 or repeated courses), rate of flare (mild to moderate and severe flare) at the end of follow-up, time to achieve the best response (complete or partial) at the end of follow-up, time to relapse after remission, and change from baseline of clinical and laboratory variables associated with lupus activity.

Complete response was defined as a Safety of Estrogens in Lupus Erythematosus National Assessment–SLEDAI (SELENA-SLEDAI) score of 2 points or less and a modified SELENA-SLEDAI Flare Index (SFI) score of 0. Partial response was defined by a reduction of at least 4 points in the SELENA-SLEDAI score with no new or worsening symptoms as measured by the SELENA-SLEDAI-SFI.

Protocol and clinical assessment

Rituximab (Mabthera®, Roche) was added to the patients' standard treatment, including premedication, according to local protocols (113 received iv. 6-Methyl-prednisolone plus Histamine-H1-antagonists or acetaminophen, and the rest received only H1-antagonists with or without acetaminophen). A follow-up of at least 10 weeks after the first course was mandatory to be included for the effectiveness analysis after the first rituximab course, but those patients who died during this period were included too. However, all patients who received at least 1 infusion of rituximab were considered in both effectiveness and safety intention-to-treat analysis at follow-up, even if they had a follow-up of less than 10 weeks. The last observation carried forward approach was used for missing data.

Baseline data were collected on lupus manifestations, previous treatment, and other associated medical problems, including the Charlson-age comorbidity index (9). A successful B-cell depletion (BCD) defined as a fall in the absolute CD19 count to $<0.005 \times 10^9$/l. Disease activity and relapses were scored using the SELENA-SLEDAI and modified SFI (10, 11). A revised SELENA flare tool that excluded the physician's global assessment component was used to define flare. The SLICC/ACR Damage Index (SDI) was used to quantify the degree of accrued irreversible organ damage (12). Physical function was measured using the Spanish version of the HAQ (13). Antiphospholipid syndrome was classified according to the Sydney revision of the Sapporo criteria (14). Lupus severity was classified using previously described criteria (15). Kidney biopsies were classified according to the International Society of Nephrology/Renal Pathology Society 2003 classification (16).

To minimize possible errors in data collection, a standard protocol was used, and all participating rheumatologists carefully discussed the protocol variables. Data collection was recorded only by the rheumatologists involved. All the investigators were familiarized with the use of disease activity indexes as most of them had been involved in clinical trials and/or trained in specific programs for the use of the SELENA-SLEDAI, SFI, and SDI provided by the SADWG-SSR. Data collection was transferred to the computerized database and on-line monitoring process was employed to analyze consistency of recorded data.

Safety assessment

Adverse events (AEs) were classified using the Medical Dictionary for Regulatory Activities Terminology (17). The type and severity of resulting AEs and their association with rituximab were evaluated. Serious AEs, infusion-related AEs, and

infection-related AEs were summarized independently. An infection was considered serious if it resulted in death, was life-threatening, required inpatient or prolonged existing hospitalization, caused persistent or significant disability/incapacity, induced a congenital anomaly/birth defect, or required intravenous antibiotic treatment.

Statistical analysis

Endpoint analyses were performed *per protocol* on the eligible sample (n = 128), and on the intention-to-treat sample, defined as all patients ever treated (n = 131). Normal distribution of variables was checked using the Kolmogórov–Smirnov test. Paired 2-sample t-test or Wilcoxon's signed-rank test was used to analyze the effect of rituximab after both the first treatment course and follow-up assessments. The McNemar test was used for the comparison of correlated proportions. Comparisons between groups were performed with χ^2 test, Student's t-test, or Mann–Whitney test, as appropriate.

Kaplan–Meier curves were used to estimate the time to best response and time to flare. The data used to calculate these metrics were those for the lowest and highest SELENA-SLEDAI scores, respectively. Univariate and bivariate logistic regression analyses were used to identify baseline factors related to response rates, severe AEs, severe infections, and mortality. Statistical analyses were performed using Stata 10.0 software (Stata Corp. USA).

RESULTS

Baseline patient characteristics

One hundred and thirty-one patients with refractory lupus were treated with at least 1 infusion of rituximab from November 18, 2003 to February 5, 2009. This sample accounts for 7.9% (range 1.5 to 9.4) of 1,659 patients followed from 19 hospitals. Three

patients were excluded for lack of a follow-up visit at the time the study was closed. Table 1 shows the baseline characteristics of the 128 selected SLE patients. Most of them were white Caucasians (92.2%), while 5.5% were Hispanics and 2.3% responded to other ethnicity. They displayed disproportionate age-related comorbidity burden and showed a Charlson-age comorbidity index of 2.2 ± 1.4 (mean ± S.D) and previous serious infections in 12 patients (9.4%), including Nocardia pneumonia, miliary tuberculosis, staphylococcal osteomyelitis, staphylococcal arthritis, or sepsis. All patients had a serious disease according to the number of ACR criteria fulfilled, the number of severely involved organs, an unusually high frequency of antiphospholipid syndrome, and the number of lupus related drugs previously taken. Kidney biopsies, according to the International Society of Nephrology/Renal Pathology Society 2003 classification, were available for 55 of 63 patients, which included class II (n = 4), class III (n = 12), class IV (n = 32), class V (n = 6), and classes IV + V (n = 1). All patients were undergoing steroid treatment and displayed clinically significant disease activity despite the use of at least 2 immunosuppressive drugs.

Drug regimens

Most of the patients were treated with 2 doses of 1,000 mg rituximab given 14 days apart or 4 weekly doses of 375 mg/m^2 rituximab (Table 2). Sixty-nine patients received repeated courses of rituximab: 30 patients (22.9%), 2 cycles; 22 patients (16.8%), 3 cycles; 11 patients (8.4%), 4 cycles; 4 patients (3.1%), 5 cycles; 1 patient (0.8%), 7 cycles; and 1 patient (0.8%), 9 cycles. Thirty-one patients received a new cycle for clinical need, while the rest were treated on an every 6 month scheduled program.

All but 2 patients received rituximab in combination with moderate to high doses of glucocorticoids (mean dose ± SD, 0.5 ± 0.8 mg/kg/day). In 32 patients (24.4%),

rituximab was given in monotherapy (n = 16) or in combination with hydroxychloroquine (n = 16) without any other immunosuppressant. In 99 (75.5%), rituximab was concomitantly used with intravenous cyclophosphamide boluses (n = 32, 0,750 mg/m^2), azathioprine (n = 25; mean dose ± SD, 108.0 ± 31.2 mg/day), methotrexate (n = 18; mean dose, 16.7 ± 9.4 mg/week), mycophenolate mophetile (n = 15), and other (n = 9).

Indications of rituximab

As shown in Table 3, most SLE patients had active disease at baseline despite treatment with moderate to high doses of corticosteroids; 75 patients (58.6%) had high or very high SLEDAI scores. Although most patients had multi-organ lupus flares, characterized by a wide range of combinations of arthritis, nephritis, and cutaneous rashes, the primary indications for rituximab were: nephritis (n = 42), arthritis (n = 27), thrombocytopenia (n = 17), neurologic manifestations (n = 11), serious general involvement (n=10), cutaneous lupus (n = 8), serious pulmonary complications (n = 5), hemolytic anemia (n = 3), and others (n = 5).

Clinical response after the first course of rituximab

One hundred and twelve patients completed a median follow-up time of 26.7 weeks (range, 11.1–42.1) after the first course of rituximab. Four patients who died before completing the minimum 10-week follow-up were also included in the analysis (n = 116).

A total of 73 SLE patients (62.9%; 95% CI, 49.3–79.1) achieved a response after the first course of rituximab; 22 patients (19.6%; 95% CI, 12.3–29.7) showed a complete response and 51 patients (45.5%; 95% CI, 36.1–55.2) a partial response. The best

clinical responses were observed in patients with arthritis (81.5%), cutaneous involvement (87.5%), nephritis (65.8%), neuropsychiatric lupus (73%), thrombocytopenia (65%), or severe generalized flare (40%).

After the first course of rituximab 21/31 (68%) patients achieved a successful B-cell depletion (CD19 count to $<0.005 \times 10^9$/l).

Furthermore, all of the clinical and laboratory baseline variables improved with rituximab treatment (Tables 3 and 4). Reductions in proteinuria and recovery in both C3 and C4 complement fraction levels were also observed. Anti-dsDNA antibody tests became negative in 49 out of 66 patients tested. Noticeably, a moderate but significant reduction in antiphospholipid antibodies was observed. Moreover, improvements were reported in physical function, SELENA-SLEDAI score, and all SFI signs and criteria. Steroid dosage requirement decreased to a mean final dose of one third compared to baseline requirements.

Effectiveness of rituximab during follow-up

Endpoint analyses were performed *per protocol* on the eligible sample (n = 128). Nonetheless, analyses based on the intention-to-treat sample, defined as all patients ever treated (n = 131), were also performed and showed similar results (data not shown). Then, 128 patients had at least one post-baseline measurement of effectiveness after a mean follow-up of 20.0 ± 15.2 months (range, 0.46–69.9) and a total follow-up of 213.46 patient-years. The clinical and biological benefits from rituximab therapy prolonged until the end of follow-up after a mean time period of 2.0 ± 1.3 rituximab cycles. As measured at the last visit, the mean dose of steroids was noticeably lower than baseline and dropped significantly in responders than in non-responders (8.5 ± 8.4

vs. 16.5 ± 17.4 mg/day; $p = 0.007$). In addition, tests for anti-dsDNA antibodies turned to were negative in 49 of the 66 patients.

One hundred and twenty-five (95.4%) patients had sufficient data to evaluate the response rate at the end of follow-up and the record from just 3 patients did not provide enough data to calculate a SELENA-SLEDAI score at the last visit. Ninety-seven patients (77.6%; 95% CI, 62.9–94.7) showed a response, with 50 patients (40.1%; 95% CI, 32.2–50.2) showing a complete response and 47 patients (38.5%; 95% CI, 29.8–47.8) showing a partial response. The median time to achieve the best response was 6.5 months (95% CI, 5.0–8.0) (Figure 1A). Thirty-seven patients (38.1%; 95% CI, 26.8–52.6) relapsed after a median of 10.8 months (95% CI, 2.8–18.7) following administration of the first course of rituximab (Figure 1B). Seven patients (7.2%; 95% CI, 2.9–1.5) had severe flares, whereas 29 patients (29.9%; 95% CI, 20.0–42.9) had mild or moderate flares, as measured by the SFI.

The best clinical responses at the end of follow-up were observed in patients with arthritis (93%), cutaneous (87.5%), nephritis (82.5%), neuropsychiatric lupus (73%), thrombocytopenia (65%), or severe generalized flare (62.5%).

Factors associated with effectiveness

As shown in Table 5, patients who were treated concomitantly with cyclophosphamide were younger, had shorter disease duration, and received lower daily doses of steroids compared with the other patients. Noticeably, they had more severe disease status at baseline when cumulative ACR criteria and SELENA-SLEDAI were considered. However, this schedule did not improve the clinical response or minimise relapses and was associated with more AEs.

Compared with 2 doses of 1,000 mg rituximab given 14 days apart, 4 weekly doses of 375 mg/m^2 rituximab was most frequently used in patients with fever (7.1% vs. 23.7%; p = 0.015), thrombocytopenia (11.8% vs. 26.3%; p = 0.043), and lupus nephritis (29.4% vs. 44.7%; p = 0.098). However, this schedule did not yield better clinical responses or fewer relapses but it was associated with more AEs (see below).

On the other hand, patients who were concomitantly treated with hydroxychloroquine exhibited less cumulative ACR criteria (1.8 ± 1.2 vs. 2.2 ± 1.4; p = 0.046), and lower SDI scores (1.3 ± 1.8 vs. 2.0 ± 2.1; p = 0.046). They had higher baseline SELENA-SLEDAI scores (16.7 ± 10.5 vs. 12.3 ± 8.4; p = 0.008), especially cutaneous lupus activity (33.9% vs. 16.7%; p = 0.002), and higher baseline numbers of mild to moderate SFI criteria (1.5 ± 0.9 vs. 1.2 ± 0.9; p = 0.030).

A number of baseline variables were associated with clinical response after the first course of rituximab, as shown by the covariates presented in Table 6. For instance, each one-point increase in the SELENA-SLEDAI score was associated with a 10% increase in the probability of response. Higher response rates were observed among patients who had at some time taken steroid doses higher than 100 mg/day, whereas patients with a previous serious hematologic disorder were 83% less likely to achieve a response.

In the multivariate logistic regression analysis at the end of follow-up, previous treatment with immunoglobulin [HR = 0.3 (95% CI, 0.1–0.9); p = 0.003], higher SLEDAI scores [HR = 1.1 (95% CI, 1.0 –1.2); p = 0.012], concomitant treatment with prednisolone plus cyclophosphamide bolus [HR = 0.1 (95% CI, 0.0–0.6); p = 0.010], and concomitant treatment with immunosuppressive agents other than cyclophosphamide [HR 3.5 (95% CI, 1.2–10.0); p = 0.022] were entered into the model.

Safety of rituximab

Fifty-one patients (38.9%) treated with at least 1 infusion of rituximab had a total of 90 AEs, and almost half of these AEs were serious (Table 7). The most common AEs were infections (22.0/100 patient-years) and infusion-associated reactions (9.3/100 patient-years). Most of the infections were serious, whereas most of the infusion-associated reactions were mild to moderate. Regarding the other AEs a great majority could not be directly related to treatment. Seventy-two AEs (84.7%) required treatment, 38 AEs (44.7%) caused hospitalization, 13 of which (15.2%) needed treatment in intensive care units.

Severe infections presented early in the study (before the 40th week), although an increase in frequency could also be observed later (after the 80th week) (Figure 2). Airways were the most frequent site of infection (3.7/100 patient-years), including pneumonia (2.8/100 patient-years), followed by herpes zoster infections (3.2/100 patient-years) and urinary tract infections (2.3/100 patient-years), including pyelonephritis (1.4/100 patient-years). Twenty-seven of 47 infectious events required intravenous antibiotics, and microorganisms were identified in 26 cases. Gram-negative bacteria were isolated in 9 cases (*E. coli*, 7 cases; *P. aeruginosa*, 1 case; *K. pneumoniae*, 1 case), Gram-positive bacteria in 5 (*Staphylococcus*, 3 cases; *L. monocytogenes*, 1 case; *Streptococcus*, 1 case), virus in 10 (6 cases of herpes zoster virus, 2 cases of HHV-1, and 2 cases of HPV), and fungi in 2 cases (*Candida* sp. and *Aspergillus*).

Six patients died; 2 fatalities were due to infection (i.e., macrophage-activated syndrome in the context of invasive aspergillosis and gangrene) and 4 complications were related to SLE (i.e., multi-organ lupus activity, acute alveolar hemorrhage, pulmonary hypertension, and acute respiratory distress syndrome).

Factors associated with severe adverse events

The patients who used 4 weekly doses of 375 mg/m^2 rituximab had a high instance of AEs compared with 2 doses of 1 g. (figure 3): total AEs 64.9/100 person-years (IRR 1.8; 95% CI 1.3 to 3.0; p = 0.002), total infections 42.3/100 person-years (IRR 3.1; 95% CI 1.7 to 5.9; p <0.001), serious infections 25.7/100 person-years (IRR 3.6; 95% CI 1.5 to 8.7; p = 0.001), sepsis 15.1/100 person-years; 95% CI 1.5 to 23.0; p = 0.003), and infections that required intensive care 7.5/100 person-years (IRR 5.3; 95% CI 1.2 to 498.4; p = 0.016).

The baseline risk factors for severe AEs, severe infections, and mortality are shown in Table 8. As determined by the multivariate analyses, the age-adjusted Charlson comorbidity index and previous treatment with a prednisolone bolus were independent risk factors for any severe AEs. Interestingly, the number of severely affected organ systems, high baseline leukocyte count, and 4 doses of rituximab were identified as risk factors for severe infection. Mortality was associated with the SDI, the number of severe SFI criteria that were met, and co-treatment with prednisolone plus cyclophosphamide bolus.

DISCUSSION

Rituximab has been widely used in patients with severe SLE to avoid potentially serious toxicities or due to the unsatisfactory response of refractory disease to immunosuppressive agents (7, 18-30). Rituximab is frequently utilized although it does not have official approval and furthermore 2 clinical trials have found insufficient efficacy for its treatment of lupus (5-7, 18). For clinical needs, the present study provides new data that rituximab can result in a high response rate in patients with refractory SLE after 10 weeks, as assessed with SELENA-SLEDAI.

The cohort analyzed here is quite sizable and could be informative enough given the severity of disease that has been treated in our sample. This great burden in our patients explains the high proportion of previous serious infections, antiphospholipid syndrome, failed drugs, resistant nephritis, etc. In fact, these patients account for less than 10% of SLE-patients treated in 19 hospitals.

We attempted to limit the bias inherent in observational studies by using a consensus protocol, monitoring data consistency, and ensuring that participant rheumatologists with a great experience in SLE have had training in specific data collection.

Rituximab was indicated when antimalarials, corticosteroids, and immunosuppressant drugs failed to control disease in patients with lupus-flare. The severity profile of the patients in this study is quite similar to previous case series, but their disease status is more severe than those in the EXPLORER (5, 6, 18) and LUNAR (7) clinical trials. Unlike the patients in the aforementioned trials, our patients achieved a first response rate of 63%, which increased to 78% by the end of the study and was accompanied by a notable decrease in steroid requirements. Overall, previous studies found that rituximab resulted in reductions in SLEDAI scores of 38–70% (24, 26, 28, 31-34), although more modest responses were observed in studies that treated patients with only mild SLE activity (33, 34). This observation was previously reported (35) and it is interesting in that it partially explains why poorer responses have been observed in clinical trials. In fact, our study found that the probability of a positive response increased by 10% for each one-point increase in baseline SELENA-SLEDAI score and was 7 times higher in patients who had at some time required very high steroid doses. Indeed, it is not surprising that a refractory disease that has failed to multiple therapies may respond poorly to rituximab. In agreement with this observation, 37.5% of patients in the present study, who had a history of severe hematologic complications that had required steroids,

danazol, immunoglobulins, or even a splenectomy, were 83% less likely to respond to rituximab.

The mean time to achieve a defined response was 3–4 months, whereas several individual parameters improved more rapidly. In our cohort, the lowest SELENA-SLEDAI scores were achieved at nearly 6 months, and patients were relapse-free for a mean of 10.8 months. These results are consistent with those of other studies (19, 23, 32, 36-39). Only 38% of responders experienced a flare (mostly mild) after the final rituximab infusion. Although a few predictors of relapse have been identified by others, including positive anti-ENA antibodies or low C3 levels (29), our study identified none. Due to retrospective collection data, the absence of a central laboratory could lead to a poorer correlation between assays and likely explain why we failed to confirm this.

In terms of efficacy and safety, and derived from its use off-label, no consensus in the dose and administration regimen of rituximab has been established for LES, and no direct comparison of 2 intravenous infusions of rituximab at 1 g versus 4 infusions of rituximab at 375 mg has been undertaken (35). Different administration schedules could be a limitation of the present study, but the analysis of this variable showed no influence in the response rate. However, regarding safety, the regimen of 4 doses of rituximab at 375 mg/m^2 was associated with a 5-fold elevated risk of serious infection compared to 2 doses of 1g in the multivariate analyze. If confirmed this important new finding could have practical impact in the management of these patients.

There are also no rules about monotherapy or combined treatment with rituximab, particularly whether cyclophosphamide or other immunosuppressant agents should be used. One study indicated that adding cyclophosphamide to rituximab does not offer additional benefits in the treatment of lupus nephritis at 48 weeks (24). In our study,

cyclophosphamide was added in patients with more severe disease status and higher baseline activity, suggesting that their rheumatologists were confident with this drug for severe lupus. Although this may be a selection bias, cyclophosphamide showed no greater benefit, and data about its combined effects have been contradictory (32, 34, 37, 40, 41). In fact, we observed that our patients concomitantly treated with other immunosuppressant drugs were 3.5 times more likely to achieve a response by the end of study. By contrast, the combination of cyclophosphamide plus steroid i.v. bolus was associated with lower efficacy at the end of the study and higher mortality, but this result may also reflect a selection bias of a more refractory disease.

Concerning re-treatment, the time regimen schedules in previous studies are also flexible but limited information is available (21, 26, 36, 38, 42, 43). In this regard, our results provide relevant information regarding this issue: 53% of patients were re-treated (1 patient even received 9 repeated courses), but less than one-third of patients received treatment every 6 months. Unlike in rheumatoid arthritis, the repeated cycle of B-cell depletion in SLE patients every 6 months would eliminate the transitional and naive B cells that typically emerge after profound depletion in SLE and could delay a late phase of improvement (44). This process could be another reason for the failure of previous clinical trials (5-7, 18) and may explain why the response rate improved over time in our patients.

Current data show a relative safety profile for rituximab in treatment of SLE patients. Our results confirm that infusion-associated AEs are mild and similar to those observed in rheumatoid arthritis patients. However, severe infections were more frequent than those observed in SLE patients in the AIR registry (26). Although the authors observed a severe infection rate of just 6.6/100 patient-years, similar to that observed for anti-TNF drugs in the treatment of RA (45, 46), they observed one additional fatal infection

and used different criteria for severe infection criteria. In our study, it is unclear whether infections observed can be attributed to rituximab given the study design and the high rate of serious infections in the group prior to rituximab. The inclusion of patients with severe lupus, even those with severely low complement levels or leukopenia and those with a large previous cytotoxic background may represent additional confounding factors. Another factor that may contribute to this high rate of infections was that having treated the patients with B cell depletion, the concomitant immunosuppressive drugs were continued in 75% of our cases. Furthermore, most risk factors for total AEs, serious infections, and mortality were linked to comorbidity and lupus severity. Accordingly, the only risk factors we have identified for serious infections that could be related to rituximab were the 4 doses of rituximab regimen as treatment with cyclophosphamide plus corticosteroid bolus might be reflecting the high risk conferred by both drugs in severely ill patients. In fact, 10% of our patients had received biologic therapy with either TNF antagonist or epratuzumab after failure of conventional treatment as some of our centers have reported benefits for this treatment (47). In our opinion, all these factors could have contributed to the higher rate of serious infections compared with other studies.

In conclusion, the LESIMAB study has demonstrated that rituximab is an effective choice in selected patients refractory to standard treatment. However, close monitoring for infections is mandatory in patients with multiple organ systems disease activity, high leukocyte counts, and a dosing schedule of 4 doses of 375-mg rituximab.

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Table 1. Baseline characteristics of 128 refractory SLE patients treated with at least one course of rituximab.

Variable	Patients
Age, mean yr ± SD	38.2 ± 12.1
Female, n (%)	115 (89.8)
Disease duration from SLE diagnosis, mean yr ± S.D.	7.3 ± 6.5
Cumulated ACR criteria, mean ± S.D.	6.5 ± 1.6
Antiphospholipid syndrome, n (%) [a]	30 (23.4)
Background serious involved organ/system, mean ± S.D. [b]	2.0 ± 1.3
Musculoskeletal, n (%)	42 (32.8)
Cutaneous-mucosal, n (%)	25 (19.5)
Kidney, n (%)	63 (49.2)
Haematological, n (%)	48 (37.5)
Nervous system, n (%)	27 (21.0)
Heart, n (%)	18 (13.7)
Pulmonary, n (%)	22 (17.1)
Other, n (%) [c]	18 (14.1)
Previous treatments, mean ± S.D.	5.3 ± 1.7
Chloroquine/Hydroxychloroquine, n (%)	113 (88.3)
Corticosteroids, n (%)	128 (100)
High dose intravenous Methylprednisolone, n (%)	75 (55.7)
Azathioprine, n (%)	88 (68.7)
Cyclophosphamide (bolus), n (%)	66 (51.5)
Mycophenolate mofetil, n (%)	54 (42.2)

Methotrexate, n (%)	48 (37.5)
Anticoagulants, n (%)	35 (27.3)
Intravenous immunoglobulin, n (%)	30 (23.4)
Anti-TNF drugs, n (%)	12 (9.4)
Cyclosporine A, n (%)	10 (7.8)
Leflunomide, n (%)	9 (7.0)
Splenectomy, n (%)	6 (4.7)
Tacrolimus, n (%)	4 (3.1)
Danazol, n (%)	4 (3.1)
Epratuzumab, n (%)	2 (1.6)
Mercaptopurine, n (%)	1 (0.8)

[a] Sydney revision of Sapporo criteria

[b] The severity of lupus was classified using the following criteria (17): Slight, when either in the past or in the present, no important organs, such as the kidneys, central nervous system, heart, or lungs, had been affected; Moderate, when only one of these organs had been or was currently affected; and Severe, when two or more of these organs had been affected or aggressive therapy had been required for complications, such as more than 50 mg of prednisone daily, or pulse therapy with steroids or cyclophosphamide boluses.

[c] Constitutional, liver, vascular, or ophthalmic.

S.D., Standard deviation.

Table 2. - Rituximab and concomitant treatment regimes in 128 refractory SLE patients.

Rituximab, n (%)	
1 g x 2	83 (64.8)
0.375 g/m^2 x 4	38 (29.7)
Others	7 (5.5)
Concomitant treatment, n (%)	
Prednisone	126 (98.4)
Cyclophosphamide i.v.	32 (24.0)
Coupled to rituximab infusions	27 (21.1)
Monthly	5 (3.9)
Prednisolone (bolus)	23 (17.0)
Prednisolone (bolus) plus Cyclophosphamide (bolus)	10 (7.8)
Azathioprine, mg/day	25 (19.5)
Methotrexate, mg/week	18 (14.1)
Mycophenolate mophetile	15 (11.7)
Other immunosuppressant agent	9 (7.0)
Hydroxychloroquine, mg/day	58 (45.3)

Table 3. Effectiveness at 6 ± 3 months after the first course (n=116[a]).

	Baseline	First course	*p* Value
HAQ (0–3), mean ± SD	1.253 ± 0.689	0.674 ± 0.556	p<0.001
SDI (0–49), mean ± SD	1.7 ± 2.1	1.8 ± 2.2	p=0.007
SELENA-SLEDAI (0–105), mean ± SD	14.6 ± 10.0	4.8 ± 4.5	p<0.001
SFI signs and criteria			
No. of mild/moderate SFI criteria, mean ± SD	1.3 ± 0.9	0.78 ± 0.06	p<0.001
No. of severe SFI criteria, mean ± SD	2.1 ± 1.3	0.37 ± 0.76	p<0.001
Prednisolone (mg/day), mean ± SD [b]	32.4 ± 57.3	11.7 ± 11.9	p<0.001
Rash, n (%) [c]	31 (24.2)	8 (7.0)	p=0.001
Nasopharyngeal ulcers, n (%)	10 (7.8)	3 (2.6)	p=0.109
Pleuritis, n (%)	8 (6.3)	1 (1.0)	p=0.031
Pericarditis, n (%)	7 (5.5)	2 (1.7)	p=0.031
Arthritis, n (%)	54 (42.2)	4 (3.5)	p<0.001
Fever (lupus), n (%)	17 (13.3)	3 (2.6)	p=0.001
Neuropsychiatric (lupus), n (%)	7 (5.5)	1 (0.9)	p=0.031
Systemic vasculitis, n (%)	6 (4.7)	2 (1.7)	p=0.063
Nephropathy, n (%)	44 (34.4)	9.0 (7.9)	p<0.001
Platelets <60 (10^9/L), n (%)	21 (16.4)	3 (2.6)	p<0.001
Haemolytic anaemia, n (%)	6 (4.7)	1 (1.0)	p=0.125
New NSAID or HCQ for SLE, n (%)	21 (16.4)	11 (9.6)	p=0.049
New immunosuppressant for SLE, n (%)	40 (31.3)	22 (19.3)	p=0.026
Hospitalisation for SLE, n (%)	62 (48.4)	17 (14.9)	p<0.001

SLE, Systemic lupus erythematosus; HAQ, Health Assessment Questionnaire; SDI, Systemic lupus international collaborating clinics American College of Rheumatology damage index; SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment-SLE disease activity index; NSAID, Non-steroidal anti-inflammatory drugs; HCQ, hydroxychloroquine; SFI, SELENA-SLEDAI Flare Index.

[a] Patients who completed at least 10 week of follow-up and patients who died before completing the minimum 10-week follow-up.

[b]Prednisone equivalent.

[c]Including cutaneous vasculitis.

Table 4. Laboratory parameters at 6 ± 3 months after the first course (n=116[a]).

	Baseline	First course	*p* Value
Leukocytes (10^9/L), mean ± SD	6.1 ± 3.2	6.4 ± 3.6	p=0.396
Neutrophils (10^9/L), mean ± SD	4.1 ± 2.6	4.1 ± 3.1	p=0.776
Lymphocytes (10^9/L), mean ± SD	1.3 ± 0.8	1.5 ± 0.8	p=0.010
Hemoglobin (g/dl), mean ± SD	11.5 ± 2.1	12.5 ± 1.7	p<0.001
Platelets (10^9/L), mean ± SD	217.9 ± 119.5	238.7 ± 93.4	p=0.024
ESR (mm), mean ± SD	34.5 ± 24.7	20.2 ± 16.0	p<0.001
CRP (mg/l), mean ± SD	12.7 ± 3	5.9 ± 10.1	p<0.001
Serum albumin (g/dl), mean ± SD	4.4 ± 5.4	4.1 ± 0.5	p<0.001
Serum creatinine (mg/dl), mean ± SD	1.0 ± 1.1	0.9 ± 0.4	p=0.123
Proteinuria (g/day), mean ± SD	1.6 ± 2.4	0.8 ± 1.2	p=0.005
C3 (mg/dl), mean ± SD	74.3 ± 30.8	92.6 ± 30.6	p<0.001
C4 (mg/dl), mean ± SD	11.6 ± 7.1	16.6 ± 8.8	p<0.001
Anti-dsDNA+, n (%)	66 (65.3)	41 (52.6)	p=0.019
aCL IgG, mean ± SD	34.0 ± 59.0	22.6 ± 48.4	p=0.025
aCL IgM, mean ± SD	14.2 ± 21.4	13.2 ± 24.8	p=0.218
Anti-β2-GPI IgG, mean ± SD	25.2 ± 62.9	12.4 ± 20.8	p=0.867
Anti-β2-GPI IgM, mean ± SD	9.1 ± 15.4	9.6 ± 14.3	p=0.601
Lupus anticoagulant, n (%)	27 (31.4)	13 (21.3)	p=0.008

ESR, Erythrocyte sedimentation rate; CRP, C-reactive protein; C3, C3 fraction of complement; C4, C4 fraction of complement; Anti-dsDNA, anti-double stranded-DNA antibodies; aCL, anti-cardiolipin antibodies; Anti-β2-GPI, Anti-β2-glycoprotein I antibodies;

[a] Patients who completed at least 10 week of follow-up plus the patients who died before completing the minimum 10-week follow-up.

Table 5.- Differences in baseline profile between lupus patients treated with and without concomitant cyclophosphamide.

	With cyclophosphamide	Without cyclophosphamide	P value
Age-incidence (yrs), mean ± SD	33.1 ± 9.8	40.3 ± 129.4	0.001
Disease duration (yrs), mean ± SD	5.2 ± 4.3	8.1 ± 6.9	0.049[a]
No. of ACR criteria, mean ± SD	7.2 ± 1.5	6.3 ± 1.6	0.003 [a]
Previous highest dose of corticosteroids (mg/day)[b], mean ± SD	74.0 ± 44.6	115.9 ± 180.8	0.036 [a]
Pleuritis, n (%)	5 (15.2)	4 (4.1)	0.044[c]
Pericarditis, n (%)	5 (15.2)	2 (2.0)	0.011 [c]
Nephritis, n (%)	16 (48.5)	29 (29.6)	0.048
SELENA-SLEDAI (0–105), mean ± SD	17.7 ± 9.7	13.2 ± 9.4	0.012 [a]
Disease flares			
SFI, mild/moderate, n (%)	0	12 (12.2)	0.037 [c]
SFI, severe, n (%)	33 (100)	84 (85.7)	0.021 [c]
No. of severe SFI criteria, mean ± SD	2.7 ± 1.1	1.9 ± 1.3	0.001 [a]

Cyclophosphamide was given intravenously (0,750 g/m^2)

SELENA-SLEDAI, Safety of Estrogens in Lupus Erythematosus National Assessment-SLE disease activity index; SFI, SELENA-SLEDAI Flare Index.

[a] Mann–Whitney U test.

[b] It refers to the highest dose ever used from the lupus diagnostic

[c] Fisher exact test.

Table 5. Uni- and multivariate logistic regression analysis of 116 lupus patients, treated with at least one course of rituximab, after the first course of treatment (6 ± 3 months). Dependent variable: complete or partial remission.

	Univariate		Multivariate [a]	
	p-value	OR (95% CI)	p-value	OR (95% CI)
Previous treatment with immunoglobulins	0.007	0.3 (0.1 – 0.7)	0.135	--
Previous treatment with prednisolone ≥100 mg/day [b]	0.032	0.3 (1.1 – 15.0)	0.010	7.3 (1.6 – 32.9)
Previous discoid rash	0.025	4.4 (1.2 – 15.8)	0.082	--
Previous severe haematologic disorder	0.003	0.3 (0.1 – 0.7)	<0.001	0.17 (0.06 – 0.46)
Baseline SLEDAI (0–105)	0.001	1.1 (1.03 – 1.2)	0.001	1.1 (1.04 – 1.16)

[a] Forward stepwise (LR); Nagelkerke's $R^2 = 0.318$.

[b] History of treatment with daily doses above 100 mg/day to control of the lupus activity. It refers to the highest dose ever used from the lupus diagnostic.

Table 6. Adverse events in SLE patients under rituximab treatment.

Adverse event type	No. of Patients	No. of Events	Incidence/100 patient-yrs (all events/patients)
	N=131 (213,46 patient-yrs)		
Total adverse events	51 (38.9)	90	42.2
Mild/moderate	25 (19.1)	47	22.0
Serious [a]	26 (19.8)	43	20.1
Fatal	6 (4.5)	6	2.8
Type of adverse event			
Infections	27	47	22.0
Mild/Moderate	10	20	9.4
Serious [a]	17	27[b]	12.6
Fatal	2	2 [c]	0.9
Infusion-associated reaction	15	20	9.3
Mild/Moderate	10	14	6.5
Serious	5	6[d]	2.8
Cervical dysplasia	1	1	0.5
Haemorrhagic cystitis	1	1	0.5
Worsening of disease	1	1	0.5
Arthralgia	1	1	0.5
Pregnancy	1	1	0.5
Thrombocytopenia	1	1	0.5
Diarrhoea	1	1	0.5
Seizures	1	1	0.5
Pyrexia	1	1	0.5
Vomiting	1	1	0.5

Breast cancer	1	1	0.5
Pulmonary haemorrhage	1	1	0.5
Pulmonary hypertension	1	1	0.5
Oedema	1	1	0.5
Multiple mononeuritis	1	1	0.5
Cutaneous lupus erythematosus	1	1	0.5
Adult respiratory distress syndrome	1	1	0.5
Retches	1	1	0.5
Anxiety	1	1	0.5
Demyelination	1	1	0.5
Headache	1	1	0.5
Peripheral ischemia	1	1	0.5
Heart failure	1	1	0.5

[a] Fatal events included.

[b] 14 sepsis.

[c] 1 sepsis.

[d] 1 anaphylactic reaction.

Table 7.- Uni- and multivariate logistic regression analysis of SLE patients under treatment with rituximab (n = 125 [a])

	Univariate		Multivariate	
	p-value	HR (95% CI)	p-value	HR (95% CI)
Severe adverse events[b]				
Age-adjusted Charlson comorbidity index	0.030	1.6 (1.1 – 2.4)	0.049	1.6 (1.0 – 2.6)
Previous treatment with steroid bolus	0.001	5.4 (2.0 – 14.8)	0.002	5.9 (1.9 – 18.4)
Baseline mild or moderate flare	0.047	0.3 (0.1 – 0.9)	0.356	--
Serum albumin, g/dL	0.047	0.4 (0.2 – 0.9)	0.159	--
Severe infection[c]				
Age-adjusted Charlson comorbidity index	0.002	1.7 (1.2 – 2.4)	0.772	--
No. of severely involved organ systems	<0.001	2.0 (1.4 – 2.9)	0.001	2.0 (1.3 – 2.9)
Baseline leukocyte count, $x10^9$/L	0.045	1.2 (1.0 – 1.3)	0.046	1.2 (1.0 – 1.4)
Baseline serum albumin, g/dL	0.005	0.3 (0.2 – 0.7)	0.509	--

Baseline serum C4 level, mg/dL	0.034	0.8 (0.8 – 0.9)	0.102	--
SDI (0–49)	0.017	1.3 (1.1 – 1.6)	0.937	--
No. of severe SFI criteria	0.019	1.6 (1.1 – 2.4)	0.856	--
Rituximab schedule				
Two doses of 1 g		Reference		
Four doses of 375 mg/m^2	0.008	3.9 (1.4 – 10.7)	0.014	5.0 (1.4 – 18.3)
Other dose	0.999	--	0.999	
Mortality [d]				
No. of severely involved systems	0.002	2.1 (1.3 – 3.4)	0.519	--
Previous treatment with immunoglobulins	0.021	7.9 (1.4 – 45.7)	0.088	
Baseline serum albumin, g/dL	0.033	0.3 (0.1 – 0.9)	0.618	--
SDI (0–49)	0.001	1.6 (1.2 – 2.2)	0.026	2.4 (1.1 – 5.2)
No. of severe SFI criteria	0.002	3.7 (1.6 – 8.6)	0.054	5.4 (0.9 – 30.6)
Baseline SELENA-SLEDAI	0.043	1.1 (1.0 – 1.1)	0.345	--
Rituximab schedule				

Two doses of 1 g		Reference		
Four doses of 375 mg/m^2	0.021	7.9 (1.4 – 45.8)	0.289	--
Other dose	0.999	--	0.889	--
Cyclophosphamide bolus therapy	0.035	6.6 (1.1 – 37.6)	0.160	--
Prednisolone plus cyclophosphamide bolus	0.002	16.7 (2.8 – 98.4)	0.042	22.3 (1.1 – 447.7)

HR, Hazard ratio; SDI, Systemic lupus international collaborating clinics American College of Rheumatology damage index; SFI, SELENA-SLEDAI Flare Index.

[a] Patients treated with at least one infusion of rituximab with a follow-up > 10 weeks.

[b] Forward Stepwise (LR); Nagelkerke's R^2 0.188

[c] Forward Stepwise (LR); Nagelkerke's R^2 0.401

[d] Forward Stepwise (LR); Nagelkerke's R^2 0.678

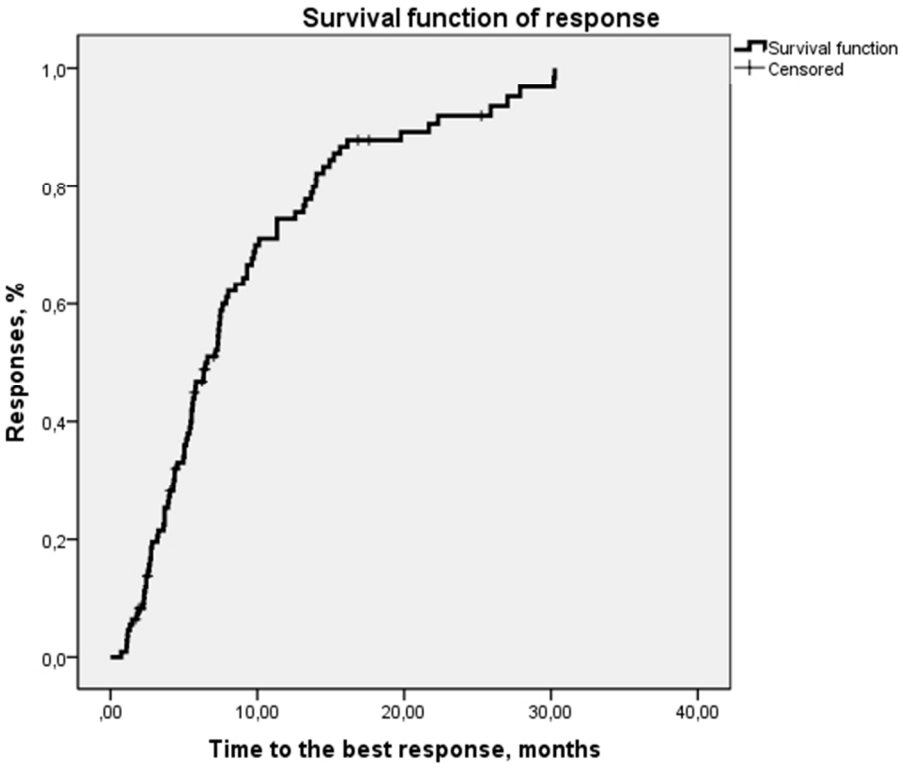

Figure 1A: Kaplan-Meier plot showing overall responses over time for 127 SLE patients after the first infusion of rituximab.
220x176mm (72 x 72 DPI)

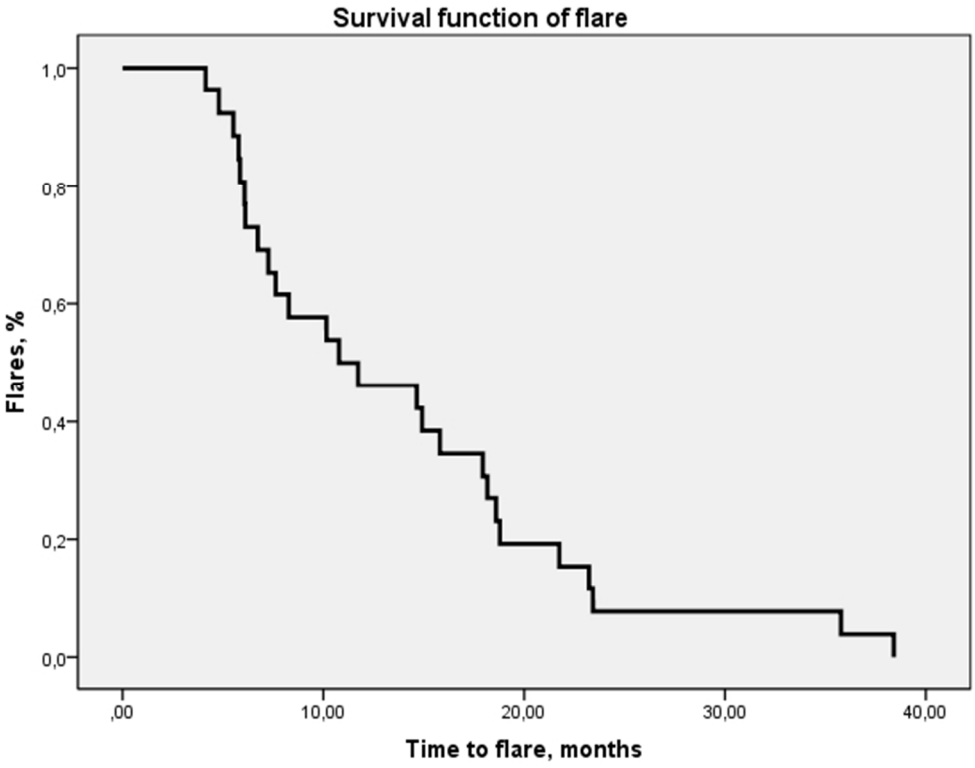

Figure 1B: Kaplan-Meier plot showing relapses over time for 127 SLE patients after a response to rituximab.
220x176mm (72 x 72 DPI)

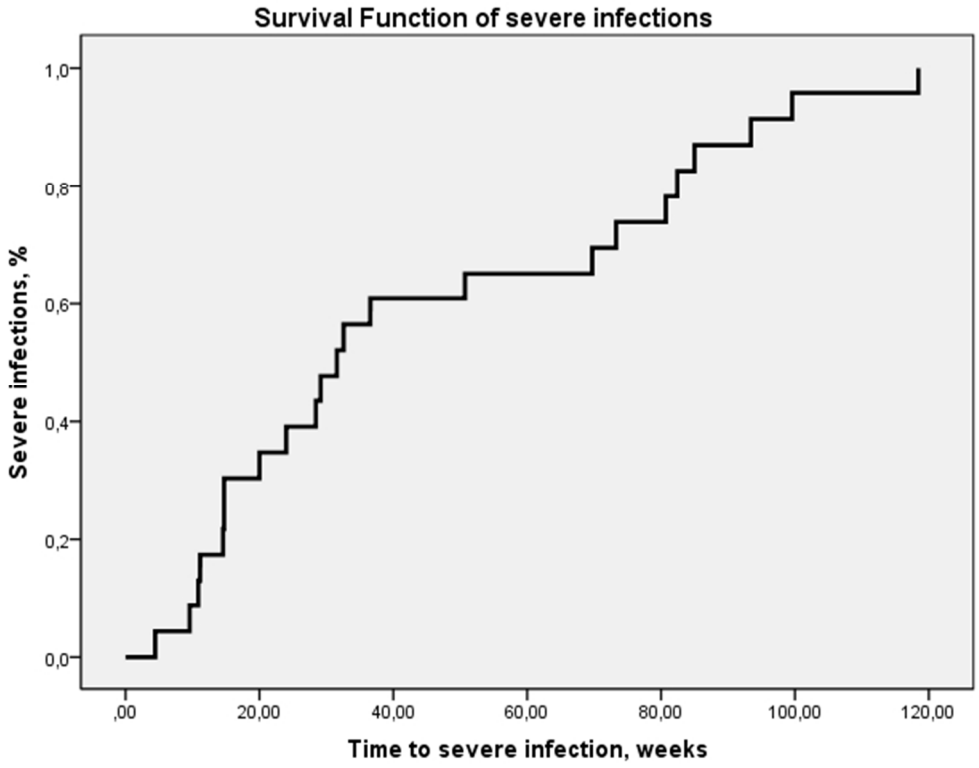

Figure 2: Kaplan-Meier plot showing severe infections events over time for all SLE patients after first infusion of rituximab.
220x176mm (72 x 72 DPI)

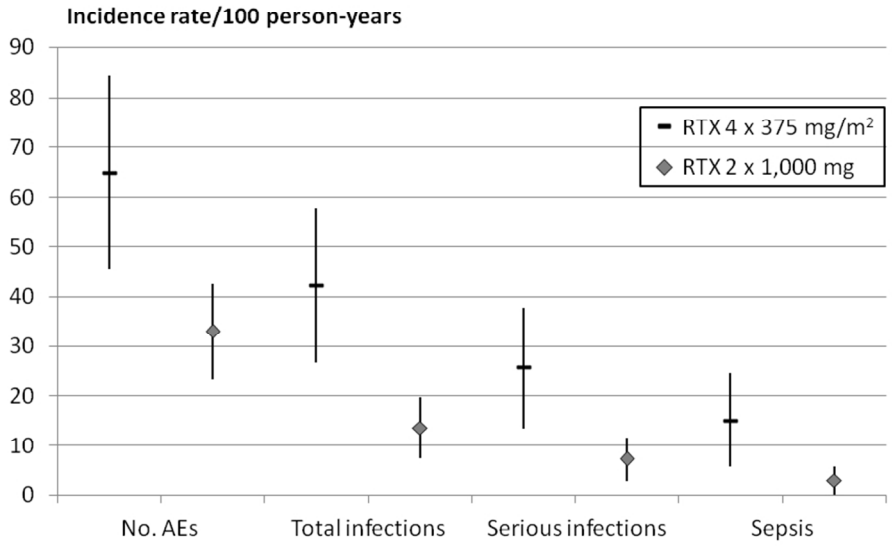

Figure 3.- Incidence of total adverse events (AEs), total infections, serious infections, and sepsis by rituximab (RTX) schedule. Point estimated incidence rate/100 person-years of 2 doses of 1,000 mg rituximab given 14 days apart (vs. 4 weekly doses of 375 mg/m2 rituximab (95% confidence interval).
254x190mm (96 x 96 DPI)

COMENTS AND CHANGES

REVIEWER 1

COMMENT/CONCERN 1:

1. **Page 6, first sentence in the paragraph about patients should read, "Patients were diagnosed with SLE according to the revised American College of Rheumatology…."**
The sentence has been corrected by adding the word "revised"

2. **Page 10, the authors should state whether the kidney biopsies were assessed by the original WHO or the newer ISN Histological classification criteria.**
Clarification about kidney biopsies assessment has been introduced in the sentence although this information had already been indicated in patients and methods. "Kidney biopsies, according to the International Society of Nephrology/Renal Pathology Society 2003 classification, were available…"

3. **Page 11, the authors have now kindly added the requested information about the concomitant immunosuppression but should supply some additional information about the cyclophosphamide which presumably was given as concomitant intravenous boluses (with the rituximab) though it could actually have been given as oral cyclophosphamide.**
Cyclophosphamide was administered intravenously (boluses). Clarification has been added: "…In 99 (75.5%), rituximab was concomitantly used with intravenous cyclophosphamide boluses…"

4. **There are a number of errors/incomplete identification of the references. For example, references 5 and 6 do not provide a volume number. Reference 7 lacks any page numbers and in reference 10, I do not know what (12 Pt1) means! References 30 and 42 lack volume and page numbers.**
The mentioned References has been corrected or completed as required :
Ref 5. Merrill J, Neuwelt C, Walace D, Shanahan J, Latinins K, Oates J. Design and baseline characteristics of patients in randomized double blind placebo controlled phase I/II study (EXPLORER) to evaluate the efficacy and safety of rituximab in patients with moderate to severely active systemic lupus erythematosus [abstract]. Ann Rheum Dis 2008 (67, suppl 2) . p. s347.

Ref 6. Arthritis and Rheum "P.L12" means "Late Abstract". He have changed the reference for the ulterior publication: 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 and Rheumatism. 2010;62(1):222-33.

Ref 8. Furie, R., Looney, R. J., Rovin, B., Latinis, Kevin M., Appel, G., Sanchez-Guerrero, J., et al; Efficacy and Safety of Rituximab in Subjects with Active Proliferative Lupus Nephritis (LN): Results From the Randomized, Double-Blind Phase III LUNAR Study [abstract]. Arthritis Rheum 2009;60 Suppl 10 :1149

Ref 10: This is the way in which the reference is cited in Pub Med: Ann Int Med 2005 Jun 21;142(**12 Pt 1**):953-62. It correlates to Supplement 1, therefore, we have changed the citation as follows:

Buyon JP, Petri MA, Kim MY et al. The effect of combined estrogen and progesterone hormone replacement therapy on disease activity in systemic lupus erythematosus: a randomized trial. Ann Intern Med Ann Intern. 2005; 142:(Suppl 1) 953-962

Ref 30. Catapano F, Chaudhry AN, Jones RB, Smith KG, Jayne DW. Long-term efficacy and safety of rituximab in refractory and relapsing systemic lupus erythematosus. Nephrol Dial Transplant. 2010 ; 25(11):3586-92.

Ref 42. Lateef A, Lahiri M, Teng GG, Vasoo S. Use of rituximab in the treatment of refractory systemic lupus erythematosus: Singapore experience. Lupus. 2010; 19(6):765-70.

5. **I suspect the editors may wish to comment but five tables and three figures seem a little excessive. I suspect that table 2 could easily be absorbed into the text.**

We presented these data in a Table because it compiles a lot of figures, which are pertinent to define accurately the concomitant medication. As this referee has also stated, this information is very relevant to understand the results of the present work, and we suspect their presentation into the text would rather be less comprehensible. As the editors have not made any comment about this issue, we understand it may be suitable for the publication, but surely we´ll be pleasant to adjust to their preferences.

6. **In the legend to table 5 (see comment above), the authors should confirm how the cyclophosphamide was given and give an indication as to the doses used).**

We have included the required information in the legend and also in the text (page 10, first paragraph, line 4)

7. **Although generally written, there are a number of typographical/English errors including the bottom two lines, page 12, which would read better as ……Anti-dsDNA antibody tests became negative in 49 out of 66 patients tested. Page 14, top two lines might be better as, "this schedule did not improve the clinical response or minimise relapses and was associated with more AEs. Page 15 bottom two lines, better to say, "The patients who used…….rituximab had a high instance of ……"**

All the suggestions has included in the text

TITLE

Multicenter longitudinal study of B-lymphocyte depletion in refractory systemic lupus erythematosus: the LESIMAB study

RUNNING FOOTLINE:

LESIMAB Study

AUTHORS

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ABSTRACT

Objective: This study aimed to investigate the effectiveness and safety of single and repeated courses of rituximab in patients with refractory lupus.

Methods: LESIMAB is a multicenter, retrospective, longitudinal study of lupus patients who have not responded to standard therapy and have been treated with rituximab. Response rates at 6 months and at follow-up were defined as efficacy outcomes. Complete response was defined as a SELENA-SLEDAI score ≤2 and a SELENA-SLEDAI Flare Index of 0. Partial response was defined as a reduction in the SELENA-SLEDAI score of ≥4 points with no new or worsening of symptoms. Adverse events were collected.

Results: Seventy-three (62.9%) of 116 patients achieved a response at 6 months (complete in 22 and partial in 51). Ninety-seven (77.6%) of 128 patients achieved a response after a mean follow-up of 20.0 ± 15.2 months (complete in 50 and partial in 47). High baseline SLEDAI score, previous treatment with ≥100 mg/day prednisone, and no history of severe hematologic flare were associated with response after the first

treatment course. The median time to response was 6.5 months (95% CI, 5.0–8.0). Thirty-seven patients (38.1%) relapsed after the first infusion. The flare was severe in 7 cases and mild to moderate in 29 cases. Serious infection rate was 12.6/100 patient-years. Four weekly doses schedule was associated with more serious infections. Six patients died; 2 of infection and 4 of lupus complications.

Conclusion: Rituximab can be an effective treatment option for refractory lupus with severe or life-threatening disease with acceptable tolerance profile.

KEYWORDS

Systematic Lupus Erythematosus ,Rituximab, Effectiveness treatment, Safety, Biological therapy, Infection.

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INTRODUCTION

Systemic lupus erythematosus (SLE) is a chronic, multisystem inflammatory disease with a wide range of clinical manifestations and characterized by the presence of auto-antibodies directed against a number of self-antigens.

In recent years, the 10-year survival rate for SLE patients has significantly improved (1), but the long-term prognosis remains poor in patients with severe SLE because they tend to suffer more complications and are exposed to more intensive immunosuppression (2). Corticosteroids and immunosuppressant drugs have traditionally been used to treat moderate to severe cases of SLE, but a substantial subset of patients either fail to achieve optimal disease control or develop severe toxicity.

Rationale for T and B cells as therapeutic targets in SLE is supported by a well documented pathogenic role of autoreactive T cells and polyclonally activated B cells in lupus manifestations (3). Rituximab is a chimeric monoclonal antibody that selectively binds the CD20 receptor and induces B-cell depletion (4). Only two controlled randomized trials of rituximab in SLE have been published(5-7). These studies detected positive signals for this treatment, but failed to detect significant differences in the primary outcomes between the active and control arms as groups. Since these results were published, many questions have been raised about the methods employed in these studies, especially regarding the bias of exclusion of severe organ manifestations. Although previous observational studies have investigated the effects of rituximab on SLE, the analysis of sizable cohorts of patients treated for clinical need is still important to improve our understanding of the potential benefit of Rituximab in SLE as used in clinical practice. The aim of this study was to investigate the effectiveness and safety of

single and repeated courses of rituximab in patients with severe refractory SLE in the clinical setting.

PATIENTS AND METHODS

Study design

LESIMAB is a multicenter, retrospective, longitudinal, observational study of SLE patients who have failed to respond to conventional therapy and have been treated with rituximab. This study was designed by the Systemic Autoimmune Diseases Working Group of the Spanish Society of Rheumatology (SADWG-SSR). Nineteen rheumatology units at Spanish university centers with substantial experience in the management of SLE agreed to participate in this study.

Patients

Patients were diagnosed with ~~of~~ SLE according to the revised American College of Rheumatology (ACR) criteria (8), who had active disease despite ongoing corticosteroid and/or immunosuppressive treatment, and completed at least 2 evaluations related to anti-CD20 infusions were eligible for the study. The Ethics and Clinical Research Committee of the Carlos Haya University Hospital approved the study as a central approval requirement, and all participants provided local Committee agreement and written informed consent from recruited patients.

Variables and Effectiveness criteria

The primary outcome for effectiveness was the rate of either complete or partial response at 6 ± 3 months after the first course of rituximab. A course was defined as a

complete set of infusions (2 doses of 1,000 mg rituximab given 14 days apart or 4 weekly doses of 375 mg/m^2 rituximab) administered in each cycle of treatment.

The secondary outcomes included the response rate at the end of follow-up (1 or repeated courses), rate of flare (mild to moderate and severe flare) at the end of follow-up, time to achieve the best response (complete or partial) at the end of follow-up, time to relapse after remission, and change from baseline of clinical and laboratory variables associated with lupus activity.

Complete response was defined as a Safety of Estrogens in Lupus Erythematosus National Assessment–SLEDAI (SELENA-SLEDAI) score of 2 points or less and a modified SELENA-SLEDAI Flare Index (SFI) score of 0. Partial response was defined by a reduction of at least 4 points in the SELENA-SLEDAI score with no new or worsening symptoms as measured by the SELENA-SLEDAI-SFI.

Protocol and clinical assessment

Rituximab (Mabthera®, Roche) was added to the patients' standard treatment, including premedication, according to local protocols (113 received iv. 6-Methyl-prednisolone plus Histamine-H1-antagonists or acetaminophen, and the rest received only H1-antagonists with or without acetaminophen). A follow-up of at least 10 weeks after the first course was mandatory to be included for the effectiveness analysis after the first rituximab course, but those patients who died during this period were included too. However, all patients who received at least 1 infusion of rituximab were considered in both effectiveness and safety intention-to-treat analysis at follow-up, even if they had a follow-up of less than 10 weeks. The last observation carried forward approach was used for missing data.

Baseline data were collected on lupus manifestations, previous treatment, and other associated medical problems, including the Charlson-age comorbidity index (9). A successful B-cell depletion (BCD) defined as a fall in the absolute CD19 count to $<0.005 \times 10^9$/l. Disease activity and relapses were scored using the SELENA-SLEDAI and modified SFI (10, 11). A revised SELENA flare tool that excluded the physician's global assessment component was used to define flare. The SLICC/ACR Damage Index (SDI) was used to quantify the degree of accrued irreversible organ damage (12). Physical function was measured using the Spanish version of the HAQ (13). Antiphospholipid syndrome was classified according to the Sydney revision of the Sapporo criteria (14). Lupus severity was classified using previously described criteria (15). Kidney biopsies were classified according to the International Society of Nephrology/Renal Pathology Society 2003 classification (16).

To minimize possible errors in data collection, a standard protocol was used, and all participating rheumatologists carefully discussed the protocol variables. Data collection was recorded only by the rheumatologists involved. All the investigators were familiarized with the use of disease activity indexes as most of them had been involved in clinical trials and/or trained in specific programs for the use of the SELENA-SLEDAI, SFI, and SDI provided by the SADWG-SSR. Data collection was transferred to the computerized database and on-line monitoring process was employed to analyze consistency of recorded data.

Safety assessment

Adverse events (AEs) were classified using the Medical Dictionary for Regulatory Activities Terminology (17). The type and severity of resulting AEs and their association with rituximab were evaluated. Serious AEs, infusion-related AEs, and

infection-related AEs were summarized independently. An infection was considered serious if it resulted in death, was life-threatening, required inpatient or prolonged existing hospitalization, caused persistent or significant disability/incapacity, induced a congenital anomaly/birth defect, or required intravenous antibiotic treatment.

Statistical analysis

Endpoint analyses were performed *per protocol* on the eligible sample (n = 128), and on the intention-to-treat sample, defined as all patients ever treated (n = 131). Normal distribution of variables was checked using the Kolmogórov–Smirnov test. Paired 2-sample t-test or Wilcoxon's signed-rank test was used to analyze the effect of rituximab after both the first treatment course and follow-up assessments. The McNemar test was used for the comparison of correlated proportions. Comparisons between groups were performed with χ^2 test, Student's t-test, or Mann–Whitney test, as appropriate.

Kaplan–Meier curves were used to estimate the time to best response and time to flare. The data used to calculate these metrics were those for the lowest and highest SELENA-SLEDAI scores, respectively. Univariate and bivariate logistic regression analyses were used to identify baseline factors related to response rates, severe AEs, severe infections, and mortality. Statistical analyses were performed using Stata 10.0 software (Stata Corp. USA).

RESULTS

Baseline patient characteristics

One hundred and thirty-one patients with refractory lupus were treated with at least 1 infusion of rituximab from November 18, 2003 to February 5, 2009. This sample accounts for 7.9% (range 1.5 to 9.4) of 1,659 patients followed from 19 hospitals. Three

patients were excluded for lack of a follow-up visit at the time the study was closed. Table 1 shows the baseline characteristics of the 128 selected SLE patients. Most of them were white Caucasians (92.2%), while 5.5% were Hispanics and 2.3% responded to other ethnicity. They displayed disproportionate age-related comorbidity burden and showed a Charlson-age comorbidity index of 2.2 ± 1.4 (mean ± S.D) and previous serious infections in 12 patients (9.4%), including Nocardia pneumonia, miliary tuberculosis, staphylococcal osteomyelitis, staphylococcal arthritis, or sepsis. All patients had a serious disease according to the number of ACR criteria fulfilled, the number of severely involved organs, an unusually high frequency of antiphospholipid syndrome, and the number of lupus related drugs previously taken. Kidney biopsies, according to the International Society of Nephrology/Renal Pathology Society 2003 classification, were available for 55 of 63 patients, which included class II (n = 4), class III (n = 12), class IV (n = 32), class V (n = 6), and classes IV + V (n = 1). All patients were undergoing steroid treatment and displayed clinically significant disease activity despite the use of at least 2 immunosuppressive drugs.

Drug regimens

Most of the patients were treated with 2 doses of 1,000 mg rituximab given 14 days apart or 4 weekly doses of 375 mg/m^2 rituximab (Table 2). Sixty-nine patients received repeated courses of rituximab: 30 patients (22.9%), 2 cycles; 22 patients (16.8%), 3 cycles; 11 patients (8.4%), 4 cycles; 4 patients (3.1%), 5 cycles; 1 patient (0.8%), 7 cycles; and 1 patient (0.8%), 9 cycles. Thirty-one patients received a new cycle for clinical need, while the rest were treated on an every 6 month scheduled program.

All but 2 patients received rituximab in combination with moderate to high doses of glucocorticoids (mean dose ± SD, 0.5 ± 0.8 mg/kg/day). In 32 patients (24.4%),

rituximab was given in monotherapy (n = 16) or in combination with hydroxychloroquine (n = 16) without any other immunosuppressant. In 99 (75.5%), rituximab was concomitantly used with intravenous cyclophosphamide boluses (n = 32, 0,750 mg/m^2–), azathioprine (n = 25; mean dose ± SD, 108.0 ± 31.2 mg/day), methotrexate (n = 18; mean dose, 16.7 ± 9.4 mg/week), mycophenolate mophetile (n = 15), and other (n = 9).

Indications of rituximab

As shown in Table 3, most SLE patients had active disease at baseline despite treatment with moderate to high doses of corticosteroids; 75 patients (58.6%) had high or very high SLEDAI scores. Although most patients had multi-organ lupus flares, characterized by a wide range of combinations of arthritis, nephritis, and cutaneous rashes, the primary indications for rituximab were: nephritis (n = 42), arthritis (n = 27), thrombocytopenia (n = 17), neurologic manifestations (n = 11), serious general involvement (n=10), cutaneous lupus (n = 8), serious pulmonary complications (n = 5), hemolytic anemia (n = 3), and others (n = 5).

Clinical response after the first course of rituximab

One hundred and twelve patients completed a median follow-up time of 26.7 weeks (range, 11.1–42.1) after the first course of rituximab. Four patients who died before completing the minimum 10-week follow-up were also included in the analysis (n = 116).

A total of 73 SLE patients (62.9%; 95% CI, 49.3–79.1) achieved a response after the first course of rituximab; 22 patients (19.6%; 95% CI, 12.3–29.7) showed a complete response and 51 patients (45.5%; 95% CI, 36.1–55.2) a partial response. The best

clinical responses were observed in patients with arthritis (81.5%), cutaneous involvement (87.5%), nephritis (65.8%), neuropsychiatric lupus (73%), thrombocytopenia (65%), or severe generalized flare (40%).

After the first course of rituximab 21/31 (68%) patients achieved a successful B-cell depletion (CD19 count to $<0.005 \times 10^9$/l).

Furthermore, all of the clinical and laboratory baseline variables improved with rituximab treatment (Tables 3 and 4). Reductions in proteinuria and recovery in both C3 and C4 complement fraction levels were also observed. Anti-dsDNA antibody tests became negative in 49 out of 66 patients tested. Noticeably, a moderate but significant reduction in antiphospholipid antibodies was observed. Moreover, improvements were reported in physical function, SELENA-SLEDAI score, and all SFI signs and criteria. Steroid dosage requirement decreased to a mean final dose of one third compared to baseline requirements.

Effectiveness of rituximab during follow-up

Endpoint analyses were performed *per protocol* on the eligible sample (n = 128). Nonetheless, analyses based on the intention-to-treat sample, defined as all patients ever treated (n = 131), were also performed and showed similar results (data not shown). Then, 128 patients had at least one post-baseline measurement of effectiveness after a mean follow-up of 20.0 ± 15.2 months (range, 0.46–69.9) and a total follow-up of 213.46 patient-years. The clinical and biological benefits from rituximab therapy prolonged until the end of follow-up after a mean time period of 2.0 ± 1.3 rituximab cycles. As measured at the last visit, the mean dose of steroids was noticeably lower than baseline and dropped significantly in responders than in non-responders (8.5 ± 8.4

vs. 16.5 ± 17.4 mg/day; $p = 0.007$). In addition, tests for anti-dsDNA antibodies turned to were negative in 49 of the 66 patients.

One hundred and twenty-five (95.4%) patients had sufficient data to evaluate the response rate at the end of follow-up and the record from just 3 patients did not provide enough data to calculate a SELENA-SLEDAI score at the last visit. Ninety-seven patients (77.6%; 95% CI, 62.9–94.7) showed a response, with 50 patients (40.1%; 95% CI, 32.2–50.2) showing a complete response and 47 patients (38.5%; 95% CI, 29.8–47.8) showing a partial response. The median time to achieve the best response was 6.5 months (95% CI, 5.0–8.0) (Figure 1A). Thirty-seven patients (38.1%; 95% CI, 26.8–52.6) relapsed after a median of 10.8 months (95% CI, 2.8–18.7) following administration of the first course of rituximab (Figure 1B). Seven patients (7.2%; 95% CI, 2.9–1.5) had severe flares, whereas 29 patients (29.9%; 95% CI, 20.0–42.9) had mild or moderate flares, as measured by the SFI.

The best clinical responses at the end of follow-up were observed in patients with arthritis (93%), cutaneous (87.5%), nephritis (82.5%), neuropsychiatric lupus (73%), thrombocytopenia (65%), or severe generalized flare (62.5%).

Factors associated with effectiveness

As shown in Table 5, patients who were treated concomitantly with cyclophosphamide were younger, had shorter disease duration, and received lower daily doses of steroids compared with the other patients. Noticeably, they had more severe disease status at baseline when cumulative ACR criteria and SELENA-SLEDAI were considered. However, this schedule did not improve the clinical response or minimise relapses and was associated with more AEs.

Compared with 2 doses of 1,000 mg rituximab given 14 days apart, 4 weekly doses of 375 mg/m^2 rituximab was most frequently used in patients with fever (7.1% vs. 23.7%; p = 0.015), thrombocytopenia (11.8% vs. 26.3%; p = 0.043), and lupus nephritis (29.4% vs. 44.7%; p = 0.098). However, this schedule did not yield better clinical responses or fewer relapses but it was associated with more AEs (see below).

On the other hand, patients who were concomitantly treated with hydroxychloroquine exhibited less cumulative ACR criteria (1.8 ± 1.2 vs. 2.2 ± 1.4; p = 0.046), and lower SDI scores (1.3 ± 1.8 vs. 2.0 ± 2.1; p = 0.046). They had higher baseline SELENA-SLEDAI scores (16.7 ± 10.5 vs. 12.3 ± 8.4; p = 0.008), especially cutaneous lupus activity (33.9% vs. 16.7%; p = 0.002), and higher baseline numbers of mild to moderate SFI criteria (1.5 ± 0.9 vs. 1.2 ± 0.9; p = 0.030).

A number of baseline variables were associated with clinical response after the first course of rituximab, as shown by the covariates presented in Table 6. For instance, each one-point increase in the SELENA-SLEDAI score was associated with a 10% increase in the probability of response. Higher response rates were observed among patients who had at some time taken steroid doses higher than 100 mg/day, whereas patients with a previous serious hematologic disorder were 83% less likely to achieve a response.

In the multivariate logistic regression analysis at the end of follow-up, previous treatment with immunoglobulin [HR = 0.3 (95% CI, 0.1–0.9); p = 0.003], higher SLEDAI scores [HR = 1.1 (95% CI, 1.0 –1.2); p = 0.012], concomitant treatment with prednisolone plus cyclophosphamide bolus [HR = 0.1 (95% CI, 0.0–0.6); p = 0.010], and concomitant treatment with immunosuppressive agents other than cyclophosphamide [HR 3.5 (95% CI, 1.2–10.0); p = 0.022] were entered into the model.

Safety of rituximab

Fifty-one patients (38.9%) treated with at least 1 infusion of rituximab had a total of 90 AEs, and almost half of these AEs were serious (Table 7). The most common AEs were infections (22.0/100 patient-years) and infusion-associated reactions (9.3/100 patient-years). Most of the infections were serious, whereas most of the infusion-associated reactions were mild to moderate. Regarding the other AEs a great majority could not be directly related to treatment. Seventy-two AEs (84.7%) required treatment, 38 AEs (44.7%) caused hospitalization, 13 of which (15.2%) needed treatment in intensive care units.

Severe infections presented early in the study (before the 40th week), although an increase in frequency could also be observed later (after the 80th week) (Figure 2). Airways were the most frequent site of infection (3.7/100 patient-years), including pneumonia (2.8/100 patient-years), followed by herpes zoster infections (3.2/100 patient-years) and urinary tract infections (2.3/100 patient-years), including pyelonephritis (1.4/100 patient-years). Twenty-seven of 47 infectious events required intravenous antibiotics, and microorganisms were identified in 26 cases. Gram-negative bacteria were isolated in 9 cases (*E. coli*, 7 cases; *P. aeruginosa*, 1 case; *K. pneumoniae*, 1 case)~~, Gram~~, Gram-positive bacteria in 5 (*Staphylococcus*, 3 cases; *L. monocytogenes*, 1 case; *Streptococcus*, 1 case), virus in 10 (6 cases of herpes zoster virus, 2 cases of HHV-1, and 2 cases of HPV), and fungi in 2 cases (*Candida* sp. and *Aspergillus*).

Six patients died; 2 fatalities were due to infection (i.e., macrophage-activated syndrome in the context of invasive aspergillosis and gangrene) and 4 complications were related to SLE (i.e., multi-organ lupus activity, acute alveolar hemorrhage, pulmonary hypertension, and acute respiratory distress syndrome).

Factors associated with severe adverse events

The patients who used 4 weekly doses of 375 mg/m^2 rituximab had a high instance of AEs compared with 2 doses of 1 g. (figure 3): total AEs 64.9/100 person-years (IRR 1.8; 95% CI 1.3 to 3.0; p = 0.002), total infections 42.3/100 person-years (IRR 3.1; 95% CI 1.7 to 5.9; p <0.001), serious infections 25.7/100 person-years (IRR 3.6; 95% CI 1.5 to 8.7; p = 0.001), sepsis 15.1/100 person-years; 95% CI 1.5 to 23.0; p = 0.003), and infections that required intensive care 7.5/100 person-years (IRR 5.3; 95% CI 1.2 to 498.4; p = 0.016).

The baseline risk factors for severe AEs, severe infections, and mortality are shown in Table 8. As determined by the multivariate analyses, the age-adjusted Charlson comorbidity index and previous treatment with a prednisolone bolus were independent risk factors for any severe AEs,. Interstingly, the number of severely affected organ systems, high baseline leukocyte count, and 4 doses of rituximab were identified as risk factors for severe infection. Mortality was associated with the SDI, the number of severe SFI criteria that were met, and co-treatment with prednisolone plus cyclophosphamide bolus.

DISCUSSION

Rituximab has been widely used in patients with severe SLE to avoid potentially serious toxicities or due to the unsatisfactory response of refractory disease to immunosuppressive agents (7, 18-30). Rituximab is frequently utilized although it does not have official approval and furthermore 2 clinical trials have found insufficient efficacy for its treatment of lupus (5-7, 18). For clinical needs, the present study provides new data that rituximab can result in a high response rate in patients with refractory SLE after 10 weeks, as assessed with SELENA-SLEDAI.

The cohort analyzed here is quite sizable and could be informative enough given the severity of disease that has been treated in our sample. This great burden in our patients explains the high proportion of previous serious infections, antiphospholipid syndrome, failed drugs, resistant nephritis, etc. In fact, these patients account for less than 10% of SLE-patients treated in 19 hospitals.

We attempted to limit the bias inherent in observational studies by using a consensus protocol, monitoring data consistency, and ensuring that participant rheumatologists with a great experience in SLE have had training in specific data collection.

Rituximab was indicated when antimalarials, corticosteroids, and immunosuppressant drugs failed to control disease in patients with lupus-flare. The severity profile of the patients in this study is quite similar to previous case series, but their disease status is more severe than those in the EXPLORER (5, 6, 18) and LUNAR (7) clinical trials. Unlike the patients in the aforementioned trials, our patients achieved a first response rate of 63%, which increased to 78% by the end of the study and was accompanied by a notable decrease in steroid requirements. Overall, previous studies found that rituximab resulted in reductions in SLEDAI scores of 38–70% (24, 26, 28, 31-34), although more modest responses were observed in studies that treated patients with only mild SLE activity (33, 34). This observation was previously reported (35) and it is interesting in that it partially explains why poorer responses have been observed in clinical trials. In fact, our study found that the probability of a positive response increased by 10% for each one-point increase in baseline SELENA-SLEDAI score and was 7 times higher in patients who had at some time required very high steroid doses. Indeed, it is not surprising that a refractory disease that has failed to multiple therapies may respond poorly to rituximab. In agreement with this observation, 37.5% of patients in the present study, who had a history of severe hematologic complications that had required steroids,

danazol, immunoglobulins, or even a splenectomy, were 83% less likely to respond to rituximab.

The mean time to achieve a defined response was 3–4 months, whereas several individual parameters improved more rapidly. In our cohort, the lowest SELENA-SLEDAI scores were achieved at nearly 6 months, and patients were relapse-free for a mean of 10.8 months. These results are consistent with those of other studies (19, 23, 32, 36-39). Only 38% of responders experienced a flare (mostly mild) after the final rituximab infusion. Although a few predictors of relapse have been identified by others, including positive anti-ENA antibodies or low C3 levels (29), our study identified none. Due to retrospective collection data, the absence of a central laboratory could lead to a poorer correlation between assays and likely explain why we failed to confirm this.

In terms of efficacy and safety, and derived from its use off-label, no consensus in the dose and administration regimen of rituximab has been established for LES, and no direct comparison of 2 intravenous infusions of rituximab at 1 g versus 4 infusions of rituximab at 375 mg has been undertaken (35). Different administration schedules could be a limitation of the present study, but the analysis of this variable showed no influence in the response rate. However, regarding safety, the regimen of 4 doses of rituximab at 375 mg/m^2 was associated with a 5-fold elevated risk of serious infection compared to 2 doses of 1g in the multivariate analyze. If confirmed this important new finding could have practical impact in the management of these patients.

There are also no rules about monotherapy or combined treatment with rituximab, particularly whether cyclophosphamide or other immunosuppressant agents should be used. One study indicated that adding cyclophosphamide to rituximab does not offer additional benefits in the treatment of lupus nephritis at 48 weeks (24). In our study,

cyclophosphamide was added in patients with more severe disease status and higher baseline activity, suggesting that their rheumatologists were confident with this drug for severe lupus. Although this may be a selection bias, cyclophosphamide showed no greater benefit, and data about its combined effects have been contradictory (32, 34, 37, 40, 41). In fact, we observed that our patients concomitantly treated with other immunosuppressant drugs were 3.5 times more likely to achieve a response by the end of study. By contrast, the combination of cyclophosphamide plus steroid i.v. bolus was associated with lower efficacy at the end of the study and higher mortality, but this result may also reflect a selection bias of a more refractory disease.

Concerning re-treatment, the time regimen schedules in previous studies are also flexible but limited information is available (21, 26, 36, 38, 42, 43). In this regard, our results provide relevant information regarding this issue: 53% of patients were re-treated (1 patient even received 9 repeated courses), but less than one-third of patients received treatment every 6 months. Unlike in rheumatoid arthritis, the repeated cycle of B-cell depletion in SLE patients every 6 months would eliminate the transitional and naive B cells that typically emerge after profound depletion in SLE and could delay a late phase of improvement (44). This process could be another reason for the failure of previous clinical trials (5-7, 18) and may explain why the response rate improved over time in our patients.

Current data show a relative safety profile for rituximab in treatment of SLE patients. Our results confirm that infusion-associated AEs are mild and similar to those observed in rheumatoid arthritis patients. However, severe infections were more frequent than those observed in SLE patients in the AIR registry (26). Although the authors observed a severe infection rate of just 6.6/100 patient-years, similar to that observed for anti-TNF drugs in the treatment of RA (45, 46), they observed one additional fatal infection

and used different criteria for severe infection criteria. In our study, it is unclear whether infections observed can be attributed to rituximab given the study design and the high rate of serious infections in the group prior to rituximab. The inclusion of patients with severe lupus, even those with severely low complement levels or leukopenia and those with a large previous cytotoxic background may represent additional confounding factors. Another factor that may contribute to this high rate of infections was that having treated the patients with B cell depletion, the concomitant immunosuppressive drugs were continued in 75% of our cases. Furthermore, most risk factors for total AEs, serious infections, and mortality were linked to comorbidity and lupus severity. Accordingly, the only risk factors we have identified for serious infections that could be related to rituximab were the 4 doses of rituximab regimen as treatment with cyclophosphamide plus corticosteroid bolus might be reflecting the high risk conferred by both drugs in severely ill patients. In fact, 10% of our patients had received biologic therapy with either TNF antagonist or epratuzumab after failure of conventional treatment as some of our centers have reported benefits for this treatment (47). In our opinion, all these factors could have contributed to the higher rate of serious infections compared with other studies.

In conclusion, the LESIMAB study has demonstrated that rituximab is an effective choice in selected patients refractory to standard treatment. However, close monitoring for infections is mandatory in patients with multiple organ systems disease activity, high leukocyte counts, and a dosing schedule of 4 doses of 375-mg rituximab.

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