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Original Research Article

Platelet count correlation: automated versus manual on peripheral smear

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Abstract

The assessment of platelet count is essential in clinical practice. The traditional method of estimating platelet counts from peripheral blood smears is a fairly accurate method and provides adequate quality assurance. Widespread use of automated analyzers based on impedance technology has resulted in an unprecedented improvement in accuracy and allows measurement of additional indices such as Plateletcrit (PCT), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW). However, both the methods have certain limitations.

Platelet counts were estimated on a 5-part Differential Automated Hematology Analyzer and manually on Leishman stained peripheral blood smears in 200 indoor patients admitted in Government Medical College Jammu to detect the correlation between the two. Estimation of MPV and PDW was done followed by a comparison with automated platelet counts.

The Mean Platelet Count, of the cases under study, given by the automated analyzer was significantly lower than the Mean Platelet Count estimated by manual method ($P < 0.001$).

However, the platelet count assessed by automated analyzer showed a strong correlation with manual platelet count (correlation coefficient, $r = 0.857$).

In thrombocytopenic patients, the platelet count assessed by automated analyzer, showed an inverse relation with Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW).

It was concluded that automated hematology analyzer is crucial for quick and accurate complete blood count evaluation but all blood samples that show abnormal results or low platelet counts on analyzers should be confirmed by manual count on peripheral smear. The platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) can point to the underlying pathology especially in cases of thrombocytopenia.

Introduction

The assessment of the Platelet Count is essential in both clinical practice and research laboratories. Clinical bleed may result from either numerical deficiency or platelet function defect. The normal range of platelet count in a healthy individual is 1,50,000 to 4,50,000/mm^3.[1,2] As long as platelet count is more than 20,000/mm^3, clinical manifestations are expected to be mild, often limited to easy bruising. With platelet count less than 10,000/mm^3, the risk of life-threatening bleeding like intracranial haemorrhage or gastrointestinal bleeding increases significantly.[3]

The common methods of platelet estimation are:

1. Manual counting using counting chamber
2. Evaluation on the peripheral smear
3. Assessment using the automated cell counters

Manual platelet counting in the Neubauer chamber, by means of a phase-contrast microscope has been recommended as a reference method for assessing the platelet number by the International Committee for Standardization in Hematology (ICSH - 1984).[4] However, it is a time consuming method which usually results in high levels of variability.

The traditional method of estimating platelet counts from peripheral blood smears is a fairly accurate method and provides adequate quality assurance. The average number of platelets counted per oil immersion field are multiplied by 20,000 to yield a platelet count estimate per µl.[5] Widespread use of automated analyzers based on impedance technology has resulted in an unprecedented improvement in accuracy. Moreover novel platelet count related indices are being estimated in addition to routine parameters. The most significant among these are Plateletcrit (PCT), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) as together they provide a priceless index to measure the functional integrity of platelets. Platelet activation, which is characterized by platelet swelling and shape change, is ultimately reflected as an increase in MPV and PDW.[6] Mean platelet volume (MPV) has been reported to be useful in differentiating thrombocytopenia due to peripheral platelet destruction from that resulting from reduced platelet synthesis. This measurement may also be used to evaluate bone marrow suppression and recovery in patients on chemotherapeutic regimens.[7] However, there are certain limitations of

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impedance counts as cell size analysis cannot discriminate platelets from other similar-sized particles.[8] Automated counting is still controversial in the case of samples from thrombocytopenic or other patients in whom other small particles could generate electrical or optical signals that are similar to platelets, such as debris and red cell fragments. On the other hand, the presence of large platelets beyond the upper threshold may lead to underestimation of the platelet counts**.** The use of multiple light scatter parameters rather than impedance alone has improved the ability to discriminate platelets.[4]

In thrombocytopenic patients, especially those with hematological neoplasms, ITP, febrile neutropenia and acute febrile illnesses (dengue, malaria, etc.) increasing the accuracy of reporting platelet counts would definitely augment clinical decision making. The index study, by analyzing the correlation between the manually performed platelet counts and the platelet counts obtained from automated analyzer, intends to address this basic yet indispensable conundrum that brings clinical and laboratory disciplines at crossroads to each other. It also aims to study the relation, if any, between the platelet count (automated) and platelet indices like Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) and to assess the possible role of these parameters in certain defined situations.

Materials and Methods

This cross-sectional study was carried out on the indoor patients of Government Medical College, Jammu and Associated Hospitals who were referred to the Hematology wing of the Department of Pathology by various clinical departments for routine Complete Blood Cell Counts. The study was prospective in nature and spanned a period of one year (November 2016 to October 2017). Clearance was obtained from the Institutional Ethics Committee. The blood samples of 200 patients were included in the study that were selected from the laboratory entry register using table of random numbers generated by OpenEpi software for windows version 3.01 available at www.OpenEpi.com. Due informed and written consent was taken from the patients.

Inclusion Criteria

1. The indoor patients who were referred to the Department of Pathology Government Medical College Jammu, from various clinical departments of the hospital and its other associated hospitals, for routine complete blood counts, irrespective of age and sex were included.

Exclusion Criteria

1. Clotted and haemolyzed samples in cases where patients could not be accessed for repeat sampling.
2. Patients who were not ready to give consent.

The EDTA anticoagulated venous blood samples (1.2mg of the anhydrous salt per ml of blood) of patients received in the laboratory were evaluated by 2 techniques for platelet estimation:-

Automated Method for the Assessment of Platelet Count

Each sample of blood was thoroughly mixed on an automated mixer and a complete blood count including the platelet count was obtained using 5-part Differential Auto Hematology Analyzer Mindray BC-5800. Platelets were counted & sized in automated counter by electrical impedance method (also known as Coulter Method).[9]

Assessment of Platelet Count on Leishman Stained Peripheral Blood Smear

Blood smears were made from the venous blood samples collected in EDTA vacutainer tubes and stained using Leishman's stain following standard protocol.[10] The number of platelets, on an average, per oil immersion field in a count of successive ten oil immersion fields was calculated which was multiplied by 20,000 for rough calculation of platelet count. It has proved to be a reliable estimation of platelets and was used to yield a platelet count estimate in lacs/mm^3.[11,12]

Normal Platelet count ranges from 150x10^9/L to 450x10^9/L.[1,2,13]

A platelet count less than 150x10^9/L was defined as thrombocytopenia while a count more than 450x10^9/L was defined as thrombocytosis.[13,14] Thrombocytopenia was further subdivided into mild (platelet count 100x10^9/L to <150x10^9/L), moderate (platelet count 50x10^9/L to <100x10^9/L) and severe (platelet count <50x10^9/L).[14]

The Mean Platelet Volume (MPV) and the Platelet Distribution Width (PDW) were also measured and the set ranges of these parameters were given by the auto analyzer Mindray BC-5800. MPV was defined as the measurement of the average size of platelets in blood as calculated by the machine while the PDW was used as a measure of platelet anisocytosis.[9]

The paired student t test was applied for comparison between manual and automated platelet counts while the unpaired student t test was used for comparison between thrombocytopenic and non-thrombocytopenic patients for both MPV and PDW. Pearson's correlation coefficient was calculated to study the correlation between automated and manual platelet count. P value $\leq$ 0.05 was taken as significant.

Results and Discussion

The age distribution of patients included in this study varied over a wide range from 1 year to 84 years. The mean age was observed to be 26.76 years (± 21.37 years). A nearly equal distribution of male and female patients was observed in the study cohort with a male to female ratio of 1.02:1.

Platelet Count

Table 1 depicts platelet counts categorized as mild, moderate, severe thrombocytopenia, normal platelet count and thrombocytosis, based on the counts obtained by the analyzer.

1. In the present study, platelet counts, on estimation by analyzer, varied from 4 x 10^9/L to 1153 x 10^9/L.
2. 78 patients (39.0%) had thrombocytopenia

3. 11.5% patients had mild thrombocytopenia.16.0% patients had moderate thrombocytopenia.
4. 11.5% patients suffered from severe thrombocytopenia.
5. 50.5% patients were observed to have normal platelet count.
6. 10.5% patients had thrombocytosis.

Table 2 depicts platelet count categorized as mild, moderate, severe thrombocytopenia, normal platelet count and thrombocytosis based on the counts obtained by manual method.

1. In the present study, the manual platelet count varied from 20 x 10^9/L to 1100 x 10^9/L.
2. 46 patients (23.0%) had thrombocytopenia.
3. 11.5% patients had mild thrombocytopenia.
4. 6.5% patients were observed to have moderate thrombocytopenia.
5. 5.0% patients suffered from severe thrombocytopenia.
6. 65.0% patients were observed to have normal platelet count.
7. 12.0% patients had thrombocytosis.

Lowest platelet counts by both the analyzer and manual method were seen in cases of aplastic anaemia, ITP, blunt trauma abdomen, dengue fever, snake bite, a case of pregnancy (33wks), acute leukaemia (AML as well as ALL), Megaloblastic anaemia, dual deficiency anaemia and anaemia of chronic disease. Highest platelet counts were seen in cases with infections, lower limb gangrene, puerperial sepsis, burns, bronchopneumonia, acute viral hepatitis, pyrexia of unknown origin, chronic myeloid leukemia, rheumatoid arthritis, hypothyroidism and endometrial carcinoma.

The mean platelet count estimated by the automated hematology analyzer was significantly lower than the mean platelet count estimated manually on peripheral smear ($P < 0.001$). However, a strong positive correlation was observed between the platelet counts assessed by automated analyzer and manual method as pointed by the Pearson's correlation coefficient (r) value of 0.857 depicted on the scatter plot (Fig. 1).

Spurious results on automated counter may be obtained due to inadequate quantity of blood sample, interference by anticoagulant(s) in peripheral blood, peculiar changes associated with the pathology in the patient, and non-adherence to technical considerations of automated analyzers. When ethylene diamine tetra-acetic acid (EDTA) is used as the anticoagulant, there is a possibility that spurious thrombocytopenia may be reported. It has been suggested that the exposure to EDTA results in divulgence of otherwise hidden epitopes in the glycoprotein alpha IIb/beta IIIa complex on platelet membrane. These epitopes are attacked by circulating (auto) antibodies which ultimately results in platelet agglutination and spurious thrombocytopenia also called EDTA-dependent pseudothrombocytopenia (EDTA-PTCP). Spuriously low platelet counts related to EDTA may also result from other less well-known mechanisms including platelet rosetting around white blood cells also known as WBC satellitism and PLT-WBC aggregates. This phenomenon appears more frequently in severely ill patients, in association with autoimmune, neoplastic, atherosclerosis-related, and liver diseases. In the majority of patients, EDTA-PTCP appears during hospitalization, indicating that the antibody is an acquired one.[15] A higher incidence of pseudothrombocytopenia has been observed in platelet counts assessed with automatic blood analyzers in patients with counts less than 100 x 10^9/L. Delay in sample processing has been identified as one of the possible causes responsible for pseudothrombocytopenia on automated analyzers.[16] It has been highlighted that precise counting of platelets is difficult particularly in the low thrombocytopenic range or when large platelets exist. Moreover Coulter counters (based on impedance technology) have been observed to yield significantly lower platelet counts in comparison to analyzers based on two-dimensional laser light scatter. This is especially true when the cause of thrombocytopenia is peripheral platelet consumption and such differences are more marked in samples from severely thrombocytopenic individuals with large platelets on the blood film.[17] A comparison of the analytical performance of the manual and of the automated counting procedure of platelets has shown that the automated count is more precise because of the higher number of platelets counted in the range >30 X 10^9plt/L. However, when platelet counts are below 30 X 10^9 /L, it is advisable to replace the automated platelet counting by the manual counting procedure. Counts lesser than 7 X 10^9plt/L (the lower limit of manual quantification) should not be reported to the physician because the imprecision below this is too high (>15%).[18] Hence in patients with thrombocytopenia and megathrombocytes, the manual platelet count is particularly beneficial in view of the lower accuracy of automated analyzers in estimating platelet counts in such patients.[4,19] The correlation between the automated and manual platelet counts has been studied by many workers who have analyzed the platelet counts in different target populations by using different automated analyzers. Table 3 summarizes the findings of these studies. In majority of these studies a strong correlation has been reported between the automated platelet count on analyzer and manual count and a similar inference was arrived at in the present study as well.

Platelet Indices: Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW)

The MPV and PDW observed in the study cohort are depicted in Table 4. The cases were categorized as thrombocytopenic and non-thrombocytopenic on the basis of the platelet count assessed on the automated analyzer.

1. The mean MPV in thrombocytopenic patients [10.36 (± 1.96)] was found to be significantly higher than in non-thrombocytopenic patients [9.61 (± 1.39)] ($P = 0.002$).
2. The mean PDW in thrombocytopenic patients [17.94 (± 0.98)] was also found to be significantly higher than in non-thrombocytopenic patients [16.41 (± 0.82)] ($P = 0.002$).

3. An inverse relationship was observed between the platelet count, as estimated on the automated analyzer, and the Mean Platelet Volume and depicted on the scatter plot (Fig. 2).

It has been widely documented that mean platelet volume shows an inverse but nonlinear relation with the platelet count in normal individuals as well as in a number of common clinical conditions and is of value in assessing platelet production in the thrombocytopenic patient.[1,7,24,25] The MPV can be used to assess the degree of bone marrow suppression and predict haematological recovery in patients having thrombocytopenia and is particularly useful in the management of thrombocytopenia due to sepsis.[25] Patients with ITP are reported to have higher values of mean platelet volume and platelet distribution width and the inverse relationship between MPV and platelet count, in these cases, is linear. Inverse relationship between MPV and platelet count is also seen in cases of anemia, thus leading to the inference that platelet parameters vary in different clinical conditions and they should be used routinely to aid in diagnosis.[6] Large platelets have been observed in peripheral smears of patients with ITP who had platelet count lower than 50 x 10^9/L.[26] Significantly increased mean platelet volume has been reported in cases with megathrombocytes in peripheral blood.[17] It has also been suggested that high MPV indicates platelet activation and may be used as an initial marker to suspect dengue fever in a case of thrombocytopenia.[27] In a univariate analysis it was revealed that after adjusting for other cardiovascular risk factors, the independent risk factors that remain significantly associated with MPV included female gender, diabetes, metabolic syndrome, serum triglyceride, hypertension, and prehypertension.[28]

Table 1: Platelet count assessed by analyzer in 200 patients

Platelet count category	**No. of patient**	**Percentage (%)**	**Mean platelet count by Analyzer (± SD) (x 10^9/L)**
Severe thrombocytopenia (<50 x 10^9/L)	23	11.5	23.91 (± 13.14)
Moderate thrombocytopenia (50 – <100 x 10^9/L)	32	16.0	76.09 (± 15.11)
Mild thrombocytopenia (100 – <150 x 10^9/L)	23	11.5	132.30 (± 12.58)
Normal platelet count (150 – 450 x 10^9/L)	101	50.5	241.48 (± 74.74)
Thrombocytosis (>450 x 10^9/L)	21	10.5	557.24 (± 155.91)
Total	**200**	**100.0**	**210.59 (± 161.65)**

Table 2: Platelet Count assessed by Manual method in 200 patients

Platelet count category	**No. of patients**	**Percentage (%)**	**Mean platelet count by Manual Method (± SD) (x 10^9/L)**
Severe thrombocytopenia (<50 x 10^9/L)	9	5.0	26.67 (± 9.54)
Moderate thrombocytopenia (50 – <100 x 10^9/L)	13	6.5	86.08 (± 36.20)
Mild thrombocytopenia (100 – <150 x 10^9/L)	24	11.5	125.25 (±13.43)
Normal platelet count (150 – 450 x 10^9/L)	130	65.0	273.82 (± 77.40)
Thrombocytosis (>450 x 10^9/L)	24	12.0	606.67 (± 123.49)
Total	**200**	**100.0**	**272.60 (± 163.81)**

Table 3: Summary of the studies comparing the automated platelet count with the manual platelet count

Study	Study population	*N*	Name of Analyzer	Mean platelet count by Analyzer (x 10^9/L)	Mean platelet count by Manual method (x 10^9/L)	P value	Correlation coefficient (r) value
Lawrence JB et al 1995.[20]	Thrombocytopenic patients (4 – 30 x 10^9/L)	20	Sysmex NE-8000	14.40	16.48	0.038	0.921
Malok M et al 2007.[5]	Randomly selected hospitalised patients	184	Coulter LH750	268	269	0.87	0.9
Ike SO et al 2010.[21]	Healthy adults as well as inpatients	60	Sysmex KX-21N	265.5 ± 18.94	251.7 ± 18.58	<0.001	0.779
Bakhubaira et al 2013.[22]	Randomly selected hospitalised patients	190	Sysmex KX-21N	245.7 ± 109.8	225.2 ± 95.4	0.44	0.563
Momani et al, 2015.[12]	Randomly selected hospitalised patients	220	Sysmex KX-21	275	269	>0.05	NA
Babadoko AA et al, 2016.[23]	Randomly selected hospitalised patients	100	Swelab Alpha, Sweden	278.10 ± 162.00	244.80 ± 171.80	0.043	0.531
Present study	**Randomly selected hospitalised patients**	**200**	**Mindray BC-5800**	**210.59 ± 161.65**	**272.60 ±163.81**	**<0.001**	**0.857**

N =Number of patients in the study; NA = not available

Table 4: Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW) in Thrombocytopenic and Non-Thrombocytopenic Patients (categorization based on Analyzer)

Category	No. of patients	Percentage (%)	Mean platelet count (± SD) (x 10^9/L)	Mean MPV (± SD) (fL)	Mean PDW (± SD)
Thrombocytopenic patients (<150 x 10^9/L)	78	39.0	77.56 (± 44.16)	10.36 (± 1.96)	17.94 (±0.98)
Non-thrombocytopenic patients (≥ 150 x 10^9/L)	122	61.0	295.79 (±151.56)	9.61 (± 1.39)	16.41 (±0.82)

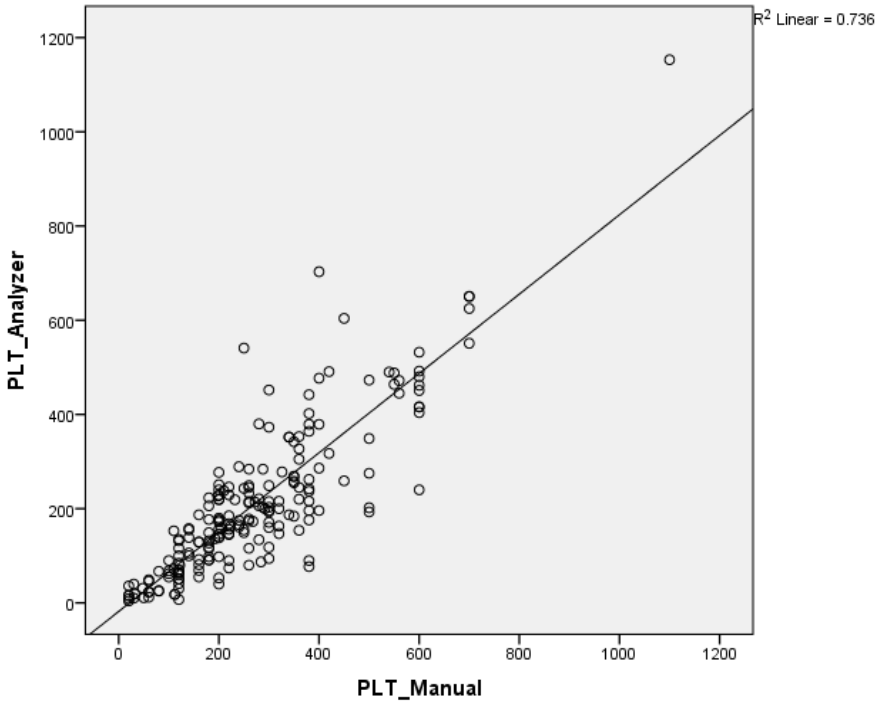

Fig. 1: Scatter plot showing strong positive correlation between manual and automated platelet counts

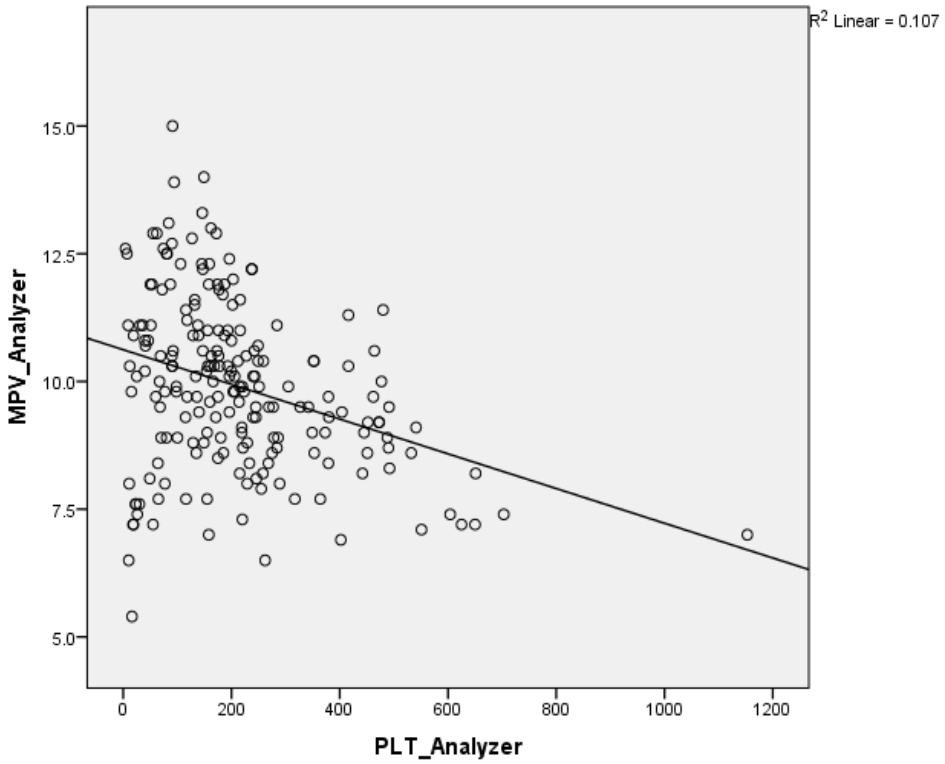

Fig. 2: Scatter plot showing inverse relation between automated platelet count and MPV

Conclusion

Thus, it can be safely concluded that a good quality hematology analyzer is crucial for quick and accurate complete blood count evaluation but at the same time all those blood samples which show abnormal results or low platelet counts on analyzers should be confirmed by manual count on peripheral smear. The platelet indices such as Mean Platelet Volume (MPV), Plateletcrit and Platelet Distribution Width (PDW) are the additional features provided by the analyzers that can indicate the pathogenesis of altered platelet count especially in cases of thrombocytopenia. Thus, the present study is an attempt also, on how to correct ourselves and the machine so that it is beneficial for patient care.

Conflicts of Interest: Nil.

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