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Original Article

Pathology Section

Analysis of Manual and Automated Platelet Count Estimation Methods: A Cross-sectional Study from a Rural Tertiary Care Centre, Maharashtra, India

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ABSTRACT

Introduction: Platelet count estimation is a critical diagnostic tool in various haematological disorders. In resource-limited settings, manual methods are often employed due to cost constraints. However, their accuracy compared to automated haematology analysers requires validation.

Aim: To assess the accuracy, reliability and agreement of manual and automated platelet count estimation methods.

Materials and Methods: A cross-sectional study was conducted at Swami Ramanand Teerth Rural Government Medical College, Ambajogai, Maharashtra, India from January to March 2024, involving 250 blood samples. Peripheral venous blood was collected in Dipotassium Ethylenediaminetetraacetic Acid (K2EDTA) tubes. Platelet counts of 250 patients were estimated using both manual methods (peripheral blood smear microscopy with Leishman stain) and an automated haematology analyser (Erba Manheim Elite 580). Data were analysed using Statistical Package for the Social Sciences (SPSS) version 29.0. Descriptive statistics, paired t-tests, Pearson correlation coefficient, Bland-Altman analysis, Intraclass Correlation Coefficient (ICC), and One-way Analysis of Variance (ANOVA) were employed. Statistical significance was set at p-value <0.05.

Results: Of the 250 cases analysed, a strong positive correlation (r=0.98, p-value <0.001) was observed between manual and automated platelet counts. Bland-Altman analysis, which assesses agreement between two methods by plotting the difference against the average of the methods, revealed a mean bias of 5.72×10³/μL (95% limits of agreement: -0.01 to 11.45×10³/μL), indicating clinically acceptable agreement. Although a statistically significant difference (p-value=0.03) was found between mean counts, its clinical relevance was minor. Agreement remained consistent across age and sex subgroups, with an ICC of 0.98 (95% CI), reflecting excellent reliability.

Conclusion: This study validates the manual platelet count estimation method as a reliable and cost-effective alternative to automated analysers in resource-constrained settings. However, rigorous training and adherence to standardised protocols are essential for accurate results. Further research is recommended to validate these findings in diverse populations and clinical scenarios, enhancing the applicability of manual methods in rural healthcare settings.

Keywords: Diagnostic accuracy, Peripheral blood smear, Statistical correlation

INTRODUCTION

Platelets, or thrombocytes, are small, disc-shaped cell fragments that are essential for blood clotting and haemostasis. These anucleate components originate from megakaryocytes in the bone marrow. Upon vascular injury, platelets rapidly adhere to the damaged endothelium, aggregate and form a haemostatic plug to prevent excessive bleeding [1,2]. The discovery of platelets dates back to the 19th century. In 1842, Alfred Donné first observed these cellular fragments using a microscope, but it was Giulio Bizzozero in the late 1800s who elucidated their role in haemostasis and thrombosis [3].

Estimating platelet counts is critical for patient treatment and is a vital component in the diagnosis of many medical disorders. Furthermore, regular platelet counts are necessary for individuals undergoing chemotherapy, as well as for those suffering from leukaemia, malaria, bacterial sepsis and pregnancy-induced hypertension [4]. The International Council for Standardisation in Haematology (ICSH) and the International Society for Laboratory Haematology (ISLH) have recommended immunoplatelet counting as a reference method for calibrating automated haematology analysers. A flow cytometer is required for this [5,6]. On rare occasions, platelet satellitism in EDTA samples may result in inaccurate results from automated cell counters [7]. In cases of severe thrombocytopenia, the results of automatic counters should not be entirely relied upon. The accuracy of platelet estimates is crucial for individuals with thrombocytopenia, particularly when platelet transfusion is being considered [8].

The aim of the study was to compare the manual methods of platelet count estimation with platelet count estimation using an automated haematology analyser at a rural tertiary care centre. To determine the accuracy, efficiency and reliability of each method in a rural healthcare setting. This comparative study aims to provide important findings on the most efficient method for estimating platelet count for pathologists working in rural areas, thus supporting improvements in clinical laboratory techniques and the overall standard of patient care in such environments.

MATERIALS AND METHODS

This cross-sectional study was conducted at Swami Ramanand Teerth Rural Government Medical College, Ambajogai, Maharashtra, India from January 2024 to March 2024 (a period of three months), assessing 250 samples. Ethical clearance was obtained with reference number 66 dated 02/05/2024.

Inclusion criteria: Patients with normal platelet counts, age range between 0-60 years and participants who provided blood samples during the study period (January to March 2024) were included in the study.

Exclusion criteria: Patients with thrombocytopenia (low platelet counts), thrombocytosis (high platelet counts), blood samples with signs of clotting or improper handling, patients with haematological disorders that severely impact platelet count estimation and patients who did not provide consent were excluded from the study.

Study Procedure

Using standard phlebotomy techniques, three milliliters of blood per person were drawn and placed in tubes containing K2EDTA anticoagulant. A peripheral blood film was prepared using a manual approach. A clean glass slide with a drop of blood on one end was used to create the blood film, which was then uniformly spread across the slide using another slide. Leishman stain was then applied to the prepared blood film, enabling the distinction of different blood cells, including platelets.

After microscopic examination of the stained slide, the number of platelets was calculated by multiplying the average number of platelets in ten oil immersion fields by 20,000 (thousand/mm^3). Momodu I research showed that, contrary to the advice of some other authors, multiplying the mean of the ten oil field platelet counts by 20,000 yielded results that were more in line with haematological analysers [9]. The five-part automated haematology analyser, Erba Manheim Elite 580, was used to perform the automated technique for estimating platelet counts. Prior to use, the equipment was calibrated using the standard calibrators provided by the manufacturer.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS 29.0. The data's normality was assessed using the Shapiro-Wilk test, Q-Q plots, and histograms. Descriptive statistics, paired t-tests, Pearson correlation, Bland-Altman plots, and ICCs were employed to evaluate agreement, correlation, and statistical differences between manual and automated platelet count methods, with a p-value <0.05 considered significant.

RESULTS

The study included 250 participants with an age range of 0 to 60 years. The mean age was 28.36 years, with 131 (52.4%) females and 119 (47.6%) males [Table/Fig-1].

Age group (years)	Male (n)	Female (n)	Total (n)
0-10	23	26	49
11-20	27	33	60
21-30	28	35	63
31-40	18	19	37
41-50	13	12	25
51-60	10	6	16

[Table/Fig-1]: Distribution of participants by age group and sex.

The mean platelet count obtained by the manual method was 338.68×10^3/µL (SD=121.83), while the mean platelet count obtained by the automated method was 332.96×10^3/µL (SD =119.35). Both manual and automated platelet count data were found to be approximately normally distributed based on graphical methods and the Shapiro-Wilk test. Therefore, a paired t-test was conducted, which revealed although statistically significant difference, a clinically insignificant between the platelet counts obtained by the manual and automated methods (t(249)=2.18, p-value=0.03 [Table/Fig-2,3].

The Pearson correlation coefficient between the manual and automated platelet counts was 0.98 (p-value <0.001), indicating a strong positive correlation. The Bland-Altman analysis assessed the agreement between the manual and automated methods. The mean difference (bias) was 5.72×10^3/µL, with 95% limits of agreement ranging from -0.01 to 11.45×10^3/µL.

Method	Mean±SD	Minimum	Median	Maximum
Manual	338.68±121.83	100	320	600
Automated	332.96±119.35	95	318	610

[Table/Fig-2]: Descriptive statistics for platelet counts (x10^3/µL).

Method	Mean difference	95% CI	t-value	p-value
Manual- Automated	5.72	(-0.01, 11.45)	2.18	0.03

[Table/Fig-3]: Paired t-test results.

The ICC for the platelet counts obtained by the two methods was 0.98 at a 95% confidence interval, indicating excellent reliability. To further explore the relationship between the two methods, platelet counts were categorised into tertiles based on the manual method. The mean platelet counts for each tertile were then compared between the two methods [Table/Fig-4].

Tertile	Manual	Automated
Low	196.88	192.56
Medium	320.00	315.32
High	485.12	475.52

[Table/Fig-4]: Mean platelet counts by tertile (x10^3/µL).

A one-way ANOVA revealed a statistically significant difference in mean platelet counts between the tertiles (F(2, 247)=356.82, p-value <0.001). Post-hoc tests (Tukey's HSD) showed that the mean platelet count was significantly lower in the low tertile compared to the medium and high tertiles for both methods (p-value <0.001). There was no significant difference in mean platelet count between the medium and high tertiles for either method (p-value >0.05).

To assess the impact of age and sex on the agreement between the two methods, Bland-Altman plots were created for different age groups and sexes. The results of [Table/Fig-5,6] suggest that the agreement between the two methods is consistent across different age groups and sexes.

Age group (years)	Bias	95% limits of agreement
0-10	6.04×10^3/µL	-2.36 to 14.44×10^3/µL
11-20	5.88×10^3/µL	-1.21 to 12.97×10^3/µL
21-30	5.52×10^3/µL	-1.86 to 12.90×10^3/µL
31-40	5.94×10^3/µL	-0.45 to 12.33×10^3/µL
41-50	5.20×10^3/µL	-2.71 to 13.11×10^3/µL
51-60	5.62×10^3/µL	-2.98 to 14.22×10^3/µL

[Table/Fig-5]: Bland-Altman analysis results by age group.

Sex	Bias	95% limits of agreement
Male	5.84×10^3/µL	-0.63 to 12.31×10^3/µL
Female	5.60×10^3/µL	-0.48 to 11.68×10^3/µL

[Table/Fig-6]: Bland-Altman analysis results by sex.

The automated haematology analyser (Erba Manheim Elite 580) was considered the standard for platelet count estimation in this study. After every 20 samples, the instrument's calibration was checked using controls provided by the company to ensure accuracy.

A strong correlation (r=0.98, pvalue <0.001) between the manual and automated methods was observed, with only a minor, although statistically significant, a clinically insignificant difference (p-value=0.03) in mean platelet counts.

DISCUSSION

The current study aimed to compare the manual and automated methods of platelet count estimation in a rural tertiary care setting. This is a pertinent issue, as platelet counts are crucial in diagnosing and managing various haematological conditions [10]. In resource-limited settings, the manual method is often the primary approach due to the cost and maintenance associated with automated

haematology analysers. However, the accuracy and reliability of the manual method are frequently questioned, necessitating a thorough comparison with the automated method [11,12].

Momodu I emphasised the value of manual platelet counts in under-resourced laboratories reporting good concordance with automated methods [9]. Similarly, studies by Webb DI et al., and Aashna et al., found strong correlations between manual and automated platelet counts [12,13]. These findings highlight the potential of manual methods as a reliable backup or primary approach in resource-constrained environments.

However, some contrasting features exist. Present study observed a in mean platelet counts between the methods, with the manual method yielding slightly higher values. This was consistent with Mishra S et al., who also reported a small but statistically significant difference in favour of the manual method [10]. Potential explanations for this discrepancy include interobserver variability in manual counting and subtle differences in counting principles between the methods.

Our findings revealed a strong positive correlation (r=0.98, p-value <0.001) between the manual and automated platelet counts, suggesting a high degree of agreement between the two methods. This aligns with previous studies that have also reported strong correlations between these methods [9,11]. The Bland-Altman analysis further supported this agreement, demonstrating a mean bias of 5.72×10^3/µL, with 95% limits of agreement ranging from -0.01 to 11.45×10^3/µL. These values fall within clinically acceptable ranges, indicating that the manual method can be used interchangeably with the automated method in most clinical scenarios.

However, while the overall agreement was high, a statistically significant difference (p-value=0.03) was observed between the mean platelet counts obtained by the two methods. The mean platelet count obtained by the manual method was slightly higher than that obtained by the automated method. This difference could be attributed to several factors, including interobserver variability in the manual method, subtle differences in the counting principles between the two methods, or potential minor calibration issues with the automated analyser. Nonetheless, the clinical significance of this small difference is likely negligible, as it falls within the expected range of variation for platelet counts [13].

Subgroup analyses by age and sex revealed consistent agreement between the two methods across different demographic groups. This suggests that the manual method can be reliably used across a wide range of patients, irrespective of their age or sex. This study provides valuable insights into the comparability of manual and automated platelet count estimation in a rural tertiary care setting. The findings suggest that the manual method can be a reliable and cost-effective alternative to the automated method, especially in resource-limited settings where access to automated analysers may be restricted. However, it is essential to ensure proper training and standardisation of the manual method to minimise interobserver variability and ensure accurate results.

Limitation(s)

It is important to note that present study had some limitations. First, it was conducted at a single centre, which may limit the generalisability of the findings to other settings. Second, the sample size was relatively small, although it was sufficient to detect a statistically significant difference between the two methods. Third, the study did not include patients with extreme thrombocytopenia or thrombocytosis, which may require additional validation of the manual method.

CONCLUSION(S)

Present study demonstrated a strong correlation and clinically acceptable agreement between manual and automated platelet count estimation methods. The manual method can serve as a viable alternative to the automated method in resource-limited settings, provided it is performed by trained personnel adhering to standardised protocols. Further research with larger sample sizes and diverse patient populations is warranted to confirm these findings and explore the potential impact of the manual method on clinical decision-making in various clinical scenarios.

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