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The diagnostic advantage of immature platelet fraction and platelet indices in thrombocytopenia cases: Tertiary care center study

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ABSTRACT

Background: Thrombocytopenia, characterized by low platelet counts, is a common hematological disorder with diverse causes, complicating diagnosis. Distinguishing between central and peripheral causes is crucial for appropriate management. Automated hematology analyzers measuring platelet indices, especially immature platelet fraction (IPF), provide valuable insights, aiding early diagnosis, preventing unnecessary transfusions, and improving patient care. **Aims and Objectives:** To evaluate the role of platelet indices, particularly the IPF, in differentiating hyper-destructive from hypo-productive thrombocytopenia and its clinical relevance in patient management. **Materials and Methods:** This hospital-based case-control study was conducted in the Department of Pathology, SNMC, Bagalkote, over 6 months (April–September 2024), with 100 participants (50 cases and 50 controls). Blood samples were analyzed using the Mindray BC-6800 to assess platelet indices, including IPF. Data were analyzed using IBM SPSS 24. **Results:** The IPF reference range in healthy controls was 0.5–5.1%. Patients with peripheral platelet destruction had a significantly higher mean IPF of 16.24%, while those with decreased production had a mean IPF of 5.95%. Statistically significant differences ($P < 0.05$) were observed between hyper-destructive, hypo-productive groups, and controls. **Conclusion:** IPF is a valuable, non-invasive, and cost-effective tool for assessing thrombocytopenia, offering a reliable alternative to bone marrow aspiration in distinguishing between central and peripheral causes.

Key words: Thrombocytopenia; Immature platelet fraction; Mean platelet volume; Reticulated platelet

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INTRODUCTION

Thrombocytopenia is the reduction of platelet count in peripheral blood and it's a most prevalent hematological anomaly often associated with severe bleeding complications.[1] Its etiology is multifactorial and poses immense challenges in diagnosing the root cause. It is crucial to determine whether thrombocytopenia is caused by increased peripheral platelet destruction or decreased platelet production for effective management.[1,2]

Automated hematology analyzers that measure reticulated platelets (RPs) as the immature platelet fraction (IPF), along with other platelet indices, are highlighted for their reliability and routine clinical accessibility. The IPF is valuable not only for differentiating between platelet consumption due to bone marrow failure or suppression and peripheral destruction but also as an early marker of platelet recovery, offering a non-invasive alternative to bone marrow examination, which can be painful for patients.[1,3,4] IPF represents the percentage of RNA-rich immature platelets in circulation and it's measured by using the reticulocyte/platelet channel of the Mindray BC-6800

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hematology analyzer through flow cytometry, where dye penetrates the platelet membrane to stain cytoplasmic RNA. IPF and other platelet indices such as mean platelet volume (MPV), platelet distribution width (PDW), plateletcrit (PCT) and platelet large cell ratio (P-LCR) help to assess the prognosis and also provide early insights. Hence, we can prevent unnecessary platelet transfusion and associated risks.[5]

Platelet count, MPV, PDW, and PCT are simple tests, that can be performed by any 3-part cell counter but IPF requires advanced equipment and which is available only in a few specialized centers. These indices are calculated by cell counter but often go unreported because of a lack of awareness about the usefulness of these parameters among pathologists and physicians as well as variability and lack of standardization in testing and reporting.[5] Platelet indices along with complete blood count may provide more valuable information regarding the underlying cause and pathogenesis of thrombocytopenia.[6]

Aims

To evaluate the clinical relevance of platelet indices, with a particular focus on the IPF, in diagnosing and monitoring thrombocytopenic patients.

Objective

To analyze their role in distinguishing between hyper-destructive and hypo-productive causes of thrombocytopenia, providing valuable insights into their diagnostic utility. By assessing these parameters, the study seeks to enhance clinical decision-making, improve patient management, and contribute to more targeted therapeutic approaches.

MATERIALS AND METHODS

The study was carried out in the Department of Pathology at S. Nijalingappa Medical College and HSK Hospital and Research Centre, Bagalkot, with institutional ethical clearance obtained before the commencement of the study (SNMC/IECHSR/2024/A-82). This was a hospital-based case-control study for a period of 6 months and a total of 100 participants were included, comprising 50 cases and 50 controls.

After taking a complete clinical history, under strict aseptic precautions, venous blood was collected in EDTA vials for hematological analysis in the Mindray BC-6800 analyzer from both controls and cases. The analysis included parameters such as IPF, platelet indices, and other routine blood cell parameters, including platelet count, which were assessed using flow cytometry. In which immature platelets were stained using RNA-specific dyes to highlight cytoplasmic RNA. IPF was identified based on fluorescence intensity, cell size, and granularity and then IPF (%) was calculated using the formula:

IPF (%) = (Immature Platelets/Total Platelet Count) × 100 by the analyzer. These values were reviewed by the pathologists.

Inclusion criteria

Cases

Patients with a platelet count below 100×10^9/L.

Controls

Healthy individuals with normal blood cell parameters, including a platelet count ranging from 150×10^9/L to 450×10^9/L.

The range gap was carefully chosen to ensure clear differentiation between thrombocytopenic patients and healthy individuals. A wider gap improves statistical reliability by eliminating gray zones where mild thrombocytopenia may occur in conditions unrelated to the study focus.

Exclusion criteria

Cases

1. Patients with pseudo-thrombocytopenia are characterized by a low platelet count on the analyzer despite the presence of platelets on the peripheral smear.

Controls

1. Individuals with ongoing fever or viral infections.
2. Individuals suffering from chronic conditions that could influence blood cell parameters.

Statistical analysis

Statistical analysis was performed using IBM SPSS 24 software. The control group was organized by age and gender, and descriptive statistics such as mean, median, and standard deviation were calculated for platelet count, IPF, MPV, PDW, PCT, and P-LCR. A reference range for normal IPF and platelet indices was established, and variations in thrombocytopenic cases were analyzed.

The cases were grouped according to age, gender, diagnosis, and the underlying cause of thrombocytopenia, with two categories: Group A1 (increased platelet destruction/consumption) and Group A2 (decreased platelet production due to bone marrow failure or suppression). Differences in IPF, MPV, PDW, PCT, and P-LCR between the two groups were assessed using the Mann-Whitney U test, considering a $P \leq 0.05$ as statistically significant. All data were kept confidential throughout the study.

RESULTS

This study included a total of 50 patients with thrombocytopenia. The age range of the case group varied from 8 to 78 years, with a mean age of 33 years, while the control group had an age range of 21–40 years, with a mean age of 26 years (Table 1). The gender distribution for both the case and control groups is shown in Figure 1.

The mean platelet count for the cases (A) was 39.8 ± 24.1 × 10^3/μL, whereas for the controls (B) it was 331.4 ± 70.2 × 10^3/μL. The mean IPF for the cases (A) was 12.53 ± 7.8%, while for the controls (B) it was 3.4 ± 1.7% (Table 2).

Statistically significant differences in platelet count and IPF were observed between the case (A) and control (B) groups, with a P<0.001. The normal reference range for IPF, based on data from 54 controls, was found to be between 0.5% and 5.1%. IPF values exceeding 5.1% were considered abnormally elevated and were significantly higher in thrombocytopenic cases compared to healthy controls.

Based on the final diagnosis and underlying pathogenesis, the 50 cases were divided into two groups:

- Group A1 (31 cases) – Increased peripheral destruction or consumption of platelets (including dengue, malaria, viral fever, ITP, and CLL cases)
- Group A2 (19 cases) – Decreased platelet production due to bone marrow failure or suppression (including megaloblastic anemia, hypoproliferative marrow, aplastic anemia, and acute leukemia cases) (Figure 2).

The mean platelet count for Group A1 was 35 ± 25.7 × 10^3/μL, while for Group A2; it was 52.3 ± 23.3 × 10^3/μL. The mean IPF for Group A1 was 16.24 ± 7.5%, compared to 5.9 ± 4.6% for Group A2 (Table 3).

A statistically significant difference in IPF values was observed between Groups A1 and A2, with a P=0.022. The IPF was notably higher in cases with increased platelet destruction (Group A1) compared to those with decreased platelet production (Group A2).

Follow-up data were available for 10 out of 50 cases over 2–3 days, revealing a decline in IPF values as platelet counts recovered. However, statistical analysis could not be performed due to the unavailability of follow-up data for the remaining cases.

Among the three groups analyzed, MPV, PDW, and P-LCR values were highest in Group A1 (hyperdestructive thrombocytopenia) compared to Group A2 (hypoproductive thrombocytopenia) and Group B (controls). These differences were statistically significant. In contrast, PCT did not show any significant variation between the groups (Table 4).

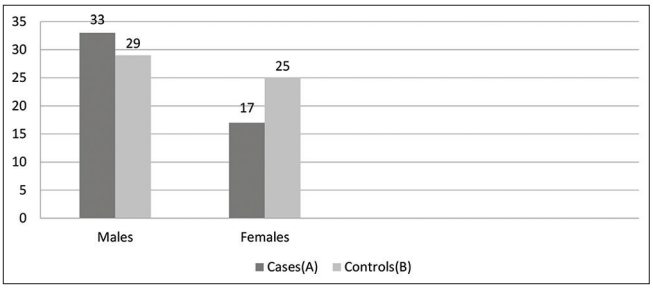

Figure 1: Gender distribution of cases and controls

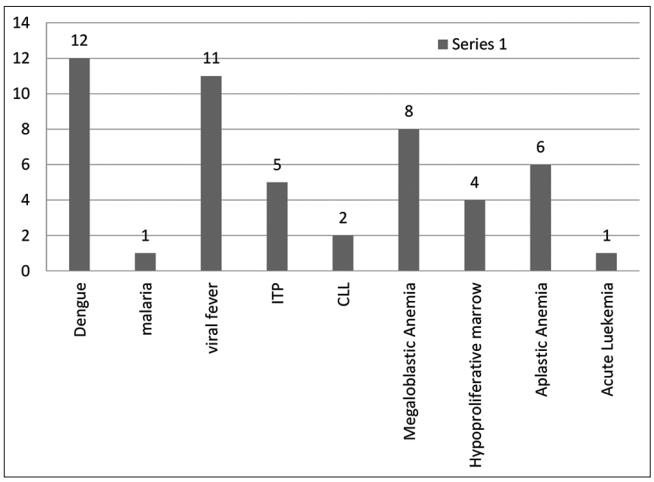

Figure 2: Cases with clinical diagnosis. ITP: Immune thrombocytopenic purpura, CLL: Chronic lymphocytic leukemia

Table 1: Age distribution of cases and control group

Group	Age range (years)	Median age (years)
Cases	8–78	33
Controls	21–40	26

DISCUSSION

The IPF serves as an indicator of thrombopoietic activity in patients with thrombocytopenia and is a valuable tool for assessing platelet dynamics in such cases. A high IPF indicates increased platelet production, whereas low IPF values reflect suppressed thrombopoiesis. In healthy individuals, the reference range for IPF is 0.5–5.1%. The findings of the present study align with those reported in previous studies[1,7,8] (Table 5).

In thrombocytopenia patients, the IPF was significantly higher (mean 12.53%) compared to healthy control (mean 3.4%), consistent with findings of Goel et al., Briggs et al., and Jung et al.[1,8,9]

Table 2: Platelet count and IPF in cases and controls

Groups	Platelet count (×10³/µL)		IPF (%)	
	Mean±Standard deviation	Range	Mean±Standard deviation	Range
Cases (A)	39.8±24.1	1–99	12.53±7.8	3.6–30.5
Controls (B)	331.4±70.2	190–500	3.4±1.7	1.1–5.1
P-value	<0.001		<0.001	

IPF: Immature platelet fraction

Table 3: Platelet count and IPF in Group A1 (increased peripheral destruction of platelets) and Group A2 (decreased platelet production)

Groups	Platelet count (×10³/µL)	IPF (%)
	Mean±Standard deviation	Mean±Standard deviation
Group A1	35.0±25.7	16.24±7.5
Group A2	52.3±23.3	5.9±4.6
P-value	<0.022	<0.022

IPF: Immature platelet fraction

Patients with thrombocytopenia from peripheral destruction exhibited a marked increase in IPF (mean 16.24%) values than patients with bone marrow suppression/failure (mean 5.9%). These findings were comparable with Naz et al., Goel et al., and Meskini et al., studies.[1,10,11]

MPV reflects the average size of platelets and serves as an indicator of their functionality and activation, measured in femtoliters (fL). MPV tends to increase when immature platelets are activated, often during hypoproduction, due to the formation of pseudopods. The normal MPV range is 7.4–10.4 fL. A high MPV combined with thrombocytopenia typically suggests peripheral platelet destruction, while a low MPV points to reduced production or bone marrow suppression, highlighting an inverse relationship between MPV and platelet count.

In cases of bone marrow suppression, an increasing MPV can signal recovery, making it a valuable marker to guide platelet transfusion decisions. MPV is calculated using the formula:

MPV (fL) = ([PCT (%)/platelet count ($\times 10^9$/L)] × 10^5).

In the present study, MPV was increased more in group A1 (Increased peripheral destruction) than in group A2 (decreased platelet production) and the control group.

PCT represents the percentage of blood volume occupied by platelets, similar to hematocrit (HCT) for red blood cells.[5] The PCT is calculated using the formula:

PCT = (Platelet count × MPV)/10,000.

The normal range for PCT is 0.108–0.282%. PCT values generally correlate with platelet count and provide information about platelet mass and function in different clinical scenarios. In this study, no significant differences were observed in PCT values.

PDW reflects the variation in platelet size and serves as an indicator of changes in platelet morphology and activity. It increases with platelet anisocytosis (size variability), often reflecting heightened platelet activation. PDW values vary widely, with a reference range between 15% and 17%. PDW and MPV exhibit a positive correlation, with higher PDW values typically aligning with increased MPV.[5,12,13]

The P-LCR represents the percentage of large platelets (>12 fL) in the blood, with a normal range of 15–35%. It serves as a marker of platelet activity and shows an inverse relationship with platelet count while being directly correlated with PDW and MPV. P-LCR is notably higher in cases of destructive thrombocytopenia compared to hypoproductive thrombocytopenia.[5,14]

Studies conducted by Kanthraj, Kuna et al., and Jeon et al., revealed that platelet indices, such as MPV, PDW, and P-LCR, were significantly higher in cases of hyperdestructive or consumptive thrombocytopenia group compared to the control group; however, differences were less pronounced than those observed for the IPF.[2,5,6,15,16] This finding is consistent with the present study (Table 6).

Thrombocytopenia in Immune Thrombocytopenia (ITP) primarily results from accelerated platelet destruction, although the exact cause remains unclear. Following acute blood loss, RPs, which contain remnants of RNA, are frequently observed. In ITP, the number of these platelets is notably elevated due to increased peripheral destruction. In this study, five cases of ITP were examined, and it was found that the IPF% was significantly higher, exceeding 7%, when compared to the normal population. This observation aligns with the findings of Naz et al. in their study.[4,10,17,18]

In the present study, 12 cases of dengue were identified, all of which exhibited elevated IPF, consistent with the findings of Kanthraj and Ahmad et al. Thrombocytopenia

Table 4: Other platelet indices in cases (Group A1 and A2) and controls

Groups	MPV		PDW		PCT		P-LCR	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Group A1	11.86	1.367	17.58	0.747	0.07	0.054	41.72	8.12
Group A2	9.38	1.33	16.01	1.07	0.06	0.03	30.75	6.90
Group B	10.0	1.27	16.14	0.455	0.32	0.05	11.90	8.475

MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, P-LCR: Platelet large cell ratio

Table 5: Reference range of IPF in healthy individuals in comparison with other studies

Studies	Reference range of IPF (%)	Mean (%)
Goel et al.	0.7–5.7	2.4
Dadu et al.	0.7–4.3	3.5
Briggs et al.	1.1–6.7	3.4
Present study	0.5–5.1	3.4

IPF: Immature platelet fraction

Table 6: Distribution and comparison of platelet indices in thrombocytopenia with other studies

Platelet indices	Kuna et al.	Nagesh et al.	Baig et al.	Present study
Hyperdestruction (Group A1)				
MPV	12.4±0.9	12.4±3.6	11.6±2.25	11.86±1.367
PDW	20.4±5.6	15.5±3.2	15.16±1.36	17.58±0.747
PCT	0.08±0.01	-	0.09±0.14	0.07±0.054
P-LCR	45.6±13.4	3.8±13	34.30±0.14	41.72±8.12
Hypoproduction (Group A2)				
MPV	8.14±1.2	9.7±0.9	8.5±1.27	9.38±1.33
PDW	18.6±1.2	13.2±2.3	14.10±1.15	16.01±1.07
PCT	0.06±0.01	-	0.08±0.12	0.06±0.03
P-LCR	14.4±1.1	25±7	31.90±3.46	30.75±6.90

MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, P-LCR: Platelet large cell ratio

in dengue is associated with multiple mechanisms, including bone marrow suppression, peripheral platelet destruction, and inhibition of hematopoiesis by bone marrow stromal cells. Furthermore, the presence of anti-platelet antibodies (anti-NS1) contributes to the premature destruction of platelets in the peripheral circulation.[5,19,20]

In aplastic anemia, the primary cause of thrombocytopenia is decreased platelet production resulting from bone marrow failure. In this study, the IPF% in aplastic anemia cases was found to be higher than in the control group, yet lower compared to groups with increased peripheral platelet destruction. These findings are in line with those observed by Sakuragi et al., Jeon et al., and Jung et al.[2,9,21]

The differences between the control group and the hypoproductive thrombocytopenia group were minimal and not statistically significant. Previous research has indicated that PDW, MPV, and P-LCR values tend to increase alongside IPF, suggesting that these indices should not be relied upon as independent markers for determining the underlying cause of thrombocytopenia.

These findings emphasize the value of IPF as a marker for thrombopoiesis, which can assist in the differential diagnosis of thrombocytopenia and potentially reduce the need for bone marrow examination. Similar results have been reported in studies by Goel et al., Naz et al., and Jung et al.[1,9,10]

The aim of this study was to assess the clinical utility of platelet indices, with a particular focus on IPF, as it provides rapid and accurate insights into megakaryocytic activity.

Limitations of the study

This study is limited by a small sample size, highlighting the need for larger, multicenter prospective studies incorporating diverse ethnic and gender groups to further establish the clinical utility of IPF in routine practice.

CONCLUSION

The measurement of IPF is a valuable tool for identifying decreased platelet production and plays a crucial role in the initial evaluation of thrombocytopenic patients. It is a rapid, simple, and cost-effective automated parameter for assessing thrombopoiesis without the need for an invasive bone marrow examination. This innovative diagnostic approach helps differentiate between thrombocytopenia caused by increased platelet destruction and that due to bone marrow failure or suppression. By providing more accurate values, it aids in distinguishing the underlying causes of thrombocytopenia, thereby supporting more targeted treatment strategies.

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Authors Contribution:
RP- Concept, design, study protocol, data collection, data analysis, manuscript preparation and submission of article; **GP and YPJ**- Design of study, literature survey, statistical analysis and interpretation, review manuscript; **PMP**- Preparation of tables, coordination and manuscript revision.

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