



## Original article

## Efficacy of rituximab versus cyclophosphamide in lupus patients with severe manifestations. A randomized and multicenter study

Lilia Andrade-Ortega,<sup>a,\*</sup> Fedra Irazoque-Palazuelos,<sup>a</sup> Ricardo López-Villanueva,<sup>b</sup> Yaneth Barragán-Navarro,<sup>a</sup> Fernando Bourget-Pietrasanta,<sup>c</sup> Maria de los Ángeles Díaz-Ceballos,<sup>d</sup> Roberto Hernández-Paz,<sup>e</sup> Adelfia Urenda-Quezada,<sup>f</sup> and Rodolfo Rivas-Ruiz<sup>g</sup>

<sup>a</sup>Servicio de Reumatología, Centro Médico Nacional 20 Noviembre ISSSTE, Mexico City, Mexico

<sup>b</sup>Hospital Regional del ISSSTE, Mérida, Yucatán, Mexico

<sup>c</sup>Hospital Vasco de Quiroga, ISSSTE, Morelia, Michoacán, Mexico

<sup>d</sup>Hospital General Dr. Darío Fernández Fierro, ISSSTE, Mexico City, Mexico

<sup>e</sup>Hospital Regional del ISSSTE, León, Guanajuato, Mexico

<sup>f</sup>Hospital Regional del ISSSTE, Chihuahua, Chihuahua, Mexico

<sup>g</sup>Hospital Infantil de México Federico Gómez, Mexico City, Mexico

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## ABSTRACT

There are no controlled studies that compare the efficacy of rituximab (RTX) with standard treatment, such as cyclophosphamide, in patients with systemic lupus erythematosus (SLE).

**Objective:** The objective of this study was to compare the efficacy of rituximab to that of cyclophosphamide in patients with severe manifestations of SLE.

**Materials and method:** This is a multicenter, randomized open and controlled trial in adults with a diagnosis of active SLE. Patients were randomized into two groups; Group 1: treated with RTX and Group 2: cyclophosphamide pulses with the same steroid scheme. We registered MEX-SLEDAI, steroid requirements and adverse events for 12 months. Descriptive and comparative statistic analyses were performed.

**Results:** Nineteen patients were included, 17 females, mean age 35.7±12.1 years and duration of disease 5.6 years (range 0.35 to 30.8 years). There were no differences at baseline regarding gender, age, duration of disease, previous treatments or disease activity between both groups. MEX-SLEDAI was reduced from 12 to 3 in Group 1 and from 9 to 2 in Group 2 (P=.80). Nevertheless, patients treated with RTX had a faster improvement. There was no difference in the cumulative steroid dose. Both groups had significant reduction in antinuclear antibody levels and similar increase in C3 levels. Adverse events were similar in both groups.

**Conclusion:** This comparative clinical study in patients with SLE shows that rituximab can be as useful as cyclophosphamide for severe manifestations, maybe showing a faster response. Adverse events were no different. Rituximab should be considered as an adequate alternative for this group of patients.

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### Eficacia de rituximab comparado con ciclofosfamida en pacientes con manifestaciones graves de lupus eritematoso generalizado. Estudio aleatorizado y multicéntrico

## RESUMEN

No existen a la fecha estudios controlados que evalúen la eficacia de rituximab (RTX) comparando con un tratamiento estándar, como ciclofosfamida, en pacientes con lupus eritematoso generalizado (LEG).

**Objetivo:** Comparar la eficacia de RTX con ciclofosfamida en pacientes con manifestaciones graves de LEG.

**Material y método:** Estudio clínico aleatorizado, multicéntrico, controlado y abierto en adultos con LEG activo. Se administró RTX o bolos de ciclofosfamida, con mismo esquema de esteroides. Se evaluó MEX-SLEDAI, dosis de esteroide y eventos adversos, durante 12 meses. Se empleó estadística descriptiva y comparativa.

## Palabras clave:

Lupus grave

Rituximab

Ciclofosfamida

Células B

\* Corresponding author.

E-mail address: [liliaandrade@prodigy.net.mx](mailto:liliaandrade@prodigy.net.mx) (L. Andrade-Ortega).

**Resultados:** Fueron 19 pacientes, 17 mujeres, con edad de: 35,7 años  $\pm$  12,1, y tiempo de evolución de 5,6 años (0,35–30,8). No hubo diferencias en género, edad, tiempo de evolución, tratamientos previos o actividad de la enfermedad al inicio entre los grupos. Se observó descenso en el MEX-SLEDAI de 12 a 3 en el grupo 1, y de 9 a 2 en el grupo 2 ( $p = 0,80$ ). El grupo que recibió RTX tuvo mejoría más rápida. La dosis acumulada de esteroide fue similar. En ambos grupos se observó reducción en niveles de anti-DNAs e incremento de C3. Los eventos adversos fueron semejantes.

**Conclusión:** Este ensayo clínico comparativo muestra que RTX puede ser tan eficaz como ciclofosfamida, para el control de manifestaciones graves del LEG, con respuesta más rápida. Los eventos adversos inmediatos y mediatos no fueron diferentes. RTX puede considerarse una opción terapéutica adecuada en este tipo de pacientes.

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## Introduction

Systemic lupus erythematosus (SLE) is the connective tissue diffuse disease considered as the prototype of autoimmune diseases. It affects virtually any organ or system, and tends to have a progressive course, alternating relapses and remissions in most patients.<sup>1</sup> About 40% of patients may present severe manifestations of the disease, which significantly affect morbidity and mortality.<sup>2</sup> Among the more severe manifestations of lupus are renal, haematological and CNS diseases and vasculitis.<sup>1</sup>

The aetiopathogenesis of SLE is complex. Within the various abnormalities observed are the loss of tolerance to autoantigens, with subsequent polyclonal activation of B cells and formation of auto-Ab, and impaired function of T lymphocytes, with production of multiple cytokines.<sup>3</sup>

In connection with the participation of B lymphocytes, patients with lupus fail to eliminate autoreactive clones at an early stage of development, implicating several genes, but especially VH4-34, which is expressed in an exaggerated manner in B cells.<sup>4</sup> In addition, sequencing of the immunoglobulin genes shows greater editions of light chains, as an unsuccessful attempt by the immune system to maintain tolerance. B cells also have a central role in antigen presentation, as they can activate and polarise T cells, thus altering the secretion of various cytokines.<sup>5</sup> There is evidence that B-cell depletion has a beneficial effect on disease activity. In murine lupus models, the B-cell-deficient mice do not develop nephritis, while the strains with intact B cells develop a severe condition.<sup>1,3</sup>

For these reasons, B-cell depletion through the use of rituximab has been considered as a potential therapeutic target in SLE. Rituximab is a chimeric monoclonal antibody (Ab), targeted against the CD20 molecule of B cells,<sup>6</sup> which depletes these cells by various mechanisms.<sup>7</sup>

Since the first publication on the use of rituximab in SLE in 2001,<sup>8</sup> numerous case reports have reported favourable responses in different manifestations of this disease.<sup>9,10</sup> However, to date there are no results from controlled clinical trials that evaluate its efficacy compared with standard treatment.

In this study, we developed a multicentre, open clinical trial to compare the efficacy of rituximab versus cyclophosphamide in boluses, which has long been considered the gold standard for severe forms of lupus.

## Material and methods

### Study design

A multicentre study was conducted in different hospitals of the Institute of Social Security and Services for State Workers (ISSSTE). The study was randomized, controlled and open, designed to evaluate the efficacy of rituximab treatment versus cyclophosphamide boluses

on patients with severe lupus manifestations. Adverse events were assessed as a secondary objective.

Informed consent was obtained from all patients, according to the guidelines of the ethics committee. Two groups were formed according to a randomization table, to receive different treatments. Group 1: IV infusion of rituximab 1g for 4h, after premedication with IV hydroxyzine, paracetamol, and dexamethasone 8mg on days 1 and 15 of the study. Re-infusion of the drug in case of relapse was administered from the sixth month. Group 2: Cyclophosphamide in boluses of 750 to 1 g/m<sup>2</sup> subcutaneously after IV hydration and administration of mesna (80% of the cyclophosphamide dose) administered every month for the first 6 months and then quarterly until the end of the study. Both groups received initial steroids at a high dose (1 g IV for 3 doses or 1 mg/kg/d) with gradual reduction, and follow-up was conducted for 12 months.

### Study subjects

The study population consisted of 19 patients over 18 years of age, diagnosed with SLE according to the classification criteria of the American College of Rheumatology, with active disease defined by Mexican Systemic Lupus Erythematosus Disease Activity (MEX-SLEDAI)=3 and renal, haematological or CNS condition or vasculitis, defined as follows: kidney function, proteinuria>0.5 g/l, erythrocytes>5/c, increased creatinine>0.5 mg/dl and/or presence of cylinders in urine; haematological activity, haemolysis with haemoglobin<12 g/dl and reticulocytes>3% or thrombocytopenia<100,000; neurological activity, psychosis, seizures, cardiovascular disease, organic brain syndrome, neuropathy and/or myelitis; and vasculitis: ulcer, stroke or nodules defined by biopsy or angiography. For patients in childbearing age, a reliable contraceptive was used. Exclusion criteria included the presence of pregnancy or lactation, history of hepatitis, the use of other immunosuppressive agents concomitantly (except antimalarial drugs), or active infection at the start of the study. Patients might have received steroids in boluses or orally depending on the degree of activity, and the required dose of steroid during the study was considered as an indicator of immunosuppressant efficacy. Patients could continue with the use of antimalarials, antihypertensives, anticoagulants, lipid lowering and anti-inflammatory drugs as necessary.

### Evaluations

Evaluations were performed monthly during the first 6 months and twice a month until a year of monitoring was completed. At each visit, there were questions on symptoms suggestive of activity and a physical examination was carried out by a rheumatologist. The MEX-SLEDAI (0–32) overall score was recorded and disease activity was defined as absent or in remission (<3), mild (3–5) or present (>5).<sup>11</sup> Haematological, blood chemistry, urinalysis, creatinine clearance, 24h urinary albumin, C3 and C4 analyses were also carried out. Anti-

double-stranded DNA Abs (anti-dsDNA) were measured by ELISA, as was CD19 lymphocyte subpopulation (absolute number/ $\mu$ l and percentage) at the initial visit, at 6 months and at 1 year.

### Statistical analysis

We used descriptive statistics, specifically the Fisher exact test for the qualitative variables and the Student t-test or Mann Whitney U test for the quantitative variables. The comparison between MEX-SLEDAI and prednisone dose was performed using the Kruskal-Wallis test. We used the SPSS-15 program.

## Results

### Characteristics of the study subjects

We included 19 patients; 17 were women, with an average age of 35.7 years $\pm$ 12.1 (mean $\pm$ SD) and the disease duration was 5.6 years (0.35 to 30.8). Demographic data and previous treatments are described in Table 1. There were no differences in gender, age, evolution time or previous immunosuppressive treatments among patients treated with rituximab (Group 1: 10) and those treated with cyclophosphamide (Group 2: 9). The degree of activity, as measured by MEX-SLEDAI was higher in patients assigned to rituximab compared to patients assigned to cyclophosphamide, but this difference was not statistically significant.

The reasons for inclusion by the type of disease activity were: kidney function in 10 patients, haematological in 5, neurological in 1 and vasculitis in 3 (Table 2). There were more patients with renal disease in Group 2 (7 vs 3), although this difference did not reach statistical significance.

### Clinical efficacy

Both groups of patients showed good clinical response, with decrease in the MEX-SLEDAI scale from 12 to 3 points in Group 1, and from 9 to 2 points in Group 2 ( $P=.80$ ) at 1 year of follow-up. The group who received rituximab presented a faster improvement, reaching significant difference in relation to the baseline visit after 4 months, against 8 months in the group treated with cyclophosphamide (Table 3 and Figure 1). Considering a MEX-SLEDAI $<$ 3, 8 patients presented remission with rituximab and 6 with cyclophosphamide at 6 months, and 9 patients with rituximab and 8 with cyclophosphamide at 1 year. One patient from the rituximab group required re-infusion of the drug at 11 months due to renal relapse.

As an indirect measurement of clinical efficacy of the immunosuppressants, the average dose of prednisone at each visit was similar in both groups. For patients in the rituximab group the decline was faster; however, there were no differences in the final cumulative dose, with this being 689.18mg for the rituximab group and 755.90 mg for the cyclophosphamide group (Figure 2).

**Table 1**  
Demographic variables and previous treatments

	Rituximab (n=10)	Cyclophosphamide (n=9)	P
<i>Clinical variable</i>			
Age ( $\pm$ SD), years	36.9 ( $\pm$ 12.18)	34.5 ( $\pm$ 12.8)	.690*
Time of evolution ( $\pm$ SD), years	7.6 ( $\pm$ 3.1)	11.1 ( $\pm$ 2.9)	.426*
Gender (female) (%)	8 (80)	9 (100)	.263**
Antinuclear antibodies + (%)	9 (90)	9 (100)	.769**
Anti-DNA antibodies + (%)	7 (70)	8 (88.8)	.750**
Baseline MEX-SLEDAI	12	9	0.28***
<i>Prior treatments</i>			
Steroids (%)	10 (100)	8 (88.9)	.474**
Chloroquine (%)	6 (60)	6 (66.7)	.769**
Cyclophosphamide	7 (70)	4 (44.4)	.641**
Azathioprine	7 (70)	7 (77.8)	.533**
Methotrexate (%)	1 (10)	3 (33.3)	.348**
Mycophenolate	2 (20)	2 (22.2)	.728**
Cyclosporine	1 (10)	0	.625**
Gamma globulin	0	0	1.00**

MEX-SLEDAI indicates Mexican Systemic Lupus Erythematosus Disease Activity; SD, standard deviation.

\*Student t test.

\*\*Fisher exact test.

\*\*\*Mann-Whitney U test.

**Table 2**  
Reason for inclusion in the study

Type of activity	Manifestations (No. of patients)	Rituximab n=10 (%)	Manifestations (No. of patients)	Cyclophosphamide n=9 (%)	P*
Haematological	Haemolytic anaemia (3)	4 (40)	Thrombocytopenia (1)	1 (11)	.33
	Thrombocytopenia (1)				
Renal	Nephritis IV (3)	3 (30)	Nephritis III (4), IV (2)	7 (77.8)	.07
	Proteinuria (3)				
	Leuko-erythrocyturia (3) > nitrogen compounds (2)				
Neurological	Cerebellar syndrome	1 (10)		0	1.0
Vasculitis	Skin lesions (2)	2 (20)	Skin lesions (1)	1 (11)	.75
Total		10		9	

No. of patients: Number of patients with the disorder.

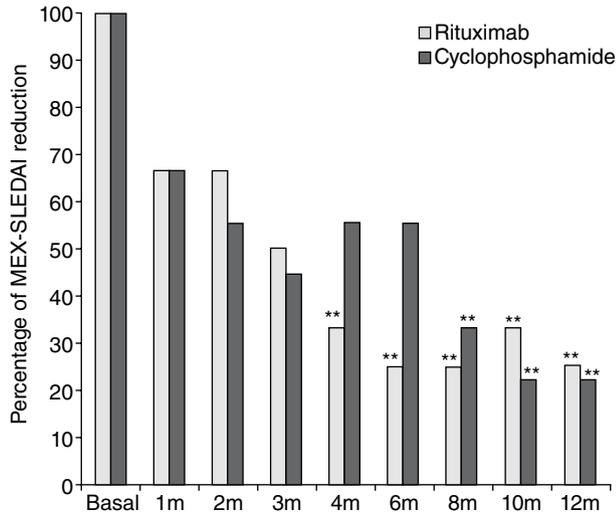
\*Fisher exact test.

**Table 3**  
Change in the MEX-SLEDAI score in the study groups

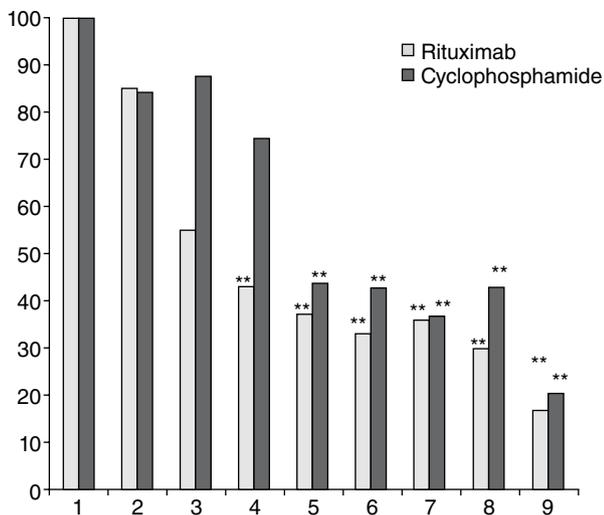
Group	Basal	1m	2m	3m	4m	6m	8m	10m	12m
Rituximab	12	8	8	6	4**	3**	3**	4**	3**
Cyclophosphamide	9	6	5	4	5	5	3**	2**	2**
P*	.28	.73	.17	.33	.52	.27	.71	.42	.80

\*Mann Whitney U test.

\*\*Kruskall-Wallis test (P<.05 vs V1).



**Figure 1.** Percentage of MEX-SLEDAI reduction in the study groups. MEX-SLEDAI indicates Mexican Systemic Lupus Erythematosus Disease Activity.



**Figure 2.** Percentage of reduction in the prednisone dose in the study groups.

**Serology variables**

We observed an increase in C3 figures in both groups compared with the baseline (57.1 to 89.4 in Group 1 vs 56.2 to 86.4 in Group 2), with no differences between the two groups.

Fifteen patients presented positive titres of anti-dsDNA (7 in Group 1 and 8 in Group 2). During follow-up, significant decrease took place in the titres, with no difference between the groups.

**Table 4**  
Serological results

	Rituximab	Cyclophosphamide	P*
Baseline C3	57.1	56.22	.933
Final C3	89.4	86.44	.635
Baseline dsDNA	333.1	378.1	.740
Final dsDNA	164.9	165.7	.789
Baseline CD19 n/µl (%)	175.4 (16.8)	137.1 (18.2)	.119
CD19 n/µl (%) at 6m	32.6 (3.1)	164.5 (16.1)	.000
Final CD19 n/µl (%)	48.8 (3.4)	128.3 (17.2)	.002

dsDNA indicates double-stranded DNA; n/µl, number of CD19 cells per microlitre.  
\*Student t test.

We measured the baseline CD19 lymphocyte subpopulation at 6 and 12 months of follow-up. None of the patients treated with cyclophosphamide decreased the CD19 levels during the study. In the group of patients who received rituximab, the average CD19 at baseline was 16.8%, at 6 months it was 3.1% and at 12 months it was 43.4% (Table 4). Of the 10 patients, only 1 did not show B-cell depletion; this finding was not related to clinical response, which was satisfactory, with a change in the MEX-SLEDAI of 11-3 at 6 months and 0 at 1 year. The patient with clinical relapse at 11 months maintained good B-cell depletion: baseline was 14%, with 4% at 6 months and 4% at 12 months.

**Safety aspects**

In general, both rituximab and cyclophosphamide were well tolerated during the study. Adverse events were divided into immediate reactions to infusion, non-serious non-immediate adverse events, infectious events and serious adverse events. There were 3 adverse events during the infusion of rituximab, which consisted of skin rash, pruritus, and tachycardia, all of which were resolved by the use steroids and antihistamines. The reactions associated with cyclophosphamide infusion (which took place in 8 infusions) were nausea, vomiting, headache and chills, mainly. A total of 23 adverse events not related to infusion were registered in patients treated with rituximab, compared with 16 in patients treated with cyclophosphamide. These were diverse, predominantly headache, dyspepsia, dizziness, skin rash and alopecia. Non-serious infections (8 in patients who received rituximab and 10 in those treated with cyclophosphamide) were urinary tract infections, upper respiratory tract infections, cellulitis and 2 cases of herpes zoster, both in patients who received cyclophosphamide. Serious adverse events in the group treated with rituximab consisted of 1 case of septic arthritis and 1 of haemarthrosis. In the group receiving cyclophosphamide, 1 anal abscess and 1 case of febrile leucopenia occurred. There were no deaths, neoplasms or opportunistic infections during the 12 months of follow-up.

**Discussion**

Although the use of rituximab has not yet been approved by the FDA for the treatment of systemic lupus erythematosus, there are presently many patients with this disease who have been treated with the drug in open clinical trials and case reports, suggesting a good response (over 90% of reported cases) and adequate tolerability in patients with refractory disease to other treatments, especially in the case of haematological, renal and nervous system manifestations.<sup>9,10</sup>

The first series of lupus cases treated with rituximab was published by Leandro et al in 2002.<sup>12</sup> From that moment, there have been numerous reports of good efficacy in patients with SLE

with various manifestations, using both the dose recommended by oncologists, and, most recently, those used in rheumatoid arthritis and in combination or not with other immunosuppressants, specifically cyclophosphamide.<sup>4,9,10</sup>

The team of Dr. Leandro has subsequently reported the results obtained in 13 patients.<sup>13</sup> They recently performed a retrospective analysis of 7 years as well. In that analysis, they found that, out of 45 patients treated with rituximab, 42% presented complete remission and 47% partial remission, all related to depletion of B cells.<sup>14,15</sup>

The Rochester group, led by Looney et al., conducted the first phase I/II study with dose escalation, finding that rituximab was generally safe and well tolerated, causing depletion of B cells that was consistent with clinical and sustained improvement.<sup>16</sup> This group has reported that variability in the response to treatment appears to be associated with the degree of B-cell depletion, the presence of human anti-chimeric Ab, polymorphisms in the expression of Fcγ receptor IIIa alleles and Afro-American descent.<sup>17,18</sup> Other authors have confirmed that patients with lupus have a B-lymphocyte depletion response less uniform than that of rheumatoid arthritis patients and with a higher percentage of formation of human anti-chimeric Ab.<sup>19</sup>

Sfikakis et al. used rituximab in patients with lupus refractory nephritis, obtaining B-cell depletion and a good clinical response.<sup>20</sup>

Vigna-Perez et al reported an open clinical study of 22 patients with lupus nephritis refractory to conventional treatment, who received rituximab, with a significant improvement in the MEX-SLEDAI index and in proteinuria. Twenty patients presented B-cell depletion and a rise in the numbers of regulatory T lymphocytes, as well as an increase in T cell apoptosis. These authors and others have suggested that good results can be obtained even with lower doses of rituximab.<sup>21</sup>

Several studies have suggested that rituximab is useful as an isolated agent for inducing remission in lupus nephritis.<sup>22</sup> Recently, the utility of rituximab alone has been compared with rituximab plus cyclophosphamide in a randomized, open study of patients with lupus nephritis. The authors found no additional benefit in the combination of rituximab with cyclophosphamide compared with the use of rituximab as single immunosuppressant.<sup>23</sup>

In relation to neuropsychiatric manifestations, Tokunaga et al. published a review of 10 patients who received rituximab in varying doses, all showing improvement in symptoms and remission that lasted between 4–35 months.<sup>24</sup> The same group reported an open multicentre study in phase I/II with 15 patients suffering from active refractory SLE. The study compared the use of rituximab, 4 weekly doses of 500 mg vs 1 g on days 1 and 15. At 28 weeks, a similar improvement in the British Isles Lupus Assessment Group (BILAG) index was found in both groups, with no evidence of difference in the required dose of prednisolone or in tolerability.<sup>25</sup>

The Karolinska Institute in Sweden have recently published their experience, which includes 16 patients with severe manifestations of lupus who received combination therapy with rituximab and cyclophosphamide. They found clinical improvement according to SLEDAI in 13 of the 16 patients and remission in 9.<sup>26</sup>

Based on these results, we present a comparative open multicentre study in a Mexican population suffering from lupus erythematosus and severe manifestations, treated comparatively with rituximab versus standard therapy with cyclophosphamide in IV boluses. We selected this face-to-face study because, even though the first reports used rituximab in combination with cyclophosphamide, recent reports did not observe advantages with this combination.<sup>23</sup> In this group of patients, we observed a clinical response, assessed by MEX-SLEDAI, that was very similar in both treatment groups, although more rapid in the group of patients who received rituximab. The percentage of patients achieving remission with this drug was 90%, comparable to that reported in the literature.<sup>10</sup>

We also observed a reduction in the specific autoAb levels and improvement in complement consumption. This serological change

was similar in both groups, resembling the findings of case series and open studies.<sup>14,15</sup>

Regarding tolerability, there was no difference in the prevalence of immediate reactions to the infusion, non-serious adverse events, infectious events and serious adverse events among patients treated with cyclophosphamide and rituximab. Although one of the suggested benefits of rituximab is that it could be associated with fewer adverse events (mainly infectious) compared with cyclophosphamide, this difference was not evident in our patients. This could be due primarily to the small number of patients as well as to the follow-up time, given that most of the patients had long-standing lupus and had previously received other immunosuppressive therapies, making it difficult to attribute immediate or late adverse events to a specific treatment. Due to the monitoring period, it was not possible to assess late adverse effects, including infertility, oncogenicity, or even a remote risk of progressive multifocal leukoencephalopathy.

Our study included a limited number of patients with different severe manifestations of lupus, whose evolution and response to treatment could be difficult to compare. That was why an overall, validated index of activity (MEX-SLEDAI), the levels of anti-dsDNA Ab, complement and the required dose of steroid were chosen as the analytical variables of efficacy.

Virtually all patients who received rituximab depleted their B cells, and this effect was long-lasting. In the only patient with reactivation during the follow-up period, there was no repletion of the CD19 levels; in another patient, there still was adequate response although it was not possible to demonstrate B-cell depletion. It has already been reported that in patients with lupus there is much greater variability in the B-lymphocyte depletion achieved with rituximab and little consistency with the clinical response.<sup>19</sup>

At present, there is no clinical trial that compares in parallel the efficacy and safety of rituximab versus cyclophosphamide, the treatment considered as the gold standard for severe manifestations of lupus. Although our study includes a limited number of patients, the results obtained with rituximab-based treatment are comparable to those obtained with the cyclophosphamide scheme. This supports what has been reported to date in case series and comparative studies in relation to B-cell depletion as an effective treatment for severe forms of lupus erythematosus.

There are currently other ongoing clinical trials of rituximab in SLE. The EXPLORER study aims to test the efficacy and safety of rituximab versus placebo plus a baseline immunosuppressive medication,<sup>27</sup> and the LUNAR study will evaluate the efficacy and safety of rituximab plus mycophenolate mofetil versus placebo plus mycophenolate in patients with lupus nephritis.<sup>28</sup> Humanized anti-CD20 Ab is also in a phase I/II study.<sup>27–29</sup>

## Conflict of interests

Dr. Andrade has been a speaker for Pizer and Roche.

Dr. Irazoque has been an external consultant to Bristol Myers Squibb, Roche, Schering-Plough and Wyeth.

Dr. Rivas has been an external consultant to IMSS, Roche, Silanes, Bayer and Abbott.

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