

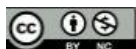
A Novel Parameter Derived from Platelet Histograms: Pre-Peak to Post-Peak Ratio and Its Correlation with Platelet Recovery in Thrombocytopenic Patients

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Abstract

Background and Aim: Automated hematology analyzers generate platelet indices and histograms that can provide insight into platelet dynamics. This study evaluates a novel parameter—the pre-peak to post-peak ratio from platelet histograms—and its associations with the percentage of giant platelet, standard platelet indices, and clinical recovery in patients with thrombocytopenia.

Methods: In an observational cohort study, 250 thrombocytopenic patients (platelet count $<150,000/\mu\text{L}$) were monitored over six days at a tertiary care center. Platelet histograms were analyzed using a 5-part automated hematology analyzer. The pre-peak/post-peak ratio was manually calculated, and its correlations with mean platelet volume (MPV), platelet distribution width (PDW), giant platelet percentage (from peripheral smears), and platelet recovery were assessed.

Results: Hyper-destructive thrombocytopenia predominated (82.4%, $n=206$), with dengue identified as the leading cause (53.9%). In these cases, giant platelet percentages surged by day 3 ($p<0.001$), positively correlating with post-peak length and inversely with the ratio. The ratio declined on day 3 and rose on day 6 in 82.4% of recovering patients. In contrast, hypo-productive cases (17.6%, $n=44$) showed no significant changes or correlations.

Conclusion: The pre-peak to post-peak ratio appears to be a simple and economical prognostic indicator for recovery in hyper-destructive thrombocytopenia, potentially guiding transfusion decisions and monitoring.

Keywords: Thrombocytopenia; Platelet histogram; Giant platelets; Mean platelet volume; Platelet distribution width; Platelet recovery; Dengue; Immune thrombocytopenic purpura

1. Introduction

Thrombocytopenia, defined as a platelet count below $150 \times 10^3/\mu\text{L}$, is one of the most common hematological abnormalities encountered in clinical practice (1). Its prevalence is approximately 1% in acute care centers but may often exceed 50% in intensive care units (2,3). Although it is broadly classified into hyper-destructive and hypo-productive forms (4), distinguishing between these mechanisms remains challenging in routine practice, especially in resource-limited settings where advanced diagnostic

modalities are unavailable. Modern automated hematology analyzers provide multiple platelet-related parameters—including mean platelet volume (MPV), platelet distribution width (PDW), platelet-large cell ratio (P-LCR), and immature platelet fraction (IPF). These parameters have been investigated across various etiologies such as immune thrombocytopenia, dengue fever, sepsis, chemotherapy-induced marrow suppression, and nutritional deficiencies. Among them, the IPF stands out as a particularly sensitive marker of marrow

thrombopoietic activity, yet its reliance on fluorescence-based technology restricts its availability in many low- and middle-income laboratories (5,6). In contrast, platelet histograms are generated universally by both three-part and five-part analyzers, making them accessible across virtually all hematology platforms (4). Previous studies have described characteristic qualitative changes in hyper-destructive thrombocytopenia, such as rightward extension of the histogram curve and increased platelet size heterogeneity due to the presence of larger immature platelets, reflecting accelerated megakaryopoiesis in response to peripheral destruction (7–10). Despite their biological plausibility [5,7,8], these histogram features have rarely been translated into standardized, quantitative metrics suitable for routine clinical use. This gap that is clinically relevant because MPV is prone to technical variability, (11) and IPF is frequently unavailable to clinicians in resource-restricted settings. Therefore, a simple, reproducible histogram-derived parameter could substantially improve diagnostic discrimination between destructive and hypo-productive thrombocytopenia; accordingly, we propose the pre-peak to post-peak (PP/PoP) ratio, a quantitative measure derived manually from routine platelet histograms. The rationale is that hyper-destructive thrombocytopenia leads to an increased population of large immature platelets, preferentially extending the post-peak region of the histogram and thereby reducing the PP/PoP ratio. In contrast, hypo-productive thrombocytopenia is not expected to produce such morphological alterations, as marrow output is diminished rather than compensatory (7,8,11,12). This study was undertaken to evaluate the diagnostic and prognostic utility of the PP/PoP ratio and to address the absence of validated histogram-based quantitative metrics in the existing literature.

2. Methods

This prospective observational cohort study was designed to evaluate the diagnostic and prognostic performance of the pre-peak to post-peak (PP/PoP) ratio derived from platelet histograms in patients with thrombocytopenia. The study was conducted at a tertiary care teaching hospital in Pune, India, where participant recruitment, clinical assessments, and laboratory investigations were performed within the Department of Pathology and associated clinical services over a six-month period from 1 January 2025 to 30 June 2025, coinciding with the region's seasonal peak of infection-related thrombocytopenia. Ethical approval was obtained from the Institutional Ethics Committee [JCDC_24_11/03], and the study complied with the principles of the Declaration of Helsinki. Participants were eligible if they were aged 15 years

or older, had a platelet count below $150 \times 10^3/\mu\text{L}$ confirmed on an EDTA sample at presentation, and were clinically stable enough to undergo serial blood sampling on days 1, 3, and 6. Exclusion criteria included EDTA-dependent pseudothrombocytopenia verified on a citrate sample, recent chemotherapy or radiotherapy within the preceding three months, ongoing treatment for established hematological malignancy, pregnancy, significant acute bleeding requiring immediate transfusion, and inability to complete follow-up sampling. Additionally, a secondary control group of 100 healthy age- and sex-matched adults with normal complete blood counts and no evidence of acute illness was included to establish reference platelet indices and histogram characteristics. All participants who completed baseline sampling on day 1 were actively followed at predefined time points (days 1 [baseline], 3, and 6). Missed evaluations were documented, and no replacements were made; all available data were included in the final analysis. The study involved no therapeutic interventions, and clinical management remained entirely at the discretion of treating physicians. The primary variable was the PP/PoP ratio, a manually derived quantitative metric of platelet histogram morphology, with the main clinical outcome defined as platelet recovery by day 6—spontaneous increase of at least $50 \times 10^3/\mu\text{L}$ from baseline without transfusion in hyper-destructive thrombocytopenia, or improvement following disease-specific therapy in hypo-productive cases—while secondary variables included mean platelet volume (MPV), platelet distribution width (PDW), proportion of giant platelets, etiological classification of thrombocytopenia, and demographic/clinical data such as age, sex, presenting symptoms, comorbidities, and bleeding manifestations. All hematological analyses were performed on a Beckman Coulter LH-750 analyzer with samples processed within two hours of collection to minimize pre-analytical variability; complete blood counts, platelet indices, and platelet histograms (2–20 fL range) were recorded at each time point, and printed histograms were manually measured using a transparent graded ruler aligned along the x-axis, with pre-peak length defined as the distance from origin to the main peak apex, post-peak length as the distance from peak to baseline return, and the PP/PoP ratio calculated by dividing pre-peak by post-peak length; measurements were performed independently by two laboratory scientists with ≥ 5 years of hematology experience, and if readings differed by $>10\%$, a third measurement was obtained, and the average of the two concordant readings was used for analysis. Each histogram was printed and measured three times to reduce variability from printing or rendering. Peripheral blood smears were prepared with

Leishman stain, examined under 100× oil immersion, and the proportion of giant platelets quantified per 100 platelets by two blinded senior hematopathologists whose interobserver agreement was assessed using Cohen's kappa, while etiological classification relied on clinical assessment, dengue NS1/IgM serology when indicated, nutritional evaluations, bone marrow examination in selected cases, and radiological investigations as required. To minimize bias, laboratory measurements followed standardized timing and analyzer protocols, histogram assessments used independent dual observers with reconciliation for discrepancies >5%, peripheral smear reviews were blinded, pre-analytical variation was controlled by fixed processing windows, and study procedures did not influence clinical decision-making. Sample size was calculated using GPower software to detect a moderate correlation coefficient of 0.30 between the PP/PoP ratio and platelet recovery, requiring a minimum of 227 participants (rounded to 250) at

$\alpha=0.05$ and 80% power.⁽¹³⁾ Quantitative variables including the PP/PoP ratio, MPV, PDW, and platelet counts were treated as continuous, with repeated measures across days 1, 3, and 6 analyzed using appropriate repeated-measures statistical tests and the PP/PoP ratio calculated consistently per standardized procedures. All statistical analyses were conducted in R software version 4.3.3 (R Foundation for Statistical Computing, Vienna, Austria), with continuous variables summarized as means \pm standard deviations and categorical variables as frequencies and percentages. Pearson's correlation coefficient was used to assess associations between continuous variables.

3. Results

The baseline characteristics and etiological pattern of the thrombocytopenia group are shown in Table 1.

Table 1. Baseline characteristics and etiological distribution of thrombocytopenia [n = 250]

| Characteristic | n [%] |
|--|------------|
| Age distribution [years] | |
| a. 15–25 | 48 [19.2] |
| b. 26–35 | 56 [22.4] |
| c. 36–45 | 42 [16.8] |
| d. 46–55 | 36 [14.4] |
| e. 56–65 | 30 [12.0] |
| f. >65 | 38 [15.2] |
| Gender | |
| a. Male | 165 [66.0] |
| b. Female | 85 [34.0] |
| Mechanism of thrombocytopenia | |
| a. Hyper-destructive | 206 [82.4] |
| b. Hypo-productive | 44 [17.6] |
| c. Hyper-destructive causes | |
| d. Dengue fever | 111 [53.9] |
| e. Other infections | 32 [15.5] |
| f. Hypersplenism [CLD with splenomegaly] | 23 [11.2] |
| g. Others | 40 [19.4] |
| Hypo-productive causes | |

| | |
|-------------------------------|-----------|
| a. Hematological malignancies | 16 [36.4] |
| b. Vitamin B12 deficiency | 14 [31.8] |
| c. Aplastic anemia | 6 [13.6] |
| d. Others | 8 [18.2] |

Most patients had hyper-destructive thrombocytopenia, and dengue fever was the most common cause, while hypo-productive thrombocytopenia was mainly linked to hematological malignancies and vitamin B12 deficiency. Peripheral smear findings supported these diagnostic groups and showed expected features such as giant platelets in

hyper-destructive conditions and typical cellular changes in megaloblastic anemia, malaria, sepsis, and leukemia. Serial measurements of platelet count, platelet indices, and histogram-derived parameters showed clear differences between hyper-destructive and hypo-productive thrombocytopenia, and these trends are presented in Table 2.

Table 2. Platelet counts and indices at baseline, day 3, and day 6

| Group | Platelet Count [$\times 10^3/\mu\text{L}$] | MPV [fL] | PDW | Giant Platelets [%] | PP/PoP Ratio |
|------------------------------------|--|-----------------|------------------|---------------------|-----------------|
| Controls [n = 100] | 212.1 \pm 59.3 | 8.18 \pm 1.12 | 15.75 \pm 0.79 | – | 0.36 \pm 0.03 |
| Hyper-destructive [n = 206] | | | | | |
| Day 1 | 42.6 \pm 18.4 | 10.5 \pm 1.4 | 16.2 \pm 0.8 | 14.2 \pm 4.3 | 0.34 \pm 0.05 |
| Day 3 | 39.2 \pm 20.7 | 11.1 \pm 1.5 | 16.8 \pm 0.7 | 18.9 \pm 5.0 | 0.30 \pm 0.06 |
| Day 6 | 94.5 \pm 36.2 | 10.9 \pm 1.3 | 17.1 \pm 0.8 | 12.7 \pm 4.6 | 0.38 \pm 0.04 |
| Hypo-productive [n = 44] | | | | | |
| Day 1 | 40.2 \pm 17.9 | 8.4 \pm 1.0 | 15.8 \pm 0.6 | 6.1 \pm 2.7 | 0.33 \pm 0.05 |
| Day 3 | 41.0 \pm 18.5 | 8.6 \pm 0.9 | 16.0 \pm 0.7 | 7.5 \pm 3.1 | 0.32 \pm 0.04 |
| Day 6 | 43.7 \pm 19.2 | 8.5 \pm 1.1 | 16.1 \pm 0.8 | 6.9 \pm 2.9 | 0.32 \pm 0.05 |

The pre-peak to post-peak ratio showed an early fall with a later rise in patients who went on to recover, while this pattern was not seen in patients with hypo-productive thrombocytopenia. Comparison of

recovered and non-recovered patients showed significant differences in several platelet parameters at key time points (Table 3).

Table 3. Comparison of recovered vs non-recovered patients with correlation coefficients

| Parameter | Recovered [n = 206] | Non-recovered [n = 44] | p-value | Correlation Coefficient [r] |
|--|---------------------|------------------------|---------|-----------------------------|
| Platelet Count [$\times 10^3/\mu\text{L}$] | | | | |
| Day 1 | 43.5 \pm 18.2 | 39.8 \pm 17.5 | 0.21 | 0.12 |
| Day 3 | 41.2 \pm 19.7 | 40.6 \pm 18.6 | 0.78 | 0.03 |
| Day 6 | 101.6 \pm 35.8 | 44.1 \pm 19.4 | <0.001 | 0.68 |
| MPV [fL] | | | | |
| Day 1 | 10.6 \pm 1.3 | 8.5 \pm 1.0 | <0.001 | 0.72 |

| | | | | |
|---------------------------------|-------------|-------------|--------|-------|
| Day 3 | 11.2 ± 1.4 | 8.6 ± 0.9 | <0.001 | 0.70 |
| Day 6 | 10.9 ± 1.2 | 8.5 ± 1.1 | <0.001 | 0.69 |
| PDW | | | | |
| Day 1 | 16.2 ± 0.7 | 15.8 ± 0.6 | 0.04 | 0.26 |
| Day 3 | 16.9 ± 0.8 | 16.0 ± 0.7 | 0.01 | 0.33 |
| Day 6 | 17.1 ± 0.8 | 16.1 ± 0.8 | 0.003 | 0.40 |
| Giant Platelets [%] | | | | |
| Day 1 | 14.6 ± 4.0 | 6.0 ± 2.5 | <0.001 | 0.71 |
| Day 3 | 19.2 ± 4.8 | 7.2 ± 3.1 | <0.001 | 0.75 |
| Day 6 | 12.5 ± 4.4 | 6.7 ± 2.7 | <0.001 | 0.63 |
| Pre-peak/Post-peak Ratio | | | | |
| Day 1 | 0.35 ± 0.04 | 0.32 ± 0.05 | <0.001 | 0.41 |
| Day 3 | 0.30 ± 0.05 | 0.32 ± 0.04 | 0.07 | -0.18 |
| Day 6 | 0.39 ± 0.04 | 0.32 ± 0.05 | <0.001 | 0.52 |

The PP/PoP ratio showed an area under the curve of 0.84, and MPV showed an area under the curve of 0.79. The combined model, which included age, sex, baseline platelet count, MPV, PDW, giant platelets, and the PP/PoP ratio, showed an area under the curve of 0.89. These values indicate moderate to good discriminative performance for identifying early recovery in hyper-destructive thrombocytopenia.

4. Discussion

This study establishes the pre-peak to post-peak (PP/PoP) ratio as a quantitative, histogram-derived marker capable of discriminating between hyper-destructive and hypo-productive thrombocytopenia while simultaneously predicting early platelet recovery in hyper-destructive states, as evidenced by the characteristic early decline in the ratio by day 3 followed by a rise by day 6 in recovering patients. This reflects accelerated megakaryopoietic activity in response to peripheral platelet destruction—a key pathophysiological feature in dengue infection and immune thrombocytopenia (9,14,15). This process is accompanied by a surge of immature, larger platelets that alter volume distribution and appear as rightward shifts in the platelet histogram, increased dispersion, and post-peak tail elongation due to young and giant platelets (4,16–18). These findings are consistent with prior observations of qualitative histogram changes in destructive thrombocytopenia where larger immature platelets cause curve broadening and right-skewing

(7–10,30). This is unlike fluorescence-based immature platelet fraction (IPF) measurements that directly quantify reticulated platelets but require advanced analyzers often unavailable in resource-limited settings (6,12,19,20). The PP/PoP ratio relies on routinely generated impedance-derived histograms accessible on standard platforms, and accumulating evidence supports IPF's utility with elevations exceeding 10% in hyper-destructive thrombocytopenia and thresholds of 8–10% reliably predicting recovery within 24–72 hours in dengue (19,21–27). Its limitations in availability underscore the value of simple surrogates like the PP/PoP ratio that quantify analogous post-peak prolongation from immature/giant platelets. It showing strong concordance with peripheral smear giant platelet enumeration and superior discriminatory performance over mean platelet volume, (4,20,28–30) It also aligns with studies demonstrating elevated MPV, PDW, and related indices in hyper-destructive vs. hypo-productive states though with variable technical reliability (1,2,30–32).

Clinically, an early PP/PoP decline signals effective marrow compensation, enabling avoidance of unnecessary platelet transfusions in hyper-destructive thrombocytopenia—particularly critical in dengue-endemic areas to mitigate risks of alloimmunization and reactions (9,14,15,31,32). Its prognostic strength increases when combined with MPV and PDW highlighting additive value among routine indices

(16–19). In hyper-destructive thrombocytopenia, such as in dengue or immune thrombocytopenia, the primary cause—peripheral platelet destruction by antibodies, immune complexes, or infection-mediated mechanisms—persists during the acute phase. However, the bone marrow responds with accelerated megakaryopoiesis and increased release of immature, larger platelets to compensate for the ongoing destruction. By day 6, in recovering patients, this robust thrombopoietic response often outpaces the rate of destruction, resulting in a net increase in circulating platelet count (typically a spontaneous rise of $\geq 50 \times 10^3/\mu\text{L}$ from baseline) even though the underlying destructive process has not yet fully resolved. This early recovery phase reflects effective marrow compensation, which is precisely what the declining-then-rising PP/PoP ratio captures through the transient predominance and subsequent normalization of large immature platelets in the histogram. Earlier histogram descriptions in thrombocytopenia were largely qualitative (e.g., base broadening or right-skew in destructive cases), limiting reproducibility (4,28). The PP/PoP ratio offers a standardized, numeric metric translatable across analyzer platforms, supported by platelet kinetic data showing young, larger platelets with elevated PDW and inverse count-volume relationships in hyper-destructive states versus minimal variation in hypo-productive ones (4,17,19). In dengue's biphasic course with nadir around days 6–7 and recovery by day 9, the ratio's temporal pattern parallels recovery dynamics, enhancing disease-specific prognostication. (23,25). The limitations are manual peak determination variability, single-center dengue-predominant cohort restricting generalizability, short follow-up unsuitable for chronic hypo-productive disorders, and absent direct IPF comparison (12,18,19). Overall, the PP/PoP ratio emerges as a cost-effective, simple, feasible tool for etiological stratification and early recovery prediction in hyper-destructive thrombocytopenia, with broader multicenter validation, extended longitudinal evaluation, and IPF benchmarking needed to solidify its routine hematological role.

5. Conclusion

The pre-peak to post-peak (PP/PoP) ratio is a simple and accessible metric that reflects platelet dynamics and identifies patients with hyper-destructive thrombocytopenia likely to recover early. It complements conventional indices like mean platelet volume and platelet distribution width, offering superior discriminatory performance in resource-limited settings. This may help guide management decisions, such as avoiding unnecessary transfusions in hyper-destructive cases. Larger multicenter studies

with longer follow-up and direct comparison with fluorescence-based indices are needed to confirm its clinical value.

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Ethical Considerations and Compliance with Ethical Guidelines

All ethical principles are considered in this article. The participants were informed of the purpose of the research and its implementation stages. They were also assured about the confidentiality of their information and were free to leave the study whenever they wished, and if desired, the research results would be available to them. A written consent has been obtained from the subjects. principles of the Helsinki Convention was also observed. This study was approved by the Ethics Committee of the Hospital, Pune [JCDC_24_11/03].

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Conflict of interest

The Authors declare that there is no conflict of interest.

AI Using Declaration

Chat GPT was used for language and grammar corrections. The final output was read and modified by all authors.

Author's contributions

All authors equally contributed to preparing this article.

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