

ORIGINAL ARTICLE

High response rate to low-dose rituximab plus high-dose dexamethasone as frontline therapy in adult patients with primary immune thrombocytopenia

David Gómez-Almaguer¹, Luz Tarín-Arzaga¹, Brizio Moreno-Jaime¹, José Carlos Jaime-Pérez¹, Adrián Alejandro Ceballos-López¹, Guillermo J. Ruiz-Argüelles², Guillermo J. Ruiz-Delgado², Olga Graciela Cantú-Rodríguez¹, Cesar Homero Gutiérrez-Aguirre¹, Mónica Sánchez-Cárdenas¹

¹Hematology Service, Hospital Universitario, Universidad Autónoma de Nuevo León, Monterrey; ²Centro de Hematología y Medicina Interna de Puebla, Clínica Ruiz, Puebla, México

Abstract

Corticosteroids as initial therapy for primary immune thrombocytopenia achieve a low rate of sustained remission. *Methods:* We prospectively evaluated the efficacy, safety, and response duration of low-dose rituximab plus high-dose dexamethasone as frontline therapy in newly diagnosed primary immune thrombocytopenia patients. One cycle of dexamethasone, 40 mg/d/intravenously for four consecutive days, plus weekly intravenous rituximab, 100 mg for four doses, was delivered. *Results:* Twenty-one consecutive adults were enrolled. The overall response at day +28 was 90.5%. Complete sustained response at 6 months and relapse rate were 76.2% and 15.8%, respectively, compared with 30% and 62.5% for a historical group who had received standard treatment with prednisone ($P = 0.005$ and $P = 0.004$). There was a 9.5% incidence of adverse effects. *Conclusions:* The combination of low-dose rituximab and high-dose dexamethasone as frontline therapy for adults with primary immune thrombocytopenia was effective and had a high overall response rate and a low incidence of relapse.

Key words immune thrombocytopenia; rituximab; low dose; dexamethasone

Correspondence David Gómez-Almaguer, MD, Servicio de Hematología del Hospital Universitario, Universidad Autónoma de Nuevo León, Madero y Gonzalitos Sn. Colonia Mitras Centro, Monterrey, C.P. 64460, México. Tel: +52 81 83486136 and +52 81 86756718; Fax: +52 81 86756717; e-mail: dr_gomez@infosel.net.mx

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Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder that involves antibody- and cell-mediated destruction of platelets as well as suppression of their production. Diagnosis requires an isolated thrombocytopenia below $100 \times 10^9/L$, no other obvious cause for the condition, and no clinically evident secondary form of ITP (1, 2). The incidence is nearly 30 new cases per million annually, and it is more prevalent in women and increases with age (3). Newly diagnosed ITP in children resolves spontaneously in over 70% of cases, whereas in adults, ITP typically has an insidious onset with no preceding viral or other illness, usually following a chronic course (4).

Corticosteroids are the initial standard therapy in adults. Prednisone at a dose of 1–2 mg/kg is given until a rise in the platelet count is obtained, which happens in about 75% of

cases, but only 5–30% will have a sustained remission (5–8). A short course of high-dose dexamethasone as frontline therapy is an alternative. In a Chinese study, the drug given as pulses of 40 mg/d for four consecutive days was initially effective in 85% of the patients; nevertheless, 50% relapsed within 6 months (9). In this setting, Mazzucconi *et al.* (10) have shown better long-term responses if four or more courses of high-dose dexamethasone are administered. On the other hand, splenectomy is the best therapeutic option for chronic ITP, but approximately 40% of splenectomized patients do not respond or relapse after surgery and are at risk of further infections (11). The thrombopoietin receptor agonists, romiplostim and eltrombopag, are both effective for the treatment of patients suffering from chronic ITP, although the response is dependent on continued administration (12, 13).

Rituximab is a chimeric monoclonal antibody directed against CD20, an antigen expressed by mature B cells (14). It was approved and licensed in 1997 for the treatment of follicular B-cell lymphoma and has since been extensively studied in various autoimmune diseases including rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, acquired hemophilia, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemia, and ITP (15, 16). Rituximab is generally administered at the dose approved for lymphomas (375 mg/m^2 weekly for 4 wk); however, the tumor burden in lymphomas is often high, whereas in ITP, the B-cell mass is normal. Therefore, low-dose rituximab (100 mg) has been used for the treatment of ITP, showing an activity almost similar to the standard dose (17).

The lack of sustained response in many patients with newly diagnosed acute ITP has prompted the search for a treatment that modifies both the underlying mechanism and the natural course of the disease. The aim of this prospective study was to evaluate the efficacy, safety, and response duration of low-dose rituximab plus short pulses of high-dose dexamethasone as frontline therapy in newly diagnosed ITP adults.

Methods

Patients

This was a non-randomized trial in patients with newly diagnosed acute ITP. Eligible patients for this prospective study were ≥ 18 yr of age with newly diagnosed ITP. Patients' inclusion criteria were active symptomatic disease with a diagnosis of ITP according to the practice guidelines of the American Society of Hematology and platelet count $<30 \times 10^9/\text{L}$ on two occasions, and no history of ingestion of drugs known to be associated to thrombocytopenia. Patients were excluded if they had active bacterial or viral infection, HIV-, HCV-, or HBsAg-positive serology, pregnancy, or concomitant malignant disease. We included a historical control group with newly diagnosed ITP patients from the same hospital registry, matched as far as possible to the trial group, according to the same baseline characteristics. None of the historical patients included had received rituximab or any previous treatment other than the standard prednisone dose.

Approval for the study was obtained from the Ethics Committee of the School of Medicine and the University Hospital of the Universidad Autónoma de Nuevo León and the Clínica Ruiz. Patients gave informed consent in accordance with the Declaration of Helsinki. This study is registered at www.clinicaltrials.gov as # NCT01107951.

Treatment

Patients received dexamethasone, 40 mg/d*i.v.* for four consecutive days (+1, +2, +3, +4), and rituximab administered

weekly at a fixed dose of 100 mg as an *i.v.* infusion for four consecutive doses (days +1, +8, +15, and +22). A second course of dexamethasone was allowed using the same schedule if the platelet count was $<20 \times 10^9/\text{L}$ before day 30.

Criteria for response and toxicity

A complete blood cell count was performed at enrollment, weekly for the first 28 d, monthly until month 6, and then every 3 months. The degree of response was defined as follows: A complete response (CR) was a platelet count $\geq 100 \times 10^9/\text{L}$; a complete sustained response (CSR) was defined if the platelet level was maintained, without additional therapy, for 6 months. A partial response (PR) was defined as a platelet level between 50 and $100 \times 10^9/\text{L}$; the overall response rate (ORR) included a partial or CR. Patients with a platelet count $<30 \times 10^9/\text{L}$ were considered non-responders (NR) and were excluded of the protocol to receive alternative treatment. The hematological improvement was also assessed evaluating time to response (TTR) and time to complete response (TCR). Relapse-free survival (RFS) included the whole period of spontaneous maintenance of the best response achieved (CR or PR), whereas treatment-free survival (TFS) was defined as the period between response to rituximab and the need for further therapy due to symptomatic or severe ($<30 \times 10^9/\text{L}$) thrombocytopenia. We also assessed the safety profile and side effect incidence according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0.

Statistical analysis

Groups were compared using the chi-square test/Fisher's exact test for categorical data and Student's *t*-test/Mann–Whitney *U* test for quantitative data. Logistic regression analysis was used to assess whether response to the combination therapy was associated with age, gender, and baseline platelet count. The same prognostic factors were correlated with RFS using the Cox regression model. The probability of RFS and TFS was calculated using the Kaplan–Meier method; the long rank test was used to compare the groups. A *P* value <0.05 was considered statistically significant, and all tests were two-sided. Statistical analysis was carried out using SPSS software version 18.0.

Results

Baseline clinical characteristics

Twenty-two consecutive patients were enrolled from December 2009 to December 2011. One patient did not complete the full scheduled treatment and was excluded. Of the 21 remaining patients, 17 were women (81.8%) and four were

men (18.2%). The median age was 51 yr (range, 18–82 yr). The median platelet count at diagnosis was $5.19 \times 10^9/L$ (range, $1–24 \times 10^9/L$). Regarding clinical features, 18 patients (80.9%) had bleeding manifestations grade 1 and 19.1% grade 2 according to the ITP Bleeding Scale (18). Six patients had systemic arterial hypertension, two diabetes mellitus, and one hypothyroidism. Background characteristics and response to therapy for the 21 patients included are shown in Table 1.

Response to therapy

All patients were followed for a median of 16.5 months (range 1.2–33). Patients with PR and CR were followed for a median of 20 months (range 9–33). At day +28, 16 patients (76.2%) had CR and three had PR (14.3%), with the ORR being 90.5%. At month six, 16 of 21 patients (76.2%) had achieved CSR and were relapse-free at the end of follow-up. Seven patients from those 16 with CSR had been retreated with a second short course of dexamethasone (one at day +8 and six at day +15). The median duration of CR was 17 months (range 9–33). The median time to reach TTR and TCR was 8 d (range 4–28). Two of the three patients who had achieved partial remission at day +28 relapsed before month six and were treated with danazol and prednisone, being subsequently splenectomized. The relapse rate was 15.8%. Cumulative 12-month RFS and TFS were 84% and 80.9%, respectively (Figs 1 and 2).

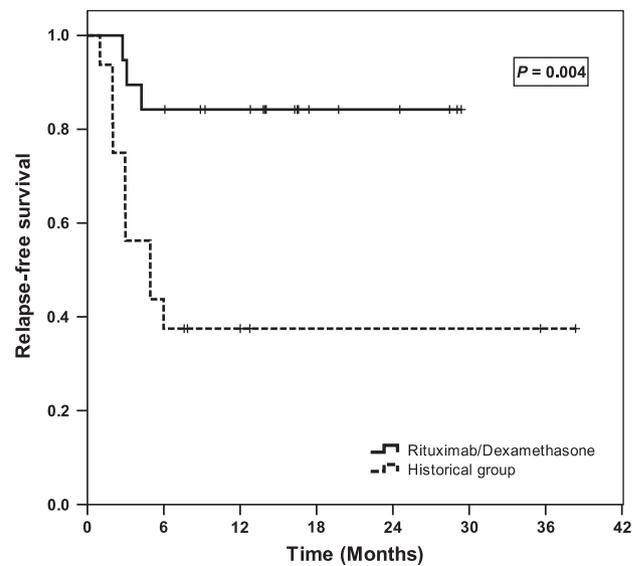


Figure 1 Cumulative relapse-free survival (RFS).

Univariate logistic regression showed no association of CR with age, OR = 1.5, 95% CI (0.195–11.5) ($P = 0.55$), gender, OR = 0.92, 95% CI (0.74–11.5) ($P = 0.69$), or baseline platelet count, OR = 2.8, 95% CI (0.325–25.7) ($P = 0.33$). Stepwise Cox regression analysis showed no relationship between RFS and age ($P = 0.53$), gender ($P = 0.45$) and baseline platelet count ($P = 0.65$).

Table 1 Salient features and response for 21 patients treated with dexamethasone and rituximab

Patient no.	Sex/Age	Basal platelets $\times 10^9/L$	Best response	Response at day 28	Time to PR/CR (wk)	Duration of response (months)	Current status	Follow-up (months)	Additional treatment
1	F/49	6	CR	CR	1/1	33	CSR	33	
2	M/54	18	CR	CR	1/1	29	CSR	29	
3	F/54	15	CR	CR	1/3	29	CSR	29	Dex
4	F/32	8	CR	CR	1/1	28	CSR	28	
5	F/56	3	CR	CR	2/2	28	CSR	28	Dex
6	F/64	18	PR	PR	1/–	–	PUR	26	P, D, S
7	F/18	18	PR	PR	1/1	–	PUR	25	P, D, S
8	F/73	5	CR	PR	1/1	–	CUR	25	D
9	F/49	4	CR	CR	1/1	24	CSR	24	
10	M/19	1	NR	NR	–/–	–	NR	5	
11	M/58	8	CR	CR	3/3	17	CSR	17	Dex
12	F/51	7	CR	CR	2/2	17	CSR	17	
13	F/58	3	CR	CR	1/1	16	CSR	16	
14	F/80	2	CR	CR	3/3	16	CSR	16	Dex
15	F/29	24	CR	CR	1/1	16	CSR	16	
16	F/62	5	CR	CR	1/4	16	CSR	16	Dex
17	F/82	1	NR	NR	–/–	–	NR	1	
18	M/41	1	CR	CR	1/1	13	CSR	13	Dex
19	F/40	5	CR	CR	1/1	9	CSR	9	
20	F/21	3	CR	CR	3/3	20	CSR	20	Dex
21	F/33	2	CR	CR	4/4	14	CSR	14	

PR, partial response; CR, complete response; CSR, complete sustained response; NR, no response; PUR, partial unsustained response; CUR, complete unsustained response; P, prednisone; D, danazol; S, splenectomy.

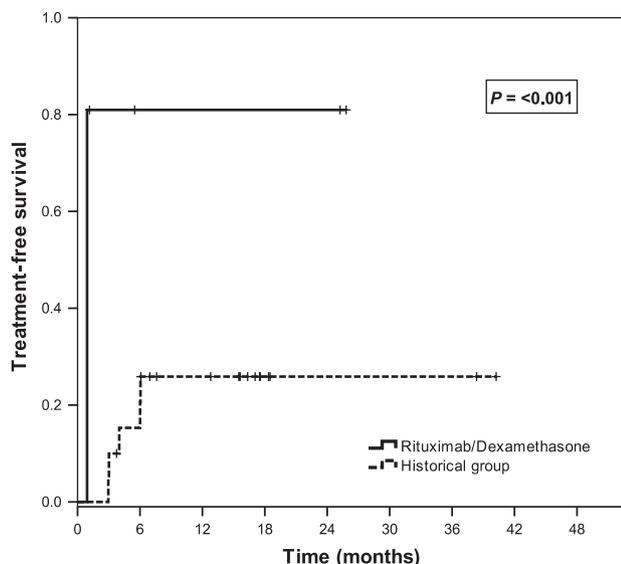


Figure 2 Cumulative treatment-free survival (TFS).

Historical control

Twenty patients with de novo presentation of ITP integrated a historical control group. They were diagnosed from January 1, 1999 to October 18, 2010. A summary of patient characteristics and platelet counts is given in Table 2. All of them had symptomatic newly diagnosed acute ITP with $\leq 30 \times 10^9/L$ platelet count. They were treated with the standard therapy for ITP, prednisone 1–2 mg/kg/d.

In the trial group, 76.2% of patients ($n = 16$) reached CSR at some point during their follow-up, in contrast to the historical group where a 30% CSR rate was obtained ($n = 6$) $P = 0.005$. Combination therapy was associated with an OR of 7.4 (CI 1.8–29.8) compared with standard therapy according to the probability of achieving sustained CR.

During their follow-up, the majority of patients in the trial group (90.5%) obtained CR/PR, in comparison with 80% in the historical group ($P = 0.001$). The use of combination therapy was associated with a HR of 2.5 (CI 1.1–5.4)

Table 2 Demographics and platelet count of patients

	Rituximab/Dex $n = 21$	Historical control $n = 20$	P
Age, median (Range)	51 (18–82)	43 (16–83)	0.640*
Gender [n (%)]			
Female	17 (81)	15 (75)	0.710**
Male	4 (19)	5 (25)	
Platelet count (Mean \pm SD)			
Diagnosis	7 (7)	13 (9)	0.042***
Day 28	177 (104)	115 (84)	0.045*
Month 6	191 (96)	139 (80)	0.068*

*Student's t -test; **Fisher's exact test; ***Mann–Whitney U test; Dex, dexamethasone.

($P = 0.016$) in relation to the probability of achieving CR. The median of days to reach CR was 8 (4–28) in contrast to a median of 30 d (8–180) in the historical group ($P = 0.004$).

In the trial group, 80.9% ($n = 17$) achieved treatment freedom after one cycle of treatment, in contrast to the historical group, in which 25% are without pharmacological treatment, $P \leq 0.001$ (Table 3). Because of the characteristics of the medication scheduled, the group treated with rituximab/dexamethasone reached freedom of treatment after day 22, contrary to the historical group in which this status was present at a median of 152 d (90–184).

The trial group presented a 15.8% relapse rate ($n = 3$) in contrast to 62.5% ($n = 10$) in the historical group ($P = 0.004$). The Cox regression analysis yielded a HR of 0.18 (CI 0.051–0.674) relapse risk in relation to the historical group ($P = 0.011$). Of the relapsed patients, two achieved PR with danazol and prednisone being subsequently splenectomized, and one patient lost his CR without further treatment.

Safety

Overall, combination therapy was well tolerated. One patient experienced mild chills during the first infusion of rituximab (CTCAE grade 1). Another patient developed a flu-like syndrome (CTCAE grade 1) after the administration of the third dose of rituximab. Infectious events were not observed.

Discussion

For more than 50 yr, conventional therapy for ITP has relied on corticosteroids as first-line treatment. However, despite the high initial therapeutic efficacy, in most cases, a drop in platelet count and a need for additional treatment follow

Table 3 Response rate and outcome after therapy

	Rituximab/Dex $n = 21$ [n (%)]	Historical control $n = 20$ [n (%)]	P
Day +28			
CR	16 (76.2)	9 (45)	0.109*
PR	3 (14.3)	5 (25)	
NR	2 (9.5)	6 (30)	
Month 6			
CR	16 (76.2)	13 (65)	0.520*
PR	1 (4.8)	3 (15)	
NR	4 (19.0)	4 (20)	
Complete sustained response	16 (76.2)	6 (30)	0.005**
Treatment-free rate	17 (80.9)	5 (25)	<0.001***
Relapse rate	3/19	10/16	0.004***

CR, complete response; NR, non-responders; PR, Partial remission. *Chi-square test; **Fisher's exact test; ***Log Rank; Dex, dexamethasone.

steroid-tapering withdrawal. Besides, long-term therapy with steroids, even at low doses, is associated with multiple known side effects.

It is now accepted that B cells have a conspicuous role in the development and perpetuation of the autoimmune response, so their depletion may be used as a treatment approach for autoimmune diseases like ITP. A complete B-cell depletion is achieved with rituximab, and detectable levels of this drug are reported at week 12 after the start of this therapy (19). The results of this study are consistent with previous reports in which a much lower dose of rituximab (nearly one-seventh of the standard dose) is sufficient to show efficacy in the treatment of active ITP (17, 19, 20).

In this study, all enrolled patients were newly diagnosed; therefore, they were all treatment naive. Previous studies have shown that the probability of successful response to rituximab is inversely proportional to the period between diagnosis and treatment (20–23); this fact may explain the low relapse rate in our study (15.8%). Clinical experience has shown a success rate of 40–60% with a median response time of 5.5 wk and a median clinical improvement time of 10.5 months (24). Our results show that 16 of 21 patients (76.2%) achieved complete and sustained response ($>100 \times 10^9/L$ for at least 6 months). Interestingly, all of these 16 patients were in CR since day +28, and all of them are nowadays relapse-free. This is an important finding, as it supports the conclusion that the majority of responses in our group are in fact due to the use of low-dose rituximab plus high-dose dexamethasone, and not the result of spontaneous remission, which has been reported to range between 9% and 30% of chronic ITP individuals either in or off treatment, splenectomized or not (25, 26). In terms of CR duration, the median time was 17 months. The median TTR of 8 d is probably related to the use of dexamethasone, which acts as a bridge until the effect of rituximab takes place. In fact, we delivered a second dose of dexamethasone in seven patients who had a platelet count $<20 \times 10^9/L$ before day +30 (one at day +8 and six at day +15), waiting for the rituximab effect; all of them had CR at day 28. In a systematic review by Arnold *et al.* (15), it was noted that response to the antibody usually occurred 3–8 wk after the first dose. It is important to note that the Italian group GIMEMA obtained good results using four courses of high-dose dexamethasone administered in the first 2 months in previously untreated ITP patients, although they included patients aged 2–70 yr of age (10). Three of our 21 patients in the trial were more than 70 yr of age; 1 (80 yr) obtained a CSR, another (73 yr) obtained a complete but unsustained response requiring danazol, and a third patient (82 yr) was a NR.

In the setting of chronic ITP, Stasi *et al.* (27) demonstrated that 10 of 25 patients responded to 375 mg/m^2 rituximab weekly during 4 wk and 7 (28%) maintained this response for at least 6 months; in México, García-Chavez

et al. (28) treated 18 patients with chronic and refractory ITP with the standard 375 mg/m^2 dose and reported a CR in five patients (28%) and a sustained response in 12 (67%). On the other hand, Zaja *et al.* (20) investigated the activity of low-dose rituximab as salvage therapy in 48 patients with previously treated symptomatic ITP; CR was achieved in 39.5%, but 55% of patients relapsed and 14 (29.2%) needed further treatment. We have previously reported a prospective trial employing a low-dose rituximab plus alemtuzumab combination for patients with steroid-refractory autoimmune cytopenias, with an ORR of 100% and a CR of 58% with a median of response duration of 46 wk (29).

In relation to acute ITP, Zaja *et al.* (30) performed a randomized trial including only newly diagnosed patients, of whom 52 were treated with dexamethasone alone for 4 d and 49 with dexamethasone plus rituximab 375 mg/m^2 weekly for 4 wk; sustained response (platelets $\geq 50 \times 10^9/L$ at month 6) was achieved in 63%. What is noteworthy in this study is that patients in both arms could receive rescue therapy with corticosteroids. In fact, 47% of patients in the rituximab group received additional steroid doses and/or intravenous immunoglobulin therapy up to day 28. Li *et al.* (31) reported that adding rituximab 100 mg weekly on days 7, 14, 21, and 28 to dexamethasone and prednisone, in previously treated and newly diagnosed ITP patients, increased the sustained response (77.4% vs. 38.7%); our data appear to confirm these results in that over 70% of ITP adults can obtain sustained response when low-dose rituximab is added to steroids. In a recent Italian study, it was suggested that the standard-dose rituximab is a better alternative for the treatment of newly diagnosed and chronic ITP; however, this conclusion is hampered by the lack of statistical significance (23). In comparison with the study of Zaja *et al.* (30), we used a fixed dose of 100 mg of rituximab, starting on day 1, and our CSR of 76.2% was slightly better. On the other hand, in contrast with the study by Li *et al.* (31), we only included treatment-naive patients, the initial infusion of rituximab was delivered on day 1, and no prednisone was added to the initial 4 d of dexamethasone. We have also demonstrated a significant improvement of CSR in the trial patients compared with the historical control group, where 62.5% of patients relapsed in contrast to 15.8% in the rituximab group ($P = 0.004$).

Our results differ from those shown by other studies in which the best response correlated with a younger age (19, 20). None of the potential predictive clinical or laboratory parameters (age, gender, baseline platelet count) were found to be significant when the logistic regression model was used. We hypothesize that this could be due to the efficacy of rituximab in preventing development of high-affinity splenic phagocytosis when administered early in treatment-naive ITP patients, allowing time for a tight immune response control to take place in the context of slower-acting steroid therapy.

Overall, the combination therapy in our group was well tolerated. There was a lower incidence of infusion-related adverse effects in this trial in comparison with the data reported in previous studies (24) (9.5% vs. 17%). This difference can be explained by the use of a considerably lower dose of rituximab. Dexamethasone was well tolerated, considering that only a single course was used in the majority of patients.

Ours and other recently published results reinforce the trend to avoid splenectomy for the treatment of steroid-resistant ITP, although controlled clinical trials directly comparing rituximab vs. splenectomy in the long term are yet to be published; the need to define the hierarchy of the available therapeutic options for ITP patients is now evident; it could be that additional studies reinforce the role of rituximab as the first choice in newly diagnosed individuals (32).

Two randomized controlled studies published within the last year found results somewhat different from ours; in the first study, no difference in response at 6 months of follow-up was documented between individuals receiving standard-dose rituximab or placebo (saline solution); however, this group differed from ours as the recruited patients were already receiving standard steroid and other treatments for several weeks before the administration of rituximab; furthermore, they included non-splenectomized adults with newly diagnosed or relapsed ITP (33); in the second report, which compared high-dose dexamethasone plus standard-dose rituximab vs. dexamethasone alone, the rituximab arm ($n = 62$) reached a 58% of sustained response vs. 37% in the dexamethasone arm ($n = 71$, $P = 0.02$) (34). Patients in this report were allowed to receive oral prednisolone for up to 1 wk prior to enrollment; thus, although not directly comparable with our smaller treatment-naïve group, this study also found a definitive superiority of combined rituximab-dexamethasone therapy.

To the best of our knowledge, this is the first trial evaluating the response to low-dose rituximab in combination with high-dose dexamethasone in newly diagnosed patients with ITP. Potential advantages of using this therapy are the high rate of CSR, a lower incidence of side effects, a long-term steroid-sparing effect, the splenectomy-sparing capacity, and important cost improvement, which is particularly beneficial in centers where patient access to this type of biological drugs is limited, as is the case in our institution. The strengths of this pilot study are its prospective methodology and the inclusion of only newly diagnosed ITP patients. Limitations include the use of a historical control group, short length of follow-up, and the small sample size.

In conclusion, the combination of low-dose rituximab and high-dose dexamethasone as frontline therapy for adults with ITP was effective and had a high ORR and a low incidence of relapse. These data need to be confirmed in a prospective randomized clinical trial including a sample size with enough power to reach statistically significant conclusions

and determine the position of this therapeutic option in the treatment of patients with newly diagnosed ITP.

Author contributions

David Gómez-Almaguer was responsible for designing the protocol and writing the manuscript. Luz del Carmen Tarin-Arzaga was responsible for reviewing the response to treatment, Table 1 and Fig. 1. Brizio Moreno-Jaime and Monica Sánchez-Cárdenas were responsible for data analysis. José Carlos Jaime-Pérez was responsible for designing and writing the protocol. Adrián Alejandro Ceballos-López was responsible for study drug administration. Guillermo J. Ruiz-Argüelles was responsible for patients' care and follow-up. Guillermo J. Ruiz-Delgado was responsible for patient recruitment and study drug administration. Olga Graciela Cantú-Rodríguez was responsible for patients' care. Cesar Homero Gutiérrez-Aguirre was responsible for patients' follow-up and drafting the manuscript. Mónica Sánchez-Cárdenas was responsible for designing the tables and figures.

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