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Original Article

Distinguishing True Thrombocytopenia from Pseudothrombocytopenia: Insights from Platelet Aggregation and Morphological Features

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ABSTRACT

Background: Automated hematology analyzers may report low platelet counts that represent either true thrombocytopenia or pseudothrombocytopenia caused by in-vitro platelet aggregation and related counting artifacts. Accurate differentiation is essential to avoid misdiagnosis, unnecessary transfusion, invasive investigation, or inappropriate clinical management. Objective: To compare demographic, hematological, and peripheral-smear morphological features between pseudothrombocytopenia and true thrombocytopenia among analyzer-flagged thrombocytopenic complete blood count reports. Methods: This retrospective observational laboratory-based study was conducted at the Department of Hematology, Fatima Memorial Hospital, Lahore, Pakistan, using complete blood count reports from January to December 2024. Reports with automated platelet counts below 150×10^9/L on the Sysmex XN-1000 analyzer were included when paired manual platelet counts and peripheral-smear findings were available. Reports correcting to $\geq150\times10^9$/L on manual verification were classified as pseudothrombocytopenia, while those remaining below 150×10^9/L were classified as true thrombocytopenia. Results: Of 2,021 reports, 244 (12.1%) were classified as pseudothrombocytopenia and 1,777 (87.9%) as true thrombocytopenia. Platelet clumping was present in 99.6% of pseudothrombocytopenia reports and 25.1% of true thrombocytopenia reports. Higher hemoglobin and female sex were independently associated with pseudothrombocytopenia, while platelet anisocytosis, nucleated red blood cells, and schistocytes were associated with lower adjusted odds of pseudothrombocytopenia. Conclusion: Peripheral-smear review and manual platelet verification are essential for distinguishing pseudothrombocytopenia from true thrombocytopenia. Platelet clumping should prompt confirmatory interpretation but should not be used alone as a diagnostic label. Keywords: Blood Platelets; Hematologic Diseases; Platelet Aggregation; Platelet Count; Pseudothrombocytopenia; Thrombocytopenia.

INTRODUCTION

Thrombocytopenia, commonly defined as a platelet count below 150×10^9/L, is a frequent hematological abnormality encountered in hospital and laboratory practice and may reflect a wide range of clinical conditions, from transient reactive changes to serious marrow, immune, infectious, or consumptive disorders (1). Because platelets have a central role in primary hemostasis, true reductions in platelet count may increase the risk of mucocutaneous bleeding, procedural hemorrhage, or life-threatening bleeding in severely affected patients (2). Accurate identification of thrombocytopenia is therefore essential, not only for estimating bleeding risk but also for guiding further diagnostic evaluation, transfusion decisions, and disease-specific management (3). A clinically important diagnostic challenge is that not every low platelet count reported by an automated hematology analyzer represents true

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thrombocytopenia. Pseudothrombocytopenia is an in-vitro laboratory artifact in which platelet aggregation, platelet satellitism, giant platelets, or analyzer-related counting limitations may produce a falsely low platelet count despite an adequate circulating platelet mass (4). Failure to recognize this artifact can lead to misclassification of patients as thrombocytopenic and may expose them to unnecessary investigations or treatments, including repeat testing, platelet transfusion, corticosteroids, immunoglobulin therapy, invasive diagnostic procedures, or avoidable delays in clinical care (5). This distinction is particularly important in high-volume diagnostic laboratories where automated complete blood count reporting is often the first basis for clinical decision-making.

Automated hematology analyzers provide rapid, reproducible, and high-throughput platelet counts using impedance, optical, or fluorescence-based technologies, but their accuracy may be reduced when platelet clumps, macroplatelets, abnormal cell fragments, or marked morphological abnormalities are present (6). In such situations, peripheral blood smear examination remains a critical confirmatory step because it allows direct visualization of platelet aggregates, platelet size variation, giant platelets, schistocytes, nucleated red blood cells, and other morphological features that may help differentiate spurious thrombocytopenia from genuine hematological disease (7). Morphological findings such as platelet clumping support an in-vitro aggregation artifact, whereas platelet anisocytosis, schistocytes, nucleated red blood cells, or other abnormal cellular features may indicate underlying marrow stress, hemolysis, fragmentation, or systemic disease processes associated with true thrombocytopenia (8,9).

Previous studies have emphasized that automated platelet counts should be interpreted alongside smear morphology and manual or confirmatory platelet assessment, especially when the automated count is unexpectedly low or inconsistent with the clinical picture (10). However, local laboratory data comparing automated platelet counts, documented manual platelet counts, and peripheral-smear morphology among analyzer-flagged thrombocytopenic reports remain limited. A large retrospective evaluation of such reports can help clarify which hematological and morphological features are most useful for distinguishing pseudothrombocytopenia from true thrombocytopenia in routine diagnostic practice. Therefore, this study aimed to compare demographic, hematological, and peripheral-smear morphological features between reports classified as pseudothrombocytopenia and those classified as true thrombocytopenia after paired automated and manual platelet assessment, with the objective of identifying practical laboratory indicators that may improve diagnostic classification and reduce unnecessary clinical intervention.

MATERIAL AND METHODS

This retrospective observational laboratory-based study was conducted at the Department of Hematology, Fatima Memorial Hospital, Lahore, Pakistan, using complete blood count reports generated between January 2024 and December 2024. The study was designed to evaluate analyzer-flagged thrombocytopenic complete blood count reports and to compare reports subsequently classified as pseudothrombocytopenia with those confirmed as true thrombocytopenia after documented manual platelet assessment and peripheral blood smear review. The unit of analysis was the laboratory report/accession rather than a prospectively enrolled clinical participant, and duplicate accessions were removed before final analysis to maintain a clean comparison dataset.

All complete blood count reports showing an automated platelet count below 150×10^9/L on the Sysmex XN-1000 automated hematology analyzer were screened for eligibility. Reports were included when they contained complete laboratory information, including the automated platelet count, documented manual platelet count, and peripheral blood smear findings. Reports were excluded if the automated platelet count was 150×10^9/L or higher, if paired automated and manual platelet count information was incomplete, if peripheral-smear evaluation was missing, or if the same accession appeared more than once in the laboratory records. Non-probability consecutive inclusion of all eligible reports within the

study period was used, allowing the sample to represent the routine diagnostic workload of thrombocytopenic reports reviewed in the laboratory during the specified year.

For each eligible report, the automated platelet count generated by the Sysmex XN-1000 analyzer was used as the initial screening value. Reports with platelet counts below 150×10^9/L underwent documented manual platelet assessment and peripheral blood film review as part of laboratory verification. Peripheral blood smears were prepared, stained with Giemsa stain, and examined by trained laboratory personnel for platelet and red-cell morphological features. The recorded smear variables included platelet clumping, platelet anisocytosis, giant platelets, nucleated red blood cells, and schistocytes. Platelet clumping was treated as a peripheral-smear morphological feature and not as a standalone diagnostic label; final classification depended on whether the documented manual platelet count corrected to the normal range or remained below the diagnostic threshold.

Reports were classified as pseudothrombocytopenia when the automated platelet count was below 150×10^9/L but the documented manual platelet count corrected to 150×10^9/L or higher. Reports were classified as true thrombocytopenia when both the automated platelet count and the documented manual platelet count remained below 150×10^9/L. This classification approach was used to distinguish reports with spurious analyzer-associated thrombocytopenia from those with persistent thrombocytopenia after manual verification. Because platelet clumping may coexist with genuinely reduced platelet counts, clumping on smear was interpreted in relation to the corrected manual platelet count rather than being used alone to define pseudothrombocytopenia.

The main outcome variable was final platelet classification, categorized as pseudothrombocytopenia or true thrombocytopenia. The primary explanatory variables included age, sex, white blood cell count, hemoglobin concentration, automated platelet count, documented manual platelet count, platelet clumping, platelet anisocytosis, giant platelets, nucleated red blood cells, and schistocytes. Automated and manual platelet counts were expressed as $\times10^9$/L, hemoglobin as g/dL, and white blood cell count as $\times10^9$/L. The classification threshold for thrombocytopenia and platelet-count correction was prespecified at 150×10^9/L.

Data were cleaned before analysis by removing duplicate accessions and excluding records with incomplete platelet-count or smear-review information. Continuous variables were assessed for distributional normality using the Shapiro–Wilk test. As the continuous variables were non-normally distributed, they were summarized as median with interquartile range and compared between groups using the Mann–Whitney U test. Categorical variables were summarized as frequencies and percentages and compared using the chi-square test, with Fisher's exact test used where expected cell counts were small. Binary logistic regression was used to identify independent predictors of pseudothrombocytopenia. Platelet clumping was reported descriptively and excluded from the multivariable regression model because its very high frequency in pseudothrombocytopenia produced quasi-complete separation and unstable adjusted estimates. Statistical analyses were performed using Python with SciPy and statsmodels and were cross-checked in SPSS. A two-sided p-value of ≤0.05 was considered statistically significant.

The sample size was determined by the number of eligible analyzer-flagged thrombocytopenic reports available during the one-year study period after application of inclusion and exclusion criteria. Because this was a retrospective laboratory-record review, no prospective sample-size calculation was performed. The study was approved by the Institutional Review Board of Fatima Memorial Hospital College of Medicine & Dentistry under IRB No. FMH-15/09/2025-IRB-1749. Laboratory records were reviewed retrospectively, and the analysis was performed on available diagnostic data after removal of duplicate and incomplete accessions to support data integrity and reproducibility.

RESULTS

A total of 2,021 eligible analyzer-flagged thrombocytopenic complete blood count reports/accessions were included after removal of duplicate and incomplete records. Based on documented manual platelet-count verification, 244 reports were classified as pseudothrombocytopenia and 1,777 reports were classified as true thrombocytopenia.

Table 1. Final Platelet Classification of Analyzer-Flagged Thrombocytopenic Reports

Final Classification	n (%)
Pseudothrombocytopenia	244 (12.1)
True thrombocytopenia	1,777 (87.9)
Total	2,021 (100.0)

Among the 2,021 thrombocytopenic reports initially flagged by automated analysis, pseudothrombocytopenia accounted for 244 reports, representing 12.1% of the final analyzed dataset. The majority of reports, 1,777 (87.9%), remained below the platelet-count threshold after manual verification and were therefore classified as true thrombocytopenia.

Table 2. Demographic and Hematological Parameters by Final Platelet Classification

Variable	Pseudothrombocytopenia Median (IQR)	True Thrombocytopenia Median (IQR)	p-value
Age, years	21.5 (0.0–38.0)	28.0 (0.1–50.0)	<0.001
WBC, $\times10^9$/L	8.7 (5.9–12.2)	8.0 (5.0–12.8)	0.051
Hemoglobin, g/dL	12.0 (9.9–14.9)	10.8 (8.8–13.0)	<0.001
Automated platelet count, $\times10^9$/L	119 (105–135)	77 (50–102)	<0.001
Manual platelet count, $\times10^9$/L	150 (150–150)	92 (60–120)	<0.001

WBC: white blood cell count; IQR: interquartile range. Continuous variables were summarized as median (IQR) and compared using the Mann–Whitney U test.

Reports classified as pseudothrombocytopenia had a lower median age than reports classified as true thrombocytopenia, although both groups showed wide age distributions. Hemoglobin was higher in pseudothrombocytopenia, with a median of 12.0 g/dL compared with 10.8 g/dL in true thrombocytopenia. The automated platelet count was also higher in pseudothrombocytopenia, with a median of 119×10^9/L compared with 77×10^9/L in true thrombocytopenia. Manual platelet verification reclassified the pseudothrombocytopenia group at the correction threshold, while the true thrombocytopenia group remained below the diagnostic threshold, with a median manual platelet count of 92×10^9/L.

Table 3. Peripheral-Smear Morphology and Sex Distribution by Final Platelet Classification

Variable	Pseudothrombocytopenia n (%)	True Thrombocytopenia n (%)	p-value
Platelet clumping	243 (99.6)	446 (25.1)	<0.001
Platelet anisocytosis	74 (30.3)	880 (49.5)	<0.001
Giant platelets	6 (2.5)	62 (3.5)	0.517
Nucleated RBCs	42 (17.2)	339 (19.1)	0.541
Schistocytes	5 (2.0)	289 (16.3)	<0.001
Female sex	121 (49.8)	792 (44.6)	0.149

RBCs: red blood cells. Categorical variables were summarized as n (%) and compared using the chi-square test or Fisher's exact test where applicable.

Platelet clumping was the dominant smear finding associated with pseudothrombocytopenia, being present in 243 of 244 reports (99.6%). However, platelet clumping was also recorded in 446 true thrombocytopenia reports (25.1%), indicating that clumping can coexist with a genuinely low platelet count and should therefore be interpreted alongside manual platelet-count correction rather than used as a standalone diagnostic label. Platelet anisocytosis and schistocytes were more frequent in true thrombocytopenia, while giant platelets and nucleated red blood cells showed no significant unadjusted group difference. Female sex was numerically more frequent in pseudothrombocytopenia, but the unadjusted comparison was not statistically significant.

Table 4. Multivariable Logistic Regression for Predictors of Pseudothrombocytopenia

Variable	Adjusted OR	95% CI	Adjusted p-value
Platelet anisocytosis	0.41	0.30–0.56	<0.001
Giant platelets	0.93	0.38–2.24	0.865
Nucleated RBCs	0.64	0.42–0.95	0.029
Schistocytes	0.12	0.05–0.29	<0.001
Hemoglobin, per g/dL	1.14	1.10–1.18	<0.001
Female sex	1.69	1.25–2.27	0.001

OR: odds ratio; CI: confidence interval; RBCs: red blood cells. Outcome variable: pseudothrombocytopenia. Odds ratios below 1 indicate lower adjusted odds of pseudothrombocytopenia and relatively greater association with true thrombocytopenia. Platelet clumping was excluded from the model because its near-complete separation produced unstable adjusted estimates.

In multivariable analysis, higher hemoglobin was independently associated with pseudothrombocytopenia, with each 1 g/dL increase corresponding to an adjusted odds ratio of 1.14. Female sex was also independently associated with pseudothrombocytopenia after adjustment, with an adjusted odds ratio of 1.69. In contrast, platelet anisocytosis, nucleated red blood cells, and schistocytes were associated with lower adjusted odds of pseudothrombocytopenia, supporting their stronger relationship with true thrombocytopenia. Schistocytes showed the strongest inverse association, with an adjusted odds ratio of 0.12. Giant platelets were not independently associated with final platelet classification.

Although platelet clumping was the most frequent smear feature in pseudothrombocytopenia, it was not entered into the multivariable regression model because it was present in nearly all pseudothrombocytopenia reports and produced quasi-complete statistical separation. Its diagnostic value is therefore best interpreted descriptively in relation to manual platelet-count correction.

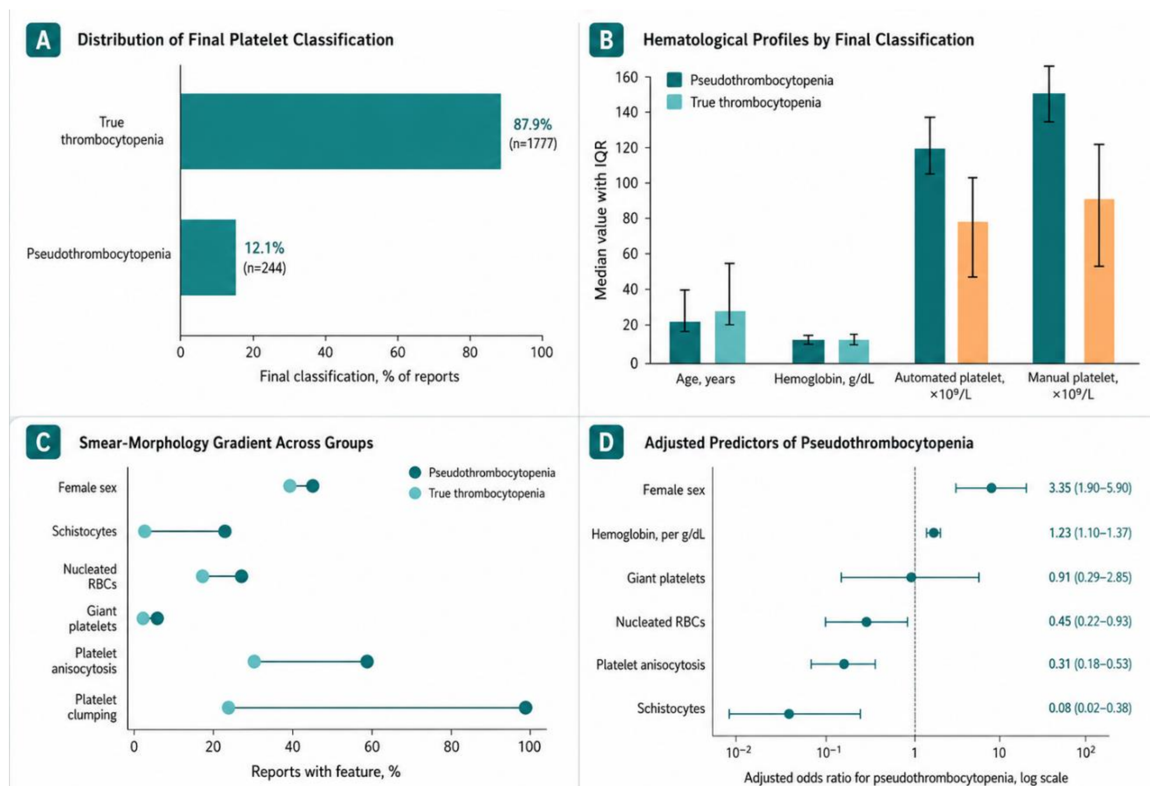

Figure 1 Platelet classification, hematological profile, peripheral-smear morphology, and adjusted predictors among analyzer-flagged thrombocytopenic reports. Among 2,021 reports, 244 (12.1%) were classified as pseudothrombocytopenia and 1,777 (87.9%) as true thrombocytopenia. Pseudothrombocytopenia showed higher median automated platelet count than true thrombocytopenia (119 vs 77×10^9/L) and higher hemoglobin (12.0 vs 10.8 g/dL), while platelet clumping was present in 99.6% versus 25.1% of reports, respectively. True thrombocytopenia showed greater frequencies of platelet anisocytosis (49.5% vs 30.3%) and schistocytes (16.3% vs 2.0%). In adjusted analysis, higher hemoglobin and female sex were associated with

pseudothrombocytopenia, whereas platelet anisocytosis, nucleated red blood cells, and schistocytes showed lower adjusted odds of pseudothrombocytopenia. Platelet clumping was presented descriptively and excluded from regression because near-complete separation prevented stable adjusted estimation.

DISCUSSION

This retrospective laboratory-based study evaluated analyzer-flagged thrombocytopenic complete blood count reports and compared cases reclassified as pseudothrombocytopenia with those confirmed as true thrombocytopenia after documented manual platelet-count verification and peripheral blood smear review. Among 2,021 eligible reports, 244 (12.1%) were classified as pseudothrombocytopenia and 1,777 (87.9%) as true thrombocytopenia, showing that a meaningful proportion of low automated platelet counts represented spurious thrombocytopenia rather than persistent platelet reduction. This finding reinforces the diagnostic importance of confirmatory platelet assessment in routine hematology practice, particularly when an automated low platelet count may trigger unnecessary clinical investigation or intervention.

The strongest morphological distinction between the two groups was platelet clumping, which was present in 99.6% of reports classified as pseudothrombocytopenia compared with 25.1% of reports classified as true thrombocytopenia. This pattern supports the role of platelet aggregation as a major smear-based indicator of pseudothrombocytopenia, but it also demonstrates that clumping alone should not be used as a standalone diagnostic label. Because one-quarter of true thrombocytopenia reports also showed platelet clumping, the finding must be interpreted together with the corrected manual platelet count. This distinction is clinically important because platelet aggregation may coexist with genuinely reduced platelet counts and may further underestimate the automated platelet count. Therefore, peripheral-smear clumping should prompt careful verification rather than automatic reclassification as pseudothrombocytopenia.

The platelet-count profile further supported this distinction. Reports classified as pseudothrombocytopenia had a higher median automated platelet count than reports with true thrombocytopenia, while documented manual platelet-count verification corrected the pseudothrombocytopenia group to the classification threshold. In contrast, the true thrombocytopenia group remained below the diagnostic threshold after manual verification. These findings are consistent with the principle that analyzer-derived platelet counts may be falsely reduced in the presence of platelet aggregates and that smear review with manual confirmation remains necessary when automated results are inconsistent with expected morphology or clinical context (11,12). However, the manual platelet-count distribution in the pseudothrombocytopenia group requires transparent reporting if values above 150×10^9/L were capped or recorded only as corrected-to-normal, because such coding would limit interpretation of the manual count as a continuous variable.

The morphology findings also showed that platelet anisocytosis, schistocytes, and nucleated red blood cells were more frequent or more strongly associated with true thrombocytopenia than pseudothrombocytopenia. Platelet anisocytosis was present in 49.5% of true thrombocytopenia reports compared with 30.3% of pseudothrombocytopenia reports, while schistocytes were recorded in 16.3% versus 2.0%, respectively. In adjusted analysis, platelet anisocytosis, nucleated red blood cells, and schistocytes showed lower odds of pseudothrombocytopenia, suggesting that these features are more consistent with genuine hematological pathology than isolated analyzer artifact. This aligns with the diagnostic role of smear morphology in identifying platelet size variation, red-cell fragmentation, and marrow-response features that may accompany true thrombocytopenia or systemic disease processes (13,14).

Higher hemoglobin was independently associated with pseudothrombocytopenia, whereas the true thrombocytopenia group had a lower median hemoglobin concentration. This pattern may reflect the greater burden of underlying marrow, hemolytic, inflammatory, infectious, or systemic illness among reports with confirmed thrombocytopenia, although the retrospective laboratory-record design does not

allow causal attribution. Female sex was also independently associated with pseudothrombocytopenia after adjustment despite a non-significant unadjusted group comparison. This finding should be interpreted cautiously because it may reflect confounding, case-mix differences, or model behavior rather than a biologically stable association. Future studies should validate this association in larger patient-level datasets with clinical diagnoses, anticoagulant details, and repeat confirmatory samples.

The present findings are consistent with previous literature emphasizing that pseudothrombocytopenia can be identified only through integration of automated analyzer data, platelet-count verification, and peripheral-smear morphology. Prior work has shown that automated platelet counts may be affected by platelet aggregation and that smear review is essential when analyzer flags or unexpected thrombocytopenia are encountered (15,16). Studies of pseudothrombocytopenia and platelet-count verification have similarly highlighted the importance of confronting analyzer results with smear findings and manual or confirmatory platelet assessment before making clinical decisions based on an isolated low automated platelet count (17,18). In the present dataset, the very high frequency of clumping in pseudothrombocytopenia supports this approach, while the concurrent presence of clumping in some true thrombocytopenia reports emphasizes that final interpretation must rely on whether platelet counts correct after verification.

This study has several strengths, including a large sample of consecutive analyzer-flagged thrombocytopenic reports, paired automated and documented manual platelet-count data, and systematic extraction of peripheral-smear morphological features. The study also reflects routine diagnostic laboratory practice in a tertiary-care setting, making the findings clinically relevant for laboratories that must rapidly distinguish spurious thrombocytopenia from true platelet reduction. However, several limitations must be acknowledged. The retrospective single-center design limits generalizability, and the unit of analysis was the laboratory report/accession rather than a prospectively characterized patient. Clinical diagnoses, bleeding status, medication exposure, transfusion history, and repeat anticoagulant-based confirmation were not reported. The manuscript also does not describe interobserver reliability for smear morphology, and routine smear documentation may be subject to observer and reporting variability. Because platelet clumping was present in nearly all pseudothrombocytopenia reports, it produced quasi-complete separation and was appropriately excluded from the multivariable model; therefore, its diagnostic value should be interpreted descriptively rather than as an adjusted independent predictor. Finally, if manual platelet counts in pseudothrombocytopenia were recorded only at the correction threshold, this should be clearly stated because it affects interpretation of platelet-count distribution and between-group comparison.

Overall, the study supports a practical diagnostic approach in which analyzer-flagged thrombocytopenia should be verified by peripheral blood smear review and documented manual platelet assessment before clinical classification. Platelet clumping is a highly important warning feature for pseudothrombocytopenia, but it should be interpreted alongside whether the platelet count corrects on manual verification. Morphological abnormalities such as anisocytosis, schistocytes, and nucleated red blood cells may support true thrombocytopenia and should prompt closer hematological interpretation. These findings can help reduce misdiagnosis, avoid unnecessary platelet transfusion or invasive workup, and improve laboratory-clinical communication in thrombocytopenia reporting (19).

CONCLUSION

Peripheral blood smear examination and documented manual platelet-count verification are essential for distinguishing pseudothrombocytopenia from true thrombocytopenia among analyzer-flagged low platelet counts. In this study, pseudothrombocytopenia accounted for 12.1% of thrombocytopenic reports, and platelet clumping was the most prominent smear feature associated with this classification. However, because clumping was also present in a subset of true thrombocytopenia reports, it should prompt confirmatory interpretation rather than serve as an isolated diagnostic label. Higher hemoglobin

and female sex were independently associated with pseudothrombocytopenia, while platelet anisocytosis, nucleated red blood cells, and schistocytes were more consistent with true thrombocytopenia. A combined approach using automated results, smear morphology, and manual platelet verification can improve diagnostic accuracy and reduce unnecessary clinical intervention in routine hematology practice.

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