

Platelet variability index: a measure of platelet count fluctuations in patients with immune thrombocytopenia

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Key Points

- The PVI score can help distinguish between ITP and nonimmune thrombocytopenia.
- Patients with thrombocytopenia and a high PVI score had a high likelihood of having the diagnosis of ITP.

Fluctuations in platelet count levels over time may help distinguish immune thrombocytopenia (ITP) from other causes of thrombocytopenia. We derived the platelet variability index (PVI) to capture both the fluctuations in platelet count measurements and the severity of the thrombocytopenia over time. Raw PVI values, ranging from negative (less severe thrombocytopenia and/or low fluctuations) to positive (more severe thrombocytopenia and/or high fluctuations) were converted to an ordinal PVI score, from 0 to 6. We evaluated the performance characteristics of the PVI score for consecutive adults with thrombocytopenia from the McMaster ITP Registry. We defined patients with definite ITP as those who achieved a platelet count response after treatment with intravenous immune globulin or high-dose corticosteroids and possible ITP as those who never received ITP treatment or did not respond to treatment. Of 841 patients with thrombocytopenia, 104 had definite ITP, 398 had possible ITP, and 339 had non-ITP thrombocytopenia. For patients with definite ITP, the median PVI score was 5 [interquartile range (IQR) 5, 6] for patients with possible ITP, the median PVI score was 3 (1, 5); and for patients with non-ITP thrombocytopenia, the median PVI score was 0 (0, 2). A high PVI score correlated with the diagnosis of definite ITP even when calculated at the patient's initial assessment, before any treatment had been administered. Platelet count fluctuations alone contributed to the specificity of the overall PVI score. The PVI score may help clinicians diagnose ITP among patients who present with thrombocytopenia for evaluation.

Introduction

Immune thrombocytopenia (ITP) is an autoimmune blood disorder characterized by a low platelet count and an increased risk of bleeding.^{1,2} In adults, ITP tends to be a chronic disease with a prevalence of 12.1 (95% confidence interval [CI], 11.1-13.0) per 100 000 adults.³ Establishing ITP as the cause of the thrombocytopenia can be challenging because there is no reliable biomarker that can differentiate ITP from non-immune thrombocytopenic disorders such as splenomegaly, or inherited thrombocytopenias; thus, ITP remains a diagnosis of exclusion, which often leads to misdiagnosis in clinical practice.⁴

The defining characteristic of ITP is a platelet count below $100 \times 10^9/L$, and the severity of the thrombocytopenia is an indicator of the severity of the disease.⁵ From a cohort of patients with ITP, we observed that the platelet count levels tended to fluctuate throughout a patient's disease course even without treatment.^{6,7} Fluctuations in platelet count level may be less evident in patients with non-immune thrombocytopenic disorders.^{8,9} Similarly, in healthy individuals, platelet count levels typically remain stable over time.¹⁰ Thus, we

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hypothesized that platelet count fluctuations, or platelet variability over time may be an indicator of the ITP diagnosis.

The development of a measure that can capture platelet variability was based on the concept of statistical volatility that is used to describe the degree of variation in financial markets as measured by the standard deviation of the logarithmic of the raw financial returns.^{11,12} Based on those principles, we derived a metric called the platelet variability index (PVI) to capture both the fluctuations in platelet count measurements over time and the severity of the thrombocytopenia. In this report, we describe the derivation of the PVI and its diagnostic application to a cohort of patients referred to a hematologist for evaluation of thrombocytopenia.

Methods

Adult patients (>18 years) who were enrolled in the McMaster ITP registry between January 2010 and January 2020 with at least 1 platelet count measurement available were included in this cohort study. The registry enrolled consecutive patients from a specialty hematology clinic at McMaster University who were referred for investigation of thrombocytopenia because of a platelet count $<150 \times 10^9/L$.^{4,13} Patients were followed every 6 to 12 months until discharge from the clinic or death, and all patients provided consent for their data to be used in emerging research. This study was approved by the Hamilton Integrated Research Ethics Board.

The diagnosis of ITP was established based on the accepted criterion of a platelet count $<100 \times 10^9/L$ with no apparent cause¹⁴ and confirmed by 2 hematologists (D.M.A. and J.G.K.) working in the clinic. For this study, we defined the subgroup of patients with definite ITP as those with a documented platelet count response after treatment with high-dose IV immune globulin (IVIG) or high-dose corticosteroids.¹⁵ We defined a platelet count response as doubling of baseline and achievement of a platelet count $\geq 30 \times 10^9/L$ per accepted criteria.¹⁶ Patients with *possible* ITP had never received treatment or did not achieve a platelet count response after treatment. Patients with non-ITP had nonimmune thrombocytopenia attributed to other conditions known to result in a low platelet count because of reduced platelet production or platelet sequestration, including myelodysplastic syndrome, liver disease, splenomegaly, or familial thrombocytopenia.¹⁷

Derivation of the PVI

We devised a statistical measure that combined platelet fluctuations over time and the severity of the thrombocytopenia into a composite index called the PVI. We used root sum squares¹⁸ to capture the variations in platelet count levels and variations in the changes between consecutive platelet count levels over time. Because platelet count fluctuation is influenced by the lowest platelet count value (eg, the lower the platelet count, the higher the possible fluctuation), we also included severity of the thrombocytopenia in the PVI measure. We used a general exponential function¹⁹ with a base parameter between 0 and 1 to the power of the lowest platelet count value to describe the thrombocytopenia severity over time. Hence, as the value of the lowest platelet count level decreased, the value of the general exponential function increased, indicating a higher level of thrombocytopenia severity. The optimal base parameter in the general exponential function for the population under investigation was determined by testing a sequence of base parameters from 0 to 1 in increments of 0.05, and selecting the base parameter that yielded the best separation in the area under the curve (AUC) between ITP and non-ITP patients.

For this study, we calculated that 236 patients would allow us to detect a 10% difference between the AUC under the null hypothesis ($AUC_0 = 75\%$) and the alternative hypothesis ($AUC_1 = 85\%$) using a 2-sided z-test at 2.5% significant level and 80% power.²⁰ Using that sample, we determined that the optimal base parameter for the general exponential function component of the PVI was 0.8.

Although the PVI can be calculated for any patient with at least 1 platelet count value, 3 or more platelet count values for any given patient were needed to measure platelet count fluctuations; thus, when fewer than 3 platelet count values were available, the PVI reflected only the thrombocytopenia severity; and when 3 or more platelet count values were available, the PVI captured both platelet count fluctuation and severity of the thrombocytopenia. The PVI can remain constant or increase over time (but cannot decrease) as new platelet count values are accumulated. The mathematical derivation of the PVI is shown in Appendix A of the supplemental material. An example of how the PVI is calculated over time with accumulating platelet count measurements in a patient with thrombocytopenia is provided in Figure 1.

The PVI score

PVI is a continuous variable that ranges from a negative value (less severe thrombocytopenia and/or low fluctuations) to a positive value (more severe thrombocytopenia and/or high fluctuations). The PVI value was converted into a 7-point ordinal score for simplicity, similar to other grading systems.²¹ The interval of each score level was determined based on the best discriminative ability using AUC for the definite ITP diagnosis.²² Because the distribution of PVI was skewed (supplemental Figure 1), the PVI intervals that provided the best discriminative power for the diagnosis of ITP were $(-\infty, -10) = \text{PVI score of } 0$, $(-10, -5) = 1$, $(-5, 0) = 2$, $(0, 5) = 3$, $(5, 8) = 4$, $(8, 12) = 5$, and $(12, +\infty) = 6$. In general, a higher PVI score indicated larger fluctuations and greater severity of thrombocytopenia over time. We calculated the PVI scores for patients with definite ITP, possible ITP, and non-ITP diagnoses.

Statistical methods

Continuous variables were reported as means and standard deviations or median and interquartile range (IQR). Categorical variables were reported as frequencies and proportions. We compared the distributions of the PVI values among patients with ITP and non-ITP. For the full cohort, we modeled the association between PVI and the ITP diagnosis using a logistic regression model and calculated sensitivity, specificity, predictive values, and AUC with corresponding 95% CIs.²³ We determined the stratum-specific likelihood ratio for each PVI score with 95% CIs²⁴ to distinguish between patients with definite ITP, possible ITP, and non-ITP. We conducted a conditional logistic regression model to evaluate the effect of the PVI score among patients with similar numbers of platelet count measurements available (<3 , 3-10, and >10 platelet counts). All analyses and modeling were performed using the R language for statistical computing.²⁵

Results

Patient characteristics

Of 919 patients with thrombocytopenia from the McMaster ITP Registry, 841 (91.5%) with at least 1 platelet count result available and a documented diagnosis were included in the analysis (Figure 2). Of those, 502 (59.7%) had ITP, including 104 (20.7%) with definite ITP and 398 (79.3%) with possible ITP. Patients with non-ITP

thrombocytopenia ($n = 339$) had the following diagnoses: mild thrombocytopenia only (platelet counts between 100 and $150 \times 10^9/L$; $n = 104$); splenomegaly ($n = 59$); familial thrombocytopenia ($n = 38$); gestational thrombocytopenia ($n = 27$); myelodysplastic syndrome ($n = 24$); thrombotic microangiopathy ($n = 19$); liver disease ($n = 15$); pseudothrombocytopenia ($n = 12$); pancytopenia with no evident cause ($n = 11$); and other ($n = 30$).

Median age at enrollment was 54.5 years (IQR 36.0, 68.0) for patients with ITP ($n = 502$) and 54.0 years (37.0, 67.0) for patients with non-ITP (Table 1). Median platelet count nadir was $16 \times 10^9/L$ (IQR 3, 46) and $78 \times 10^9/L$ (IQR 43.5, 113), respectively. For patients with ITP, median disease duration was 5.6 years (IQR 1.9, 11.1) from diagnosis to the end of follow-up, and 375 patients (74.7%) had received prior ITP treatments. The median number of platelet count measurements per patient was 17 (IQR 7, 42) for patients with ITP and 8 (IQR 4, 21) for patients with non-ITP.

PVI

The PVI index was calculated for each patient using all available platelet counts (Table 2). The median PVI was significantly higher for patients with ITP (6.4; IQR -2.9, 10.7) compared with patients with non-ITP (-10.5; IQR -18.8, -1.6; $P < .001$), which translated to a median PVI score of 4 vs 0, respectively (Figure 3). For the diagnosis of ITP, the PVI had a sensitivity of 70.3% (95% CI, 66.3-74.3) and specificity of 77.6% (73.1-82.0), positive predictive value was 82.3% (78.7-85.9), negative predictive value was 63.8% (59.2-68.5), and AUC of 80.0% (77.0-83.0). For the diagnosis of definite

ITP, the sensitivity of the PVI was 98.1% (95% CI, 95.4-100), and specificity was 86.1% (82.5-89.8) (Table 3). The positive predictive value was 68.5% (61.0-75.9), negative predictive value of 99.3% (98.4-100), and AUC of 96.8% (95.3-98.2).

PVI score

For each stepwise increase in PVI score, we observed a corresponding increase in the proportion of patients with definite ITP (Table 4). The PVI score had a sensitivity of 93.3% (95% CI, 88.5-98.1) and a specificity of 89.4% (86.1-92.7) for the diagnosis of definite ITP. The positive predictive value was 72.9% (95% CI, 65.4-80.5), negative predictive value was 97.7% (96.1-99.4), and the AUC was 96.2% (94.5-97.7). A similar increase in the proportion of patients with possible ITP was observed with increasing PVI scores (Table 4). Illustrative examples of the platelet count levels over time and the associated PVI scores for patients with ITP and non-ITP diagnoses are shown in Figure 4.

PVI score at initial assessment

We determined the PVI score for patients on the day of enrollment into the registry to simulate a new hematology referral for a patient referred for evaluation of thrombocytopenia. Of the 837 evaluable patients at the time of enrollment, 542 (64.8%) had never received ITP treatment. The median initial PVI score was 5 (IQR 4,5) for patients who were ultimately diagnosed with definite ITP ($n = 104$; 12.4%), 2 (0,4) for patients with possible ITP ($n = 397$; 47.4%), and 0 (0,2) for patients with non-ITP

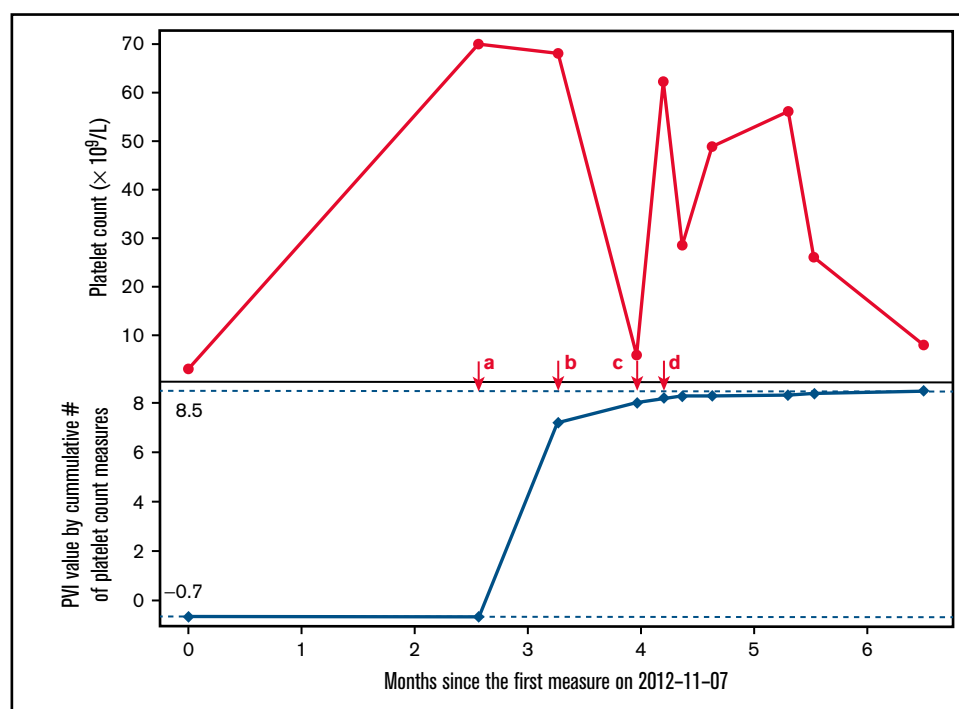


Figure 1. Illustrative example of the platelet variability index (PVI) over time in a patient referred for investigation of thrombocytopenia. At timepoint a, 2 platelet count values were available ($3 \times 10^9/L$ and $70 \times 10^9/L$) and the lowest platelet count value was $3 \times 10^9/L$; thus, the PVI was calculated by $\log(0.8^3) = -0.7$. At timepoint b, the third platelet count ($67 \times 10^9/L$) was added, which allowed for the measurement of platelet count fluctuation to be included into the overall PVI calculation, measured as $\log(0.8^3 \times 2630.2) = 7.2$. At timepoint c, the fourth platelet count was added ($7 \times 10^9/L$) and the fluctuation component increased; thus, PVI at this timepoint was $\log(0.8^3 \times 5892.9) = 8.0$, translating to a PVI score of 5 (high likelihood of definite ITP). After timepoint d, the lowest platelet count did not change and further changes in platelet count fluctuations were relatively small; hence, the PVI value remained stable.

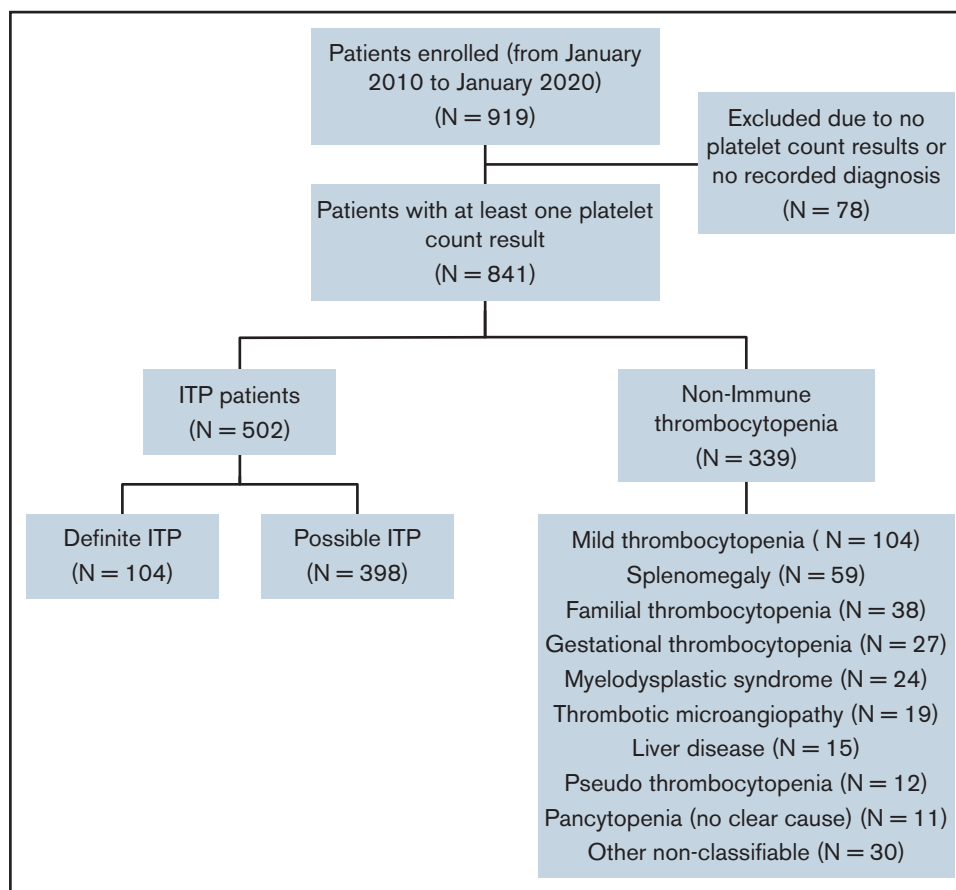


Figure 2. Patients in the McMaster ITP Registry included in this study.

thrombocytopenia ($n = 336$; 40.1%). The PVI score was similar for patients with primary ITP and secondary ITP (median score 3 [1-5]); for example, among the 12 patients with drug-induced ITP, the median PVI score was 4 (2,5) at initial assessment. At the time of initial assessment, the PVI score had a sensitivity of 97.1% (95% CI, 93.9-100), specificity of 74.1% (69.4-78.8), positive predictive value of 53.7% (46.6-60.9), negative predictive value of 98.8% (97.5-100), and AUC of 91.8% (88.9-94.7) for the diagnosis of definite ITP (Table 2). Of the 837 evaluable

patients at the time of enrollment, 609 (71.9%) had 3 or more platelet counts; thus, the PVI value was a function of both the thrombocytopenia severity and platelet count fluctuations.

We evaluated the initial PVI score for the subgroup of patients who had never received treatment ($n = 542$). Of those, 14 (2.5%) were ultimately diagnosed with definite ITP; 238 (43.9%) were diagnosed with possible ITP and 290 (53.5%) had non-ITP thrombocytopenia. Initial PVI scores for each of these groups were 3 (2,5), 1 (0,2), and

Table 1. Demographics of patients with ITP and non-ITP thrombocytopenia syndromes

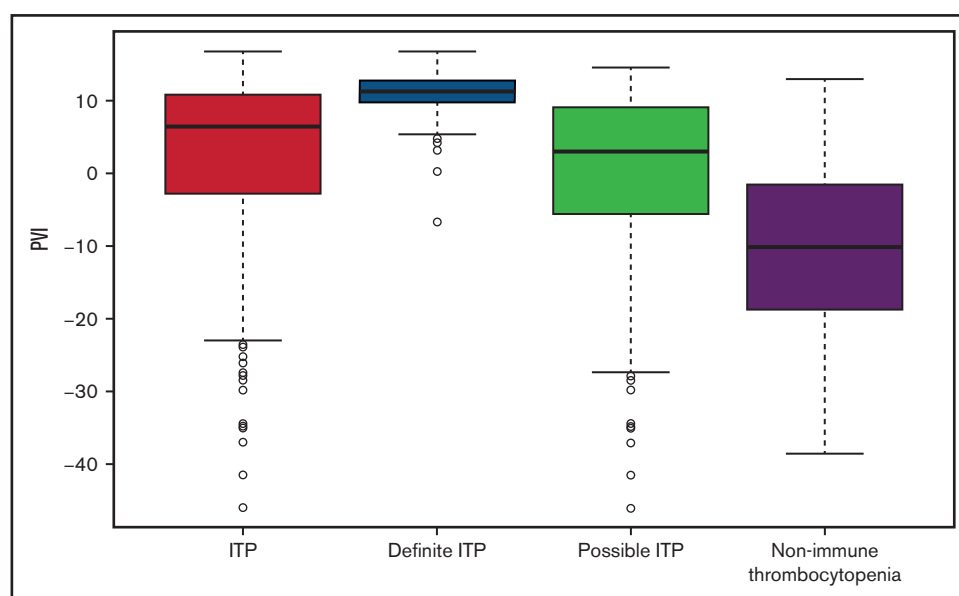
	ITP			Non-ITP
	Definite ITP subgroup ($n = 398$)	Possible ITP subgroup ($n = 502$)	Total	$n = 339$
($n = 104$)				
Age at enrollment, y; median (IQR)	52.5 (34.8, 67.0)	55.0 (36.0, 68.8)	54.5 (36.0, 68.0)	54.0 (37.0, 67.0)
Female, n (%)	64 (61.5)	231 (58.0)	295 (58.8)	172 (50.7)
Received previous ITP treatment, n (%)	104 (100.0)	271 (68.1)	375 (74.7)	75 (22.1)
Follow-up, y; median (IQR)	9.8 (5.3, 13.0)	4.2 (1.5, 10.1)	5.6 (1.9, 11.1)	3.7 (1.3, 8.4)
Nadir platelet count (median, IQR) $\times 10^9/L$	3 (1, 10)	23 (5, 58)	16 (3, 46)	78 (44, 113)
Platelets $< 20 \times 10^9/L$ ever, n (%)	62 (59.6)	148 (37.2)	210 (41.8)	25 (7.4)
Number of platelet count measures per patient	54.0 (30.0, 85.3)	12.0 (5.0, 28.8)	17.0 (7.0, 41.8)	8.0 (4.0, 21.0)

Definite ITP was defined as patients with ITP who had a documented response to intravenous immune globulin, prednisone, or dexamethasone. A treatment response was defined as doubling of the baseline platelet count and having at least 1 measure of platelet counts above $50 \times 10^9/L$ within 4 weeks after treatment.

Table 2. PVI by disease category

Diagnosis	At initial assessment		At final assessment	
	PVI (Continuous; median, IQR)	PVI score (0-6; median, IQR)	PVI (Continuous; median, IQR)	PVI score (0-6; median, IQR)
ITP (entire cohort)	0.5 (−8.1, 8.9)	3 (1, 5)	6.4 (−2.9, 10.7)	4 (2, 5)
Definite ITP	8.9 (5.8, 11.0)	5 (4, 5)	11.1 (9.7, 12.7)	5 (5, 6)
Possible ITP	−1.1 (−10.3, 7.3)	2 (0, 4)	3.0 (−5.6, 9.0)	3 (1, 5)
Non-immune TCP	−14.3 (−21.7, −4.9)	0 (0, 2)	−10.5 (−18.8, −1.6)	0 (0, 2)
Splenomegaly	−14.1 (−18.9, −5.6)	0 (0, 1.5)	−9.8 (−16.6, −2.6)	1 (0, 2)
Liver disease	−8.5 (−10.2, 1.5)	1 (0, 3)	−0.9 (−4.7, 2.1)	2 (2, 3)
Familial TCP	−11.7 (−17.4, −4.5)	0 (0, 2)	−7.0 (−12.0, −2.0)	1 (0, 2)
MDS	−1.5 (−11.0, 2.1)	2 (0.8, 3)	1.7 (−1.9, 4.4)	3 (2, 3)

PVI scores and associated PVI values: 0 (−∞, −10); 1 (−10, −5); 2 (−5, 0); 3 (0, 5); 4 (5, 8); 5 (8, 12); 6 (12, +∞). Brackets are inclusive of the value limit.
MDS, myelodysplastic syndrome; TCP, thrombocytopenia.

**Figure 3. Boxplot of the platelet variability index (PVI) by diagnosis category.**

0 (0,1), respectively. Among this cohort, the PVI score had a sensitivity of 92.9% (95% CI, 66.1-99.8), a specificity of 83.1% (78.3-87.2), a positive predictive value of 21.0% (16.5-26.3), a negative predictive value of 99.6% (97.3-99.9), and an AUC of 89.4% (80.6-98.3) for the diagnosis of definite ITP at the time of enrollment. After controlling for the number of platelet count measurements, the PVI score had a sensitivity of 85.7% (95% CI, 67.4-100), a specificity of 86.6% (82.6-90.5), a positive predictive value of 23.5% (11.9-35.2), a

negative predictive value of 99.2% (98.1-100), and an AUC of 91.2% (85.0-97.4) for the diagnosis of definite ITP.

Contribution of platelet count fluctuation to the overall PVI score

To determine the contribution of each of the components of the PVI (platelet count fluctuation and severity of thrombocytopenia), we evaluated the diagnostic performance of platelet count fluctuation alone, thrombocytopenia severity alone or both combined into the overall PVI. The sensitivities of each component for the diagnosis of definite ITP (compared with non-ITP) were: fluctuation alone, 97.1% (95% CI, 93.9-100); severity alone, 92.3% (95% CI, 87.2-97.4); and overall PVI, 98.1% (95% CI, 95.4-100). Specificities were 83.2% (79.2-87.2), 87.3% (83.8-90.9), and 86.1% (82.5-89.8), respectively.

To demonstrate the contribution of platelet fluctuations to the overall PVI score, we calculate the PVI score for patients with moderately reduced platelet count nadirs (between 30 and 60 × 10⁹/L). In this subgroup of 149 patients, the median PVI score was 3 (IQR 3, 3)

Table 3. Operating characteristics of the PVI for patients with definite ITP

Operating characteristics	PVI (Continuous variable)	PVI score (Ordinal score 0-6)
Sensitivity (95% CI)	98.1% (95.4-100)	93.3% (88.5-98.1)
Specificity (95% CI)	86.1% (82.5-89.8)	89.4% (86.1-92.7)
Positive predictive value (95% CI)	68.5% (61.0-75.9)	72.9% (65.4-80.5)
Negative predictive value (95% CI)	99.3% (98.4-100)	97.7% (96.1-99.4)
AUC (95% CI)	96.8% (95.3-98.2)	96.2% (94.5-97.7)

Table 4. Stratum-specific likelihood ratios of the PVI score for patients with definite and possible ITP

Definite ITP					
PVI score	PVI interval	Definite ITP (n = 104)	Non-ITP (n = 339)	Proportion with definite ITP	Stratum-specific likelihood ratios (95% CI)
0	$PVI \leq -10$	0	172	0/172 (0.0%)	$LR^- = 0$ (0-0)
1	$-10 < PVI \leq -5$	1	45	1/46 (2.2%)	$SSLR^+ = 0.07$ (0.01-0.36)
2	$-5 < PVI \leq 0$	0	47	0/47 (0.0%)	$SSLR^+ = 0$ (0-0)
3	$0 < PVI \leq 5$	6	39	6/45 (13.3%)	$SSLR^+ = 0.5$ (0.23-1.12)
4	$5 < PVI \leq 8$	8	14	8/22 (36.4%)	$SSLR^+ = 1.86$ (0.82-4.22)
5	$8 < PVI \leq 12$	52	20	52/72 (72.2%)	$SSLR^+ = 8.47$ (5.34-13.44)
6	$PVI > 12$	37	2	37/39 (94.9%)	$SSLR^+ = 60.3$ (17.08-212.88)
Possible ITP					
PVI score	PVI interval	Possible ITP (n = 398)	Non-ITP (n = 339)	Proportion with possible ITP	Stratum-specific likelihood ratios (95% CI)
0	$PVI \leq -10$	57	172	57/229 (24.9%)	$LR^- = 0.28$ (0.22-0.37)
1	$-10 < PVI \leq -5$	44	45	44/89 (49.4%)	$SSLR^+ = 0.83$ (0.57-1.23)
2	$-5 < PVI \leq 0$	62	47	62/109 (56.9%)	$SSLR^+ = 1.12$ (0.79-1.59)
3	$0 < PVI \leq 5$	60	39	60/99 (60.6%)	$SSLR^+ = 1.31$ (0.9-1.9)
4	$5 < PVI \leq 8$	51	14	51/65 (78.5%)	$SSLR^+ = 3.1$ (1.76-5.46)
5	$8 < PVI \leq 12$	87	20	87/107 (81.3%)	$SSLR^+ = 3.71$ (2.34-5.86)
6	$PVI > 12$	37	2	37/39 (94.9%)	$SSLR^+ = 15.76$ (4.42-56.22)

LR^- , likelihood ratio for a negative result; $SSLR^+$, stratum-specific likelihood ratio for a positive result within the stratum indicated.

for patients who were ultimately diagnosed with definite ITP (n = 5; 3.4%), 2 (2,3) for patients with possible ITP (n = 83; 55.7%), and 2 (2,3) for patients with non-ITP (n = 61; 40.9%). Among this cohort with similar platelet count nadirs, the overall PVI had a sensitivity of 80% (95% CI, 44.9-100), specificity of 95.1% (89.7-100), positive predictive value of 57.1% (20.5-93.8), negative predictive value of 98.3% (95.0-100), and AUC of 91.1% (77.2-100) for the diagnosis of definite ITP.

Number of platelet count measurements needed for the PVI

To determine the optimal number of platelet count measurements required to calculate the PVI, we examined the AUC and 95% CI for patients with definite ITP using varying numbers of platelet count values per patient (supplemental Figure 2). The AUC increased from 67% with 1 platelet count measurement to 80% with 3 platelet count measurements. With 8 or more platelet count measurements, the lower bound of the 95% CI for AUC was >85% for definite ITP.

Discussion

In this study, we describe a novel metric to characterize patients with thrombocytopenic disorders. The PVI (reported as a continuous scale) or PVI score (reported as an ordinal scale) reflects the degree of platelet fluctuations over time and the severity of the thrombocytopenia. We showed that the PVI score can provide additional information to help clinicians arrive at a diagnosis of ITP. Higher PVI scores correlated with the diagnosis ITP, especially among patients who had demonstrated a platelet count response following ITP treatments (definite ITP). In that group, the sensitivity and specificity of the PVI score for the diagnosis of definite ITP was 93.3% and 89.4%. Even when the PVI score was determined at the time of initial evaluation and before any treatment had been given (64.8% patients were

treatment-naïve patients at the time of enrollment), the PVI score had a sensitivity and specificity of 97.1% and 74.1% for the diagnosis of definite ITP. The PVI score was also helpful in differentiating ITP vs non-ITP thrombocytopenia with high specificity in the group of patients with moderately reduced platelet count levels ($30\text{--}60 \times 10^9/\text{L}$), which is commonly the group in which the diagnosis is most uncertain; however, sensitivity was only 80%. The PVI measure consists of 2 components: the degree of platelet count fluctuation and the severity of the thrombocytopenia. We showed that both components contributed to the performance of the overall PVI score.

Other biomarkers have been investigated to distinguish ITP from other causes of thrombocytopenia. Platelet autoantibodies targeting platelet glycoprotein (GP) receptors have been studied extensively over the past several decades. In a systematic review of prospective studies that tested for antibodies directly bound to the platelet surface, the sensitivity of platelet autoantibodies, either anti-GPIIb/IIIa or anti-GPIbIX, for the diagnosis of ITP was 53% and 90%, respectively.²⁶ In a recent evaluation of platelet antibody testing in which the analysis was restricted to patients who fulfilled established clinical¹⁴ and testing criteria,²⁷ sensitivity and specificity of platelet antibody testing was 90% and 78%, respectively. This high sensitivity was not consistent across other studies.²⁶ Furthermore, platelet antibody testing using recommended methods is not widely available and has not been adopted into clinical practice.

Immature platelet fraction was recently evaluated in a retrospective study of 272 children. In that study, a high immature platelet fraction (>8.45%) discriminated between ITP and other causes of thrombocytopenia, with a sensitivity of 80.4% and specificity of 79.9%.²⁸ Similar findings have been reported in adults.^{29,30} Platelet activation has also been compared between groups of patients with thrombocytopenic syndromes. In a study of platelet aggregometry adjusted for platelet count, patients with ITP had increased expression of bound fibrinogen

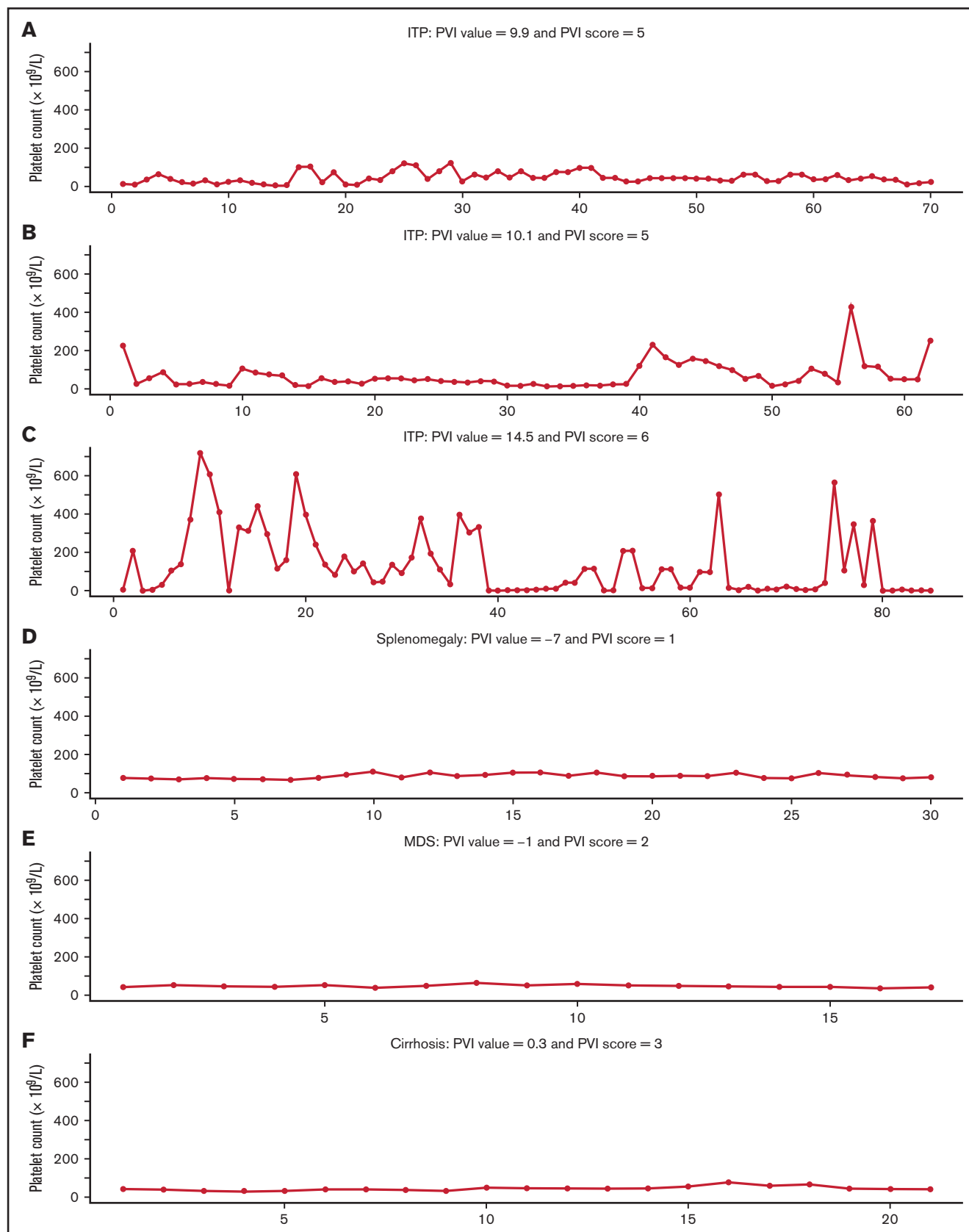


Figure 4. PVI score for 6 patients with thrombocytopenia because of ITP (A-C) and non-ITP thrombocytopenia (D-F).

and CD63 following activation, compared with patients who had thrombocytopenia in the context of cancer.³¹ The performance characteristics of the PVI was similar or better than these other biomarkers and could be assessed simply based on routine platelet count values, which are typically available at the time of a new hematology referral. Furthermore, the PVI is a dynamic measure whose accuracy improves as more platelet count values accumulate over time. It is likely that other variables will be needed in conjunction with the PVI to develop a robust clinical prediction model.

The cause of platelet count instability in patients with ITP is likely related to immune dysregulation caused by autoantibodies, cellular cytotoxicity, complement, or other immunological pressures causing premature platelet destruction or impaired platelet production.^{2,32-34} Rapid platelet count changes have been observed in patients with ITP in the setting of infection, vaccinations, or other immune stimuli.³⁵ Conversely, patients with thrombocytopenia from nonimmune causes tend to have stable platelet count levels, and the thrombocytopenia is related to a new equilibrium in circulating platelet numbers that balance platelet removal, sequestration, and reduced platelet production. For example, platelet count levels in patients with cirrhosis are lower than normal because of increased sequestration in an enlarged spleen and/or decreased production due to reduced levels of endogenous thrombopoietin.^{8,9}

We used patients with definite ITP as the gold standard for this validation study. These patients demonstrated a platelet count response to ITP-specific treatments and were identified as having ITP based on the best available information after consensus of 2 experienced hematologists. This criterion has previously been used in a blinded agreement study¹⁵ and endorsed in a recent international consensus report.³⁶ Platelet antibody tests were not used to categorize "definite" ITP because the low sensitivity²⁶ and the poor correlation with IVIG response.³⁷ Although a response to treatment is a useful marker to identify patients with ITP, it can only be used retrospectively and thus is not a useful criterion for the clinician evaluating a new patient with thrombocytopenia. In this study, we showed that the PVI score can serve as a surrogate for treatment response and help differentiate ITP from non-ITP patients based on historical platelet count values. For the PVI score to provide information on platelet fluctuation, at least 3 and ideally 8 platelet count measurements are needed. In this study, the vast majority of patients had 3 or more platelet counts at the time of enrollment into the registry, corresponding to the time of initial hematology assessment. Thus, for most patients presenting to a

hematologist for the evaluation of thrombocytopenia, the PVI score is a feasible metric that reflects both the thrombocytopenia severity and platelet count fluctuation.

Strengths of this study are the immediate clinical need for a simple diagnostic tool that can be readily applied in practice. The concept of the PVI was based on observations and mathematical principles that have been used and widely tested in financial markets, where fluctuations are common. Limitations of the PVI is that it does not account for the time horizon over which the platelet count measurements were collected. The PVI would not be useful at differentiating ITP from cyclical thrombocytopenia, although, this rare thrombocytopenic syndrome may also have an immune mechanism.³⁸ This study was done in an academic practice setting, and may not be generalizable to community clinics. Although the derivation of the PVI involved advanced mathematical functions, these can be programmed into a user-friendly application that is readily accessible.

In conclusion, this study highlights the concept of platelet count fluctuation as a feature that may distinguish ITP from other causes of thrombocytopenia. We described the derivation of the PVI score and how it may be applied in clinical practice. Further studies using the PVI score in conjunction with other patient variables is warranted to improve the diagnosis of ITP.

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Authorship

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References

- Cooper N, Bussell J. The pathogenesis of immune thrombocytopaenic purpura. *Br J Haematol*. 2006;133(4):364-374.
- Zufferey A, Kapur R, Semple JW. Pathogenesis and therapeutic mechanisms in immune thrombocytopenia (ITP). *J Clin Med*. 2017;6(2):16.
- Terrell DR, Beebe LA, Neas BR, Vesely SK, Segal JB, George JN. Prevalence of primary immune thrombocytopenia in Oklahoma. *Am J Hematol*. 2012;87(9):848-852.
- Arnold DM, Nazy I, Clare R, et al. Misdiagnosis of primary immune thrombocytopenia and frequency of bleeding: lessons from the McMaster ITP Registry. *Blood Adv*. 2017;1(25):2414-2420.
- Williamson DR, Albert M, Heels-Ansdell D, et al; PROTECT collaborators, the Canadian Critical Care Trials Group, and the Australian and New Zealand Intensive Care Society Clinical Trials Group. Thrombocytopenia in critically ill patients receiving thromboprophylaxis: frequency, risk factors, and outcomes. *Chest*. 2013;144(4):1207-1215.
- Tarantino MD, Fogarty PF, Shah P, Brainsky A. Dental procedures in 24 patients with chronic immune thrombocytopenia in prospective clinical studies of eltrombopag. *Platelets*. 2015;26(1):93-96.

7. Tsai CH, Bussel JB, Imahiyerobo AA, Sandler SI, Ogunka BA. Platelet count control in immune thrombocytopenic purpura patient: optimum romiplostim dose profile. *J Process Contr*. 2016;45:76-83.
8. Gschwamler M, Vavrik J, Gebauer A, et al. Course of platelet counts in cirrhotic patients after implantation of a transjugular intrahepatic portosystemic shunt—a prospective, controlled study. *J Hepatol*. 1999;30(2):254-259.
9. Al-Huniti A, Kahr WH. Inherited platelet disorders: diagnosis and management. *Transfus Med Rev*. 2020;34(4):277-285.
10. Segal JB, Moliterno AR. Platelet counts differ by sex, ethnicity, and age in the United States. *Ann Epidemiol*. 2006;16(2):123-130.
11. Figlewski S. Forecasting volatility. *Financ Mark Inst Instrum*. 1997;6(1):1-88.
12. Lopez JA. Evaluating the predictive accuracy of volatility models. *J Forecast*. 2001;20(2):87-109.
13. Arnold DM, Clare R, Salib M, et al. The McMaster ITP Registry: assessing the prevalence, clinical and laboratory features of immune thrombocytopenia [Abstract]. *Blood*. 2014;124(21):5008.
14. Neunert C, Terrell DR, Arnold DM, et al. American Society of Hematology 2019 guidelines for immune thrombocytopenia [published correction appears in *Blood Adv*. 2020;4(2):252]. *Blood Adv*. 2019;3(23):3829-3866.
15. Salib M, Clayden R, Clare R, et al. Difficulties in establishing the diagnosis of immune thrombocytopenia: An agreement study. *Am J Hematol*. 2016;91(8):E327-E329.
16. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: report from an international working group. *Blood*. 2009;113(11):2386-2393.
17. Gauer RL, Braun MM. Thrombocytopenia. *Am Fam Physician*. 2012;85(6):612-622.
18. Everitt B. The Cambridge Dictionary of Statistics. 2nd ed. Geoderma. Cambridge, UK: Cambridge University Press; 2004.
19. Abramson J. Power functions and polynomial functions. Precalculus. Houston, TX: Openstax; 2021:353-369.
20. Obuchowski NA, McClish DK. Sample size determination for diagnostic accuracy studies involving binormal ROC curve indices. *Stat Med*. 1997;16(13):1529-1542.
21. Warkentin TE, Sheppard JI, Smith JW, et al. Combination of two complementary automated rapid assays for diagnosis of heparin-induced thrombocytopenia (HIT). *J Thromb Haemost*. 2020;18(6):1435-1446.
22. Barrio I, Arostegui I, Rodríguez-Álvarez MX, Quintana JM. A new approach to categorising continuous variables in prediction models: proposal and validation. *Stat Methods Med Res*. 2017;26(6):2586-2602.
23. Mercaldo ND, Lau KF, Zhou XH. Confidence intervals for predictive values with an emphasis to case-control studies. *Stat Med*. 2007;26(10):2170-2183.
24. Peirce JC, Cornell RG. Integrating stratum-specific likelihood ratios with the analysis of ROC curves. *Med Decis Making*. 1993;13(2):141-151.
25. R Core Team. R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing; 2018. Available from: <https://www.r-project.org/>. Accessed 15 July 2021.
26. Vrbensky JR, Moore JE, Arnold DM, Smith JW, Kelton JG, Nazy I. The sensitivity and specificity of platelet autoantibody testing in immune thrombocytopenia: a systematic review and meta-analysis of a diagnostic test. *J Thromb Haemost*. 2019;17(5):787-794.
27. Arnold DM, Santoso S, Greinacher A; Platelet Immunology Scientific Subcommittee of the ISTH. Recommendations for the implementation of platelet autoantibody testing in clinical trials of immune thrombocytopenia. *J Thromb Haemost*. 2012;10(4):695-697.
28. McDonnell A, Bride KL, Lim D, Paessler M, Witmer CM, Lambert MP. Utility of the immature platelet fraction in pediatric immune thrombocytopenia: differentiating from bone marrow failure and predicting bleeding risk. *Pediatr Blood Cancer*. 2018;65(2):E26812.
29. Ali I, Graham C, Dempsey-Hibbert NC. Immature platelet fraction as a useful marker in the etiological determination of thrombocytopenia. *Exp Hematol*. 2019;78:56-61.
30. Jeon K, Kim M, Lee J, et al. Immature platelet fraction: a useful marker for identifying the cause of thrombocytopenia and predicting platelet recovery. *Medicine (Balt)*. 2020;99(7):E19096.
31. Skipper MT, Rubak P, Stentoft J, Hvas AM, Larsen OH. Evaluation of platelet function in thrombocytopenia. *Platelets*. 2018;29(3):270-276.
32. Tólti LJ, Nazy I, Jafari R, Arnold DM. Piecing together the humoral and cellular mechanisms of immune thrombocytopenia. *Semin Thromb Hemost*. 2011;37(6):631-639.
33. Shrestha S, Nazy I, Smith JW, Kelton JG, Arnold DM. Platelet autoantibodies in the bone marrow of patients with immune thrombocytopenia. *Blood Adv*. 2020;4(13):2962-2966.
34. Audia S, Mahévas M, Samson M, Godeau B, Bonnotte B. Pathogenesis of immune thrombocytopenia. *Autoimmun Rev*. 2017;16(6):620-632.
35. Rinaldi M, Perricone C, Ortega-Hernandez OD, Perricone R, Shoenfeld Y. Immune thrombocytopenic purpura: an autoimmune cross-link between infections and vaccines. *Lupus*. 2014;23(6):554-567.
36. Provan D, Arnold DM, Bussel JB, et al. Updated international consensus report on the investigation and management of primary immune thrombocytopenia. *Blood Adv*. 2019;3(22):3780-3817.
37. Rogier T, Samson M, Mourey G, et al. Antiplatelet antibodies do not predict the response to intravenous immunoglobulins during immune thrombocytopenia. *J Clin Med*. 2020;9(6):1998.
38. Kyrle PA, Eichinger S. How I manage cyclic thrombocytopenia. *Blood*. 2021;137(2):178-184.