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ORIGINAL ARTICLE

A retrospective analysis of 122 immune thrombocytopenia patients treated with dapsone: Efficacy, safety and factors associated with treatment response

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Abstract

Background: The optimum second-line treatment or best sequence of treatments for immune thrombocytopenia (ITP) are yet to be determined. Our institution has accumulated extensive experience regarding the use of dapsone as second-line therapy for ITP.

Objectives: We aimed to assess the efficacy rate and safety of dapsone treatment in ITP patients.

Patients/Methods: Here we report our experience in a retrospective study, including 122 patients, with a median treatment duration with dapsone of 6 months and a median follow-up period of 3.4 years.

Results: The overall response rate in this cohort was 66%, including 24% of complete responses. Among responders, in 24% a relapse occurred while on treatment. Therefore, a sustained response was observed in 51% of patients. Interestingly, 81% of the responders maintained the response after the interruption of treatment, for a median time of 26 months. Side effects were reported in 16% of the patients in this cohort and treatment was interrupted due to side effects in 11% of patients. The main cause in these cases was hemolytic anemia and methemoglobinemia. Reductions in hemoglobin levels during the use of dapsone were seen in 94% of the patients. Responders presented significantly greater reductions in their hemoglobin levels than nonresponders did: median hemoglobin drop of 1.9 g/dl vs. 1.2 g/dl (p = .004).

Conclusions: Our findings suggest that dapsone has adequate efficacy and is well tolerated. Although the mechanism of action is still unclear, our observation that the degree in the drop of hemoglobin is greater in responders suggest a possible role of the blockage of the reticuloendothelial system in the therapeutic effect of the drug.

de Paula and Annichino-Bizzacchi are joint senior authors.

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KEYWORDS
dapsone, hemolysis, immune thrombocytopenia, methemoglobinemia, splenectomy

1 | INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune disorder that assumes a chronic form in more than 60% of the affected adults.[1,2] Around 70% to 90% of adult ITP patients need medical treatments because of very low platelet counts or bleeding, usually in the first year of the diagnosis.[1,3,4] Corticosteroids are the standard first-line therapy, but only 20% of patients will have long-term responses.[5,6] Therefore, the vast majority of patients end up requiring second-line therapies. The best second-line therapy for ITP is currently considered a challenge. Splenectomy has historically been the standard second-line therapy, but with the advent of new therapeutic options such as rituximab and thrombopoietin receptor agonists (TPO-RA) in the past few years, the actual performing rate has declined significantly.[7,8] These new drugs have encouraging efficacy reports, but an important limitation in their use is their cost, which enforce a rational use of these options, particularly in countries with limited resources.

Despite the increase in new treatment possibilities, most international guidelines make no recommendations as to which should be considered the best sequence of treatments because of lack of evidence.[9,10] These guidelines emphasize the main therapeutic options for second-line therapies splenectomy, rituximab and TPO-RA, and the choice between them should be based on patient characteristics.[9] In specific situations, second-line recommendation suggests TPO-RAs as first choice.[9] A systematic review on the topic and the latest guideline from the American Society of Hematology concluded that because of the absence of good-quality evidence there is need for additional research in this setting.[9,11] In view of these considerations, many experts believe there is still space for older and inexpensive alternative drugs in ITP treatment, such as dapsone, mycophenolate mofetil, azathioprine, or hydroxychloroquine, especially in cost-restricted scenarios.[12–16]

Dapsone is mainly used in infectious diseases such as leprosy and malaria because of its antimicrobial action, which occurs in the same way as for sulfonamides, through inhibition of synthesis of dihydrofolic acid.[17] It is also used for its anti-inflammatory effect in several chronic dermatological conditions such as dermatitis herpetiformis, cutaneous lupus erythematosus, and others.[18] The first published report of its successful use in the treatment of ITP was in an incidental finding, when the drug was used to treat discoid skin lesions of a patient with systemic lupus erythematosus.[19] Since then, a few prospective open-label studies and small retrospective analyses have been published showing its potential use in ITP.[20] It is included in the international consensus report as a possible second-line treatment for ITP.[5,10] In our center, we have been using dapsone as standard second-line therapy for ITP patients for the past 25 years and have accumulated extensive data regarding this drug.[21] In this study, we aimed to assess the efficacy rate, frequency, and severity of side effects of dapsone therapy in ITP patients. As a secondary endpoint, we wanted to evaluate possible clinical factors associated with a treatment response.

Essentials

- The best second-line therapy for immune thrombocytopenia (ITP) is a matter of debate.
- Retrospective review of 122 ITP patients treated with dapsone in a single institution.
- The response rate observed was 66%, including 24% of complete responses.
- Responders presented a greater fall in hemoglobin levels than nonresponders.

2 | METHODS

This was a single-center retrospective study, in which medical charts of all ITP patients in follow-up at our outpatient clinic in the period of 2011 through 2017 were reviewed for identification of patients treated with dapsone. Our center is a reference center for treatment of mainly adult ITP patients, but we occasionally receive pediatric patients older than age 10 years. The institution's ethics committee approved the study and informed consent was obtained of participating patients. A standard reporting questionnaire was used for the collection of information regarding demographic data, clinical data of ITP diagnosis and classification, previous treatments, dapsone treatment information, dapsone-related side effects, and treatments received after the withdrawal of dapsone. Platelet counts at initiation, throughout treatment, and at last follow-up were recorded for all patients. Available data regarding methemoglobin levels and hemolysis markers (hemoglobin, hematocrit, reticulocyte counts, lactate dehydrogenase levels, haptoglobin, and indirect bilirubin) at the beginning and during dapsone treatment were also recorded. All of the study information was collected by two investigators and verified by a third investigator.

2.1 | Eligibility criteria

Patients were included in the study if they met the inclusion criteria: (1) diagnosis of primary or secondary ITP, (2) age over or equal to 10 years, and (3) dapsone treatment for a period of at least 15 days. Exclusion criteria were concomitant use of any other therapies for ITP, important missing data (precluding definition of treatment response), or significant hypersplenism. Patients in use of low doses of corticosteroids at dapsone initiation were excluded, because we considered this as concomitant medication. The recommendations

from the International Working Group were used for the definitions of ITP diagnosis, duration, and classification.[22] All of the patients underwent routine diagnostic studies for excluding secondary causes for thrombocytopenia.

2.2 | Response criteria

This study used the criteria from the International Working Group for assessing treatment response to dapsone.[22] Response (R) was defined as platelet counts over or equal to 30×10^9/L and at least a 2-fold increase from baseline values; complete response (CR) was defined as platelet counts over or equal to 100×10^9/L. Platelet counts had to be confirmed in two separate occasions, at least 1 week apart, and performed while patients were on treatment with dapsone. Patients achieving R or CR were classified as responders. Patients were only classified as R in the absence of treatment with anything other than dapsone in the 30 days preceding the initiation of the drug. Patients that did not meet criteria for R or CR were classified as nonresponders (NR).

Loss of response (LR) was defined as platelet counts falling below 30×10^9/L or bleeding symptoms or indication of a treatment intervention. Duration of response was calculated as the time since first achievement of response or complete response to the time of loss of response.

Definitions for maintenance of response were the following: patients were considered to have sustained responses if they did not experience a loss of response, regardless of being on dapsone or not. Treatment-free response (TFR) was defined as patients maintaining response criteria for a minimum of 2 months after the termination of dapsone treatment.

2.3 | Dapsone treatment and follow-up

In our center, we use dapsone as the standard second-line therapy for ITP patients that fail to respond to corticosteroids or are corticosteroid dependent since 1996. Occasionally, it is used later on in treatment of our ITP patients (as third- or fourth-line therapy) or as first-line therapy if a patient has a contraindication for the use of corticosteroids. The starting dose is routinely a fixed dose of 100 mg/d. We usually wait until completion of 2 months of treatment to consider a patient an NR. At that time, treatment is discontinued in the majority of these patients. In some NR patients, we occasionally increase the dosage to a maximum of 150 mg/d. In the responders, treatment is generally maintained for a minimum of 6 months and then dose tapering is initiated, with reductions to 25 to 50 mg/d. If a patient experiences a LR during dose tapering, the dosage is usually increased to 100 mg/d. In these cases, treatment can be prolonged for long periods, in the absence of adverse events. Blood counts are performed at all outpatient visits, usually the first evaluation is performed between 3 and 6 weeks after initiation of treatment. We routinely measure hemolysis markers and methemoglobin levels 1 month after the initiation of dapsone since May 2014. Before that, these tests were only performed if a patient presented with symptoms suggestive of methemoglobinemia or developed anemia. Because patients with G6PD deficiency have an increased sensitivity toward dapsone-induced hemolytic anemia and are prone to having a severe hemolytic crisis,[23] screening for G6PD deficiency has been performed before initiation of dapsone treatment since 2014 to avoid exposing a patient with G6PD deficiency to the drug.

2.4 | Statistical analyses

Interval data were described as median, interquartile range (IQR), and in some cases, the minimum and maximum values. Categorical data were described as absolute numbers and frequencies. Comparisons between groups of patients with different outcomes were performed with Mann-Whitney *U* test for interval data and for categorical data with chi-square or Fisher's exact test when necessary. To compare pre- and posttreatment interval data Wilcoxon matched-pair test was used. To determine which baseline characteristics were independent predictors of response we performed a multivariable analysis. *p* values below .05 were considered significant. Statistical analyses were performed using SAS System for Windows (version 9.4.) and SPSS 26 (IBM SPSS version 26).

3 | RESULTS

3.1 | Patients' characteristics

Medical charts from 272 ITP patients were reviewed, with the identification of 147 patients treated with dapsone during the follow-up period. Eight patients did not meet inclusion criteria because of short duration of dapsone treatment. Seventeen patients were excluded from the study because of: concomitant use of other therapies for ITP ($n = 8$), important missing data ($n = 7$), and significant hypersplenism ($n = 2$). The final total of evaluated patients was 122. Baseline characteristics of these patients are shown in Table 1. We included four pediatric patients in this study, with ages ranging from 11 to 17 years. Among the 21 patients with secondary ITP, the main associated causes were viral infections (human immunodeficiency virus and hepatitis C) in 11 patients and lupus in six patients.

3.2 | Treatment efficacy

The overall response rate in this cohort was 66% ($n = 81$), including 24% of CRs ($n = 29$). The best platelet count achieved during treatment among responders was a median of 104×10^9/L (IQR 75–145×10^9/L). Median platelet counts observed during the first year of treatment in the three patient groups are shown in Figure 1.

Among the adult patients with primary ITP ($n = 97$) the response rate was 64% ($n = 62$), with 23% ($n = 22$) of CRs. If we analyze only

the response rate of dapsone when used as second-line therapy in adult patients with primary ITP ($n = 77$), the response rate was 71% ($n = 55$), with 25% of CRs ($n = 19$).

TABLE 1 Baseline characteristics of study patients

Baseline Characteristics of Study Patients	All Patients ($N = 122$)
Age - years, median (range)	50 (11–84)
Female sex, *n* (%)	89 (73)
Primary ITP, *n* (%)	101 (83)
Phases of the disease	
Newly diagnosed ITP, *n* (%)	22 (18)
Persistent ITP, *n* (%)	33 (27)
Chronic ITP, *n* (%)	67 (55)
Time since ITP diagnosis – years, median (range)	1.1 (0.3–25)
Number of prior ITP treatments, *n* (%)	
None	11 (9)
One - corticosteroids	94 (77)
Two - corticosteroids (first line), splenectomy (second line)	9 (7)
Three or more[a]	8 (7)
Previous splenectomy, *n* (%)	17 (14)
Baseline platelet count – 10^9/L, median (IQR)	19 (10–25)
Baseline hemoglobin level – g/dl, median (IQR)	14.0 (12.9–15.2)

Abbreviations: IQR, interquartile range; ITP, immune thrombocytopenia.
[a]Previous treatments were the following: corticosteroids (first line), splenectomy (second line), and rituximab (third line) in five patients; corticosteroids (first line), splenectomy (second line), azathioprine (third line), and cyclosporine (fourth line) in one patient; corticosteroids (first line), splenectomy (second line), and eltrombopag (third line) in one patient; corticosteroids, rituximab, and azathioprine in one patient.

Twenty percent ($n = 8$) of the NRs had their dosage escalated to 150 mg/d, the median time for this dose escalation was 2.4 months (IQR 1.7–2.3 months). None of these patients responded to the dose escalations, and it resulted in occurrence of symptomatic methemoglobinemia in one patient, leading to the withdrawal of the drug.

The median time to achieve a response was 31 days (IQR 21–48 days), varying from a minimum of five up to 132 days. If we look at time points of 30, 60, and 90 days, the percentage of responders that achieved a response at these time points were 50%, 75%, and 92%, respectively. It is important to keep in mind that these are retrospective data, and patients returned for their first evaluation after initiation of treatment in different time intervals, which were highly variable. Among responders, 76% had achieved a response in their first evaluation after initiating treatment, so it is possible that the median time for a response could be shorter than what we observed.

In this cohort, 80% ($n = 98$) of patients completed at least 3 months of treatment and the efficacy rate at 3 months was 65% ($n = 79$), including 24% ($n = 29$) of CRs. Fifty-nine percent of patients ($n = 72$) completed at least 6 months of treatment, and the efficacy rate at 6 months was 63% ($n = 77$), including 24% ($n = 29$) of CRs. Detailed information regarding response characteristics and classifications are shown in Table 2.

3.3 | Possible baseline clinical factors associated with treatment response

We analyzed if any of the baseline clinical characteristics were associated with a greater chance of treatment response, and saw that patients that had been submitted to a splenectomy before dapsone treatment ($n = 17$) had a higher chance of treatment failure when compared with patients that had not had a splenectomy ($p = .003$;

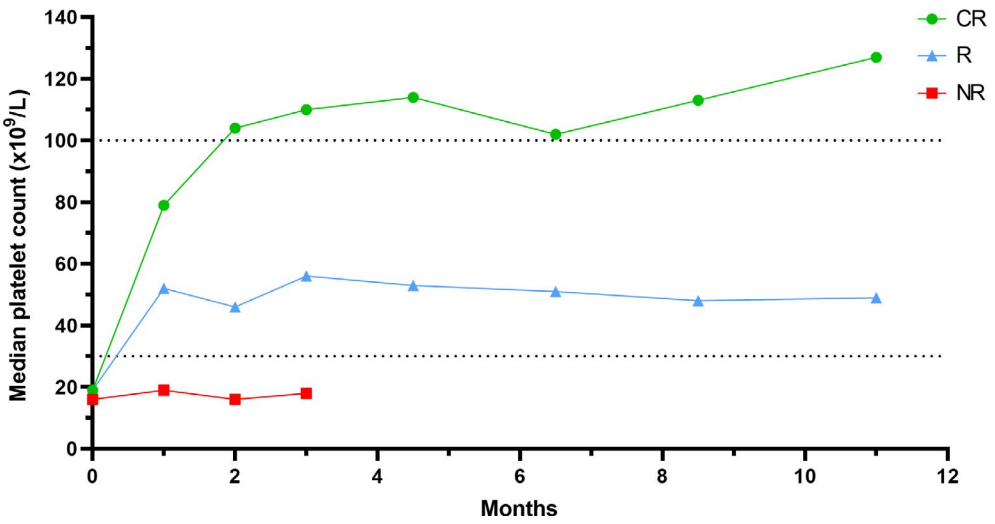

FIGURE 1 Platelet counts during dapsone treatment. Median platelet counts over time during the first year of treatment for the three groups – patients with a complete response (CR), response (R) and nonresponders (NR). Treatment lasted a median of 3 months in nonresponders

TABLE 2 Response characteristics and classifications

	All Patients $n = 122$	Nonresponders (NR) $n = 41$	Responders (CR+R) $n = 81$	Complete Response (CR) $n = 29$	Response (R) $n = 52$	p
Time since ITP diagnosis (y)[a]	1.1 (0.4–4.7)	1.5 (0.5–5.6)	1.0 (0.4–4.3)	0.7 (0.2–1.3)	1.6 (0.5–4.9)	**.03**[f]
Duration of treatment (m)[a]	5.7 (2.9–13.3)	2.8 (1.6–4.6)	9.6 (4.5–16.9)	9.2 (4.0–14.7)	10.7 (4.7–26.5)	.46[f]
Follow-up after suspension of dapsone (y)[a]	2.7 (0.9–4.4)[b]	2.0 (0.8–3.7)	2.9 (1.1– 4.8)	3.2 (2.2– 4.8)	2.5 (0.7– 4.8)	.24[f]
Total study follow-up (y)[a]	3.4 (1.3–4.9)	2.5 (1.0–3.7)	4.0 (2.0–5.6)	4.0 (2.1–5.4)	3.8 (1.6–5.7)	.92[f]
Time to response (days)[a]	\	\	32 (21–48)	28 (21–53)	34 (22–47)	.59[f]
Proportion of patients with response at 3 months, *n* (%)	79/122 (65%)	43/122 (35%)[c]	79/122 (65%)	29/122 (24%)	50/122 (41%)	\
Proportion of patients with response at 6 months, *n* (%)	77/122 (63%)	45/122 (37%)[d]	77/122 (63%)	29/122 (24%)	48/122 (39%)	\
LR during dapsone treatment, *n* (%)	\	\	19 (23%)	2 (7%)	17 (33%)	**.01**[g]
LR after cessation of dapsone, *n* (%)	\	\	9 (17%)[e]	3 (13%)	6 (21%)	.47[g]
Duration of response under treatment (m)[a]	\	\	7.0 (3.8–15.3)	7.8 (3.1–13)	6.1 (4.1–24.9)	.54[f]
Duration of TFR (m)[a]	\	\	26 (5–48)	38 (7–52.5)	22 (2–41.5)	.16[f]

Abbreviations: CR, complete response; IQR, interquartile range; ITP, immune thrombocytopenia; LR, loss of response; m, months; NR, nonresponders; R, response; y, years.

Bold indicates statistically significant p values.

[a]Data expressed as median (IQR).

[b]The number of patients that interrupted treatment during study follow-up was 109, including 40 nonresponders and 69 responders (26 CR and 43 R).

[c]This number ($n = 43$) includes the 41 nonresponders, one patient with a response (R) that presented a loss of response (LR) at 3 months of treatment (LR on drug) and one patient with a response (R) that presented a LR after the suspension of dapsone (LR off drug).

[d]This number ($n = 45$) includes the 41 nonresponders, the 1 patient with a response (R) that presented a LR off drug, and 3 patients with a response (R) that presented a LR on drug at 3, 4, and 5 months of treatment.

[e]The number of patients with sustained responses during dapsone treatment and that ended their treatment during study follow-up was 52, including 24 CR patients and 28 R patients.

[f]Mann-Whitney test, two-tailed (CR vs. R).

[g]Fisher's exact test, two-sided (CR vs. R).

chi-square test). The response rate observed in splenectomized patients was only 35% (vs. 71% in nonsplenectomized patients).

When we compared time since ITP diagnosis between NRs and Rs (CR+R), there was no significant difference ($p = .25$; Mann-Whitney); however, we found a difference in time since ITP diagnosis between patients with a CR and those with an R ($p = .03$; Mann-Whitney), being shorter in the former (median of 8 vs. 19 months) (Table 2).

To try to determine which baseline characteristics (age, sex, time since ITP diagnosis, and previous splenectomy) were independent predictors of response, we performed a multivariable analysis. Previous splenectomy was the only baseline characteristic independently associated with a response to dapsone (OR 0.06; 95% CI 0.004–0.921), meaning that nonsplenectomized patients had a 94% greater chance of responding to dapsone, in comparison to the previously splenectomized patients. However, among the 17 patients with previous splenectomy, in 94% ($n = 16$) dapsone was used as a third-line therapy. So, we added in the multivariable analysis dapsone as third-line therapy as a predictor of response, and it was not possible to analyze the independent effect of dapsone as third-line therapy on treatment response because these predictors (previous splenectomy and dapsone as third-line) are redundant.

3.4 | Long-term remissions

Median treatment duration was 6 months for the entire cohort, with a median duration of 3 months in NRs and 10 months in Rs. The median study follow-up period was of 3.4 years, varying from a minimum of 1.3 months up to 17.6 years. Total duration of response was a median of 24 months, being significantly longer in patients with CR when compared with those with R (median of 34 vs. 12 months; $p = .05$; Mann-Whitney test).

Dapsone treatment had been definitely interrupted in 89% ($n = 109$) of the patients and 11% ($n = 13$) were still in treatment with the drug at last study follow-up. Reasons for cessation of dapsone treatment in this cohort were: absence of response ($n = 36$; 36%), tapering in responsive patients ($n = 25$; 27%), noncompliance ($n = 19$; 17%), loss of response ($n = 14$; 13%), and adverse events ($n = 12$; 13%). At last follow-up, 15% ($n = 12$) of the responders were still in treatment with dapsone.

Dose tapering was performed in 33% of responders ($n = 27$). Median time to initiate dose tapering was 4.9 months. Six of these 27 patients that initiated dose tapering during the study period, were still in use of dapsone at the last follow-up. In the 21 patients that completed the tapering and suspended the drug during the study period, the total duration of dose tapering was a median of 6 months (IQR 2.4–14.8 months). This great variation in the duration of dose tapering was due to decreases in platelet counts during tapering, which led to new escalations of dosages.

Among all responders ($n = 81$), 23% ($n = 19$) experienced a loss of response while on dapsone therapy. Therefore, a sustained response was observed in 51% ($n = 62$) of the study cohort. In only 16% ($n = 3$) of the patients with relapses this loss of response occurred during dose tapering of the drug. Median duration of response under treatment in patients with relapses was of 7 months. Patients with a CR had a much lower relapse rate than those achieving response, of only 7% in the former and 33% in the latter ($p = .01$; Fisher's exact test). A flowchart of the study cohort showing the classifications of response is shown on Figure 2.

Among the responders with sustained responses that ended treatment during study follow-up ($n = 52$), 17% ($n = 9$) experienced a loss of response within 2 months of dapsone withdrawal,

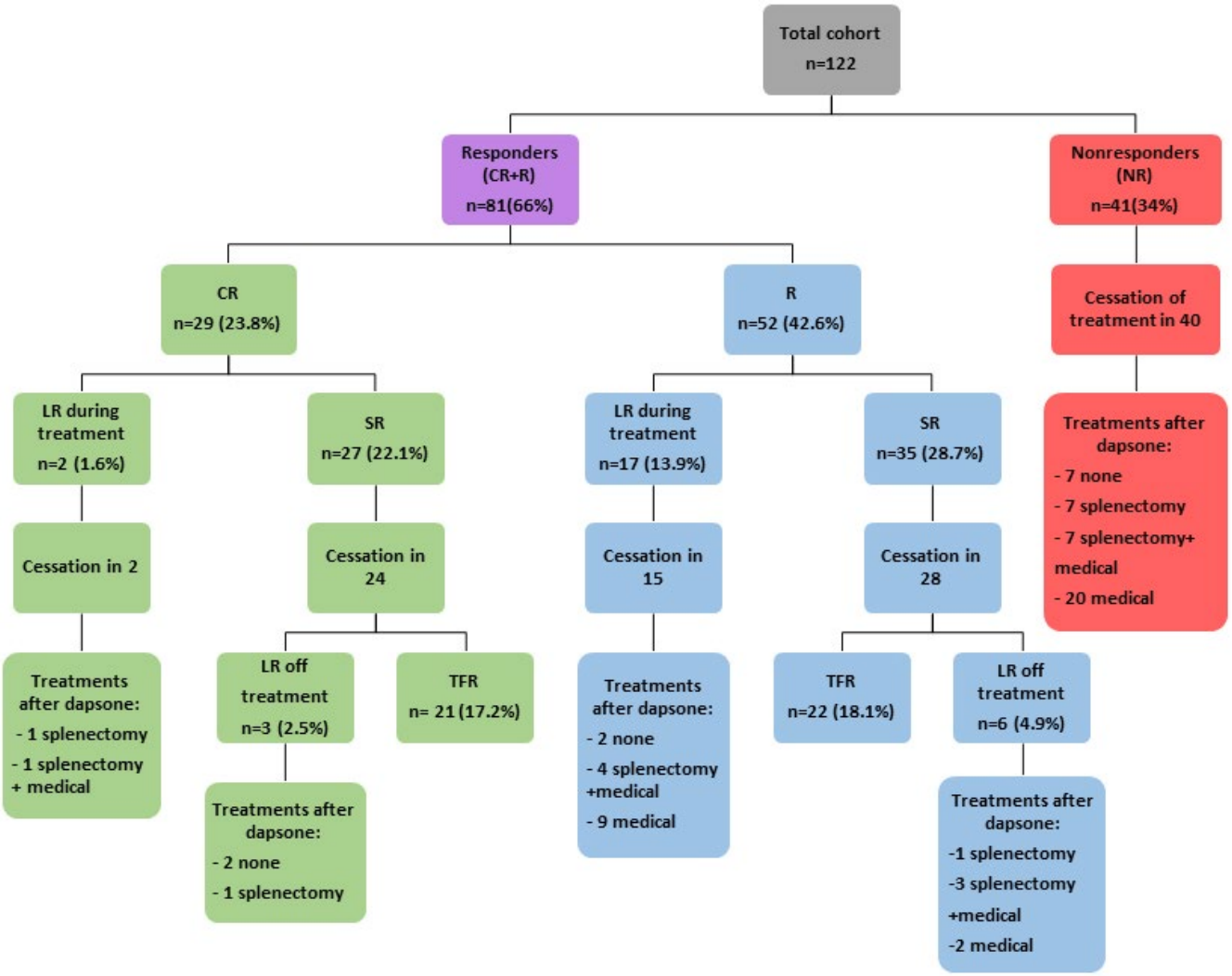

FIGURE 2 Flowchart of responses to dapsone during study follow-up. Flowchart of responses in dapsone-treated patients, during treatment and after cessation of the drug. Median follow-up was 3.4 years for the entire cohort; 2.5 years for the nonresponders; and 4.0 years for responders. The percentages shown are related to the entire cohort. Abbreviations: CR, Complete response; LR, loss of response; NR, nonresponders; R, response; SR, sustained response; TFR, treatment-free response (maintenance of response after suspension of dapsone)

21% (n = 11) lost response later on during study follow-up and 62% (n = 32) still maintained remission of ITP at their last follow-up, without any additional treatments (Figure 2; Table 2). Thirty-five percent of the entire cohort (n = 43/122) was able to maintain response off drug. The median duration of this TFR was 26 months (Table 2). The cumulative probability of TFR shown on the Kaplan-Meier curve, was higher in patients that had achieved CRs during dapsone treatment (p = .02; log-rank test) (Figure 3).

As expected, splenectomy was performed later on in only 16% of the responders, a much lower rate than observed in NRs (47%; p < .0001; log-rank test) (Figure 4). Other medical therapies were indicated later on in 23% of the responders, 46% did not need any other treatments, and 15% were still in treatment with dapsone at last follow-up.

3.5 | Safety

Symptomatic adverse events were seen in 16% of the patients in this cohort: symptomatic hemolytic anemia (n = 5), symptomatic methemoglobinemia (n = 5), gastrointestinal symptoms (n = 4), skin rash (n = 2), pruritus (n = 1), headache (n = 1), and hepatic toxicity (n = 1). Treatment was interrupted because of side effects in 11% (n = 13) of the patients, the main causes in these cases were hemolytic anemia (n = 5) and methemoglobinemia (n = 5).

Of the 122 patients in this study, 54 had methemoglobin measurements during dapsone treatment and elevations of methemoglobin levels were observed in 83% of the evaluated patients (n = 45), but these elevations were usually mild, with a median methemoglobin level of 2.6% (IQR 1.9–4.6%; varying from a minimum of 0.6% up to 16.1%). The median time of measurement of methemoglobin level was 1.2 months after the initiation of dapsone (IQR 0.9–2.9 months). In 21 patients, we have paired measurements of methemoglobin, at initiation and during dapsone treatment. In 19 of these patients, there were significant elevations in methemoglobin levels (p < .0001; Wilcoxon signed-rank test). Methemoglobinemia occurred more often in older patients, with median age of 53 years (IQR 30–69 years) in these patients vs. 29 years (IQR 21–52 years) in patients without elevated levels of methemoglobin (p = .03; Mann-Whitney test). Only five patients in the cohort had symptomatic methemoglobinemia, symptoms observed in these cases were cyanosis, weakness, and headaches. In all symptomatic patients, treatment with dapsone was immediately interrupted, with rapid resolution of symptoms without any need of additional treatments. Median levels of methemoglobin were higher in symptomatic patients (7%; IQR 2.3–12%) when compared

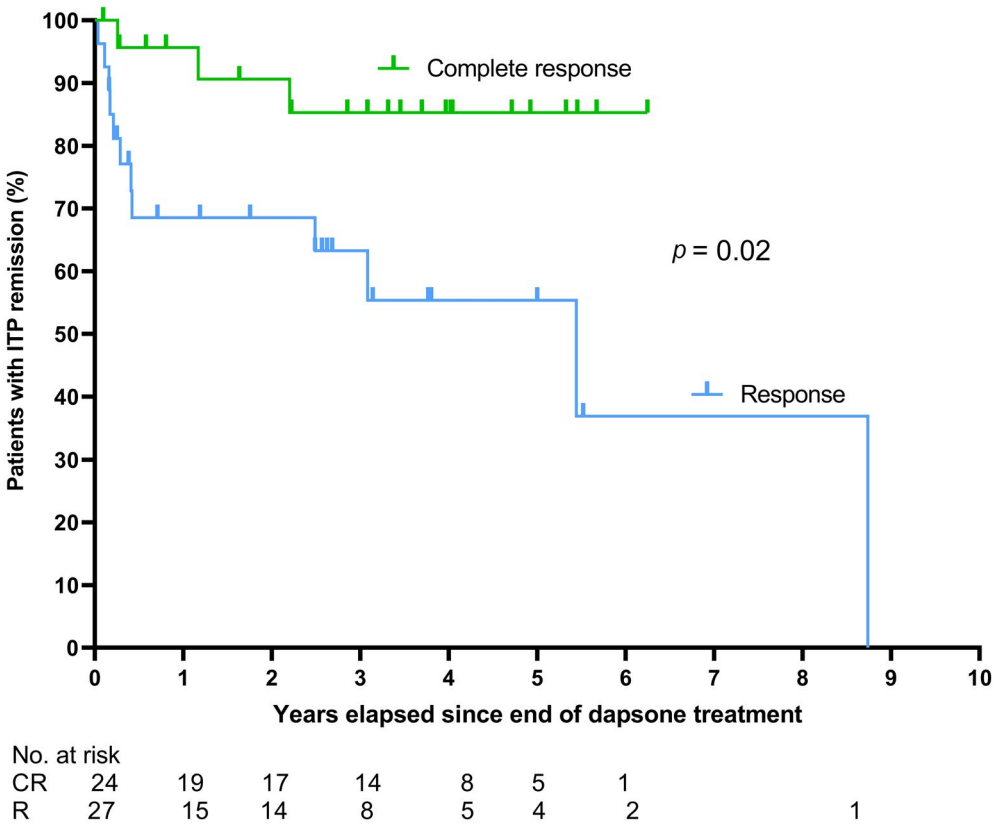

FIGURE 3 Maintenance of response after cessation of dapsone treatment. Kaplan-Meier curve showing the cumulative probability of maintenance of response after cessation of dapsone treatment in the two groups of responders – patients with a complete response (CR) and with a response (R). Patients with a complete response had a greater probability of maintaining their response after the end of treatment when compared with those with a response (p = .02; log-rank rest)

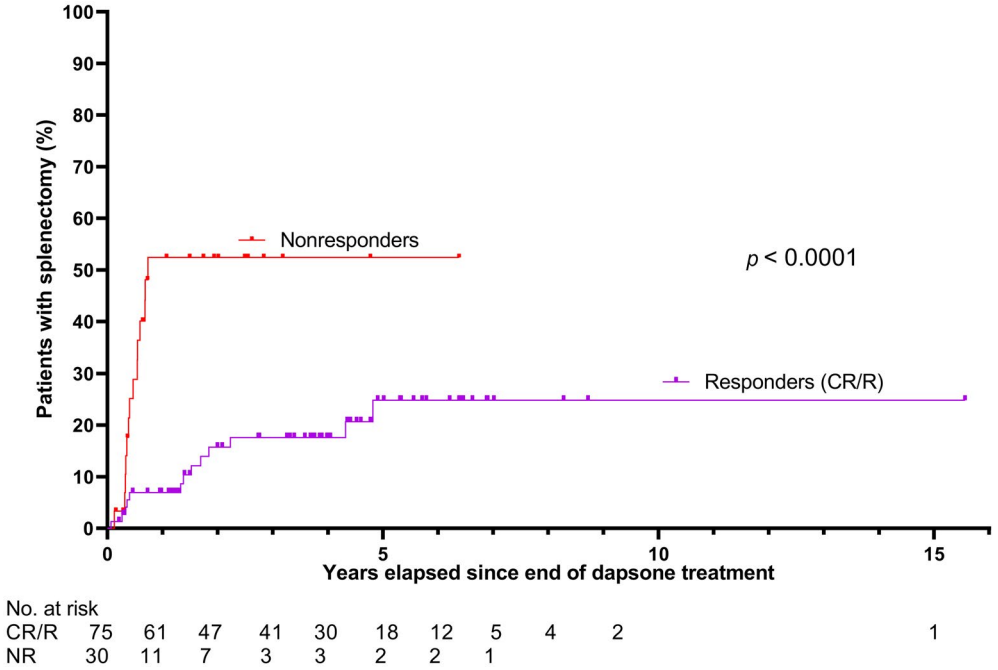

FIGURE 4 Splenectomy rates during study follow-up. Kaplan-Meier curve showing the cumulative probability of splenectomy during study follow-up. The probability of having a splenectomy was much higher in the nonresponders when compared with the responders ($p < .0001$; log-rank test). Abbreviations: CR, complete response; NR, nonresponders; R, response

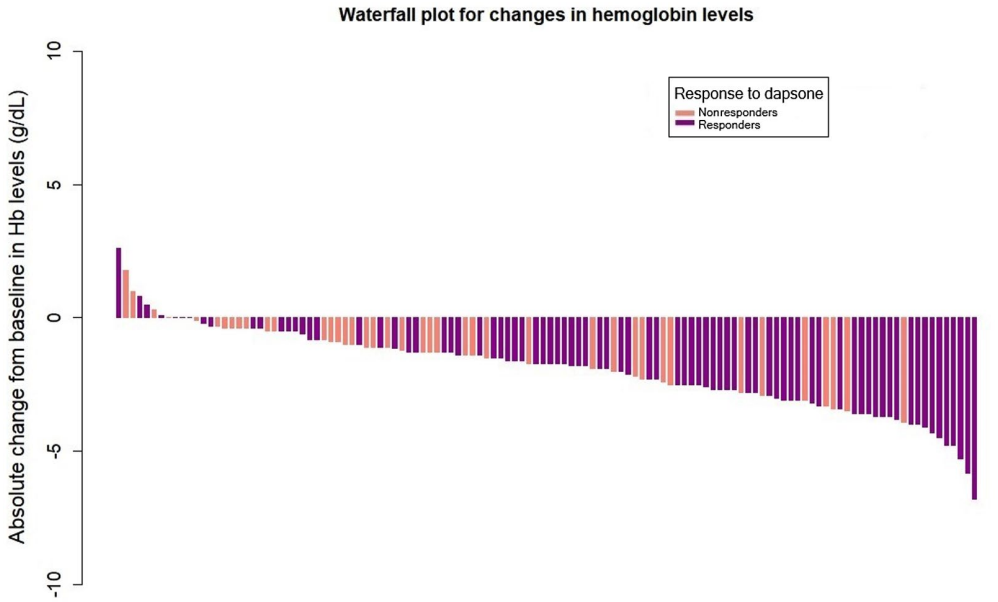

FIGURE 5 Waterfall plot for changes in hemoglobin levels. Waterfall plot showing the maximum variation in hemoglobin levels in relation to the baseline value during treatment with dapsone. Each column represents an individual patient. Responders include patients with complete response (CR) and response (R)

with the asymptomatic (median of 2%; IQR 1.8–4.6%) ($p = .04$; Mann-Whitney test).

All patients had complete blood counts performed before and throughout treatment, and 94% of the patients presented reductions in hemoglobin levels during the use of dapsone. Median baseline hemoglobin level in this cohort was 14.0 g/dl (IQR 12.9–15.2 g/dl) and the median level of the lowest hemoglobin reached during dapsone treatment was 12.0 g/dl (IQR 11.0–13.3 g/dl). Median hemoglobin fall was 1.7 g/dl (IQR 0.9–2.9 g/dl) and only 49% of the patients reached hemoglobin levels consistent with a diagnosis of anemia, in these cases the median hemoglobin level was 11.0 g/dl (IQR 10.4–11.7 g/dl). Maximum falls in hemoglobin levels per individual patient are shown on Figure 5. The median time to achieve the lowest hemoglobin level in this cohort was

2 months (IQR 1.0–5.0 months). In 64% of the patients, we measured hemolysis markers during dapsone treatment and in 90% alterations in one or more markers were detected: 66% presented elevation of total reticulocyte counts, 59% had reduced levels of haptoglobin, 30% had elevated lactate dehydrogenase levels, and 23% presented hyperbilirubinemia. Only 22% of the patients were screened for G6PD deficiency before dapsone initiation. Symptomatic anemia was seen more frequently in older patients, with median age of 64 years (IQR 56–76 years) in symptomatic vs. 48 years (IQR 29–61 years) in asymptomatic (p = .03; Mann-Whitney test). Only five patients in the cohort had symptomatic anemia, symptoms observed were mainly weakness, headaches, and palpitations. No patient required blood transfusions because of anemia. In all symptomatic cases, dapsone treatment was immediately interrupted, with rapid resolution of the anemia. Median hemoglobin fall in symptomatic patients was more than double of what was observed in asymptomatic patients (4.1 vs. 1.7 g/d, IQR 2.3–5.6 vs. 0.8–2.8 g/dl) (p = .01; Mann-Whitney test).

There was no difference between Rs and NRs with regards to the incidence of symptomatic adverse events.

3.6 | Hemolysis and dapsone efficacy

The reduction in hemoglobin levels occurred in both of the patient groups, the Rs and the NRs, but the fall was greater in the responders. We evaluated the variation of hemoglobin level per individual patient with what we called delta hemoglobin, which we calculated as the value of hemoglobin at baseline minus the lowest level of hemoglobin achieved during treatment. This drop was more significant in the responders: they had median delta hemoglobin of 1.9 g/dl (IQR 1.3–3.2 g/dl) vs. 1.2 g/dl (IQR 0.5–2.3 g/dl) in the NRs (p = .004; Mann-Whitney test) (Figure 6). There was also a weak negative correlation between the delta of platelet counts (platelet counts at baseline and best value achieved) and delta hemoglobin (r = −.2, p = .01, Spearman correlation test).

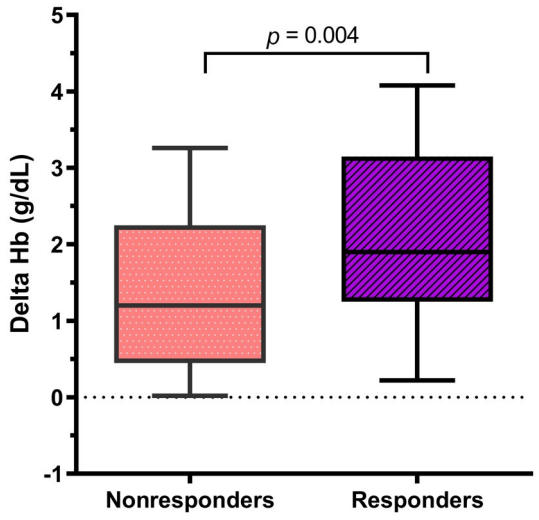

FIGURE 6 Decrease in hemoglobin levels during dapsone treatment. Delta hemoglobin was calculated as the value of hemoglobin at baseline minus the lowest level of hemoglobin achieved during treatment. The decrease was more significant in the responders when compared with nonresponders (p = .004; Mann-Whitney test)

4 | DISCUSSION

In this large cohort of ITP patients treated with dapsone, our results show that the drug has an adequate efficacy rate, comparable to a series of other available treatments used in ITP. This study also allowed us to report on original safety data, regarding the effect of dapsone on hemolysis and metahemoglobinemia. The overall response rate in this cohort was 66%, including 24% of complete responses, and 51% of sustained long-term responses that lasted for more than 2 years after the cessation of the drug. If we compare these efficacy rates with those of drugs recently approved by the US Food and Drug Administration for ITP such as fostamatinib or avatrombopag, the response rates seen with dapsone are, at least, comparable.[24,25] These drugs were tested in more chronic and refractory ITP populations than the population evaluated in this cohort, but if we compare our efficacy results with those of rituximab, an established second-line treatment option, dapsone has very similar initial response rates (approximately ~60% for rituximab) and superior long-term results (~40% of remission maintenance at 2 years for rituximab).[26–28]

The overall response rate of 66% seen in this cohort is slightly higher than what has been previously described in the literature, which varies from 46.2% to 55%, considering only studies that used the same criteria for evaluating response (platelet cutoff of 30 $\times$ 10^9/L).[29–31] The rates reported for CR in these studies are very variable, from 20% up to 40.5%. We believe the great variability in the reported efficacy rates is due to the small numbers of patients included in each of these previous studies (ranging from 20 to 42 patients). There are older studies with bigger cohorts being evaluated, the two largest reach a total of 66 and 55 adult patients, but comparisons are difficult to make because they used different criteria for evaluating response (platelet cutoff of 50 $\times$ 10^9/L).[32,33]

We have few pediatric patients in follow-up at our center, but we included these patients (n = 4) in the cohort because we wanted to include all patients in follow-up at the center that met the eligibility criteria. Because we know that pediatric ITP patients may have a different natural history than adult patients, we performed a sensitivity analysis, excluding these four pediatric cases from the cohort and we verified that this did not alter the response rates and classifications (data not shown). Older age at presentation (age ≥11 years) is one of the main predictors of chronicity of ITP in children, and this is the age range included in our cohort.[34]

The average dapsone dosage used in previous reports is variable, in some the drug is used in a dose range of 1 to 2 mg/kg/d.[30,33,35] In others, it is used in fixed dosages, ranging from 33 mg/d to 100 mg/d.[21,31,32,36–38] In this study, we used an initial fixed dosage of

100 mg/d, but in cases with previous reports of dose-related side effects we started with 50 mg/d. In 20% of the NRs, we escalated the dosage, reaching 150 mg/d, but none of these patients achieved response during the dose escalation. From this experience, we believe that dose escalations to 150 mg/d may not be advisable because it may increase the occurrence of dose-related side effects and with no apparent benefit in response rate.

The rate of relapse while on treatment in this study was 23%, but even in these patients with transient responses, the response to dapsone lasted a median of 7 months, reaching up to 3.7 years. Relapses occurred very rarely in patients that achieved CR. Previous reported rates for relapse while on treatment are very variable, ranging from 0 up to 21.7%.[29–33] Many have reported that most patients relapsed after interruption of treatment; thus, an unexpected finding in this cohort was the fact that responding patients presented long-term responses, with 62% of patients maintaining their response for more than 2 years after the withdrawal of the drug.

As expected, responding patients had a much lower chance of having an indication for splenectomy later on and the majority had no need for any further medical treatments. Thus, when used as a second-line therapy, dapsone had a role as a splenectomy-sparing agent in our cohort. In our center, the usual sequence of treatments in ITP patients is corticosteroids followed by dapsone and then splenectomy or other medical treatments as third-line. Both baseline characteristics (previous splenectomy and use of dapsone as third-line therapy) reflect almost the same population in our cohort and it is not possible determine the role of each one individually in the response to dapsone. However, the biological plausibility that previous splenectomy is the main predictor of outcome is in line with the leading hypothesis regarding the mechanism of action of dapsone in ITP (a reduction of platelet sequestration due to competitive inhibition of the reticuloendothelial system by the red cell destruction). A trend toward fewer responses in splenectomized patients (30% vs. 58%) has been previously reported.[32] Our data showed that previously splenectomized patients had a much lower response rate to the drug (35%); therefore, we consider that they should not be candidates for treatment with dapsone. Furthermore, our patients that used dapsone earlier on in the disease (median duration of 8 months), had a greater probability of achieving a complete response. This finding is in agreement with two previous studies, and further supports the use of the drug as second- or third-line therapy, before splenectomy is performed.[29,31]

Dapsone is metabolized in the liver by N-acetylation and N-hydroxylation. Its metabolite hydroxylamine reacts with oxyhemoglobin (Fe^{2+}) to form methemoglobin (Fe^{3+}).[17] Methemoglobinemia occurred in some extent in almost all patients in this cohort, but it was usually mild and asymptomatic. In only five cases (4%), treatment had to be stopped because of this side effect, and symptoms resolved rapidly after interruption. We currently evaluate methemoglobin levels routinely in all patients treated with dapsone, and most patients present mild to moderate elevations. There are no data in the literature to compare this with, for all of the previous studies report on methemoglobin levels only in patients with symptoms. In our center, we do not consider asymptomatic elevations reason to interrupt treatment. If long-term asymptomatic elevations in methemoglobin levels have any clinical consequences in patients treated for extensive periods is not known. It is possible that methemoglobinemia becomes less pronounced as treatment is continued, due to and adaptive increase in NADH-dependent reductase activity in the erythrocyte.[17] This has been previously reported by other authors,[29] but we did not evaluate in this study because we did not perform serial mensuration of methemoglobin in this cohort. There are small studies in healthy volunteers and in dermatitis herpetiformis patients showing that concomitant use of cimetidine in patients treated with dapsone can lower methemoglobin levels by approximately 30%.[39,40] This was not attempted in our cohort, but it is a possible alternative in patients having significant methemoglobin elevations that lead to interruption of treatment.

Dose-related hemolytic anemia is another very frequent side effect seen in patients treated with dapsone, but in our experience, it rarely limits its usage. Almost all patients presented reductions in their hemoglobin levels, but in only one-half, the levels were below the normal range, and even in these cases, anemia was very mild. In only five cases (4%) treatment had to be interrupted because of symptomatic hemolytic anemia. This is comparable to what has been previously described in the literature, with 1% to 3% of patients requiring withdrawal of treatment resulting from this side effect.[30,32,33] Older patients were more likely to experience symptomatic hemolytic anemia, as well as methemoglobinemia, and therefore they might be candidates for using lower dosages of the drug (50–75 mg/d).

An important finding in this study was the relationship between hemolysis and platelet response. We observed that responders to dapsone treatment presented a more pronounced drop in their hemoglobin levels, with a median fall of almost 2 g/dl. This finding also supports the hypothesis for the mechanism of action of the drug in this setting; a reduction of platelet sequestration in the reticuloendothelial system from the red cell destruction. The lower response rate observed in splenectomized patients of this cohort also corroborates this theory. Our finding confirms the results of a previous study were the authors had observed a greater fall in hemoglobin among the responders to dapsone, also with a median of 2 g/dl.[32]

This is the largest study of our knowledge of dapsone treatment in ITP patients, but it has several limitations, mainly related to its retrospective design. Although we have used a routine protocol, patients in this cohort were treated according to their individual necessities and physicians made adjustments according to their clinical judgment and thus, some heterogeneity in treatment was observed. Patients treated with dapsone were evaluated at different time points, making it difficult to determine the average time to achieve a response to the drug. We also cannot make any affirmations regarding the timing of occurrence of methemoglobinemia and its behavior over time. The optimum duration of therapy and dosage in responders are yet to be determined. Prospective studies are needed to clarify these issues.

In conclusion, we believe dapsone is a useful treatment option in ITP patients, with good tolerability and low cost. It can be proposed as an alternative to conventional second-line therapies in cost-restricted scenarios in patients who are steroid dependent or refractory. When used as a second-line therapy, it has a role as a splenectomy-sparing agent. Although the mechanism of action is still unclear, our observation that the degree in the drop of hemoglobin is greater in responders suggests a possible role of the blockage of the reticuloendothelial system in the therapeutic effect of the drug.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTION

Marina P. Colella: study design, revision of the collected data, statistical analyses and interpretation, drafting of the manuscript, and revision of manuscript; Fernanda A. Orsi: statistical analyses and interpretation, drafting of manuscript, revision of manuscript; Elizio C.F. Alves: collection of data; revision of the manuscript; Gabriela F. Delmoro: collection of data, revision of manuscript; Gabriela G. Yamaguti-Hayakawa: statistical analyses and interpretation, revision of manuscript; Erich V. de Paula: study design, interpretation, revision of manuscript; Joyce M. Annichino-Bizzacchi: study design, interpretation, revision of manuscript. All authors have read and approved the final version of the manuscript.

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