Utility of automated cell counter histogram in diagnosis of thrombocytopenia and pseudothrombocytopenia

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Background: Automated hematology analyzers have become mainstream of complete blood count (CBC) during the past two decades; they are potential, more accurate to produce results of CBCs. However, often automated analyzers can give false results too, specially, falsely low platelet count due to platelets aggregates, which have to be confirmed on peripheral smears. Aims and Objectives: The aim and objective of the study was to diagnose thrombocytopenia (TCP) cases and differentiate them from pseudothrombocytopenia (PTCP) cases by analyzing platelet histogram and to determine the sensitivity and specificity of platelet histograms for diagnosing TCP and PTCP. Materials and Methods: The present study was conducted in the Department of Pathology at Shyam Shah Medical College, Rewa, M.P., after obtaining ethical clearance from the Institutional Ethics Committee. It was prospective study conducted for a period of 15 months from January 2021 to May 2022 on 1000 samples. Results: The sensitivity and specificity of platelet histogram flags and presence of multiple peaks to diagnose TCP and PTCP cases were calculated as 73.60% and 93.60%, respectively. The present study also interpret that mean platelet volume and platelet distribution width cannot use as indicator to differentiate between TCP and PTCP. Conclusion: The present study concludes that analysis and interpretation of histogram more specifically platelet histogram flagging provide clue for early detection of PTCP cases prior peripheral smear examination and also helpful in differentiating them from true TCP cases. These platelet histogram flagging can be used as a screening parameter for the detection of PTCP and these are helpful in preventing unnecessary stress for clinician, patients, and their relatives. However, the peripheral smear examination will remain the gold standard to differentiate TCP from, PTCP. Key words: Ethylenediaminetetraacetic acid; Thrombocytopenia; Pseudothrombocytopenia
count given by automated hematology analyzers, while the actual platelet count is within normal range for the same patient. The reason behind falls low platelet count is that the hematology analyzers count the platelet clumps (CLP) as a single giant platelet or as small lymphocytes. Its prevalence is reported to vary between 0.1% and 2% among hospitalized patients and 15–17% among out patients evaluated for isolated thrombocytopenia (TCP). It can lead to diagnostic error, overtreatment, and further unnecessary testing. Clinical consequences with potential life-threatening events (Unnecessary platelet transfusion, inappropriate treatment including corticosteroids) are still observed when PTCP is not readily detected.

Ethylenediaminetetraacetic acid (EDTA) is introduced in 1950s in laboratories. Since then, it is widely used as anticoagulant in laboratories. One of the most important disadvantages of EDTA is that it causes platelet aggregation. Most often the clumping is caused by the presence of agglutinating anti-platelet antibodies that react with platelets in blood. Platelet clumping leads to PTCP, which has to be examined and confirmed by microscopic examination of peripheral smear of that sample.

The present study is conducted to find out cases with falsely low platelet. It can be only revealed by conventional blood smear inspection and consequent correlation with different values produced by automated hematology analyzers. Visual evaluation of blood smears is regarded as gold standard for the detection of EDTA-induced PTCP.

**Aims and objectives**
1. To diagnose TCP and pseudo-TCP using automated cell counter histogram
2. To determine the sensitivity and specificity of platelet histograms for diagnosing TCP and PTCP.

**MATERIALS AND METHODS**

The present study was conducted in the Department of Pathology at Shyam Shah Medical College, Rewa, M.P., after obtaining ethical clearance from the Institutional Ethics Committee vide no IEC/MC/2020/463. It was prospective study conducted for a period of 15 months from January 2021 to May 2022 on 1000 samples. All samples were collected from different wards of Sanjay Gandhi Memorial and Gandhi Hospital Rewa (M.P). Samples were collected in EDTA vials and were processed in automatic hematology analyzer (SYSMEX KX-21). Platelets parameters, changes in platelet histogram such as PU flag, and multiple peaks CLP flag were studied in the samples having platelets count less than 1 lakh were selected and smears prepared for same.

After drying, smears examined in microscope at low power and oil immersion. If platelet count on peripheral smears is found to be <1 lakh, then it will be counted as true TCP and if the platelet count on peripheral smears is found to be more than 1 lakh, then it will be counted as PTCP. After counting platelets on peripheral smears, consequently, we have correlated various changes in platelet histogram such as PU flags and multiple peaks (CLP flag), with peripheral smears findings. Sensitivity, specificity, positive predictive value, and negative predictive value of platelet histogram were calculated using SPSS software.

**Inclusion criteria**
1. Platelet count <1 lakh
2. Known cases of immune thrombocytopenic purpura
3. Patients with prolonged bleeding time.

**Exclusion criteria**
1. Platelets count more than 1 lakh
2. Known cases of leukemia
3. Clotted sample
4. Dilute sample
5. Patients with deranged coagulation profile.

**RESULTS**

In our study, it seems that, out of 1000 cases, only 53% (530/1000) of cases showed true TCP, whereas 47% (470/1000) cases are belong to PTCP category (falsely low platelet count given by cell counter) and it is merely a laboratory artifact, which is primarily due to EDTA-induced platelet clumping. Most of the PTCP cases showed abnormal platelet distribution curve such as the presence of multiple peaks (CLP flag) or presence of PU flag (curve not end at base line). These changes are shown below in the cases which are observed during study.

**Cases of PTCP observed during study**

**Case 1**

Figure 1.

**Case 2**

In the present study, it is observed that the cases in which platelet count is falsely low, i.e., PTCP cases most of them shows multiple peaks/Pu flag in platelet distribution curve which comprises 44% (440/1000) of all studied samples and 93% (440/470) of all PTCP cases (Table 1). The present study also interpret that most of the true TCP cases, i.e., 73% (390/530), did not showed any of above given abnormality in platelet distribution curve; however, it is also observed that 26% (140/530) of true TCP cases showed multiple peaks/Pu flag in platelet distribution curve of complete histogram [Figure 2].
In the present study, it is observed that only 19% (100/530) of total cases of TCP shows increase in MPV, while remaining 81% (430/530) cases of TCP have MPV of normal range. Similarly, 19% (90/470) cases of PTCP showed increase in MPV, while remaining 81% (380/470) of cases did not show change in MPV (Table 2). Thus, according to the present study, MPV cannot be used as an indicator to differentiate TCP from PTCP.

In the present study, it is observed that 64% (340/530) cases of TCP showed increase in platelet distribution width (PDW), whereas in 36% (190/530) of cases, it remains in normal range. Similarly, 72% (340/470) cases of PTCP showed increase in PDW, whereas in remaining 28% (130/470) cases, PDW remains in normal range (Table 3). Thus, according to the current study, it seems that PDW alone cannot be used as a marker to differentiate TCP from PTCP.

After analyzing various changes in platelet distribution curve such as PU flag and the presence of multiple peaks and their correlation with platelet count given by automated hematology analyzer and platelet count on peripheral smears, the sensitivity, specificity, positive predictive value, and negative predictive value were found to be 73.60%, 93.60%, 92.85, and 75.90, respectively.

DISCUSSION

Among various parameters of platelet histogram (MPV, PDW, PU flags, and multiple peaks), we assessed that the presence of multiple peaks (CLP flag) and PU flag have potential ability to detect the PTCP cases. The sensitivity of platelet histogram is found to be 73.60% and specificity is 93.60%, positive predictive value is 92.85, and negative predictive value is 75.90. The sensitivity and specificity may vary on different automated hematology analyzers, to our knowledge, there is no study done on SYSMEX KX 21 analyzers; however, few studies done on different analyzers are summarized in Table 4 with their sensitivity and specificity.

Almost similar study conducted by Hawkins et al.,\textsuperscript{10} using Sysmex XE-5000 analyzer on 600 EDTA-anticoagulated blood specimens, The sensitivity of platelet abnormal distribution flag and platelet CLP flag was found to be 42% and 57%, respectively. The specificity of platelet abnormal distribution flag and platelet CLP flag was 57% and 99%, respectively.

A study Conducted by Schuff-Werner et al.\textsuperscript{11} considered platelet distribution curve and changes observed in it, like curve is flattened and serrated like a saw blade. Sensitivity was calculated at 33.2%, specificity at 97.3%, and efficiency at 96.7%. The sensitivity of platelet histogram in this study is far low (33.2%) as compare to our study (73.60%), but specificity is nearly similar to the present study, which is 93.60% in the present study.

In a study by Xu et al.,\textsuperscript{12} on the samples with low platelet counts showed that the total sensitivities of the platelet, CLP

<table>
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<th>Table 1: Correlation of thrombocytopenia and pseudothrombocytopenia with presence of multiple peaks/PU flag</th>
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<tbody>
<tr>
<td>TCP</td>
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<tr>
<td>Number</td>
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<tr>
<td>140</td>
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</table>

TCP: Thrombocytopenia, PTCP: Pseudothrombocytopenia, CLP: Clump flag

<table>
<thead>
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<th>Table 2: Correlation of thrombocytopenia and Pseudothrombocytopenia with MPV</th>
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<td>TCP</td>
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<tr>
<td>Number</td>
</tr>
<tr>
<td>430</td>
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</tbody>
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| PTCP | Normal MPV | Increased MPV |
| Number | Percent | Number | Percent |
| 380 | 81 | 90 | 19 |

MPV: Mean platelet volume

<table>
<thead>
<tr>
<th>Table 3: Correlation of thrombocytopenia and pseudothrombocytopenia with PDW</th>
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<tr>
<td>TCP</td>
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<tr>
<td>Number</td>
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<tr>
<td>190</td>
</tr>
</tbody>
</table>

| PTCP | Normal PDW | Increased PDW |
| Number | Percent | Number | Percent |
| 130 | 28 | 340 | 72 |

PDW: Platelet distribution width, TCP: Thrombocytopenia, PTCP: Pseudothrombocytopenia
Table 4: Comparison of sensitivity and specificity of histogram in different type of studies

<table>
<thead>
<tr>
<th>Study conducted by</th>
<th>Year</th>
<th>Parameters included</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
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<tr>
<td>Hawkins et al.</td>
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<td>PAD</td>
<td>For PAD- 42%</td>
<td>83</td>
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<tr>
<td></td>
<td></td>
<td>CLP</td>
<td>For CLP- 57%</td>
<td>99</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>For PAD and Or CLP=73</td>
<td>82</td>
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<td>Schuff-Werner et al.</td>
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<td>Xu et al.</td>
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<td>94 and 91</td>
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<tr>
<td>Present study</td>
<td>2020-22</td>
<td>Platelet distribution curve and its PU flag</td>
<td>73.60</td>
<td>93.60</td>
</tr>
</tbody>
</table>

PAD: Platelet abnormal distribution, CLP: Platelet clump flag, IMI: Immature cell information, PU flag: Platelet upper discriminator flag

Figure 1: (a) Platelet count in this histogram is $29 \times 10^{3}/\mu L$ and platelet distribution curve shows multiple peaks and not ending at base line (PU flag). (b) Peripheral smear (Leishman stain, ×100): - Peripheral smear of corresponding case shows multiple CLP of platelets, platelet count found to be $190 \times 10^{3}/\mu L$, and total leukocyte count found to be $18 \times 10^{3}/\mu L$. Thus, from above given histogram and peripheral smear findings, we can say that it is a case of PTCP

Figure 2: (a) In above Histogram, the platelet distribution curve shows multiple peaks (saw tooth appearance of graph) and platelet distribution curve does not ending at base line (PU flag). (b) Peripheral smear (Leishman stain, ×100): The corresponding peripheral smear shows large CLP of platelets. Platelet count of this case on peripheral smear is found to be 180000 per cubic mm. Thus, from above given histogram and peripheral smear findings, we interpret that it is a case of PTCP
and platelet abnormal flags on platelet histogram/platelet distribution curve were 80.01% and 100%, respectively.

**Limitations of the study**
This study is done on Sysmex KX-21 automated analyser, the results may vary on different model of automated hematology analysers. So multicentric studies, with large sample size are needed to establish the sensitivity and specificity of platelet histogram flagging for detection of thrombocytopenia and pseudothrombocytopenia.

**CONCLUSION**

The present study concludes that analysis and interpretation of histogram, more specifically platelet histogram flagging, provide clue for early detection of PTCP cases prior peripheral smear examination and also helpful in differentiating them from true TCP cases. These platelet histogram flagging can be used as a screening parameter for the detection of PTCP and these are helpful in preventing unnecessary stress for clinician, patients, and their relatives. However, the peripheral smear examination will remain the gold standard to differentiate TCP from PTCP.

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**REFERENCES**


**Authors Contribution:**

HM- Concept and design of the study, prepared first draft of manuscript; SKS- Concept, coordination, statistical analysis and interpretation, preparation of manuscript and revision of the manuscript; RQU- Preparation of manuscript, interpretation, and revision of the manuscript; RJ- Interpreted the results; reviewed the literature and manuscript preparation; AG- Manuscript preparation; RG- revision of the manuscript; VKS- revision of manuscript; PSR- Revision of manuscript; SS- Revision of manuscript.

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