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ORIGINAL ARTICLE

Rituximab therapy for chonic and refractory immune thrombocytopenic purpura: a long-term follow-up analysis

Jaime Garcia-Chavez · Abraham Majluf-Cruz · Laura Montiel-Cervantes · Miriam García-Ruiz Esparza · Jorge Vela-Ojeda · The Mexican Hematology Study Group

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Abstract The aim of this study was to evaluate the long-term response to rituximab in patients with chronic and refractory immune thrombocytopenic purpura (ITP). Adults with ITP fail to respond to conventional therapies in almost 30% of cases, developing a refractory disease. Rituximab has been successfully used in these patients. We used rituximab at 375 mg/m^2, IV, weekly for a total of four doses in 18 adult patients. Complete remission (CR) was considered if the platelet count was $>100\times10^9$/l, partial remission (PR) if platelets were $>50\times10^9$/l, minimal response (MR) if the platelet count was $>30\times10^9$/l and $<50\times10^9$/l, and no response if platelet count remained unchanged. Response was classified as sustained (SR) when it was stable for a minimum of 6 months. Median age was 43.5 years (range, 17 to 70). Median platelet count at baseline was 12.5×10^9/l (range, 3.0 to 26.3). CR was achieved in five patients (28%), PR in five (28%), MR in four (22%), and two patients were classified as therapeutic failures (11%). Two additional patients were lost to follow-up. The median time between rituximab therapy and response was 14 weeks (range, 4 to 32). SR was achieved in 12 patients (67%). There were no severe adverse events during rituximab therapy. During follow-up (median, 26 months; range, 12 to 59), no other immunosuppressive drugs were used. In conclusion, rituximab therapy is effective and safe in adult patients with chronic and refractory ITP. Overall response rate achieved is high, long term, and with no risk of adverse events.

Keywords Chronic ITP · Rituximab · Anti-CD20 · Refractory ITP

J. Garcia-Chavez · L. Montiel-Cervantes · M. G.-R. Esparza · J. Vela-Ojeda
Departamento de Hematología, UMAE "Dr. Antonio Fraga Mouret", Hospital de Especialidades CMN "La Raza", Mexico City, Mexico

A. Majluf-Cruz (✉)
Unidad de Investigación Médica en Trombosis, Hemostasia y Aterogénesis, Hospital General Regional Gabriel Mancera, Instituto Mexicano del Seguro Social,
Apartado postal 12-1100, Mexico City, México 12 DF, Mexico
e-mail: amajlufc@gmail.com

Introduction

In immune thrombocytopenic purpura (ITP), platelets are coated with an IgG autoantibody that prompts its premature destruction and, as a result, different grades of peripheral thrombocytopenia and clinical bleeding become evident [5, 23]. Steroids are the first-line treatment for acute ITP. In case of failure to steroids, splenectomy induces a 70 to 80% response rate [10]. However, almost 30% of adults with ITP fail to respond to conventional therapies [steroids, IV immunoglobulin (IVIg), splenectomy, or immunosuppressive drugs] and, eventually, they develop a chronic refractory disease [5, 12, 23]. Refractory patients are defined as those who failed standard-dose steroids and splenectomy, requiring further treatment due to unsafe platelet counts ($<30\times10^9$/l) or clinical bleeding. These patients are unlikely to be cured, although spontaneous remissions sometimes occur [17]. If a patient becomes refractory, some alternatives are available such as danazol, dapsone, azathioprine, high-dose steroids, or chemotherapy combinations like CHOP regimen. However, these options are not quite useful because if response is achieved it is but for a brief period and sustained responses are scarce [23]. Hematopoietic stem cell transplantation has been occasionally used in some cases, but this option represents a high-risk procedure [14]. At the end, these patients may respond

poorly to subsequent therapy, may have significant morbidity from the disease and its treatment (bleeding and infections secondary to immunosuppressive therapy), and the mortality rate at 10 years may rise up to 20% [5, 11]. On the other hand, these patients have higher hemorrhagic risk imposed by trauma or surgery. Therefore, new treatment strategies for these patients are needed.

Rituximab is an anti-CD20 chimeric murine-human monoclonal antibody. CD20 is a B-lymphocyte membrane protein absent on other normal cells. This monoclonal IgG-kappa antibody is used in non-Hodgkin lymphomas because it induces apoptosis or direct lysis of B cells [19]. In ITP, B cells are responsible of autoantibody production and the subsequent platelet opsonization that allows its destruction. Therefore, if B cell clones are eliminated, thrombocytopenia could be reverted.

There are some reports informing that rituximab is useful in refractory ITP showing a high overall response rate, with complete remission (CR) and partial remission (PR) rates ranging from 48 to 54% [4, 8, 9, 18, 20–22]. We report herein our long-term experience with rituximab therapy in chronic and refractory ITP.

Materials and methods

In a 5-year period, we prospectively administered rituximab to all patients with chronic and refractory ITP who were unresponsive to prednisone, azathioprine, vincristine, danazol, and high-dose dexametasone, among other therapies, including those unsuccessfully splenectomized. Patients must have a platelet count of $<30\times10^9$/l to be considered for this therapy.

Rituximab was given in doses of 375 mg/m^2, diluted in 500 ml 5% dextrose–water solution in a 4-h continuous IV infusion. This regimen was indicated weekly for four doses after prophylactic application of antipyretic and antihistamine premedication administered 30 min before rituximab infusion (paracetamol, 500 mg, PO; diphenhydramine, 30 mg, PO; and hydrocortisone, 100 mg, IV). Outpatient follow-up was scheduled monthly for 6 months. Long-term follow-up was performed every 2 months.

Response was classified as complete (CR) if the platelet count was $>100\times10^9$/l, partial (PR) if platelet count $>50\times10^9$/l, minimal response (MR) if platelet count was $<50\times10^9$/l and $>30\times10^9$/l, and no response (NR) if there was no change from the baseline platelet count. Response was sustained (SR) when it was maintained for a minimum of 6 months.

Numerical variables were expressed using the median and range number. The probability to achieve more than 50×10^9/l and 100×10^9/l platelets and the median time from response to end of follow-up was calculated using the Kaplan and Meier method.

Before starting treatment, all patients signed an informed consent authorization. The procedures done in this protocol were in accordance with the Helsinki Declaration of 1975. The protocol was approved by the Ethics Committee of the participating hospitals.

Results

Eighteen patients were candidates for this therapy, and all of them were suitable for evaluation because they received the planned four doses of rituximab. Patient characteristics and response to rituximab are shown in Table 1. Median age for the whole group was 43.5 years (range, 17 to 70). Median platelet count at baseline was 12.5×10^9/l (range, 3.0 to 26.3). Median follow-up was 26 months (range, 12 to 59). Mean number of treatment regimens received before rituximab was 5.5 (range, 3 to 8). Fifteen patients (83%) had failed to splenectomy.

CR was achieved in five patients (28%), PR was obtained in another five (28%), MR was seen in four (22%) patients, and two patients were considered treatment failures (11%). Two patients were lost to follow-up (11%). Ten patients (55%) achieved $>50\times10^9$/l platelets. Median time from the first rituximab dose to achievement of any response was 14 weeks (range, 4 to 32). Twelve patients (67%) showed SR beyond the sixth month of follow-up. As expected from the results depicted in Table 1, we were unable to find any correlation between the ITP duration and the type of response to rituximab. Figure 1 shows the median platelet counts during follow-up in three different groups of patients classified according to their pattern of response (CR, PR, and MR). The median time to achieve $>50\times10^9$/l platelets was 5 months (95%CI=0.5 to 11.6 months), and the median time to achieve $>100\times10^9$/l platelets was not reached (Fig. 2). One patient relapsed 22 months after the first course of rituximab. She received steroids for another 14 months; she did not obtain response, and a second course of rituximab was given using the regimen described previously. She achieved a new PR 6 months after stopping therapy. After 22 months of follow-up, PR still remains in this patient.

Median time of response duration was 54 months (95% CI=15–93 months) for patients with CR, 18 months (95% CI=8–28 months) for patients with partial response, and 12 months (95%CI=7–17 months) for those individuals with minimal response. Difference was statistically significant between patients in CR vs those achieving PR or MR ($p<0.05$). No difference was observed between PR and MR (Fig. 3).

Table 1 Patient characteristics and outcomes

Patient/age/gender	Evolution[a] (months)	Previous treatments	PC at baseline ($\times 10^9$/l)	PC at 12 months ($\times 10^9$/l)	Time to response (weeks)	Response type	SR
1/42/F	37	P, S, Az, D, IFN	3.0	146	24	CR	Yes
2/43/F	84	P, S, Az, D, IFN, V	5.0	123	24	CR	Yes
3/30/F	48	P, S, Az, D, IFN, V	12.5	34. 5	16	MR	Yes
4[b]/27/F	38	P, S, Az, D, IFN	24.4	78	16	PR[b]	Yes[b]
5/70/M	60	P, Az, D, V	7.8	NR	NR	NR	No
6/43/F	96	P, S, Az, D, IFN, V	10.1	37.7	8	MR	Yes
7/53/F	96	P, S, Az, D, IFN, V	12.5	54	12	PR	Yes
8/36/F	264	P, S, Az, D, IFN, V, OE, IVIg	5.0	30.6	4	MR	Yes
9/17/F	38	P, S, Az, D, IFN, V, OE	6.5	129	4	CR	Yes
10/57/F	26	P, Az, D	8.3	64	8	PR	Yes
11/36/F	25	P, S, Az, D, IFN, V	7.0	49	20	MR	No
12/63/F	120	P, Az, D, V	26.3	NR	NR	NR	No
13/53/F	348	P, De, D, S, Az	22.0	112	8	CR	Yes
14/22/F	266	P, S, D, IFN, IVIg	12.6	67	12	PR	No
15/56/F	137	P, D, S, Az	18.4	223	4	CR	Yes
16/35/F	38	P, D, Az, S	23.2	11.4	0	NR	No
17/52/F	41	P, D, S	16.5	51.9	4	PR	Yes
18/24/F	60	O, D, S	24.0	NR	NR	NR	No

PC Platelet count, *P* prednisone, *S* splenectomy, *Az* azathioprine, *D* danazol, *IFN* interferon alpha 2b, *V* vincristine, *De* dexamethasone, *OE* opsonized erythrocytes

[a] Time from ITP diagnosis to rituximab therapy

[b] A second course of rituximab was given

Side effects related to the first dose of rituximab such as fever, chills, and respiratory symptoms were common (8 out of 18 patients, 43%). There were no severe adverse events during drug administration. Neither hemorrhagic events nor infections were recorded. Neutropenia, an occasional long-term side effect of rituximab therapy, was never recorded in our group of patients (Fig. 4). In fact, the lower neutrophil count recorded during the study was 1.3×10^9/l in patient 7 after 6 weeks of treatment with rituximab. No patient needed additional immunosuppressive therapy

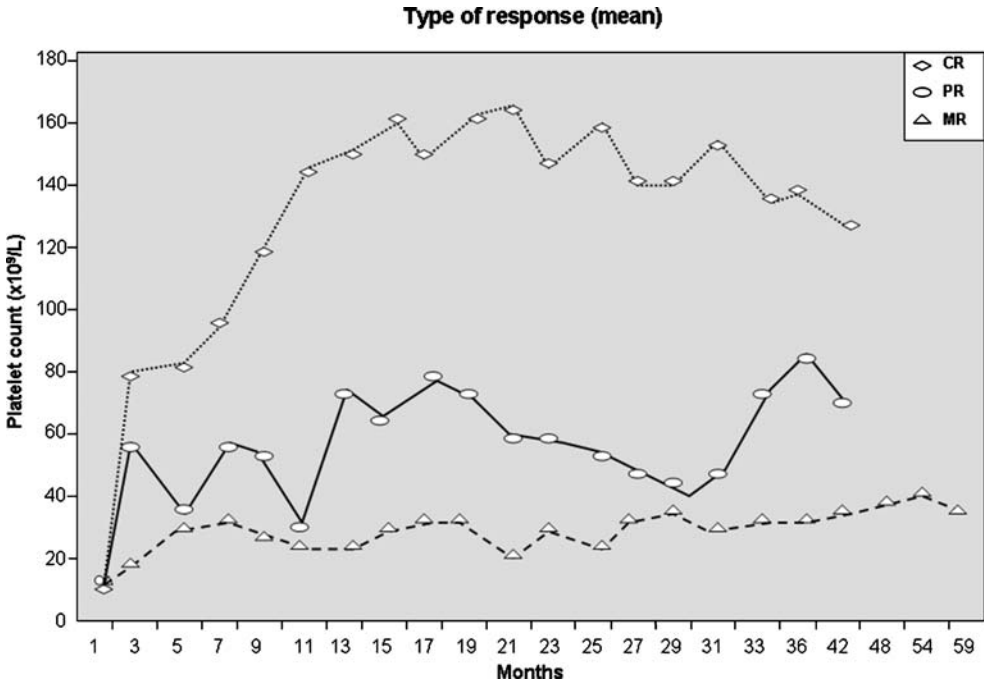

Fig. 1 Platelet count achieved after first dose of rituximab (*time 0* first dose of rituximab). *Diamonds*, CR; *ovals*, PR; *triangles*, MR

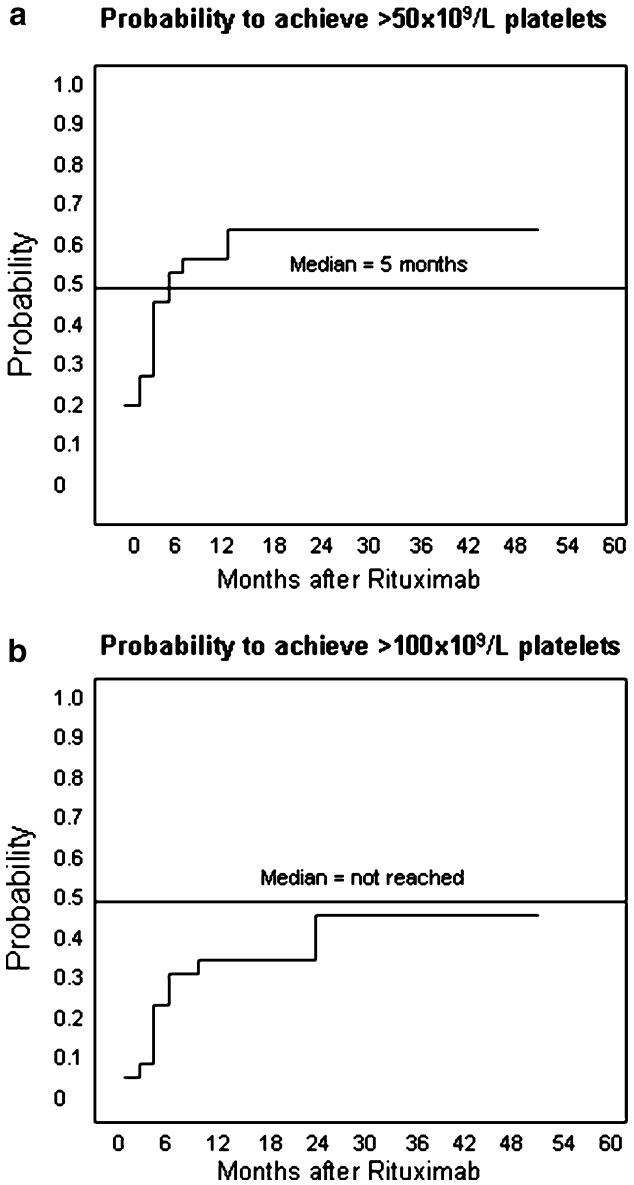

Fig. 2 Time required to obtain platelet counts $>50\times10^9$/l (**a**) or $>100\times10^9$/l (**b**) after first dose of rituximab in adult patients with chronic and refractory ITP

other than rituximab. During the whole follow-up period, no other illnesses have been recorded in this group of patients.

Discussion

Because almost 30% of individuals with ITP do not respond to the first- and second-line therapies, they live with low platelet counts, a situation that carries a high risk of hemorrhage and, eventually, a short life expectancy. It has been largely demonstrated that ITP with persistent low platelet counts carries a grave prognosis [6, 13]. Efforts to increase the platelet count to at least 30×10^9/l include IVIg,

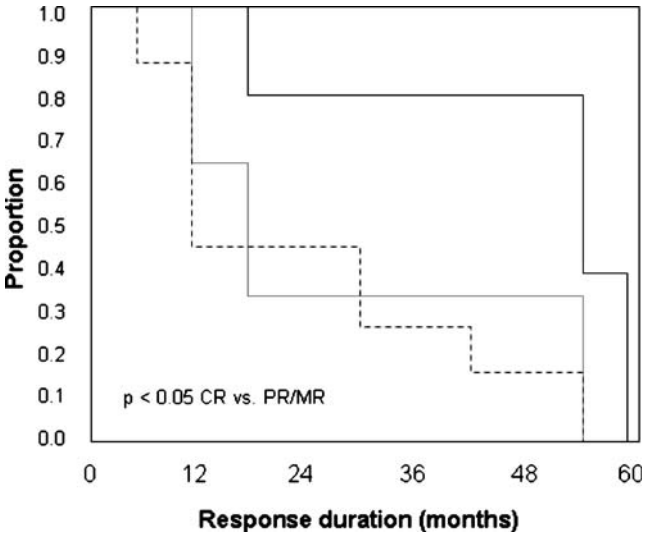

Fig. 3 Median duration of response for patients who achieved complete remission (*CR*; 54 months, 95%CI=15–93 months), partial remission (*PR*; 18 months, 95%CI=8–28 months), or minor response (*MR*; 12 months, 95%CI=7–17 months)

anti-D immunoglobulin, cyclophosphamide, polychemotherapy such as the CHOP regimen, thrombopoietin, interleukin-11, dapsone, alpha interferon, plasma exchange, and bone marrow transplantation [23]. Using these therapies, the response rate is low and the patient is exposed to unnecessary risks [10]. When the first ITP patient successfully treated with rituximab was informed, it drew the attention of physicians because this regimen offered high response rate and low toxicity. In subsequent reports, researchers have found similar results.

The aim of this study was to evaluate the response rate achieved in 18 patients with chronic and refractory ITP after rituximab treatment as well as the evolution of these patients to learn about the possible long-term side effects associated with the use of this drug, information that is

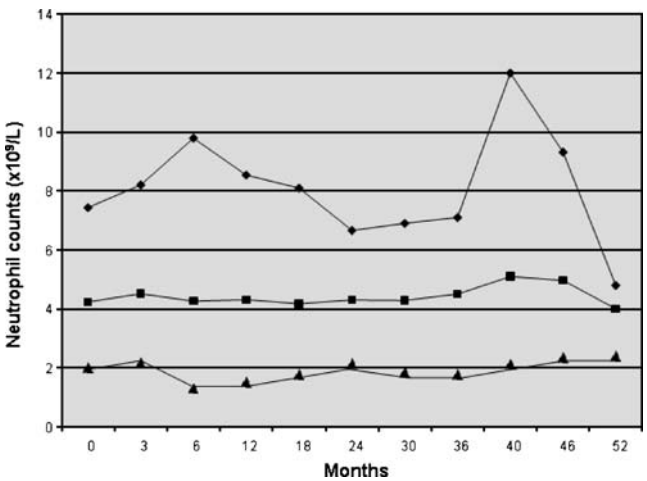

Fig. 4 Mean (*squares*), maximum (*diamonds*), and minimum (*triangles*) neutrophil counts through the follow-up of patients receiving rituximab for chronic and refractory ITP

almost inexistent in the literature. We observed 12 favorable responses (67%) and at least a stable clinical evolution in two additional patients (11%), for a global response rate of 78%. Based on the characteristics of the evolution of our group of patients, we may emphasize some points of interest: (1) Response rate obtained was quite satisfactory considering the history of chronicity and refractoriness of the patients; (2) the monoclonal antibody was well tolerated and caused only mild allergic reactions that could be easily managed with antihistaminics and paracetamol; (3) most of the patients achieve SR; and (4) increase of platelet count was not always immediate (median time to response=14 weeks), a fact that slightly disagrees with previous reports that found shorter time to response (3 to 8 weeks; Table 2) [4, 8, 20]. In fact, in a recent systematic review about the efficacy of rituximab in adult patients with ITP, it was found that median time to response was 5.5 weeks (ranges 2 to 18 weeks) [2]. We do not have an explanation for this discrepancy. After a careful analysis, we did not find significant differences between the results obtained in the systematic review and our results in terms of the variables that may affect the time to response to rituximab, namely, age, sex, previous use of corticosteroids, number of treatments before rituximab, rituximab dose and schedule, period between diagnosis and rituximab administration, duration of ITP before rituximab, and pretreatment platelet counts. Although splenectomy may not be a significant predictor of response to rituximab as previously suggested [2, 4, 8], it should be noted that 83% of the patients included in our report had had splenectomy as compared with 50.5% of the patients in the systematic review. Therefore, history of splenectomy seems to be the only factor related to a different time to response to rituximab in our series.

Of course, we have no data about all possible variables influencing the pattern of response to rituximab. For example, we do not show information about B-cell counts and platelet autoantibodies before and after rituximab therapy, two variables that may influence the response as observed in other trials. The expected therapeutic effect of rituximab is a reduction in specific platelet-associated autoantibodies and the consequent increase in platelet counts. Although rituximab has not been always associated with a reduced load of platelet autoantibodies and most of the publications reporting this effect are small series of cases [1], the high overall response rate obtained in our study allows us to believe that rituximab effectively decreased the B-cell counts as well as the levels of platelet autoantibodies.

Three patterns of response to rituximab have been proposed: early (before the fourth dose of rituximab), intermediate (7 to 11 weeks after rituximab), and delayed (>13 weeks after rituximab) [8]. In our study, the probability to achieve a platelet count $>50\times10^9$/l occurred at a median of 5 months, so we speculate that the complete inhibition of antibody formation and restoration of platelet counts with rituximab may occur after at least 5 months (95%CI=0.5 to 11.6 months) from the first dose of the antibody (Fig. 2). However, some patients may achieve a quite delayed response (as long as 1 year after therapy), a situation in which it is important to wait a reasonable time period before another treatment is planned.

A previous report informed the results obtained in patients treated with the same regimen as we used [4]. After a median follow-up of 47 weeks, the authors observed a lower overall response rate of 44% (CR=18%, PR=15%, MR=10%); most of the responses were sustained. They observed two response patterns: (1) an early-response group in which responses appeared within the first 2 weeks after the first dose of rituximab; (2) a late-response group characterized by an increase in platelets several weeks after rituximab. Finally, after a median follow-up of 72.5 weeks, the response rate was 54% with a majority of SR [8]. In a prospective trial performed in pediatric patients with chronic ITP, treatment with rituximab produced an increase of $>50\times10^9$/l platelets in 11 of 36 children (31%) [3]. Median time to response was 1 week (range, 1 to 7 weeks); however, a 6% incidence of serum sickness was observed. More recently, one of the largest experiences in ITP patients treated with rituximab was published [15]. An increase of $>50\times10^9$/l platelets was observed in 55% of the patients (CR=46%, SR=35%). The only predictive factor for SR was to achieve CR. Patients who were treated more intensively (more than three different treatments) and those with a longer ITP duration (>10 years from diagnosis) had the worse response. In this study, non-splenectomized patients had a higher early response rate than those splenectomized. Although some of these results are similar

Table 2 Patterns of response in patients with chronic and refractory ITP (from [1, 6, 18])

Reference	Number of patients	OR (%)	CR (%)	PR (%)	Median time to response (weeks)
Mayo clinic	12	50	42	8	?
USA–Italy	57	53	31	22	8
Denmark	35	33	18	15	2–8
Peru	22	68	40	27	?
Mexico	18	56	28	28	14

OR Overall response, *CR* complete response, *PR* partial response

to those reported in our study, we believe that contrasting results seen in our study are partially explained by differences in the inclusion and response criteria used, in the evolution time of the disease, and in the number and type of treatments given before rituximab.

As early relapses may be seen in ITP patients treated with rituximab and because re-treatment with this monoclonal antibody offers good results [16], this drug has been used as a long-term maintenance treatment. Based on long-lasting remissions achieved using one or two doses of rituximab [7] and the results of late responders, we feel that a single dose of 375 mg/m^2 every 6 months could be an alternative for this purpose.

On the other hand, because of the mechanism of action of rituximab and its interference with the immune system, we searched for possible long-term complications associated with its use, namely, neoplasias, chronic infections, or autoimmune diseases. Immune status is a major concern in patients treated with rituximab being the induction of low CD20+ counts and hypogammaglobulinemia (specifically low levels of immunoglobulin M), two previously reported secondary effects. Although we do not have data about these two immunological variables, the lack of severe infectious diseases during the long-term follow-up of our patients allows us to speculate that no severe immune abnormalities were developed in our series. As we previously stated, during the follow-up period, no associated illnesses or pathological phenomena were observed. To our knowledge, this seems to be the first report about the lack of long-term complications associated with rituximab.

In conclusion, rituximab is a long-term, safe, and effective alternative treatment of chronic and refractory ITP patients. Prospective randomized clinical trials are needed to elucidate the efficacy of the drug in comparison to splenectomy in early stages of the disease

Acknowledgment The authors declare that the treatments described in the article comply with the current law of the Mexican Republic including all ethical aspects.

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