Estimation of platelet count from peripheral blood smear based on platelet: red blood cell ratio. A prospective study in a tertiary care hospital

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Abstract

Introduction: Accurate and reproducible platelet counts is essential for patient management. There are many methods for counting platelets. Study was conducted with the objectives of estimation of platelet count indirectly from peripheral blood smear (PBS) on the basis of platelet: red blood cell (RBC) ratio and using automated RBC count; and to verify the reliability of this method in comparison to automated platelet counts.

Materials and Methods: A prospective study was conducted in a tertiary care hospital on 200 Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples. Samples were evaluated by automated hematology analyser using impedance counting method and by examination of PBS. Number of platelets/1000 RBC in PBS was multiplied by automated RBC count in 10^6/uL to get an estimate of platelet count in 10^3/uL.

Results: Two sample T test showed no significant difference between the two methods. Pearson correlation of the two methods showed high correlation.

Conclusion: Estimation of platelet count on the basis of platelet: RBC ratio is a reliable technique and can be used to validate automated platelet counts.

Key words: Peripheral blood smear, Platelet count, Platelet: red blood cell ratio.

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Introduction

Platelets play a key role in hemostasis and thrombosis. Platelet count is one of the critical parameters in patient care. Normal range of platelet count in a healthy individual is 150 - 400 x10^3/uL. The methods commonly used for estimating platelet count are:

1. Manual method using counting chamber.
2. Examination of a peripheral blood smear (PBS).
3. Using automated hematology analyzers.

Accurate and reproducible platelet counts is essential for patient management. Manual method is time consuming, subjective and tedious with high levels of imprecision[1]. Automated hematology analyzers produce erroneous results in the presence of particles of similar size and/or light scatter like fragmented red blood cells (RBC), microcytic RBC, apoptotic white blood cell fragments and in the presence of giant platelets and platelet clumps[2,3]. Estimation of platelet count from PBS based on average number of platelets in an oil immersion field (OIF) is approximate and does not give the real number of platelets[2].

Objectives of the study

1. Estimation of platelet count from PBS indirectly by counting the number of platelets per 1000 RBC in PBS and then calculating the platelet count on the basis of platelet: RBC ratio using the automated RBC count.
2. To verify the reliability of this technique by comparing the platelet count obtained by this method with the automated platelet count.

Materials and Methods

This is a prospective study conducted in a tertiary care hospital on blood samples received in Central laboratory during October-November 2015. Institutional ethical committee approval was taken for the study. Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples sent to Central laboratory for platelet count from subjects of any age and gender, and with any diagnosis during the study period were included in the study.

Hemolysed and clotted samples were excluded.

Sample size: 200.

The samples were analyzed by two methods:

1. Samples were analyzed in automated hematology analyzer Sysmex XP100 using impedance method to get complete blood count (CBC).
2. Air dried thin smears were made from all samples and stained with Leishman stain.

These PBS were examined under light microscope using x100 oil immersion lens. In a monolayer zone of the smear, platelets were counted simultaneously with RBC till 1000 RBC were counted. The number of platelets per 1000 RBC thus obtained was multiplied by

automated RBC count in 10^6/uL to get an estimation of platelet count in 10^3/uL.

Data was processed using SPSS Program v17.

Results

Two sample T test was done. Automated platelet count ranged from 20 – 688 x 10^3/uL and had a mean value of 308 x 10^3 / uL. Platelet count estimated by the method used in this study had a range of 15 – 695 x 10^3/ uL and the mean was 309 x 10^3 /uL. (Fig. 1). P value $\leq$ 0.05 was considered as statistically significant. P value was 0.928 and T value was - 0.09. Hence, two sample T test showed no significant difference between the two methods.

Pearson correlation of two methods was done and gave a value of 0.978 and P value of < 0.001. Thus the two methods are highly positively correlated.

Discussion

Accurate and reproducible platelet counts are essential for the management of thrombocytopenic patients at risk of bleeding[4]. Platelet count is important to evaluate the risk of occurrence of spontaneous bleeding in a patient[1]. If there is confidence in the platelet count values at low levels, it is possible to reduce platelet transfusions to those that are clinically necessary[1].

Many methods for counting platelets have been published and the number of alternative methods is due to difficulties in counting small cells which are activated easily, aggregate and the difficulty in differentiating platelets from extraneous matter[1].

With the development of sophisticated automated blood cell analyzers, the proportion of blood count samples which require a blood smear has steadily diminished. Nevertheless, examination of blood smear is a crucial diagnostic tool.

Analysers using the standard impedance measurement are able to provide an accurate platelet count upto 20 x 10^9/L[1].False increases in platelet count will occur when red cell or white cell fragments, microcytic red cells, immune complexes, bacteria or cell debris are included in the count[5]. False decrease in platelet count will occur in the presence of large platelets and if there is platelet clumping[1].

An estimation of whether the platelet count is normal, low or high and any abnormalities of platelet morphology can be made out by examining a PBS.

It is a standard procedure that all abnormal platelet values generated by cell counters should be confirmed by manual examination of Leishman stained PBS[6,7]. Each time the automated count is erroneous, the platelet count must be systematically estimated from blood smears because even the most expensive and effective machine is not able to replace human judgement[8].

Estimation of platelet count from PBS is done by counting the number of platelets in 10 OIF and multiplying the average number of platelets in an OIF by 15000 or 20000. This count is reasonably close to automated machine counts[9-12]. If RBC by a semi-automated counter is available, it is possible to obtain an approximation of the platelet count by counting the proportion of platelets to red cells in a thin part of a film made from an EDTA blood sample using the x100 oil immersion objective[13]. Using this method, Theml H and also Brahimi et al have estimated the number of platelets relative to 1000 RBC and found it to be reliable[14,2].

The traditional 'gold standard' method for platelet count was manual phase contrast microscopy[2,15,16]. But, this method is time consuming and imprecise at low counts. It still offers a relatively inexpensive, simple and viable means to enumerate platelets in non-specialized laboratories[1].

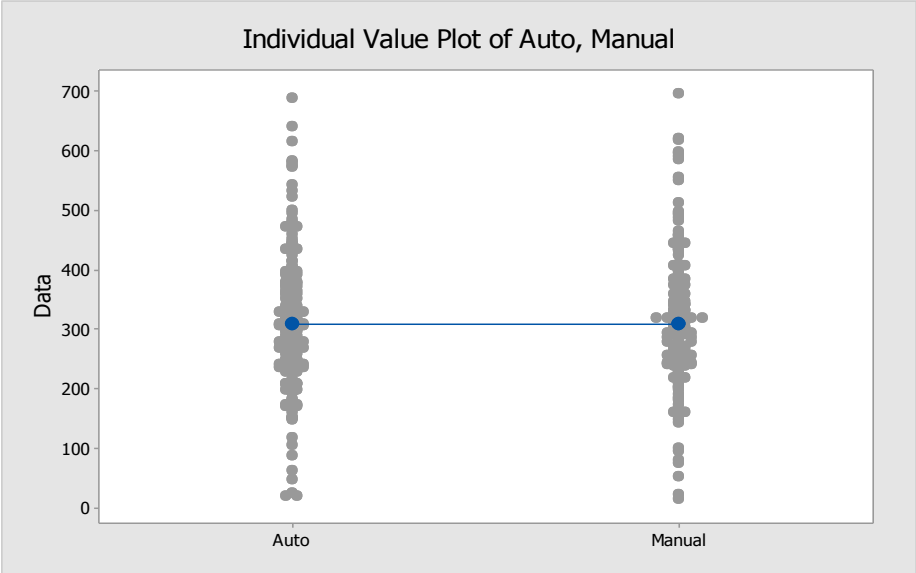
Now, the International Reference Method (IRM) for platelet count is flow cytometry[17-19]. The proposed International Society of Laboratory Haematology (ISLH) reference method uses specific monoclonal antibodies to platelet cell surface antigens (e.g., anti CD41 and anti CD61) which are conjugated to a fluoroscent substance. Flow cytometric analysis of the ratio of fluorescent platelets to non-fluorescent red cells gives a highly accurate and precise platelet counts[17,18]. This offers a suitable comparator for platelet count methods.

In the present study, PBS was examined to determine the platelet: RBC ratio. Using this value and the automated RBC count, estimation of platelet count was done. Platelet counts by this method were not significantly different from automated platelet counts.

Conclusion

Platelet count estimated from PBS based on platelet: RBC ratio were not significantly different from platelet count estimated by automated hematology analyser. This is a reliable technique and can be used for microscopic validation of automated platelet counts.

Table 1: Data shows platelet count in 10^3/ uL



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Conflicts of interest: Nil

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