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Edited by Yogesh Singh Bisht & Ruby Singh
E-mail: yogeshbishttech@gmail.com & bioinformationruby@gmail.com
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Role of platelet histogram in differentiating true thrombocytopenia from pseudothrombocytopenia: A prospective study

Neha Kirar*, Arjun Singh, Nidhi Rai, Anand Bhadkariya, Minu Bakna & Vandana Pahadiya

Department of Pathology, Government Medical College, Datia, Madhya Pradesh, India; *Corresponding Author

Affiliation URL:
https://datiamedicalcollege.edu.in/

Author contact:
Neha Kirar - E-mail: nehakirar93@gmail.com
Arjun Singh - E-mail: dr_arjun12@yahoo.co.in

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Nidhi Rai - E-mail: nidhirai18sep@gmail.com
Anand Bhadkariya - E-mail: anandbhadkaria@gmail.com
Minu Bakna - E-mail: minubakna@gmail.com
Vandana Pahadiya - E-mail: vandapahadiya@gmail.com

Abstract:
Pseudothrombocytopenia (PTCP) is an *in vitro* artifact caused by EDTA dependent platelet aggregation, leading to falsely low platelet counts. Therefore, it is of interest to evaluate the platelet histogram patterns for differentiating PTCP from true thrombocytopenia in 1,000 EDTA-anticoagulated blood samples. Peripheral smear identified 613 (61.3%) cases of true thrombocytopenia and 387 (38.7%) cases of PTCP, with abnormal histogram patterns more frequent in PTCP (78.3% vs. 18.1%). Platelet histogram analysis showed 78.3% sensitivity, 82.9% specificity, 74.3% positive predictive value, 85.8% negative predictive value and 81.1% diagnostic accuracy. Thus, platelet histogram evaluation provides a rapid screening approach for PTCP, while peripheral smear examination remains essential for definitive diagnosis.

Keywords: Pseudothrombocytopenia (PTCP), platelet histogram, EDTA, thrombocytopenia

Background:
Platelets are essential for haemostasis and abnormalities in their count or function are commonly linked with bleeding disorders. Accurate platelet estimation is therefore a key component in diagnosing and managing various clinical conditions **[1]**. However, Pseudothrombocytopenia (PTCP) represents an *in vitro* artifact where automated hematology analyzers falsely report low platelet counts due to platelet clumping **[2]**. This phenomenon was first described in 1969 **[3]**. PTCP is an *in vitro* artefact with several underlying causes, such as anticoagulant-induced platelet aggregation, platelet satellitism, cold-related agglutination, the presence of large platelets, formation of small clots and errors in blood collection technique. Among these factors, it is most commonly linked to the use of ethylenediaminetetraacetic acid (EDTA), a routinely employed anticoagulant in hematology, which can trigger platelet clumping in certain susceptible individuals **[4].** PTCP occurs due to EDTA induced antiplatelet antibodies that promote *in vitro* platelet agglutination, leading analyzers to miscount clumps as single large platelets or even small lymphocytes **[5, 6]**. Failure to recognize PTCP may result in misdiagnosis, unnecessary investigations and inappropriate treatments, including unwarranted platelet transfusions or corticosteroid therapy. Its prevalence is reported to vary between 0.1% and 2% among hospitalized patients1-3 and 15–17% among out patients evaluated for isolated thrombocytopenia (TCP) **[7]**. Automated analyzers generate platelet histograms, which normally show a smooth bell-shaped curve within a size range of 2–30 fL **[8]**. In PTCP, these histograms often appear abnormal, with irregular curves, distorted peaks, or tailing. Such deviations, along with analyzer flags, provide important clues and should prompt confirmation through peripheral blood smear examination, which remains the gold standard for diagnosis **[9]**. Therefore, it is of interest to evaluate the role of platelet histogram patterns in differentiating true thrombocytopenia from EDTA-dependent pseudothrombocytopenia using peripheral blood smear examination as the reference standard.

Materials and Methods:
Study design and settings:
This prospective observational study was undertaken in the Department of Pathology at Government Medical College, Datia, Madhya Pradesh, after receiving clearance from the Institutional Ethics Committee. It followed a prospective design and was carried out over a period of one year, from April 2024 to March 2025. During this time, a total of 1000 complete blood count (CBC) samples were included for analysis.

Inclusion criteria:
[1] Automated Platelet count <1 lakh/µL
[2] Samples received during the study period with adequate blood volume and proper anticoagulation

Exclusion criteria:
[1] Patients with leukemia or other hematological malignancies
[2] Known case of immune thrombocytopenia (ITP).
[3] Patients with inherited or acquired bleeding disorders.
[4] Clotted, hemolyzed, or inadequately collected blood samples.

Sample collection and laboratory analysis:
Blood samples were collected in EDTA vacutainers and processed on an automated hematologyanalyzer (Sysmex XN-550) as per standard laboratory guidelines. In samples showing platelet counts below 1 lakh per µL, platelet indices and histogram abnormalitiessuch as PU flags and multiple peaks (CLP flags) were carefully evaluated. Peripheral blood smears were prepared for all such cases and stained with Leishman stain. Once the smears were adequately stained and dried, they were examined under a light microscope using both low-power and oil immersion objectives. Platelet counts were performed in ten oil immersion fields and the average value was calculated. This average was then multiplied by 15,000 to obtain the estimated platelet count per microliter. The count derived from peripheral smear examination was considered the reference (gold standard) value. The smear-based platelet count was compared with the automated analyzer results. Cases with platelet counts below 1 lakh per µL on smear examination were classified as true thrombocytopenia (TCP). In contrast, samples that showed higher platelet counts on smear but low values on

the analyzer were categorized as PTCP. Final classification into PTCP or true thrombocytopenia was based on the platelet count obtained from peripheral smear examination.

Statistical analysis:

Data were analyzed using IBM SPSS Statistics software (Version 26.0; IBM Corp., Armonk, NY, USA).Continuous variables were expressed as mean ± standard deviation (SD), whereas categorical variables were summarized as frequencies and percentages. Associations between categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Diagnostic performance of platelet histogram abnormalities was evaluated by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall diagnostic accuracy, considering peripheral blood smear examination as the gold standard. A two-tailed p-value <0.05 was considered statistically significant.

Results:

The majority of participants belonged to the 21–60 years age group (44.5%), with a slight male predominance (52.3%). A statistically significant association was observed between age groups and the distribution of TCP and PTCP in both genders, indicating that these conditions vary across different age categories. Based on peripheral smear examination, which was considered the gold standard, 613 cases (61.3%) were diagnosed as true TCP, while 387 cases (38.7%) were identified as PTCP (**Table 1).** Abnormal platelet histogram findings, characterized by the presence of multiple peaks or PU flags, were significantly more frequent in pseudothrombocytopenia than in true thrombocytopenia (78.3% vs. 18.1%; χ^2 = 351.75, p < 0.001), indicating a strong association between platelet aggregation and EDTA-dependent pseudothrombocytopenia **(Table 2).** Increased MPV was observed more frequently in pseudothrombocytopenia than in true thrombocytopenia (45.2% vs. 34.3%). This difference was statistically significant (χ^2 = 11.58, p < 0.001), suggesting that elevated MPV is associated with platelet aggregation **(Table 3).** Increased PDW was significantly more common among patients with pseudothrombocytopenia than those with true thrombocytopenia (70.3% vs. 63.5%; χ^2 = 4.63, p = 0.031), indicating greater platelet size variability in platelet aggregation **(Table 4).** The diagnostic performance of platelet histograms, when compared with peripheral smear findings, showed good reliability **(Table 5).** Peripheral smear of corresponding case shows scattered platelets, platelet count found to be 360×10³ per µl. Peripheral smear of corresponding case shows multiple CLP of platelets, platelet count found to be 340×10³ per µl. Peripheral smear of corresponding case shows multiple CLP of platelets, platelet count found to be 380×10³ per µl**.** Peripheral smear of corresponding case shows few platelets, platelet count found to be 30×10³ per µl**.**

Table 1: Distribution of cases based on peripheral blood smear findings

Peripheral smear diagnosis	Number (n=1000)	Percentage (%)
True thrombocytopenia (TCP)	613	61.3
Pseudothrombocytopenia (PTCP)	387	38.7

Table 2: Correlation of thrombocytopenia and pseudothrombocytopenia with presence of multiple peaks/pu flag

Platelet histogram finding	TCP (n=613)	TCP (%)	PTCP (n=387)	PTCP (%)	p value
PU flag/Multiple peaks present	111	18.11	303	78.29	<0.001
PU flag/Multiple peaks absent	502	81.89	84	21.71	

Table 3: Correlation of thrombocytopenia and pseudothrombocytopenia with mean platelet volume (MPV)

MPV status	TCP (n=613)	TCP (%)	PTCP (n=387)	PTCP (%)	p value
Normal MPV	403	65.74	212	54.78	<0.001
Increased MPV	210	34.26	175	45.22	

Table 4: Correlation of thrombocytopenia and pseudothrombocytopenia with platelet distribution width

PDW status	TCP (n=613)	TCP(%)	PTCP(n=387)	PTCP(%)	p value
Normal PDW	224	36.54	115	29.72	<0.001
Increased PDW	389	63.46	272	70.28	

Table 5: Sensitivity and specificity of platelet histograms for diagnosing TCP and PTCP

Parameter	Value (%)
Sensitivity	78.29%
Specificity	82.87%
PPV	74.26%
NPV	85.81%
Accuracy	81.10%

Discussion:

The present study showed that 61.3% of patients with an automated platelet count below 100×10³ per µL had true thrombocytopenia, whereas 38.7% represented pseudothrombocytopenia (PTCP) confirmed by peripheral blood smear examination. This relatively high proportion of PTCP emphasizes that spurious thrombocytopenia remains a frequent laboratory challenge when only EDTA-anticoagulated automated counts are considered. The higher frequency observed in our study is likely attributable to the selective inclusion of thrombocytopenic samples rather than an unselected laboratory population. Recent reviews have similarly highlighted that EDTA-dependent platelet aggregation is one of the commonest causes of falsely decreased platelet counts and may result in unnecessary investigations, platelet transfusions, or inappropriate therapy if not promptly recognized **[6, 7].** The principal finding of this study was the strong association between abnormal platelet histogram patterns (multiple peaks or platelet clump flags) and pseudothrombocytopenia. Nearly four-fifths of PTCP cases demonstrated abnormal histogram patterns compared with less than one-fifth of true thrombocytopenia cases (p<0.001). This finding supports previous studies showing that platelet histograms and analyzer-generated platelet clump flags are reliable indicators of EDTA-induced platelet aggregation. Xu *et al.* **[7]** reported that platelet abnormal flags generated by the Sysmex XN series showed excellent performance for detecting platelet clumping, while So *et al.* **[8]** emphasized that abnormal histogram morphology provides an important warning signal requiring microscopic confirmation.

The distorted histogram pattern observed in PTCP results from platelet aggregates being recognized as giant platelets or small leukocytes rather than individual platelets, thereby producing falsely reduced automated platelet counts. Another important observation was the significantly higher prevalence of increased mean platelet volume (MPV) among PTCP cases. Elevated MPV probably reflects the presence of large platelet aggregates and giant platelets that alter volume-based impedance measurements. Similar observations have been reported in recent laboratory studies evaluating EDTA-dependent pseudothrombocytopenia, where platelet aggregation artificially increases platelet size indices. Although MPV alone cannot differentiate PTCP from true thrombocytopenia, its interpretation together with platelet histogram abnormalities improves diagnostic confidence **[9]**. Similarly, platelet distribution width (PDW) was significantly increased in pseudothrombocytopenia, indicating greater heterogeneity in platelet size. Platelet aggregation produces variable-sized platelet clumps, resulting in wider platelet size distribution and consequently elevated PDW values. Previous studies have reported comparable increases in PDW among samples exhibiting platelet clumping, suggesting that PDW should be regarded as a supportive rather than an independent diagnostic marker. Therefore, PDW should always be interpreted alongside platelet histogram findings and peripheral smear examination **[6, 8]**. The present study further demonstrated that platelet histogram analysis achieved a sensitivity of 78.3%, specificity of 82.9%, positive predictive value of 74.3% and negative predictive value of 85.8% and overall diagnostic accuracy of 81.1% for identifying pseudothrombocytopenia. These values are comparable with recent reports by researchers who reported diagnostic accuracy approaching 74% and Xu *et al.*, who observed excellent diagnostic performance of analyzer-generated platelet clump flags. The slightly higher diagnostic accuracy observed in the present study may be explained by simultaneous evaluation of histogram morphology, platelet clump flags, platelet indices and systematic confirmation by peripheral smear. These findings indicate that automated platelet histograms provide an effective first-line screening tool but cannot replace microscopic confirmation **[7, 10]**. From a clinical perspective, early recognition of pseudothrombocytopenia has important implications. Failure to identify EDTA-induced platelet aggregation may expose patients to unnecessary repeat investigations, bone marrow examinations, corticosteroid therapy, platelet transfusion, or delays in surgical procedures **[11, 12]**. Incorporating routine review of platelet histograms and analyzer flags before reporting severe thrombocytopenia may substantially improve laboratory quality assurance and patient safety, particularly in resource-limited settings where rapid clinical decisions frequently depend on automated hematology analyzer results **[13]**.

Conclusion:

Automated haematology analysers are valuable in peripheral health settings where advanced laboratory facilities are limited. Platelet histograms rapidly screen for thrombocytopenia and flag platelet clumping, but peripheral smear examination is essential for confirmation. Combining automated counts, histogram analysis and smear review improves diagnostic accuracy in resource-limited settings.

References:

[1] Milevoj Kopčinović L *et al. Biochem Med (Zagreb)*. 2024 **34**:030504. [PMID: 39435172]
[2] Yilmaz S *et al. Medicine (Baltimore)*. 2023 **102**:e35395. [PMID: 37832120]
[3] Woo S *et al. Int J Lab Hematol*. 2025 **47**:79. [PMID: 39434430]
[4] Yamade K *et al. Int J Lab Hematol*. 2021 **43**: 927. [PMID: 33548102]
[5] Lardinois B *et al. J Clin Med*. 2021 **10**:594. [PMID: 33557431]
[6] Cattaneo M. *Haematologica*. 2025 **110**:1677. [PMID: 40145275]
[7] Xu P *et al. Clin Chem Lab Med*. 2022 **60**:748. [PMID: 35212492]
[8] So MK *et al. Ann Lab Med*. 2024 **44**:478. [PMID: 38572537]
[9] Meena H *et al. Asian Journal of Medical Sciences*. 2023 **14**:166. [DOI: 10.3126/ajms.v14i7.53334]
[10] Baccini V *et al. J Clin Med*. 2020 **9**:808. [PMID: 32188124]
[11] Dhanabalan RT J *Clin of Diagn Res*.2025 **19**:EC06. [DOI: 10.7860/JCDR/2025/79987/22166]
[12] Giovannelli A *et al. Clin Chim Acta*. 2026 **578**:120514. [PMID: 40730297]
[13] Dönmez E & Kaya Z. *Turk J Pediatr*. 2024 **66**:666. [PMID: 39582446]

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