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Interpretation of Platelet Histograms and Its Correlation with Peripheral Smear in Data Showing Thrombocytopenia

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Abstract

Objective: To analyze the significance of platelet histograms and peripheral smears in understanding thrombocytopenia and compare them for the incidence of pseudothrombocytopenia.

Methods: This prospective study was conducted in the Department of Pathology at a tertiary care medical institute. The study included 200 cases of thrombocytopenia. Platelet parameters (PCT, PDW, MPV) were obtained using an autoanalyzer, and peripheral smears were examined manually. Thrombocytopenia cases were classified into hyper-destructive, hypo-productive, and abnormal pooling categories based on etiology and platelet histogram patterns. The incidence of pseudothrombocytopenia was also compared. A p-value of less than 0.05 was considered statistically significant.

Results: The gender distribution showed a male preponderance (56% male, 44% female). The mean age of the patients was 26.8 years. The study found hyper-destructive thrombocytopenia to be the most common type, with viral fever, sepsis, and malaria being the common etiologies for this type of thrombocytopenia. Histogram analysis revealed distinct patterns for different types of thrombocytopenia. The study also noted a higher incidence of pseudothrombocytopenia in automated analysis compared to manual methods, with a statistically significant difference.

Conclusion: Platelet histograms combined with peripheral smear analysis provide crucial information about the etiology and nature of thrombocytopenia. This integrated approach enhances diagnostic accuracy and aid in effective patient management.

Keywords: Peripheral smear, platelet histogram, pseudo-thrombocytopenia, thrombocytopenia

Introduction

Thrombocytopenia is a common condition characterized by a reduced platelet count below 150,000/cumm in the circulating blood. It is linked to various diseases, and understanding its etiology is crucial for patient management.[1] The condition can be caused by hypoproduction, hyper destruction, or abnormal platelet pooling. Recent advancements in automated hematology analyzers have revolutionized laboratory practices by providing not only platelet counts but also valuable platelet indices such as Mean Platelet Volume (MPV), Plateletcrit (PCT), and Platelet Distribution Width (PDW). These indices, especially when analyzed through platelet histograms, offer a deeper insight into the nature and underlying causes of thrombocytopenia.[2]

The study of platelet histograms, specifically the maximum or peak of the volume distribution curve, has become an important parameter in assessing thrombocytopenia. By combining this approach with peripheral smear analysis, a deeper understanding of this condition can be achieved. Peripheral smears, a traditional method in hematology, involve

examining blood smears under a microscope to identify and quantify different blood cells. When used in conjunction with automated indices, it offers a comprehensive view of the patient's hematological profile.[3]

Recent research has emphasized the significance of these indices in different clinical scenarios. Infections like dengue fever, malaria, and rickettsial diseases often exhibit thrombocytopenia with notable changes in platelet indices such as MPV and PDW. These platelet indices could not only serve as potential indicators for these diseases but also indicate the worsening condition of the affected patient as thrombocytopenia becomes more severe.[4]

Platelet indices can also be used to determine the etiology of thrombocytopenia to some extent. In order to better understand the pathophysiological mechanisms of thrombocytopenia in specific diseases, it can be categorized into three main groups: reduced production, increased destruction, and abnormal pooling..[5] Since different etiologies have distinct pathophysiological mechanisms in the development of thrombocytopenia, a fairly accurate diagnosis can be made by using these indices in conjunction with relevant investigations such as serological tests and bone marrow examinations in selected cases.[6]

Furthermore, platelet indices also play a crucial role in diagnosing hyper-destructive thrombocytopenia, such as Immune Thrombocytopenic Purpura (ITP), hemolytic anemia, drug toxicities, and infections.[7] Indices like MPV and PDW are generally higher in ITP patients compared to those with hypo-productive thrombocytopenia. Understanding these differences in various platelet indices and the basic mechanisms of thrombocytopenia development in a specific case is essential for effectively managing patients with thrombocytopenia.[8]

In the context of diseases such as viral fever, malaria, and autoimmune conditions causing thrombocytopenia, Pseudothrombocytopenia poses a diagnostic challenge. Pseudothrombocytopenia is a laboratory artifact that is often misinterpreted as true thrombocytopenia.[9] Automated analyzers are efficient in high output settings, but they may occasionally misclassify pseudothrombocytopenia caused by platelet clumping or size variability. On the other hand, manual analysis offers the advantage of direct visualization, which allows for more accurate identification of platelet aggregates and other anomalies. However, it is a labor-intensive process and is subject to human error.[10]

Taking into consideration all of these factors, a study was undertaken to analyze the correlation between the platelet histogram and peripheral smear in cases of thrombocytopenia.

Methods

This was a prospective study conducted in the Department of Pathology from April 2023 to December 2023. The Institutional Ethical Committee approved the study (Ref BV (DU) MC &H/Sangli/IEC/510/23, dated 08/03/2023). The sample size was calculated using the formula

$$N = (Z_{\alpha}^{2}) \text{ X } SD^{2} / d^{2}$$

OPENEPI software version 3 was used for this study, based on pilot studies conducted on the topic of surgical management of perianal fistula. Using a power of 90% and a confidence interval of 95%, a sample size of 200 was determined. As a result, 200 cases with thrombocytopenia were included in the study. All samples received in the hematology lab displaying thrombocytopenia were included, and there were no specific exclusion criteria.

Demographic details of all patients, including age and gender, were noted. The clinical details of the patients were reviewed from their medical records. Special attention was given to their fever history, intake of any drugs that could cause thrombocytopenia, and history of any autoimmune disorders. If any relevant history was missing, the treating physician was contacted to obtain the necessary information. Serological tests that could help determine the cause of thrombocytopenia, such as Dengue IgG, IgM, or NS1, as well as serological tests for typhoid or rickettsial fever, were also examined and reviewed.

In the study, all the samples_received in hematology lab with thrombocytopenia were studied. EDTA anti-coagulated venous samples were processed within four hours of sample collection in the autoanalyzer.[11] Platelet parameters obtained includes– PCT, PDW and MPV. A peripheral smear stained with Leishman stain was reviewed with a specific purpose to rule out pseudo-thrombocytopenia cases. The analyzer was regularly calibrated for accuracy as well as precision using standardized quality control material.

The reports were divided into patients

Table 1 Age Distribution of the Studied Cases

Age Category	Number of Cases n=200	Percentage
0–20	82	41.00
20–40	78	39.00
>40	40	20.00

Mean Age: 26.8 +/- 14.2 years

with hyper-destructive thrombocytopenia, hypo-productive thrombocytopenia, and thrombocytopenia due to abnormal pooling.[12] The histograms of all cases with thrombocytopenia were analyzed using an automated hematology analyzer. The histograms were categorized into Normal curve, Curve not touching baseline, Bimodal, short peak, Saw tooth, and broad based on the shape of the histogram. A correlation between platelet histograms and peripheral smear was conducted in all cases.[13] Additionally, pseudothrombocytopenia, an artifact resulting from platelet aggregation primarily due to the anticoagulant EDTA in automated analyzers, was identified. Cases of suspected pseudothrombocytopenia were confirmed by manually examining the peripheral blood smear for evidence of clumping and comparing these observations with the automated counts.

The statistical analysis was done by SPSS version 21.0 software. Mean and standard deviation were used to present quantitative data, such as the age of patients and platelet count. On the other hand, qualitative data was presented as tables showing incidence and percentages for the causes of thrombocytopenia. A p-value less than 0.05 was considered as statistically significant.

Results

The analysis of patient gender distribution revealed that out of 200 cases of thrombocytopenia, there were 112 (56%) males and 88 (44%) females. There was a higher prevalence of males, with a male-to-female ratio of 1:0.78. The most commonly affected age group was below 20 years, with 82 (41%) patients. Additionally, 78 (39%) patients were between 20-40 years old, while 40 (20%) patients were above 40 years of age. The mean age of the cases was 26.8 +/- 14.2 years (Table 1).

The analysis of the type of thrombocytopenia showed that hyper-destruction, constituted the largest group with 152 cases, accounting for

Table 2 Type of Thrombocytopenia and Etiological Causes

Etiology	Causes	Number of cases n=200	Percentage
Group A (Hyper-destruction)	Viral fevers	75	37.50
	Sepsis	24	12.00
	Malaria	15	7.50
	Liver disease	11	5.50
	Renal disease	9	4.50
	Cardiac disease	5	2.50
	ITP	13	6.50
Group B (Hypo-production)	Anemia	15	7.50
	MDS	6	3.00
	Leukemia	16	8.00
	Pancytopenia	3	1.50
Group C (Abnormal pooling)	Splenomegaly	8	4.00

Table 3 Distribution of Various Histogram Curves in Various Groups

Etiology	Type of Curves on Histogram	Number of cases n=200	Percentage
Group A (Hyper-destruction)	Normal curve	5	3.29
	Normal curve not touching the baseline	83	54.61
	Broad base	14	9.21
	Bimodal	11	7.24
	Short peak	36	23.68
	Saw tooth	3	1.97
Group B (Hypo-production)	Normal curve	10	25.00
	Normal curve not touching the baseline	6	15.00
	Broad base	4	10.00
	Bimodal	5	12.50
	Short peak	12	30.00
	Saw tooth	3	7.50
Group C (Abnormal pooling)	Normal curve	1	12.50
	Normal curve not touching the baseline	2	25.00
	Broad base	1	12.50
	Bimodal	1	12.50
	Short peak	1	12.50
	Saw tooth	2	25.00

76% of the total. Within this group, the major causes included Viral fevers (37.50%), Sepsis (12%) and Malaria (7.5%). Hypo-production group included 26 cases, making up 13% of the total. The causes in this group were Anemia (7.5%), leukemia (8%) Myelodysplastic Syndromes (MDS) (3%), and Pancytopenia (1.5%). Lastly thrombocytopenia due to

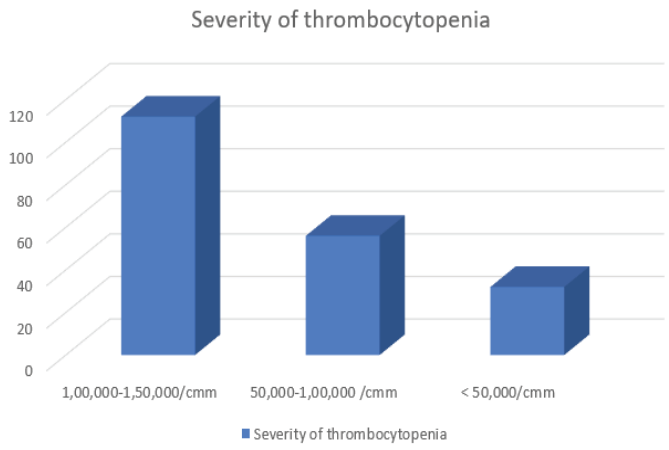

Fig. 1 Severity of Thrombocytopenia in Studied Cases

Table 4 Pseudothrombocytopenia in Manual Method Versus Automated Analysis

	Thrombocytopenia	Pseudothrombocytopenia	Total
Manual	197	3	200
Automated	189	11	200

p=0.03 (Significant)

abnormal pooling was seen in 8 (4 %) cases, predominantly caused by Splenomegaly (Table 2).

The analysis of the severity of thrombocytopenia showed that the majority of the patients had a platelet count between 100,000-150,000/cmm (56%), followed by 50,000-100,000/cmm (28%), and <50,000/cmm (16%) (Fig. 1).

The analysis of histograms revealed that in group A (Hyper destruction), out of 152 patients, the most common pattern was a normal curve that did not touch the baseline, which was seen in 83 (54.61%). The other common patterns were a short peak (23.68%) and a broad-based (9.21%) histogram. Among the 40 patients in group B, the most common histogram patterns were a short peak (30.00%) and a normal curve (25.00%). Finally, among the 8 patients in group C, the most common histogram patterns were a normal curve that did not touch the baseline (25.00%) and a sawtooth (25.00%) pattern (Table 3).

All samples were analyzed for the presence of pseudothrombocytopenia using both manual and automated methods. Out of the 11 (5.5%) samples analyzed using automated analysis, pseudothrombocytopenia was detected, while only 3 samples showed pseudothrombocytopenia when analyzed manually. The incidence of pseudothrombocytopenia was found to be higher in the automated analysis compared to the manual method, and this difference was statistically significant (p<0.03) (Table 4).

Discussion

It is crucial to analyze the relationship between automated hematology analyses and traditional microscopic examination. Studies have shown that automated cell counters may not always capture the complete hematological profile particularly in cases of anemia and thrombocytopenia.[14] The interpretation of platelet histograms offers a detailed view of platelet size distribution which is helpful in understanding the underlying pathology of thrombocytopenia.[15] The correlation between platelet histograms and peripheral smear findings is significant. For instance, a study by Sandhya *et al*.[16] demonstrated a strong correlation between RBC histograms and peripheral smear in anemia typing suggesting a similar potential in thrombocytopenia cases. This correlation is also significant in diagnosing and categorizing thrombocytopenia whether it is hyper destructive, hypo productive or due to abnormal pooling.

In this study, the most common etiology of thrombocytopenia was viral fever (37.50%), sepsis (12%), leukemia (8%), and malaria (7.5%). Abnormal pooling of platelets was found to be the cause of thrombocytopenia in 8 (4%) cases. Patel *et al.* conducted a study to analyze the etiology of thrombocytopenia. In this study, the most common causes of thrombocytopenia were malaria, viral fever, and dengue. Non-infectious causes included cirrhosis, neonatal septicemia, gestational thrombocytopenia, iron deficiency anemia, preterm neonate, megaloblastic anemia, pneumonia, ITP, DIC, acute myeloid leukemia, and chronic lymphoid leukemia. The study found malaria to be the leading cause of thrombocytopenia, followed by viral fever (other than dengue) and dengue fever, with cirrhosis and neonatal septicemia also being significant contributors.[17] Viral fever and malaria were also common etiologies in this study. Similar etiological causes were also reported by Gauer *et al*[18] and Fountain *et al*.[19]

The histograms of all samples were analyzed. In group A (Hyper-destruction), the most common pattern was a normal curve that did not touch the baseline, which was observed in 83 cases (54.61%). Among the 40 patients in group B, the common histogram patterns were a short peak (30.00%) and a normal curve (25.00%). Lastly, among the 8 patients in group C, the common histogram patterns were a normal curve that did not touch the baseline (25.00%) and a saw tooth pattern (25.00%). Shetty A *et al*. analyzed the variations of histograms in various mechanisms of thrombocytopenia. In this study, adults with thrombocytopenia

were divided into four groups based on the mechanism of thrombocytopenia, and variations in platelet histograms were studied in all groups. The following variations in histograms were observed in this study: a normal curve (8.03%), a curve not touching/reaching the baseline (43.75%), a broad-based curve (10.71%), a bimodal curve (7.14%), a curve with a short peak (25.9%), and a saw-tooth appearance of the curve (4.47%).[20] Similar histogram findings were also reported by Walle *et al*.[21] and Bhola *et al*.[22]

In this study, the comparison of manual and automated analysis for pseudothrombocytopenia showed that the incidence of pseudothrombocytopenia was higher in automated analysis compared to the manual method. The difference was significant (P<0.03). A similar study conducted by Lardinois *et al.*[23] also found an increased risk of pseudothrombocytopenia (PTCP) in automatic blood analyzers. The authors recommended that PTCP be considered in the assessment of low platelet count particularly when automated blood analyzers were used.

Combining platelet histograms with peripheral smear analysis is an effective strategy for diagnosing thrombocytopenia and increasing diagnostic accuracy. This integrated approach not only improves accuracy, but also aids in effective patient management. The study also emphasizes the significance of manual analysis in detecting pseudothrombocytopenia, which is often overlooked in automated analysis. In conclusion, it is important to understand platelet morphology changes to differentiate the causes of thrombocytopenia and identify cases of pseudothrombocytopenia.

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