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Comparison of Platelet Count by Automated and Manual Methods in Thrombocytopenia Patients

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Abstract

Objective: This study aimed to investigate platelet count variations in adult thrombocytopenia patients by comparing automated platelet counts with manual counting methods.
Methodology: A comparative cross-sectional study was conducted at the Hematology department of King Edward Medical University, Lahore from January 2022 to June 2022, involving 60 patients with thrombocytopenia. Complete blood counts were performed using an Automated Hematology Analyzer, and peripheral smears were prepared and manually examined by two experts to verify platelet counts.
Results: The study included 31 females and 29 males, with a mean age of 43.7 years. The mean platelet count obtained from automated analyzers was 58 ± 28×10^9/L, while the manually verified platelet count on peripheral smears was 117 ± 13×10^9/L, with a significant p-value of <0.001. Pseudo-thrombocytopenia was observed in 52% of patients, primarily due to platelet clumps (42%) and giant platelets (39%).
Conclusion: The study underscores the importance of manual verification of platelet counts in thrombocytopenic patients, as automated counts tended to underestimate platelet levels. Peripheral smears remain the gold standard for accurate platelet counting, helping prevent unnecessary investigations and ensuring appropriate patient care
Key words: Diagnostic accuracy, automated haematology analyser, sysmexXN-1000, malaria, microscopy.

Introduction

Platelets are discoid cells without a nucleus having a diameter of 1-3 µm. These are synthesized by cytoplasmic fragmentation of one of the hematopoietic stem cells megakaryocytes.[1] Almost 1000-3000 platelets are formed from a single megakaryocyte.[2] A typical platelet has a lifespan of 7-12 days before being destroyed by macrophages in the spleen.[3] In blood clotting, platelets contribute to both structural and molecular functions. These are involved in thrombosis, hemostasis, and wound repair.[4] The typical platelet count for adults is 150–400 10^9/L. The accuracy of the platelet count is important in several critical patients.[5] Thrombocytopenia can be caused by a variety of disorders, including dengue fever, malaria, malignancy, severe sepsis, DIC, etc.[6] For the management of thrombocytopenia in clinical care, a timely and exact platelet count is essential.[7] In a hematology laboratory, there are numerous methods for counting platelets. It can be done either via an automated hematology analyzer or manually under a microscope by viewing peripheral blood smears or through a Neubauer chamber.[8] Over the last decade, automated hematology analyzers have made significant progress in the detection and characterization of blood cells.[9] On the other hand, manual approaches have gradually lost their utility in the laboratory to decrease the burden on humans, and financial resources, and minimize the turnaround time.[10] Although hematology analyzers normally produce an accurate platelet count, their accuracy has been brought into question while enumerating low platelet counts, platelet abnormalities, or platelet-like fragment interference.[11]

Currently, the majority of automated hematology analyzers count platelets using optical density and electronic impedance concepts. Recently, certain

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analyzers were made with the ability to count platelets using flow cytometry. As a result, these analyzers are capable of calculating platelet counts using optical, immunological, and impedance techniques.[12] The immunological method by flow cytometry in conjunction with a semi-automated impedance counter with a single-channel aperture has been recommended as the reference method for counting platelets by the International Council of Laboratory Hematology till 2001[13] Although these methods are widely used still manual verification of platelet count remains the "gold standard", though it might be time-consuming.[14] Mostly platelet count is done on an automated analyzer but just like other machines it also has its drawbacks, especially while analyzing decreased platelet counts. The machine gives flags for platelet clumps, fragmented RBCs, giant platelets, and small RBCs which can interfere with platelet count, hence accurate count cannot be generated. In such cases, manual verification of platelet count is of utmost importance for critical care and evaluation of thrombocytopenic patients which can lead to life-threatening bleeds.[15]

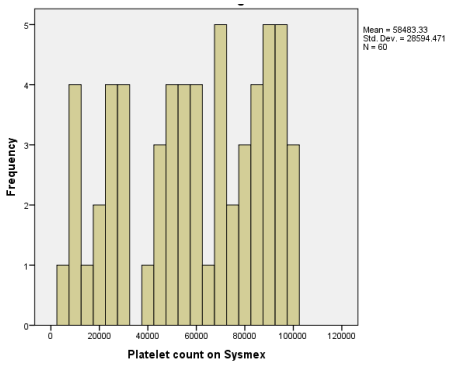
Peripheral blood film is highly useful in the diagnosis of unexplained thrombocytopenia i.e pseudo-thrombocytopenia and also in monitoring the therapeutic responses.[16] It can be used as the quality control for the verification of results generated by automated instruments.[17]

Methodology

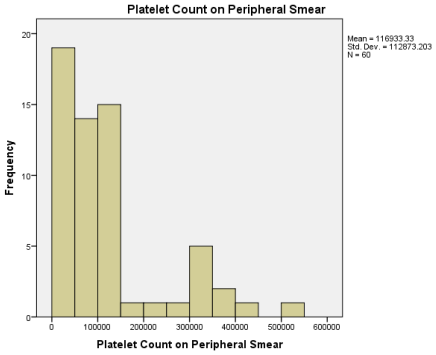
It was a comparative cross-sectional study, carried out at King Edward Medical University, Lahore from January 2022 to June 2022. Inclusion criteria were all the samples presented at Mayo hospital laboratory, having thrombocytopenia with platelet count less than 100,000/uL on automated blood cell counter. Samples excluded were the hemolysed or clotted ones. Under aseptic methods, 3ml of EDTA whole blood was collected from all the patients. After the collection of whole blood, samples were analyzed by using complete blood counter based on florescence method. A peripheral smear for each sample was prepared following the SOPS and was stained with routine Giemsa stain. Platelet count was done manually by two experts viewing the same smear, to minimize inter observer difference. An average was taken out if no significant difference in platelet count was present. Platelets were counted on the slide under an oil immersion lens (100X) in 10 fields and multiplied by 15,000 to get the estimated platelet count.[18] Data were analyzed using SPSS version 26. Qualitative variables were described as frequency, and quantitative variables were measured as mean and standard deviation and keeping the 95% confidence interval and p-value of $\leq$0.05. T-test was applied to compare the mean platelet count obtained by both methods.

Results

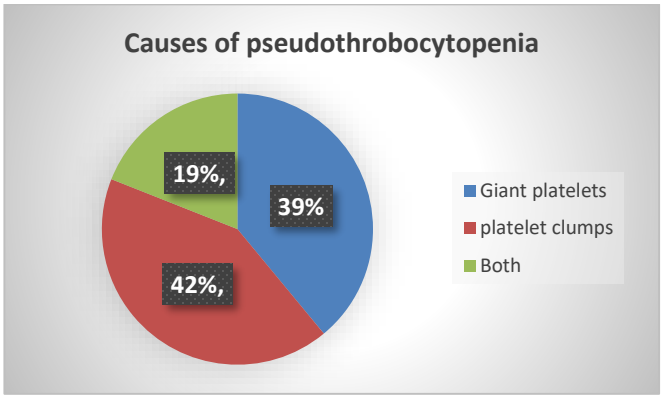
Our study included 60 adult patients including 31 females and 29 males with a male-female ratio of 1:1. The mean age was 43.7 years, The mean platelet count on automated analyzers was 58 ± 28×10^9/ L whereas the platelet count verified on peripheral smear was 117±13×10^9/L with a significant p-value of <0.001. (graph 1 and 2) Out of 60 patients 58% had true thrombocytopenia whereas 52% had pseudo thrombocytopenia. However, when examined on peripheral smear the actual platelet count was actually higher. The causes of pseudo thrombocytopenia when confirmed on peripheral examination were the following: 42% of the patients had platelet clumps, 39% of the patients had giant platelets, and 19% of patients had both giant platelets and platelet clumps. (Graph 3)



Graph 1. (Histographical distribution of platelet counts on Analyzer and their frequencies)



Graph 2: (Histological distribution of platelet count on peripheral smear with their frequencies)



Graph 3: (Pie Chart showing percentages of causes of pseudo thrombocytopenia)

Discussion

Over the last decade, significant improvements have been made in automated hematology analyzers, which are used for both analytical purposes and the description of whole blood cells. Manual procedures in hematology labs have been gradually losing their importance in regular testing because of it.[19] Although hematology analyzers often produce precise platelet counts, their precision has been called into doubt while counting low platelet counts, in the context of platelet anomalies, or when intrusion from fragments that are like platelets, has been seen.[20] When an automated platelet count is low or flagged, the calculation of platelet counts from the manual method counting by examining blood smears should be the gold standard, since no machine, no matter how costly or effective, can completely replace human judgment. [21]

Our results showed a male-female ratio of 1:1 however in another study by Berkman N there was a reverse ratio of 2.1:1 for male to female.[22] In our study for those samples showing thrombocytopenia, platelet count on an automated analyzer was compared to the platelet count on peripheral smear. The results on the smears were higher than automation. Out of 60 patients, 25 had pseudo thrombocytopenia. It was mostly due to presence of platelet clumps and giant platelets. In a study by Silvestri Fsimilar results were seen. Pseudothrombocytopenia was seen in 25 % of the patients with thrombocytopenia.[23] Pseudothrombocytopenia a relatively common finding in clinical laboratories, can lead to diagnostic errors, overtreatment, and further (even invasive) unnecessary testing. Clinical consequences with potential life-threatening events (e.g., unnecessary platelet transfusion, inappropriate treatment including splenectomy or corticosteroids are still observed when pseudothrombocytopenia is not readily detected.

Moreover, the clinical decision to proceed with prophylactic platelet transfusions is widely based on trigger points for platelet counts being equal to 20, 10, or even 5* 10^9/L. The problems of counting imprecision in the low thrombocytopenic range on automated analyzers can be minimized by reviewing the smears as it remains the “gold standard” for platelet counting.

Conclusion

In thrombocytopenia, it is crucial to make a peripheral smear to confirm the platelet count before treatment. It may save patients from unnecessary investigation. Thus the peripheral smear remains the gold standard for accurate platelet count.

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