



## ORIGINAL PAPER

# Study of platelet kinetics in immune thrombocytopenia to predict splenectomy response

Ana Mendoza<sup>1</sup>  | María Teresa Álvarez-Román<sup>1,2,3</sup>  | Elena Monzón-Manzano<sup>1,2</sup> |  
 Paula Acuña<sup>1,2</sup> | Elena G. Arias-Salgado<sup>1,2</sup> | Isabel Rivas-Pollmar<sup>1,2</sup> |  
 Mónica Martín-Salces<sup>1,2</sup> | Bárbara Martínez de Miguel<sup>4</sup> | Elena Martínez Montalbán<sup>4</sup> |  
 Víctor Jiménez-Yuste<sup>1,2,3</sup> | Nora Butta<sup>1,2</sup>

<sup>1</sup>Department of Haematology, La Paz University Hospital, Madrid, Spain

<sup>2</sup>IdiPAZ, Madrid, Spain

<sup>3</sup>Autonomous University of Madrid, Madrid, Spain

<sup>4</sup>Radiopharmacy Unit, La Paz University Hospital, Madrid, Spain

## Correspondence

María Teresa Álvarez-Román, Department of Haematology, La Paz University Hospital, Paseo La Castellana 261, Madrid 28046, Spain.  
 Email: [mariat.alvarez@uam.es](mailto:mariat.alvarez@uam.es)

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## Summary

Despite the efficacy of splenectomy for chronic immune thrombocytopenia (ITP), its considerable failure rate and its possible related complications prove the need for further research into potential predictors of response. The platelet sequestration site determined by <sup>111</sup>In-labelled autologous platelet scintigraphy has been proposed to predict splenectomy outcome, but without standardisation in clinical practice. Here, we conducted a single-centre study by analysing a cohort of splenectomised patients with ITP in whom <sup>111</sup>In-scintigraphy was performed at La Paz University Hospital in Madrid to evaluate the predictive value of the platelet kinetic studies. We also studied other factors that could impact the splenectomy outcome, such as patient and platelet characteristics. A total of 51 patients were splenectomised, and 82.3% responded. The splenic sequestration pattern predicted a higher rate of complete response up to 12 months after splenectomy ( $p=0.005$ ), with 90% sensitivity and 77% specificity. Neither age, comorbidities, therapy lines nor previous response to them showed any association with response. Results from the platelet characteristics analysis revealed a significant loss of sialic acid in platelets from the non-responding patients compared with those who maintained a response ( $p=0.0017$ ). Our findings highlight the value of splenic sequestration as an independent predictor of splenectomy response.

## KEYWORDS

immune thrombocytopenia, platelet glycome, platelet sequestration kinetic, splenectomy

## INTRODUCTION

Primary immune thrombocytopenia (ITP) is an acquired autoimmune disorder affecting children and adults. Its underlying pathophysiology involves several pathways implicated in the reduced blood platelet count, such as autoantibody-mediated and T cell-mediated platelet clearance, combined with impaired platelet production.<sup>1–3</sup> The spleen plays a key role, given that opsonised platelets are phagocytosed by splenic macrophages expressing fragment-crystallisable gamma

receptors, which bind to antibody-covered platelets. The spleen also serves as the principal site of anti-platelet antibody production.<sup>4</sup> Recent advances have been made in developing new treatment options targeting these various paths, including thrombopoietin receptor agonists (TPO-RAs), Syk inhibitors and neonatal Fc receptor inhibitors, among others.<sup>5</sup> Given the heterogeneity that characterises this disease, it remains unclear which mechanism is responsible for thrombocytopenia. It is therefore challenging for physicians to select an individualised therapeutic regimen for

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each patient with ITP, and further efforts have been made to design specific assays to determine thrombocytopenia's predominant underlying mechanism.

Splenectomy is a conventional treatment for ITP patients, with response rates of up to 80% reported, approximately 60% of which are sustained over time.<sup>6,7</sup> Similar results have been found in the post-TPO-RAs and rituximab eras.<sup>8</sup> The risk of intrabdominal haemorrhage and long-term infections or cardiovascular complications must be considered prior to its performance.<sup>9</sup> Secondly, current guidelines recommend reserving splenectomy for those chronic cases (more than 12 months of disease)<sup>10</sup> in which at least one second-line therapy has failed (including corticosteroids, intravenous immunoglobulin, TPO-RAs or rituximab).<sup>11–13</sup> Thus, the latest American Society of Hematology guidelines and the updated international consensus report advise splenectomy as a third-line option.<sup>11,12</sup> As a result, its use has significantly decreased in the last decade, especially since the arrival of TPO-RAs.

Several studies have been conducted to explore possible predictive factors of response to identify the patients most likely to respond to splenectomy, with disparate results in the literature.<sup>14–16</sup> Only age and platelet count within the first week after splenectomy were uniformly associated with response.<sup>17,18</sup> Attempts to create a predictive model to optimise the splenectomy indication with the previous factors have failed.<sup>19</sup> Platelet sequestration studies, which differentiate the site of platelet destruction in patients with ITP,<sup>20–22</sup> have also been proposed as promising predictors of splenectomy outcome.<sup>23–28</sup> Different scanning techniques employing radiopharmaceuticals have been employed over time, with the <sup>111</sup>In-labelled autologous platelet scintigraphy emerging as a useful tool to determine the patients with a splenic elimination pattern who might respond better to splenectomy compared with those with a non-splenic pattern. Previous literature has explored this hypothesis without achieving consistent results,<sup>14,15,29–31</sup> with three main groups providing recent robust data on its utility in this scenario.<sup>24,26,27</sup> A feasible explanation for the heterogeneity of the previous studies could be the lack of standardisation about the included population, follow-up, definition of response or isotopic agents used. Because of its technical limitations, <sup>111</sup>In-labelled platelet scintigraphy is not widely available; hence, the ITP guidelines do not establish a universal recommendation for its performance. This highlights the need for further research to validate this test and identify the most suitable cases for splenectomy.

The importance of platelet glycans in the platelet senescence and apoptosis processes has been demonstrated in numerous studies.<sup>32–34</sup> In particular, the platelet lifespan is determined by sialic acid loss as a result of exposure to intrinsic sialidases.<sup>35</sup> Desialylated platelets expose underlying galactose residues that are recognised by the hepatic Ashwell-Morell receptor (AMR), triggering platelet clearance at the liver.<sup>36–38</sup> The change in glycome composition of platelets in patients with ITP could be implicated in the lack of response to certain treatments.

This study aimed to evaluate whether the platelet sequestration site impacts the response to splenectomy. Secondary objectives include calculating the sensitivity and specificity of the scintigraphy test and investigating further factors that could explain treatment failure among patients with splenic clearance.

## METHODS

### Study design

A retrospective cohort study was performed. Patients with ITP who underwent <sup>111</sup>In-labelled platelet scintigraphy at La Paz University Hospital between 2005 and 2021 were included. This sequestration study was routinely practised in patients for whom splenectomy was indicated (chronic ITP with failure of at least one previous line of treatment).<sup>11–13</sup> We included those patients who, based on clinical history, had been splenectomised. Data from the patients were collected from the electronic medical records.

Patients were followed for at least 12 months after splenectomy. The response to splenectomy was assessed in terms of blood platelet count at 3, 6 and 12 months after surgery, according to the ITP Spanish Group 2020 guidelines.<sup>13</sup> Complete response (CR) was defined as a platelet count greater than  $100 \times 10^9/L$  without any signs of bleeding; and partial response (PR) was defined as a platelet count that it ranged from 30 to  $100 \times 10^9/L$  and was twice the baseline count. Patients with a platelet count less than  $30 \times 10^9/L$  without a previous response were considered refractory or relapsed if there was a previous response. The need for additional treatment following a splenectomy was also recorded. Patients were divided into responders (CR and PR) and non-responders (refractory or relapsed within 3 months) for the analysis.

### Autologous <sup>111</sup>In-labelled platelet scintigraphy protocol

The <sup>111</sup>In-labelled platelet study was performed according to the method recommended by the International Committee for Standardization in Hematology Panel on Diagnostic Application of Radionuclides.<sup>39</sup> A 50 mL of whole-blood sample was obtained from the patient, and platelet labelling with <sup>111</sup>In-oxine was conducted ex-vivo. Autologous labelled platelets were injected, and subsequent blood samples were taken at 15 and 30 min and 1, 2, 3, 4, 24, 48, 72 and 96 h after injection. Platelet survival time was calculated from radioactive counts measured by a gamma counter (which registers peaks at 150–330 keV), employing a multiple hit method to estimate the mean platelet half-clearance time. Scintigraphy dynamic images with a gamma camera were acquired to calculate the mean platelet lifespan (MPLS) and the splenic:liver (S:L) ratio. The S:L ratio was validated in previous cohorts to assess the platelet sequestration site.<sup>22</sup> The sequestration

patterns were categorised as purely splenic if the S:L ratio was greater than 2; predominantly splenic if the S:L ranged from 1.4 to 2; mixed if the S:L went from 0.8 to 1.4; and hepatic if the S:L was less than 0.8. The sequestration site was dichotomised for the analysis between splenic, if purely splenic or predominantly splenic, and non-splenic, if mixed or hepatic.

## Platelet characteristics

The platelet characteristics of a group of patients with splenic sequestration were prospectively examined after splenectomy to investigate whether they might have influenced the response. A healthy control group was also included for the sake of comparison. Platelet activation markers, active caspases-3, -7, -8 and -9, the fibrinogen (alpha IIb/beta III: CD41b/CD61) and von Willebrand (CD42a/CD42b) receptors and surface sialic acid exposure were evaluated by flow cytometry as described in Appendix S1.

## Statistical analysis

The categorical variables were described as frequencies and percentages, and the continuous variables as means, medians and interquartile range (IQR, 25%–75% percentile). For the categorical data, the chi-squared or Fisher's exact test was applied, as appropriate. Logistic regression models were applied for the multivariate analysis. The one-way analysis of variance (ANOVA), the Kruskal–Wallis or the Mann–Whitney test was employed for comparing the continuous variables. Survival curves were constructed by the Kaplan–Meier method to calculate the relapse-free survival in the splenectomised patients. The log-rank test and hazard ratio (HR) were applied to compare the results. A *p*-value less than 0.05 was considered significant, and 95% confidence intervals (95% CIs) were used for the odds ratio (OR) and HR calculation. Statistics were performed with IBM SPSS software version 21. This study was authorised by the local medical ethics committee of La Paz University Hospital in Madrid (Spain). Informed consent was obtained from those patients in whom the platelet characteristics were analysed after splenectomy for the purpose of the study.

## RESULTS

### Baseline characteristics

Autologous <sup>111</sup>In-labelled platelet scintigraphy was conducted for 80 patients, 51 of whom underwent laparoscopic splenectomy and were eligible for the analysis. The baseline characteristics of the splenectomised patients are included in Table 1. Most (52.9%) patients were younger than 40 years, with a mean age of 32 years at the time the splenectomy was performed; 68% of the cohort was female. The majority had no comorbidities. The median number of previous lines

**TABLE 1** Characteristics of ITP patients who were splenectomised.

	All ( <i>n</i> = 51)	Splenic sequestration ( <i>n</i> = 40)	Non-splenic sequestration ( <i>n</i> = 11)
Age (years), <i>n</i> (%)			
<40	27 (52.9%)	21 (52.5%)	6 (54.5%)
40–70	22 (43.1%)	18 (45%)	4 (36.4%)
>70	2 (3.9%)	1 (2.5%)	1 (9.1%)
Gender, <i>n</i> (%)			
Female	35 (68.6%)	26 (65%)	9 (81.8%)
Male	16 (31.4%)	14 (35%)	2 (18.2%)
Comorbidities <sup>a</sup> , <i>n</i> (%)			
No	32 (62.7%)	26 (65%)	6 (54.5%)
Yes	19 (37.3%)	14 (35%)	5 (45.5%)
Number of treatment lines, <i>n</i> (%)			
1–2	18 (35.3%)	15 (37.5%)	3 (27.3%)
>2	33 (64.7%)	25 (62.5%)	8 (72.7%)
Previous response to other therapies <sup>b</sup> , <i>n</i> (%)			
No	21 (41.2%)	15 (37.5%)	6 (54.5%)
Yes	30 (58.8%)	25 (62.5%)	5 (45.5%)

Note: No significant differences in the baseline characteristics were found between patients with and without splenic sequestration (*p* > 0.05).

<sup>a</sup>Comorbidities were defined as relevant systemic comorbidity (cardiovascular or lung disease, kidney failure, malignancy or autoimmune disease).

<sup>b</sup>Previous therapies included corticosteroids, intravenous immunoglobulin (IVIG), thrombopoietin receptor agonists (TPO-RAs) or rituximab.

of treatment was 2 (IQR 1–2), and 58% of the patients had previously responded to other therapies. Most of them had been heavily pretreated at the time of the splenectomy, with more than two lines of previous therapies (Table 1). Splenic sequestration was present in 40 of 51 patients (78%), including purely and predominantly splenic clearance. The mean S:L ratio in the splenectomised cohort was 3.31 (±2.12), and the median platelet count at the time of the scintigraphy was  $110 \times 10^9/L$  (IQR  $61$ – $160 \times 10^9/L$ ). When classified according to the sequestration pattern, there were no differences in terms of baseline characteristics between subgroups (Table 1). The site of platelet sequestration did not significantly differ between the patients treated and untreated with TPO-RAs (*p* = 0.81). The reason for performing splenectomy in those cases with a non-splenic pattern was patient preferences. Among the 29 patients in whom splenectomy was not performed, only 9 presented with a non-splenic pattern; thus, the sequestration site was the reason for not performing splenectomy only in a minority of patients. Patient preference was the main reason for avoiding splenectomy in the 20 remaining patients with splenic sequestration.

The response rate to splenectomy was 82.3%. Nine (17.7%) patients were refractory or had an early relapse within the first 3 months. CR at 3 months post-splenectomy was achieved in 78.4% of patients, and it was maintained at 12 months. The median follow-up was 6 years (IQR 4–10). At the last follow-up, 72.5% showed a sustained response, with the remaining 27.5% requiring additional treatments after

splenectomy. Among the 40 patients with splenic sequestration, 90% achieved CR at 3 months, with 87.5% maintaining it at 12 months. In contrast, only 45.5% of the non-splenic sequestration group maintained a complete response 12 months after splenectomy. Three (6%) patients presented complications secondary to splenectomy: portal venous thrombosis, mesenteric bleeding and thrombocytosis-induced priapism. There was only one non-haemorrhagic fatality event reported in the cohort: an acute myocardial infarction. The outcomes of the 29 non-splenectomised patients showed a 65.5% CR rate with an alternative treatment, with further treatment required in 44.8% of them. Among the 20 patients with splenic sequestration, 70% achieved CR without splenectomy, compared to the 90% of patients with splenic sequestration who reached CR after splenectomy. There were no significant differences according to the response to other therapies different from splenectomy between the patients with splenic and non-splenic sequestration ( $p=0.496$ ).

## Platelet kinetics and response to splenectomy

As shown in Table 2, the response to splenectomy was not statistically related to age, comorbidities, lines of treatment or previous response to them. The splenic sequestration pattern was the only factor associated with a higher response rate regarding CR at 3, 6 and 12 months. In contrast, no significant differences were observed in terms of sequestration when analysing the need for further treatment at the last follow-up ( $p=0.131$ ). A multivariate regression model was designed to explore the previous results (see Table S1

**TABLE 2** Characteristics of patients with ITP, stratified by response to splenectomy.

	Responders ( <i>n</i> = 42)	Non-responders ( <i>n</i> = 9)	<i>p</i> -value
Age (years), <i>n</i> (%)			
<40	23 (54.8%)	4 (44.4%)	0.451
40–70	18 (42.9%)	4 (44.4%)	
>70	1 (2.4%)	1 (11.1%)	
Comorbidities, <i>n</i> (%)			
No	27 (64.3%)	5 (55.6%)	0.623
Yes	15 (35.7%)	4 (44.4%)	
Number of treatment lines, <i>n</i> (%)			
1–2	16 (38.1%)	2 (22.2%)	0.366
>2	26 (61.9%)	7 (77.8%)	
Previous response to other therapies, <i>n</i> (%)			
No	16 (38.1%)	5 (55.6%)	0.334
Yes	26 (61.9%)	4 (44.4%)	
Platelet sequestration, <i>n</i> (%)			
Splenic	38 (90.5%)	2 (22.2%)	<b>0.000</b>
Non-splenic	4 (9.5%)	7 (77.8%)	

Note: Chi-squared test or Fisher's exact test was applied, as appropriate. Significant differences ( $p < 0.05$ ) are marked in bold.

in Appendix S1). Similarly, the splenic pattern was statistically different between responders and non-responders at 3 (OR=36.77,  $p=0.001$ ) and 6 or 12 months of follow-up (OR=22.68,  $p=0.005$ ). The need for additional treatment at the last follow-up was not associated with the sequestration pattern. The relapse-free survival according to the site of platelet sequestration in the 51 splenectomised patients is shown in Figure 1. Patients with non-splenic sequestration had three times increased risk of relapse (HR=3.12, 95% CI 1.05–9.71), compared with those with a splenic pattern ( $p=0.021$ ).

In our cohort, the previously proposed S:L cut-off of 1.4 for considering the sequestration as splenic predicted the response to splenectomy with 90% sensitivity and 77% specificity.

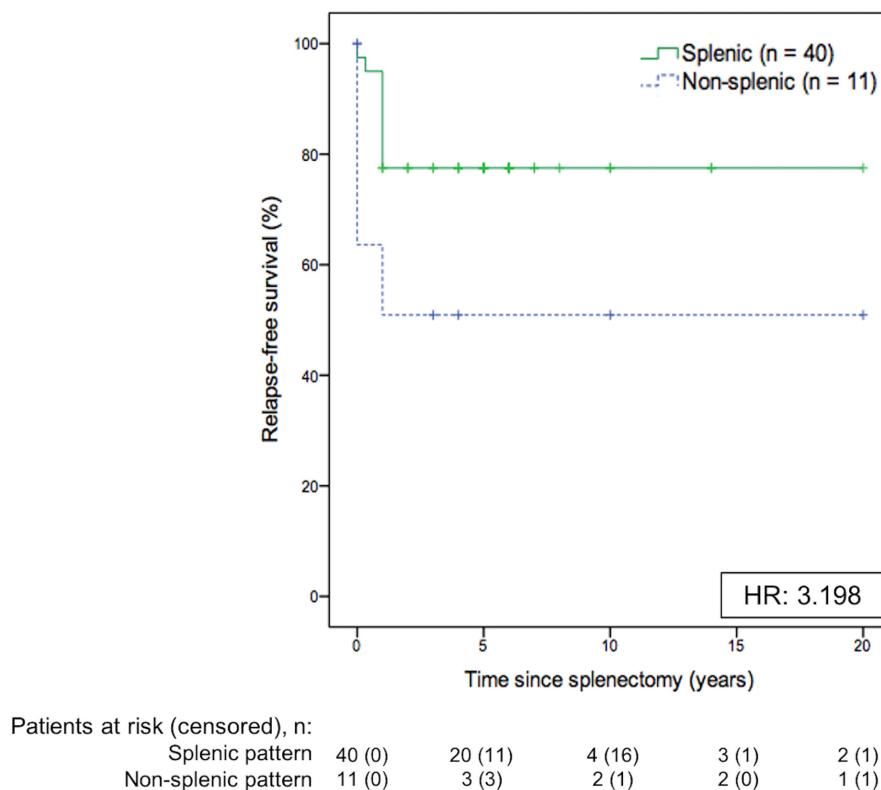
## Platelet characteristics and response to splenectomy

Among the group of patients with ITP who had splenic sequestration, the platelet characteristics of 7 patients with ITP who failed to respond to splenectomy (2 non-responders and 5 with relapse beyond 3 months) were compared with those of 16 responders, as well as those of 20 healthy controls. There were no significant differences between the groups for most of the characteristics studied (Table 3), except for the sialic acid exposure, which was decreased in the non-responding patients ( $p=0.0017$ ). *Ricinus communis* agglutinin (RCA) binding to the platelet surface of the non-responders was greater compared with the patients who maintained response (Figure 2). No significant differences were found in the baseline characteristics of these patients according to the RCA-binding status, neither in the platelet count at the time the analysis was performed, the time between the splenectomy and the study, nor in the previous received treatments or the response to them (see Table S2 in Appendix S1).

## DISCUSSION

Our results confirm the predictive value of  $^{111}\text{In}$ -labelled platelet studies prior to splenectomy, with splenic and predominantly splenic sequestration patterns associated with higher rates of complete response at 3, 6 and 12 months after splenectomy. The response rates and complications secondary to splenectomy in this study were consistent with the previous literature.<sup>6,9,19</sup>

The relevance of these findings relies on the ability of the scintigraphy to identify patients who might respond to splenectomy, showing benefit even in those cases with a lower probability of success, such as multirefractory patients. The impact of the sequestration site as an independent predictor of splenectomy outcome retained statistical significance even with the addition of baseline characteristics as covariates in the analysis, with no association between the



**FIGURE 1** Relapse-free survival after splenectomy. Relapse-free survival in the 51 splenectomised patients stratified by sequestration pattern: splenic (solid line) versus non-splenic (dotted line). Log-rank test and HR were applied. Patients with non-splenic sequestration had three times increased risk of relapse at 20 years (HR = 3.12, 95% CI 1.05–9.71), compared with those with a splenic pattern ( $p = 0.021$ ). HR, hazard ratio.

sequestration pattern and the other analysed factors. Some previous studies had shown age as a presurgical predictor of response<sup>15,17,24,31</sup>; However, the current study did not demonstrate any association between age and splenectomy outcome. One possible reason for these disparate findings is the uniformity of our cohort regarding the characteristics of the patients, including age. More than 95% of the patients in this study were younger than 70 years of age. The heterogeneous inclusion criteria as well as the lack of baseline characteristic stratification might explain the variable results from the majority of previous studies. We stratified the baseline features of the included patients in the current study to avoid potential confounding factors. Differences in response definitions also contributed to the limited standardisation. In addition, we analysed the need for additional treatment at the last follow-up, and no significant differences were found in terms of sequestration site. Previously published studies had revealed an increased likelihood of CR at last follow-up in patients with a splenic pattern of platelet sequestration, although only two had investigated the treatment reliance.<sup>24,40</sup> Future lines of investigation should include this variable in the analysis to improve the relevance of the research.

A recent meta-analysis reviewed the available data about the association between post-splenectomy platelet response and sequestration pattern.<sup>28</sup> Remarkably, only 4 of the 23 included studies were published after 2010.<sup>26,27,31,40</sup> Eight studies provided data on splenectomy outcome stratified

by sequestration pattern, with four of them indicating that splenic sequestration was associated with a better response after splenectomy.<sup>23,24,27,28</sup> These 4 studies used indium as the isotopic agent, with 8 of the 23 including ones employing different radionuclides. Amini et al.<sup>28</sup> reported a higher response rate among patients with a splenic pattern in the pooled analysis (OR = 14.21, 95% CI 3.65–55.37), compared to the non-splenic pattern. They suggested the need for further studies with multivariate analysis, including possible confounders, to avoid the heterogeneity of the previous ones. Just one of the previous studies performed multivariate comparisons<sup>24</sup>; consequently, our results add consistent data to the existing evidence about the utility of <sup>111</sup>In-platelet sequestration studies before splenectomy. Furthermore, the utility of this test in patients treated with TPO-RAs was only validated in one previous study.<sup>41</sup> Our results confirm that the TPO-RAs treatment did not significantly modify the site of platelet destruction.

To our knowledge, only Roca et al.<sup>26</sup> had directly analysed the predictive value of the scintigraphy test. The S:L index at 30 min after injection of autologous <sup>111</sup>In-labelled platelets predicted the splenectomy outcome with the highest sensitivity and specificity (100%) compared with the other platelet kinetic parameters (initial platelet recovery, spleen:heart or liver:heart index). The S:L ratio based on the MPLS is the current standard method employed to measure the platelet lifespan, with reproducible results, but its predictive value

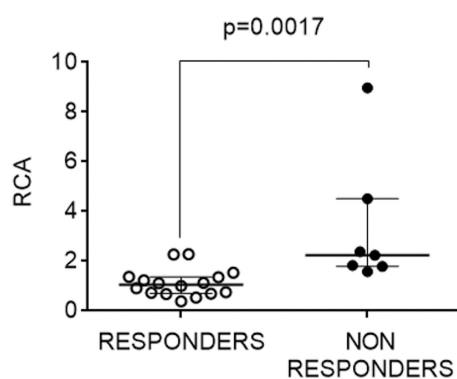


TABLE 3 Platelet characteristics stratified by response to splenectomy.

	Responders (n = 16)	Non-responders (n = 7)	Healthy controls (n = 20)
Basal PAC1-binding (Median % of positive cells, IQR)	0.50 (0.30–0.83)	0.50 (0.30–0.83)	0.23 (0.07–0.71)
TRAP-induced PAC1-binding (Mean % of positive cells $\pm$ SD)	60.46 $\pm$ 20.76	<b>50.11 <math>\pm</math> 19.49<sup>†</sup></b>	<b>72.85 <math>\pm</math> 15.35</b>
ADP-induced PAC1-binding Columns 1–2 (Median % of positive cells, IQR) Column 3 (Mean % of positive cells $\pm$ SD)	71.00 (62.43–76.30)	67.00 (38–78)	67.33 $\pm$ 18.71
Basal P-selectin exposure Columns 1–2 (Median % of positive cells, IQR) Column 3 (Mean % of positive cells $\pm$ SD)	2.43 (0.70–4.98)	2.86 (1.00–3.60)	2.71 $\pm$ 2.95
TRAP-induced P-selectin exposure (Mean % of positive cells $\pm$ SD)	77.01 $\pm$ 10.17	79.04 $\pm$ 8.06	79.59 $\pm$ 12.23
ADP-induced P-selectin exposure (Mean % of positive cells $\pm$ SD)	51.80 $\pm$ 16.58	47.13 $\pm$ 11.42	43.11 $\pm$ 16.49
Basal CD63 exposure Columns 1–2 (Median % of positive cells, IQR) Column 3 (Mean % of positive cells $\pm$ SD)	0.12 (0.10–1.39)	0.30 (0.20–0.70)	0.19 $\pm$ 0.24
TRAP-induced CD63 exposure (Mean % of positive cells $\pm$ SD)	57.91 $\pm$ 13.20	58.69 $\pm$ 12.18	52.64 $\pm$ 14.73
CD41b (Mean fluorescence $\pm$ SD)	630.50 $\pm$ 136.90	639.12 $\pm$ 112.90	583.50 $\pm$ 136.21
CD61 (Mean fluorescence $\pm$ SD)	202.50 $\pm$ 92.44	201.50 $\pm$ 92.44	183.00 $\pm$ 115.30
CD42a (Mean fluorescence $\pm$ SD)	258.70 $\pm$ 76.04	239.80 $\pm$ 20.65	230.40 $\pm$ 49.44
CD42b (Mean fluorescence $\pm$ SD)	174.00 $\pm$ 119.70	142.01 $\pm$ 44.72	151.60 $\pm$ 93.06
Caspase-3 -7 (Mean % of positive cells $\pm$ SD)	55.52 $\pm$ 14.38	56.39 $\pm$ 11.92	49.92 $\pm$ 10.79
Caspase-8 (Mean % of positive cells $\pm$ SD)	54.31 $\pm$ 12.87	56.83 $\pm$ 8.77	52.45 $\pm$ 14.90
Caspase-9 (Mean % of positive cells $\pm$ SD)	58.41 $\pm$ 9.37	57.30 $\pm$ 11.66	52.21 $\pm$ 10.01
RCA-binding (Mean fluorescence $\pm$ SD)	<b>399.60 <math>\pm</math> 138.80</b>	<b>917.60 <math>\pm</math> 226.30<sup>**†</sup></b>	<b>394.10 <math>\pm</math> 251.40</b>

Note: Characteristics of platelets from ITP patients with splenic sequestration who had responded or failed to splenectomy and from healthy controls. Results are expressed as absolute values with mean  $\pm$  SD if normal distribution or medians (IQR, 25%–75% percentile) if skewed distribution. One-way ANOVA or the Kruskal–Wallis and post hoc Dunn's multiple comparison tests were applied for comparisons among groups. Significant differences ( $p < 0.05$ ) are marked in bold with \* (responders vs. non-responders) or <sup>†</sup> (non-responders vs. healthy controls) when they were found.

Abbreviations: ADP, adenosine diphosphate; ANOVA, analysis of variance; IQR, interquartile range; RCA, *Ricinus communis* agglutinin; SD, standard deviation; TRAP, thrombin receptor-activating peptide.



**FIGURE 2** RCA-FITC binding to platelets according to response to splenectomy. RCA-binding was determined in quiescent platelets from patients with ITP who responded or failed to respond to splenectomy. RCA-FITC binds to galactose residues from washed platelets, which are exposed when sialic acid residues are lost. Results are expressed as the ratio between mean fluorescence values from the ITP patients and the mean values from a healthy control group ( $n = 20$ ). Horizontal lines represent mean values. Mann–Whitney test was applied. The patient from the non-responder group with the highest RCA-binding compared with the rest of the group, was excluded for the statistical analysis to avoid any bias. FITC, fluorescein isothiocyanate; RCA, *Ricinus communis* agglutinin.

has not been previously evaluated. Thus, we established the sensitivity and specificity of this parameter. Our findings showed an increased sensitivity (90%) over specificity (77%) regarding the S:L ratio, revealing a potential negative predictive value of this test not previously described in the literature. These results raise the possibility of alternative therapies in those cases with a non-splenic pattern. Nevertheless, Palandri et al.<sup>27</sup> question the value of the non-splenic pattern to identify those cases with a lower likelihood of response. This observation was based on a similar probability of a stable response rate in splenectomised patients with non-splenic uptake compared with a historical cohort of patients treated with rituximab instead of surgery.<sup>42</sup> Therefore, future studies should be specifically designed to establish the benefit of splenectomy versus pharmacological treatment for these patients.

The relatively high rate of false positives concerning the site of platelet sequestration, with 22% of patients with splenic uptake and no response to splenectomy, proves an unmet need for assessing new predictors of response. The AMR-mediated platelet degradation at the liver provides a potential explanation for the splenectomy failure.<sup>34,37,38,43</sup> Nevertheless, desialylation may not only reflect the risk of

splenectomy failure but also the multirefractory nature of these patients. Several studies have shown the determinant role of desialylation in ITP refractory patients, which may be implicated in the lack of response to certain treatments.<sup>44,45</sup> Our results showed enhanced platelet desialylation in those refractory patients with splenic clearance, without association with the other analysed characteristics in this subgroup of patients, supporting the existing evidence of the potential role of the platelet sialylation pattern as a response biomarker.<sup>3,44–46</sup> We cannot exclude the desialylation pattern reflecting the multirefractory nature of our included patients rather than the splenectomy failure by itself, but neither the previous therapies nor the response to them were associated with desialylation in this study. Furthermore, the correlation of the platelet desialylation with the hepatic sequestration pattern has not yet been elucidated, and because of the limited sample size and the measurement of the platelet characteristics performed after splenectomy, we could not assess this association. Similarly, additional underlying mechanisms different from desialylation might be responsible for splenectomy failure. For instance, Canales-Herrerias et al.<sup>47</sup> demonstrated that high-affinity autoreactive plasma cells persist in the bone marrow of ITP patients with immediate failure to splenectomy. In our cohort, all seven non-responders with splenic sequestration in whom the RCA-binding was analysed presented less sialic acid exposure; hence, no differences can be established between the two refractory ones and the five with early relapse. The comparison between these subgroups is beyond the scope of this study due to the limited number of patients in each group. Therefore, all the above hypotheses should be explored in prospective studies.

The correlation between the anti-platelet antibodies and the platelet sialic acid pattern has been discussed in the literature as well.<sup>44,45</sup> Revilla et al.<sup>44</sup> reported an association of the desialylation state with the antibodies against the glycoprotein (GP) Ib/IX, which were overexpressed in refractory patients. The antibody-mediated desialylation has been suggested as the cause of treatment refractoriness in other studies, even independently of the antibody specificity.<sup>45,48,49</sup> A recent work has studied the correlation of the anti-platelet antibodies with platelet sequestration,<sup>50</sup> showing no clear association with the anti-GPIb/IX or anti-GPIIb/IIIa specificities, but interestingly, they found an association between the anti-GPV antibodies and the splenic clearance, whose significance remains to be elucidated. Unfortunately, we did not measure the anti-platelet antibodies in our study, so we cannot establish conclusions about this aspect, while future lines of investigation should address this variable.

The main limitation of our study is its retrospective nature, with the analysis of platelet glycome performed after splenectomy, which might have interfered with the interpretation of the results. Moreover, a potential selection bias secondary to the unblinded design must be carefully considered. The scintigraphy result conditioned the decision to conduct a splenectomy, resulting in a higher risk of overestimating the findings and providing a feasible

explanation for the lower specificity that we found.<sup>26</sup> Lastly, the patients included were recruited and studied in a single centre, ensuring the standardisation of the evaluation criteria and scintigraphy methodology rather than dismissing relevance.

In summary, this study confirms the value of the <sup>111</sup>In-platelet kinetic studies to select suitable candidates for splenectomy. To increase its efficiency and safety, a routine selection of patients who would benefit from surgery should be considered. In this regard, new biomarkers of response, such as platelet desialylation, might be implemented in the near future.

## AUTHOR CONTRIBUTIONS

Ana Mendoza, María Teresa Álvarez-Román and Nora Butta designed the study protocol; Ana Mendoza, María Teresa Álvarez-Román, Isabel Rivas-Pollmar, Mónica Martín-Salces and Nora Butta collected the data from the included patients; Bárbara Martínez de Miguel and Elena Martínez Montalbán provided the scintigraphy data; Elena Monzón-Manzano, Paula Acuña, Elena G. Arias-Salgado and Nora Butta performed the platelet analysis; Ana Mendoza and Nora Butta carried out the statistical analysis and interpreted the data; Ana Mendoza wrote the manuscript; María Teresa Álvarez-Román, Víctor Jiménez-Yuste and Nora Butta critically reviewed the manuscript; and all the authors approved the final version of the manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest.

## DATA AVAILABILITY STATEMENT

For original data, please contact [maria.t.alvarez@uam.es](mailto:maria.t.alvarez@uam.es). Additional supporting information can be found online in the Supplementary material file.

## ETHICS STATEMENT

This study was authorised by the local medical ethics committee of La Paz University Hospital in Madrid (Spain).

## PATIENT CONSENT STATEMENT

Informed consent was obtained from those patients in whom the platelet characteristics were analysed after splenectomy for the purpose of the study.

## PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Granted.

## ORCID

Ana Mendoza  <https://orcid.org/0009-0006-5248-8708>

María Teresa Álvarez-Román  <https://orcid.org/0000-0003-3296-4288>

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## SUPPORTING INFORMATION

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