https://doi.org/10.33472/AFJBS.6.11.2024.1181-1198

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

COMPARATIVE STUDY OF PLATELET COUNT ESTIMATION BY MANUAL AND AUTOMATED METHODS IN A TERTIARY CARE HOSPITAL IN INDIA

Dr. Madhusmita Samal[1*], Dr. V. Eswari[2],

[1*]Final Year Post Graduate, Dept. Of Pathology, Meenakshi Medical College Hospital & Research Institute, Kanchipuram, Tamil Nadu.

2Professor & Hod, Dept. Of Pathology, Meenakshi Medical College Hospital & Research Institute, Kanchipuram, Tamil Nadu.

Corresponding Email: m.samal07@gmail.com

Article Info

Volume 6, Issue 11, July 2024

Received: 23 May 2024

Accepted: 20 June 2024

Published: 09 July 2024

doi: 10.33472/AFJBS.6.11.2024.1181-1198

ABSTRACT:

Context:
Platelet count estimation is an essential element of routine hemogram. It is crucial in the management of hemorrhagic disorders. Manual platelet count correlation using a peripheral smear or Neubauer chamber is one of the methods used when automated platelet counts are found to be abnormal (especially in cases of severe thrombocytopenia). It is neither practicable nor economical to routinely corroborate abnormal platelet values by immunological platelet estimation (flowcytometric analysis using antibodies against platelet cell surface antigens such as CD41, CD42, CD61)[1]. Hence, manual platelet count estimation remains a more feasible alternative for review of low machine counts across laboratories around the world.

Aim:
We sought to comparatively analyze the platelet estimation derived from manual modality with respect to that derived from automated haematology analyzer.

Methods:
This prospective cross sectional study included both in-patients and out-patients who were admitted in or presented to Meenakshi Medical College Hospital & Reserch Institute (MMCHRI), Kanchipuram, Tamil Nadu between Mar 2024 and May 2024. Blood samples were retrieved from 500 patients of both paediatric and adult age groups and either gender. Samples were collected in EDTA vacutainers and peripheral smears were prepared from the same using Leishman's stain. Platelet enumeration was done both by automated and manual slide method and the results compared.

Conclusion:
We noted an excellent correlation between the platelet counts obtained by traditional slide method and automated cell counter method at normal ranges of platelet values. However, at markedly high (Platelet count $\geq 700\times10^3/\mu L$) and low counts (Platelet count $\leq 20x10^3/\mu L$) and in cases with high mean platelet volume (MPV >14 fL), relatively poor correlation between both the methods was noted. Hence, this study underscores the importance of manual verification of platelet counts in thrombocytopenic patients, especially those with values close to the threshold for prophylactic platelet transfusion ($\leq 20\times10^3/\mu L$).

Keywords: Platelet count. Automated platelet count. Manual platelet count. Peripheral blood smear. Automated cell counter. Automated haemocytometer. Haematology analyzer. Impedance. Thrombocytopenia. Thrombocytosis

1. INTRODUCTION

At the end of the nineteenth century, Italian pathologist Giulio Bizzozero identified platelets as an important blood component and observed their fundamental role in the mechanism of blood clotting.[2] He implicated their role in the formation of white thrombus capable of arresting hemorrhage. Another important milestone in the history of hematology was the discovery of the hematopoietic function of bone marrow by Giulio Bizzozero and Ernst Neumann in 1868. A lot has changed in the world of hematology and laboratory diagnosis ever since. We have an array of sophisticated machines such as hematology analyzers, flow cytometers, coagulation analyzers and automated slide stainers at our disposal now. However,

much still rests on the quality of smears and staining and manual microscopic method when it comes to platelet enumeration.

Platelets (aka thrombocytes) are the smallest blood cells measuring 1.5-3 µm in diameter, i.e., about one fifth the diameter of a mature RBC. They are anucleated, discoid/plate shaped circulating fragments of megakaryocytes with a short lifespan of ~7-10 days. Structurally, they have a phospholipid bilayer plasma membrane that is the site of expression of various surface markers like CD9, CD36, CD41, CD42, CD63, GPCR, IIbIIIa, and GLUT-3.[3] These surface receptors trigger the release of α granules which mediate various biological platelet functions.[3] Functionally, platelets are important physiologic mediators of hemostasis and vascular repair. But their biological functions are considered to be far beyond that. Their role has been implicated in inflammation, atherosclerosis, antimicrobial host defense, angiogenesis, wound healing and oncogenesis.[4]

There are various methods of platlet estimation in practice such as, manual Neubaeur chamber counting, counting by peripheral blood smear, automated hemocytology analyzer counting, immunoplatelet counting and counting by radioisotope labeling technique. International council for standardization in hematology (ICSH) and International society for laboratory in hematology (ISLH) have recommended immuonoplatelet counting as the gold standard reference method for the calibration of automated hematology analyzers.[5] However, platelets are conventionally counted by two methods in routine everyday practice, manual microscopic method and automated analyzer method. It is standard protocol in most hematology laboratories the world over to perform a manual microscopic review of Leishman stained peripheral blood smears for abnormal platelet values generated by automated cell counters.

Manual counting of platelet is done in three ways.

The first employs counting of platelets in the Neubauer chamber after dilution with a suitable diluting fluid (e.g., 1% ammonium oxalate).

The second uses Leishman's stained peripheral blood smear for platelet enumeration.

The third method involves counting platelets simultaneously with RBCs till a count of 1000 RBCs is reached. The number of platelets per 1000 RBCs thus obtained is multiplied by the automated RBC count ($x10^6$ cells/µl) to give an approximate manual count ($x10^3$ cells/µl).

The use of phase contrast microscope for manual counting of platelets was considered the gold standard once but has since been discontinued as it was not only time-consuming and cumbersome but also imprecise at lower values.

Automated platelet enumeration involves one or more of the following principles : electrical impedance, conductivity, optical scattering (light diffraction or fluorescence techniques) and immunologic flow cytometry (monoclonal antibodies directed against the platelet membrane glycoproteins). The automated 5-part analyzer that we employed for the study measures platelets by flow cytometry based principles of light scattering. Platelets are identified by their size (low angle light scatter) and refractive index (high angle light scatter). Giant platelets are similarly identified on the basis of their size and refractive index. Thus they can sometimes get misread as RBCs by the hemocytometer due to their large size and lack of nucleus. Besides platelet count, it also provides other platelet indices like mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW).

Although automated methods are generally deemed to be more accurate, they are prone to interferences from particles of similar sizes and/or light scatter properties such as RBC fragments, apoptotic WBC fragments, bacteria, fungi, lipids and cryoglobulins etc.[6] They also suffer from preanalytical pitfalls like platelet clumping and an array of other such spurious parameters. Hence, it is standard protocol in most hematology laboratories to review abnormal/suspicious platelet values generated by automated cell counters by using an alternate method (such as manual examination of Leishman stained peripheral blood smears).

Conversely, even though manual haemocytometry is cost effective and easily achievable in resource limited laboratory set-ups, it can be time-consuming, cumbersome and requires skilled expertise. It also is non-standardisable and imprecise, more so at lower and higher values of platelet counts.[1] Due to the inherent subjectivity involved in manual platelet estimation, a significant inter-observer variability has also reported (coefficient of variations in the range of 15-40% as per various studies[7]).

A knowledge of the limitations of both methods of platelet estimation (automated versus manual) is essential for management of thrombocytopenia. It would also aid in facilitating clinical consensus regarding prophylactic platelet transfusion thresholds and management of blood product inventory in the blood bank.

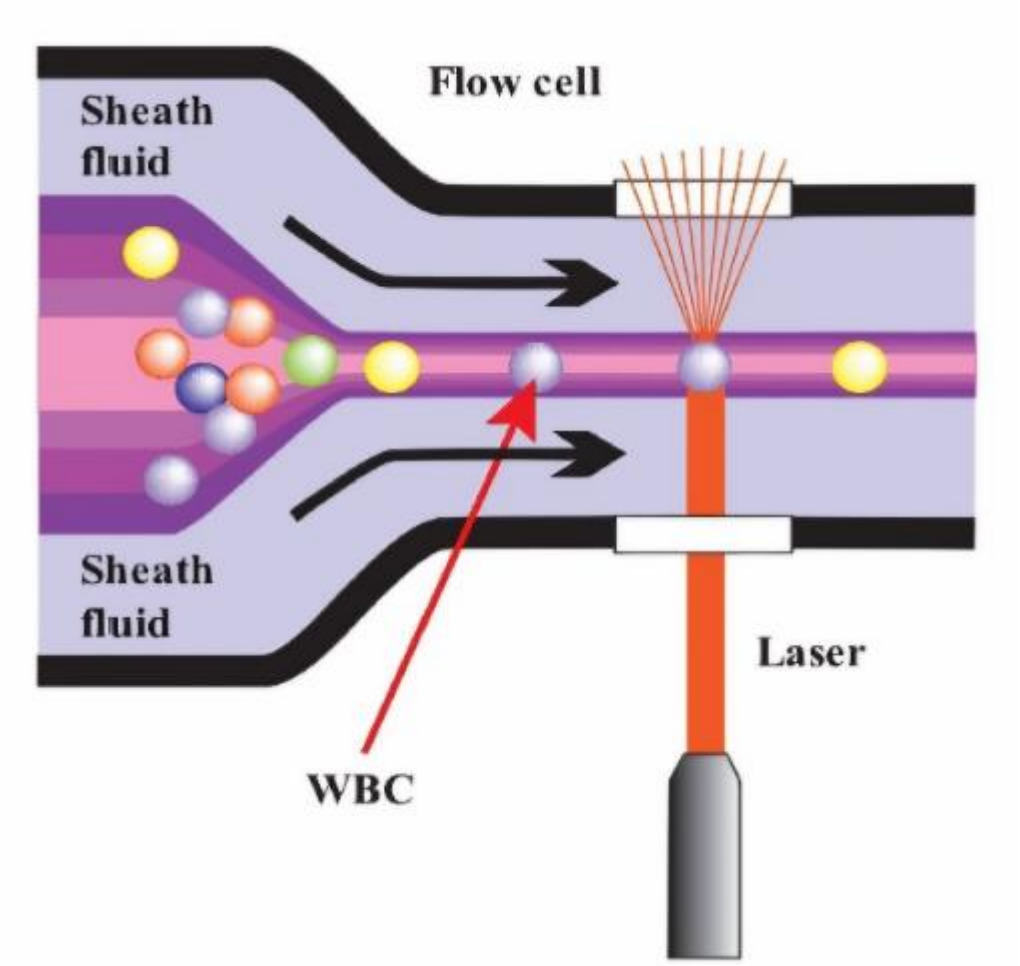

Fig.1 - Working principle of Erba H560 hematology analyzer

Source: Internet. Preface to clinical case study for Mindray haematology analyser.

Aims & Objectives

1. The present study aimed to comparatively analyse the accuracy of platelet estimation derived by manual modality (Leishman stained thin peripheral smear) with respect to that derived from impedance based automated haematology analyzer.
2. Additionally, we also tried to ascertain the inter-observer variability between two independent observers for manual platelet counts at high, low and normal ranges.

2. MATERIALS AND METHODOLOGY

1. Study Design : Cross sectional prospective study.
2. Study Area : Clinical Pathology Laboratory, Department Of Pathology, MMCH&RI
3. Study Population : Patients admitted in the in-patient department (medical ward, pre and post-operative wards, ICUs) and out-patients undergoing routine/serial blood sampling at our hospital.
4. Age Group : Our study was not age specific and included venous blood samples both from paediatric and adult populations of either gender.
5. Sample Size : 500
6. Study Period : March 2024 to May 2024

Inclusion Criteria

1. Patients of all ages (paediatric, geriatric, normal adult population) and either gender with normal, low or high platelet counts were included in the study.

Exclusion Criteria

1. Blood samples meeting the sample rejection criteria of the laboratory were excluded from this study. Clotted/hemolysed samples, samples sent in inappropriate blood collection tube and unlabeled/mislabeled samples were rejected.
2. Smears showing platelet clumping on peripheral smear were excluded.
3. Patients with a previous recorded history of EDTA-dependent pseudothrombocytopenia (PTCP) were excluded from the study.
4. Samples established to have gram positive coccemia on peripheral smear examination were excluded as the cocci (0.5-2.0 µm) can lead to spuriously high automated platelet counts.[8]

Work Flow

1. This study was conducted in the clinical pathology laboratory of a tertiary care hospital in south India (Department of Pathology, MMCHRI) after obtaining ethical clearance from the institutional ethics committee.
2. The study group of 500 patients included both in-patients and out-patients of either gender who were being sampled for routine or serial complete blood count (CBC) analysis as part of their treating physician's advice.
3. The blood samples were collected by the phlebotomist/nurse at the collection centre/ward/ICU of MMCHRI as per routine practice.
4. The peripheral venous sample was collected in EDTA (ethylenediamine-tetraacetic acid) containing vacutainers. All precautions were taken to ensure adequate mixing of the blood with the anticoagulant.
5. Leishman stained thin peripheral smears were prepared for manual counting of platelets under 100x oil immersion fields (OIFs). The peripheral smears were prepared by the standard 'wedge' procedure.
6. The counting of platelets (both by manual and machine) was performed within 2 hours of collection and the values recorded.
7. The automated counts were performed by a calibrated and adequately quality-controlled (both internal and external) Erba H560 5-part hematology analyser.
8. All machine derived platelet indices such as mean platelet volume (MPV), plateletcrit (PCT) and platelet distribution width (PDW) were recorded for all the samples.
9. Leishman stained peripheral smears were observed under Olympus CH20i light microscope using 100x oil immersion lens.

10. Manual platelet count estimation was done by counting the number of platelets in 10 OIFs in an appropriate area (junction of body and tail where cells are monolayered).
11. The formula used to derive manual platelet count was [15,000 x Average platelet count in 10 OIFs] lacs/µL.
12. The results derived from automated hemocytometry were categorized into three groups :
13. Group A - Thrombocytopenia (<1.5 lacs/µL)
14. Group B - Normal (1.5-4.5 lacs/µL)
15. Group C - Thrombocytosis (>4.5 lacs/µL).
16. Platelet count derived from manual method was statistically compared with that derived from automated hemocytometry using statistical tools.
17. Additionally, manual platelet counts obtained by two different observers (blinded) were compared across all ranges.
18. An acceptable difference in results between the automated analyser counts and manual peripheral smear based counts was deemed to be up to 25% as suggested by Clinical Laboratory Improvement Amendments 2019 (CLIA).[9]

Data Analysis

The data was entered into Microsoft excel sheet and exported to SPSS 23.0 for analysis. Central tendencies (Mean, median and mode) and standard deviation of quantitative variables were calculated and compared. The sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio were calculated for the machine and automated counts. Correlation analysis was done by Pearson correlation method to see the association between both the variables. And for 'p' value, one way analysis of variance was done. A p-value of <0.05 was considered to be statistically significant. Simple linear regression analysis and coefficient of determination (R2) for correlation analysis between the two methods was used. Independent't' test was done to determine if there is a significant difference between the means of the manual platelet counts as observed by two different observers and how they are related. All tests were applied at a 99% level of significance. Agreement between the platelet count estimates of two different observers was assessed using Bland-Altman's plot.

3. RESULT

Our study constituted of 500 cases (422 adults and 78 children). The cases were categorised into 3 different groups based on their automated analyzer platelet count.

Group	Sample Character	Platelet Count	No. of Patients
A	**Thrombocytopenia**	**<1.5 lacs/µL**	**210**
B	**Normal Platelet Count**	**1.5 lacs - 4.5 lacs/µL**	**214**
C	**Thrombocytosis**	**>4.5 lacs/µL**	**76**

Table 1 - Categorisation of patients into different groups based on their platelet count.

The following were our sample sources: In-patients - 53.2% (n=266), preoperative check-ups - 15% (n=75), post-operative patients - 6.6% (n=33), OPD consultations - 22% (n=110) and routine health check-ups/ health schemes - 3.2% (n=16) (Fig.2).

The reason for platelet count estimation in the 266 in-patient admissions (both ward and ICU) as mentioned in the laboratory requisition forms were as follows : Dengue and other viral fevers - 19% (n=95), Scrub typhus - 3.2% (n=16), Malaria - 5.4% (n=27), Acute bacterial infections (MC - UTI followed by LRTI) - 12.4% (n=62), Sepsis with/without sepsis related complications like shock, MODS and DIC - 6.2% (n=31), CLD - 2.6% (n=13), CKD - 3.8%

(n=19), bleeding/clotting disorders - 0.6% (n=3). No specific pathologies were considered for exclusion from the study.

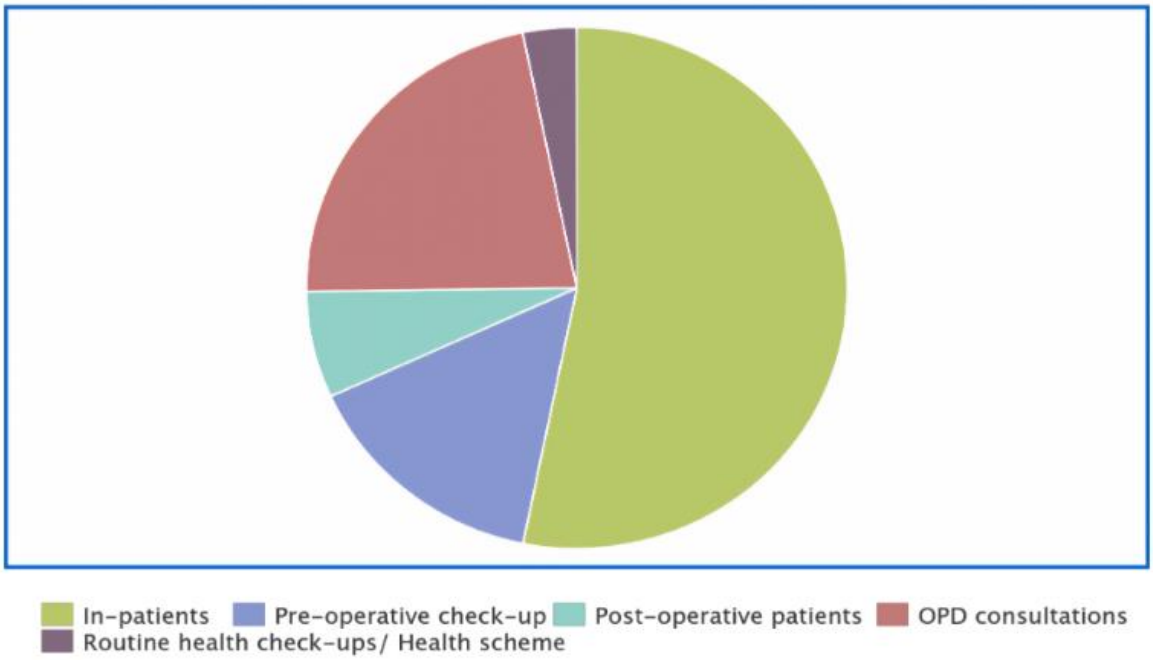

Fig.2 - Percentage distribution of sample sources

The median age of the study population was 38 years with an interquartile range of 34 years. The median age for Group A (Thrombocytopenia) was 30 years and the interquartile range was 33 years. The median age for Group B (Normal platelet count) was 36 years and the interquartile range was 35 years. The median age for Group C (Thrombocytosis) was 49 years and the interquartile range was 15 years.

Mean $\overline{x}$	**38.123**
Median $\widetilde{x}$	**38**
Mode	**60**
Range	88
Minimum	1
Maximum	89
Count *n*	500
Sum	19061.5
Quartiles	Quartiles: Q_1 --> 20 Q_2 --> 38 Q_3 --> 54
Interquartile Range IQR	34

Table 2 - Descriptive statistics for age in the study population

Mean $\overline{x}$	**36.228571428571**
Median $\widetilde{x}$	**30**
Mode	**60**
Range	82
Minimum	7
Maximum	89
Count *n*	210
Sum	7608
Quartiles	Quartiles: Q_1 --> 19 Q_2 --> 30 Q_3 --> 52
Interquartile Range IQR	33
Outliers	none

Table 3 - Descriptive statistics for age in Group A (Thrombocytopenia)

Result Details & Calculation: Group A

X Values
∑ = 172.7
Mean = 0.822
∑(X - Mx)2 = SSx = 30.125

Y Values
∑ = 180.735
Mean = 0.861
∑(Y - My)2 = SSy = 48.504

X and Y Combined
N = 210
∑(X - Mx)(Y - My) = 33.07

R Calculation
r = ∑[(X - My)(Y - Mx)] / √[(SSx)(SSy)]

r = 33.07 / √[(30.125)(48.504)] = 0.8651

Meta Numerics (cross-check)
r = 0.8651

Mean $\overline{x}$	**36.81308411215**
Median $\widetilde{x}$	**36**
Mode	**2**
Range	76
Minimum	1
Maximum	77
Count *n*	214
Sum	7878
Quartiles	Quartiles: Q_1 --> 20 Q_2 --> 36 Q_3 --> 55
Interquartile Range IQR	35
Outliers	none

Table 4 - Descriptive statistics for age in Group B (Normal Platelet Count)

Result Details & Calculation: Group C

X Values
∑ = 454.17
Mean = 5.976
∑(X - Mx)2 = SSx = 120.634

Y Values
∑ = 498.495
Mean = 6.559
∑(Y - My)2 = SSy = 426.517

X and Y Combined
N = 76
∑(X - Mx)(Y - My) = 197.454
R Calculation

r = ∑([X - My)(Y - Mx)] / √[(SSx)(SSy)]

r = 197.454 / √[(120.634)(426.517)] = 0.8705
Meta Numerics (cross-check)
r = 0.8705

Result Details & Calculation: Group B

X Values
∑ = 611.848
Mean = 2.859
∑(X - Mx)2 = SSx = 123.639

Y Values
∑ = 620.73
Mean = 2.901
∑(Y - My)2 = SSy = 117.186

X and Y Combined
N = 214
∑(X - Mx)(Y - My) = 108.229

R Calculation
r = ∑[(X - My)(Y - Mx)] / √[(SSx)(SSy)]

r = 108.229 / √[(123.639)(117.186)] = 0.8991

Meta Numerics (cross-check)

Mean $\overline{x}$	**47.092105263158**
Median $\widetilde{x}$	**49**
Mode	**46**
Range	70
Minimum	1
Maximum	71
Count *n*	76
Sum	3579
Quartiles	Quartiles: Q_1 --> 45 Q_2 --> 49 Q_3 --> 60
Interquartile Range IQR	15
Outliers	1, 2

Table 5 - Descriptive statistics for age in Group C (Thrombocytosis)

89.2% (446/500) of all manual platelet counts were found to lie within the acceptable error limit prescribed by CLIA (within 25% of the automated machine value). Singh et al[10] observed similar values (86%) in their study. In our study, the highest concordance between automated and manual values was seen in Group B - normal platelet count (94.39%).

Group	No. within acceptable error	Percentage within acceptable error
All cases (Group A+Group B+Group C)	**446/500**	**89.2%**
Group A (Thrombocytopenia)	**178/210**	**84.76%**
Group B (Normal platelet count)	**202/214**	**94.39%**
Group C	**66/76**	**86.84%**

(Thrombocytosis)		

Table 6 - Manual platelet counts within acceptable error limits (as per CLIA) across different groups

The median machine platelet count was 215 x 10^3/µL while median manual platelet count was 233 x 10^3/µL. The coefficient of variation for automated machine counts vis-à-vis manual counts were 0.46 vs 0.56, 0.27 vs 0.26 and 0.21 vs 0.36, p<0.001 for Group A, Group B and Group C respectively. The sensitivity, specificity, positive predictive value, negative predictive value and likelihood ratio were calculated for the machine and automated counts and showed concordance.

Method	Mean	Median	Standard Deviation	Coefficient of Variation
Automated	**0.82**	**0.88**	**0.38**	**0.46**
Manual	**0.86**	**0.81**	**0.48**	**0.56**

Table 7 - Descriptive summary of platelet counts in Group A (Thrombocytopenia)

Method	Mean	Median	Standard Deviation	Coefficient of Variation
Automated	**2.86**	**2.77**	**0.76**	**0.27**
Manual	**2.90**	**2.7**	**0.74**	**0.26**

Table 8 - Descriptive summary of platelet counts in Group B (Normal platelet count)

Method	Mean	Median	Standard Deviation	Coefficient of Variation
Automated	**5.98**	**5.33**	**1.27**	**0.21**
Manual	**6.56**	**5.4**	**2.38**	**0.36**

Table 9 - Descriptive summary of platelet counts in Group C (Thrombocytosis)

The correlation (R) between manual and machine platelet count in the thrombocytopenic group, normal count group and thrombocytosis group were .865, .899 and .870 respectively, signifying a very good positive correlation. The p value for all 3 groups (Group A - Thrombocytopenia, Group B - Normal count and Group C - Thrombocytosis) was < .00001.
Discrepancies were seen only in cases of marked thrombocytopenia (Plt count ≤ 20x10^3/µL) or those flagged as high MPV (>14 fL) by the hematology analyser.
The correlation between manual and machine count in cases of severe thrombocytopenia (Plt count ≤ 20×10^3/µL) was relatively poorer. R value was .685 (p value <.001) in those with counts between 10 - 20 x 10^3/µL, and .482 (p value .097) in those with counts < 10 x 10^3/µL.
At normal values of platelet counts, an inverse relationship was observed between platelet count and mean platelet volume (MPV). Other studies have shown similar associations.[11]
MPV >12 fL was detected more commonly in patients with thrombocytopenia (79.56%, n=78/102), followed by those with normal counts (21.57%, n=22/102) and only in 2 (1.96%) patients with thrombocytosis.
It was also noted that correlation between manual platelet count and automated count was relatively poor (r=.724, p<.001) in patients with high MPV (>14 fL) as compared to those with normal MPV.

Group	Pearson's Correlation Coefficient (r)	p Value

Group A -Thrombocytopenia (Overall)	**.865**	**<.001**
Marked Thrombocytopenia (10 - 20x10³/µL)	**.685**	**<.001**
Marked Thrombocytopenia (≤ 10x10³/µL)	**.482**	**0.97**
Group B - Normal Platelet Count (1.5 lacs - 4.5 lacs/µL)	**.899**	**<.001**
Group C - Thrombocytosis (>4.5 lacs/µL)	**.870**	**<.001**
Thrombocytosis (>7 lacs/µL)	**.788**	**<.001**
High Mean Platelet Volume (>14 fl)	**.724**	**<.001**

Table 10 - Correlation between manual and automated hematology analyzer platelet counts across different groups

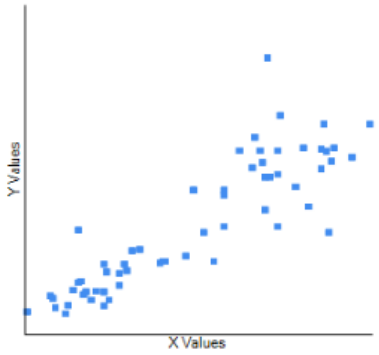

Fig. 3 - Regression analysis scatterplot of group A comparing automated (X axis) to manual (Y axis) platelet counts and showing moderate dispersion

Interpretation: Group A

The Pearson's correlation coefficient (R value) was calculated at .865 for Group A (Thrombocytopenia) which signifies a very strong positive correlation. It is indicative of the strength of the relationship/association between the two variables such that high X variable scores go with high Y variable scores (and vice versa) and low X variable scores go with low Y variable scores (and vice versa).

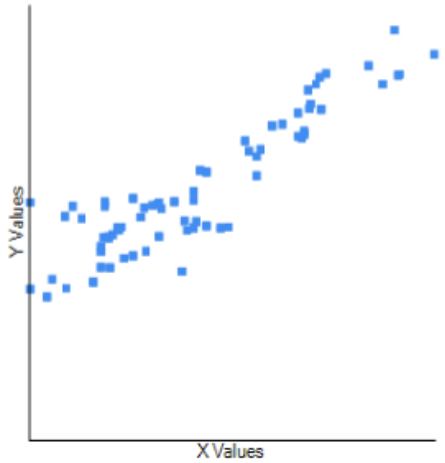

Fig. 4 - Regression analysis scatterplot of group B comparing automated (X axis) and manual (Y axis) platelet counts and showing mild dispersion

Interpretation: Group B

The Pearson's correlation coefficient (R value) was calculated at 0.899 for Group B (Normal platelet count) which signifies a very strong positive correlation. It is indicative of the strength of the relationship/association between the two variables such that high X variable scores go with high Y variable scores (and vice versa) and low X variable scores go with low Y variable scores (and vice versa).

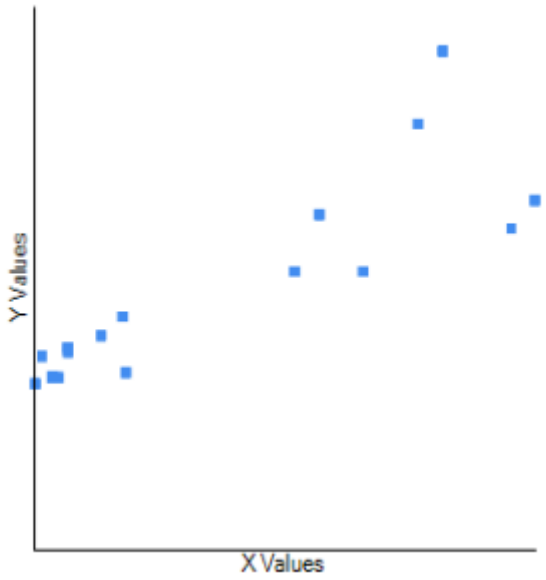

Fig.5 - Regression analysis scatterplot of group C comparing automated (X axis) and manual (Y axis) platelet counts and showing moderate to wide dispersion

Interpretation: Group C

The Pearson's correlation coefficient (R value) was calculated at 0.870 for Group C (Thrombocytosis) which signifies a very strong positive correlation. It is indicative of the strength of the relationship/association between the two variables such that high X variable

scores go with high Y variable scores (and vice versa) and low X variable scores go with low Y variable scores (and vice versa).

Observer	Group	Mean	Median	Standard Deviation	Coefficient of variation
Observer 1	All samples	2.6	2.33	2.2	0.85
	Group A	0.86	0.81	0.48	0.56
	Group B	2.9	2.7	0.74	0.26
	Group C	6.56	5.4	2.38	0.36
Observer 2	All samples				
	Group A	0.9	0.9	0.42	0.47
	Group B	2.9	2.7	0.73	0.25
	Group C	6.44	5.39	2.01	0.31

Table 11 - Comparison between the manual platelet counts derived by two different observers across different groups

Difference Score (T value) calculation

Observer 1 :
N1 : 500
df1 = N - 1 = 500 - 1 = 499
M1 : 2.6
SS1 : 2202.76
s21 = SS1/(N - 1) = 2202.76/(500-1) = 4.41

Observer 2 :
N2 : 500
df2 = N - 1 = 500 - 1 = 499
M2 : 2.6
SS2 : 2438.16
s22 = SS2/(N - 1) = 2438.16/(500-1) = 4.89

T-value Calculation

s2p = ((df1/(df1 + df2)) * s21) + ((df2/(df2 + df2)) * s22) = ((499/998) * 4.41) + ((499/998) * 4.89) = 4.65

s2M1 = s2p/N1 = 4.65/500 = 0.01
s2M2 = s2p/N2 = 4.65/500 = 0.01

t = (M1 - M2)/√(s2M1 + s2M2) = 0/√0.02 = 0

Interpretation:
The t value is 0.00387. The p value is .498456. And the result is not significant at p<.05.

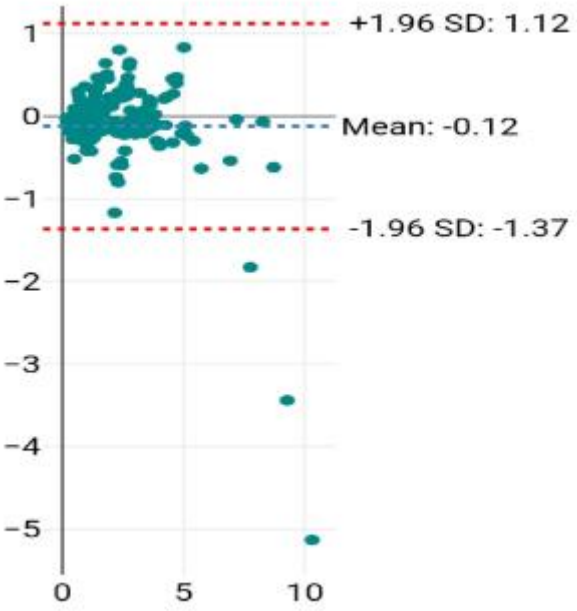

Fig. 6 - Bland-Altman plot showing agreement between manual platelet count values of two different observers

Interpretation:
Most values in the Bland-Altman plot are concentrated around the mean and within the 1.96SD. This signifies a strong agreement between the two measurements (observer 1 and observer 2) of the same variable (manual platelet count).

X axis - Mean of manual platelet counts as observed by two different observers

Y axis - Difference of manual platelet counts as observed by two different observers

4. DISCUSSION

Mean platelet counts (both automated and manual) were found to be higher in adult women than in adult men for a given age interval. It was also noted that the mean platelet volume (MPV) was lower in women as compared to men across age groups.
We concluded from our study that for platelet enumeration done by peripheral smear method, a standard multiplication factor of 15,000 on oil immersion field gives results that are reasonably close to counts derived from automated analyzers. Singh et al[10] also established in their study that a multiplication factor of 15,000 gives the most accurate result.
There was no significant difference between manual platelet counts done by peripheral smear method and automated method except in cases of severe thrombocytopenia (Plt count $\leq 20x10^3/\mu L$).This was possibly because of interference by the presence of giant platelets. Giant platelets are indicative of compensatory response of marrow to low platelet levels and a consequent increase in platelet turnover in thrombocytopenia.

A linear positive relationship was noted between the degree of thrombocytopenia below $20x10^3/\mu L$ and falling R values, signiying poorer association at lower counts. Segal et al[12] and Kunz[13] also concluded in their study that automated counts have lower accuracy and precision at thrombocytopenic range.

As was expected, higher mean platelet volumes were typically found to be associated with almost all clinical causes of thrombocytopenia (Dengue, ITP, Sepsis, DIC, preeclampsia etc.) except for cases of aplastic anemia. A relatively poorer correlation between manual and automated hemocytometry values was also noted at higher MPV values (>14 fL).

The interobserver coefficient of variations (CV) between the platelet values estimated by two independent observers was found to be in the range of 7 to 26%. The independent t test shows no significant variance in the two set of values. Bland-Altman plot showed a strong agreement between both the set of values.

5. CONCLUSION

1. Manual platelet count estimation of Leishman stained peripheral smears under oil immersion field employing multiplication factor of 15,000 was found to have comparable accuracy to automated analyser counts.
2. 89.2% of manual platelet counts were found to lie within the acceptable error limit prescribed by CLIA (within 25% of the automated machine value).
3. The correlation between automated and manual counts was excellent in normal values of platelet count. However, the correlation was relatively poor in patients with severe thrombocytopenia (i.e., platelet counts $\leq 20 \times 10^3/\mu L$) and marked thrombocytosis (i.e., platelet count $\geq 700 \times 10^3/\mu L$).
4. As was expected, a higher mean platelet volume (MPV) was detected in cases of thrombocytopenia (79.56%) followed by normal counts (21.57%) and thrombocytosis (1.96%).
5. Correlation between manual platelet count and automated count was relatively poor in those with high MPV (>14 fL) as compared to patients with normal MPV. This is presumably due to interference by giant platelets (pollution of the platelet distribution curve) that are read as RBCs by the analyzer.
6. Within the normal ranges of platelet counts (Group B), there was an inverse correlation noted between platelet count and MPV.
7. Analytical variability between the manual platelet counts as observed by independent observers showed a high degree of agreement and correlation. The highest inter-observer variability was seen in the thrombocytopenia group followed by the thrombocytosis group.
8. The threshold platelet count of $20 \times 10^3/\mu L$ for prophylactic platelet transfusion may need a revision given the fact that automated analyzers tend to underestimate platelet counts at lower values, as was evidenced in our study.
9. Hence, this study underscores the importance of slide review of platelet counts in all thrombocytopenic patients, especially those with values close to the prophylactic transfusion threshold.

Conflict Of Interest

This research project is self-sponsored without any financial aid from any institution/ company. The authors have no competing/conflicting interests associated with this publication.

6. REFERENCES

1. Baccini, V., Geneviève, F., Jacqmin, H., Chatelain, B., Girard, S., Wuilleme, S., … Bardet, V. (2020). Platelet Counting: Ugly Traps and Good Advice. Proposals from the French-Speaking Cellular Hematology Group (GFHC). Journal of Clinical Medicine, 9(3), 808. doi:10.3390/jcm9030808
2. Mazzarello P, Calligaro AL, Calligaro A. Giulio Bizzozero: a pioneer of cell biology. Nat Rev Mol Cell Biol. 2001 Oct;2(10):776-81. doi: 10.1038/35096085. PMID: 11584305.
3. Ghoshal K, Bhattacharyya M. Overview of platelet physiology: its hemostatic and nonhemostatic role in disease pathogenesis. ScientificWorldJournal. 2014 Mar 3;2014:781857. doi: 10.1155/2014/781857. PMID: 24729754; PMCID: PMC3960550.
4. Cerletti C, Tamburrelli C, Izzi B, Gianfagna F, de Gaetano G. Platelet-leukocyte interactions in thrombosis. Thromb Res. 2012 Mar;129(3):263-6. doi: 10.1016/j.thromres.2011.10.010. Epub 2011 Nov 9. PMID: 22075180.
5. International council for standardization in haematology expert panel on cytometry; International society of laboratory hematology task force on platelet counting. Platelet counting by the RBC/platelet ratio method. A reference method. Am J Clin Pathol. 2001;115(3):460-4.
6. Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count. Int J Lab Hematol. 2007 Apr;29(2):77-91. doi: 10.1111/j.1751-553X.2007.00909.x. PMID: 17474881.
7. Kaufman, R. M., & Sloan, S. R. (2007c). Transfusion to bone marrow or solid organ transplant recipients. In Elsevier eBooks (pp. 539–550). https://doi.org/10.1016/b978-0-443-06981-9.50044-2
8. Kakkar, N. (2004). Spurious rise in the automated platelet count because of bacteria. Journal of Clinical Pathology, 57(10), 1096–1097. doi:10.1136/jcp.2004.016758
9. Madison WI Westgard QC 2019. CLIA Requirements for Analytical Quality. [Online]. 2019 [cited 2021 Jul 16]; [about 5 screens]. Available from: https://westgard.com/clia-a-quality/quality-requirements/125-clia.html
10. Singh, N., Tiwari, A., Pal, S., Anthony, M., Chowdhury, N., & Rao, S. (2022). Platelet count estimation on peripheral smear: What should be an acceptable "multiplication factor"? Journal of Medical Evidence, 3(2), 119. https://doi.org/10.4103/jme.jme_36_20
11. Jansson, L. (1985). Relationship of mean platelet volume to platelet count in morphologic evaluation of thrombopoiesis. Scandinavian Journal of Haematology, 35(3), 363–366. https://doi.org/10.1111/j.1600-0609.1985.tb01721.x
12. Segal, H. C., Briggs, C., Kunka, S., Casbard, A., Harrison, P., Machin, S. J., & Murphy, M. F. (2005). Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. British Journal of Haematology, 128(4), 520–525. https://doi.org/10.1111/j.1365-2141.2004.05352.x
13. Kunz D. Possibilities and limitations of automated platelet counting procedures in the thrombocytopenic range. Semin Thromb Hemost. 2001 Jun;27(3):229-35. doi: 10.1055/s-2001-15252. PMID: 11446656.