

Estimation of Platelet Count on the Basis of Red cell: Platelet Ratio

Balsam F Abid MBChB; MSc. Path.

Abstract

Background: Modern haematology analyzers are able to produce platelet counts with great precision and accuracy. However, in certain cases these analyzers produce erroneous platelet results. Therefore, the estimation of platelet count from blood smears should be systematic each time the automated count is erroneous. However, in comparison with the procedure for an automated count, the examination of a blood smear using a counting Neubauer chamber is a labor-intensive and therefore relatively expensive investigation.

Objective: Verification of the reliability of the estimation technique of platelet count on the basis of red cell: platelet ratio.

Material and Methods: In the period between January 2006 and March 2006 one hundred platelet counts were executed in the National Center for Haematological Diseases by two laboratory methods: an automated count using an impedance cell counter and then a manual method by reviewing microscopic blood smears. The number of platelets per 1000 erythrocytes was multiplied by the automated RBC ($\times 10^6$ cells/ μl) to give an approximate manual count ($\times 10^3$ cells/ μl). Two-paired t-test was used for comparison of the two methods.

Results: Platelet count using the manual method was as follow: the range was 100-499 $\times 10^3/\mu\text{l}$, the mean count was $263.11 \pm 104.07 \times 10^3/\mu\text{l}$, and the median was

247.5 $\times 10^3/\mu\text{l}$. using the automated method, platelet count ranged between 95-484 $\times 10^3/\mu\text{l}$, the mean was 258.43 $\times 10^3/\mu\text{l}$, and the median was 242.5 $\times 10^3/\mu\text{l}$. There was no significant difference in results of platelet count using both methods ($P < 0.05$). Regression analyses gave the following equation by comparing the automated (y) to the manual method (x): $y = 0.9893x - 1.8621$ ($r = 0.966$). The paired t-test showed no significant difference between the two methods ($p < 0.05$). The ICC was equal to 0.988. The plot of the differences between the automated and manual values against their means showed that the difference mean was 2.116 with a standard deviation $SD = 40.215$. It was noticed that 93% of the differences were within the agreement limits ($\text{mean} \pm 2SD$).

Conclusion: Red blood cell:platelet ratio method requires only an accurate RBC count performed on a calibrated hematology analyzer to calculate platelet count. This method is precise, simple, and consumes less time than using a counting chamber, and therefore, potentially should supersede ordinary manual counting.

Key words: red cell: platelet ratio, platelet count

IRAQI J MED SCI, 2009; VOL.7 (3):40-45

Introduction

The estimation of platelet count from blood smears must be systematic each time the automated count is erroneous because even the most expensive and most effective machine is not able to replace human judgement. With the development of sophisticated automated blood-cell analyzers, the proportion of blood-count samples that require a blood smear has steadily diminished and in

many clinical settings is now 10 to 15 percent or less. Nevertheless, the blood smear remains a crucial diagnostic aid⁽¹⁾. Modern haematology analyzers are able to produce platelet counts with great precision and accuracy. However, in certain cases these analyzers produce erroneous platelet results, for example pseudothrombocytopenia⁽²⁾, or pseudothrombocytosis or at least obvious overestimation of the real number of platelets as in patients with acute leukaemia. Because of their shape and size, haematology analyzers add several undefined particles to the platelet cluster. In some cases, this

Dept. Pathology, College of Medicine, AL-Nahrain University,

Address Correspondence to: Dr Balsam F Abid E-mail: dr.balsam@yahoo.com

Received: 20th May 2009, Accepted: 23rd August 2009.

may even lead to the masking of a (possible life threatening) thrombocytopenia, and consequently the withholding of proper medication or other crucial supportive measures⁽³⁾.

The International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Hematology (ISLH) recommend the counting of specifically labeled platelets relative to the RBCs with a fluorescence flow cytometer, together with an accurate RBC count determined with a semiautomated, single-channel aperture-impedance counter as a reference method for the enumeration of platelets⁽⁴⁾.

Aim of the study

Verification of the reliability of the estimation technique of platelet count manually on the basis of red cell : platelet ratio.

Material and Methods

Blood sample collection and processing:

A total of 100 blood specimens were obtained from patients between January 2006 and March 2006 in the National Center for Haematological Diseases. Specific diseases or conditions of a patient were not considered for inclusion or exclusion in the study.

All venous blood specimens were collected into tubes containing ethylenediaminetetraacetic acid (EDTA) and then were stored at room temperature until analyzed within four hours.

Notation was made if clots were seen in the blood sample or if the amount of blood in the tube was grossly inadequate such that a high concentration of EDTA would be present; these samples were excluded from the study.

Automated Method: After thorough mixing of each blood sample on an automated mixer for 10 min, a

complete automated blood count was performed using an impedance cell counter (Coulter Counter), which was maintained and calibrated as recommended by the manufacturer.

Manual Method: Thin air-dried blood smears made after thorough mixing of each sample were stained manually, using Leishman stain, and examined under light microscopy with a X100 oil-immersion lens. The slides were entirely scanned for platelet aggregates and/or macrothrombocytes and, if any, the samples were excluded from the study.

The red cell: platelet ratio was calculated in the monolayer zone of the smear as follows: The number of erythrocytes observed in a quarter of the oil-immersion field was multiplied by four instead of counting all the erythrocytes in the field. Then all the platelets in the same field were counted.

Other fields were examined in the same way until a minimum number of 1000 erythrocytes was reached. The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count (RBC) ($\times 10^6$ cells/ μ l) to give an approximate manual count ($\times 10^3$ cells/ μ l)⁽⁵⁾.

Statistical Method

The mean, median, and range of platelet count using the two laboratory methods were calculated. Simple linear regression plot was used to compare the manual with the automated platelet counts.

Intra-class Correlation Coefficient (ICC) was calculated in order to identify the degree of correspondence and the agreement between the two methods. The ICC value is measured on a scale of 0 to 1, good reliability was assumed as an ICC > 0.75. A paired t-test was performed, a statistically significant difference in platelet level was set at a level of $p < 0.05$.

Results

Results of platelet count using the manual method were as follows: the range was between $100-499 \times 10^3/\mu\text{l}$, the mean platelet count was $263.11 \pm 104.07 \times 10^3/\mu\text{l}$, and the median was $247.5 \times 10^3/\mu\text{l}$. By using the automated method, platelet count ranged between $95-484 \times 10^3/\mu\text{l}$, the mean was $258.43 \pm 103.13 \times 10^3/\mu\text{l}$, and the median was $242.5 \times 10^3/\mu\text{l}$ (table 1). The report of evaluation with the two laboratory methods gave the following equation by comparing the automated (y) to the manual method (x):

$y = 0.9893x - 1.8621$ ($r = 0.966$) (Figure 1).

The paired t-test showed no significant difference between the two methods ($p < 0.05$). The ICC was equal to 0.988.

The plot of the differences between the automated and manual values against their means showed that the difference mean was 2.116 with a standard deviation $SD = 40.215$ (Figure 2). It was noticed that 93% of the differences were within the agreement limits ($\text{mean} \pm 2SD$).

Table 1: Results of platelet count using manual and automated methods

	Manual platelet count method ($\times 10^3/\mu\text{l}$)	Automated platelet count method ($\times 10^3/\mu\text{l}$)	P-value
Range	100-499	95-484	<0.05
Mean $\pm$ SD	263.11 ± 104.07	258.43 ± 103.13	
Median	247.5	242.5	

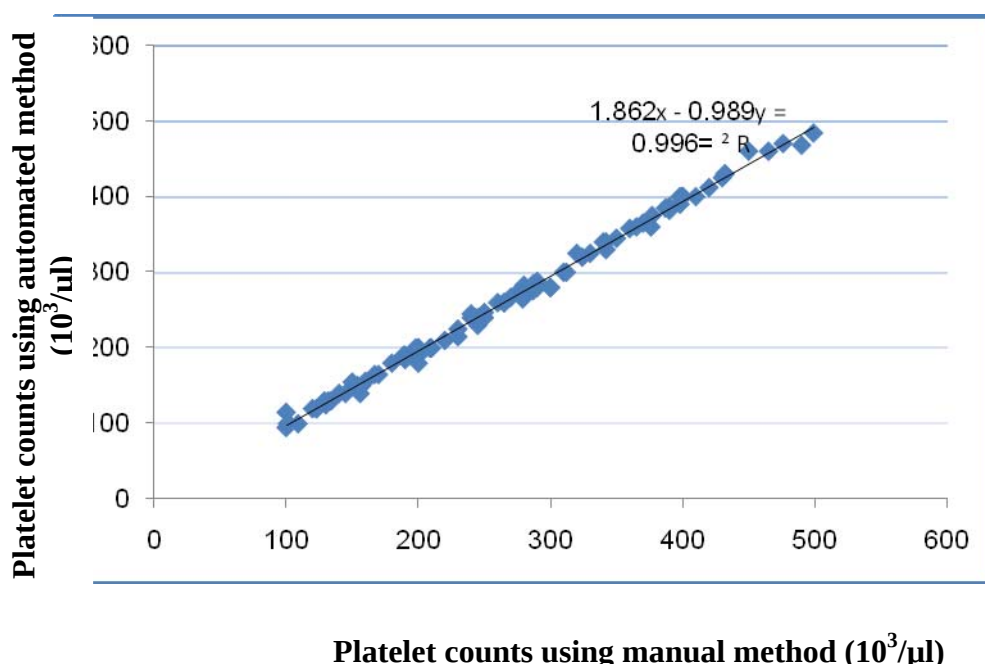


Figure 1: The regression analyses for the entire data set using manual and automated methods

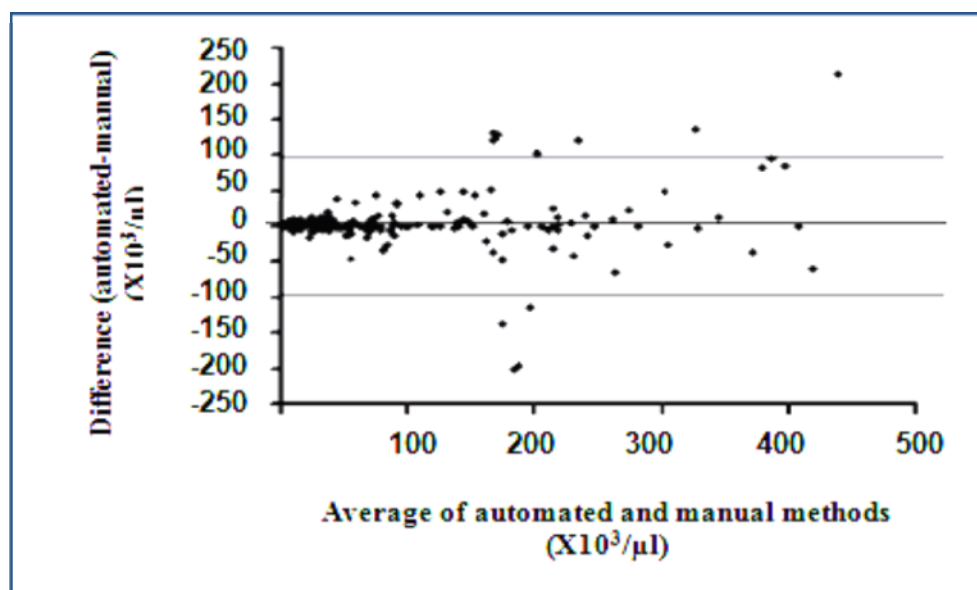


Figure 2: Difference versus mean plots for automated and manual platelet counts. The middle solid line is the mean of the difference; the outer solid lines are the upper and lower limits of agreement (mean $\pm$ 2SD)

Discussion

Even in the age of molecular analysis, the blood smear remains an important diagnostic tool. Physicians should request a blood smear when there are clinical indications for it. If error is to be avoided, sophisticated modern investigations of hematologic disorders should be interpreted in the light of peripheral-blood features as well as the clinical context. In comparison with the procedure for an automated count, the examination of a blood smear is a labor-intensive and therefore relatively expensive investigation. A request for a blood smear is usually the result of an abnormality in the complete blood

count or a response to "flags" produced by an automated instrument⁽¹⁾.

Obtaining an accurate platelet count by using an automated hematology analyzer may be complicated by the presence of particles of similar size and/or light scatter properties (red cell fragments, microcytic red cells, apoptotic white blood cell fragments) and by giant platelets and platelet clumps^(6,7). Falsely low platelet counts may be the result of small clots, platelet clumping, platelet satellitism, or abnormally large platelets. Underlying causes that may be revealed by the blood smear include the May-Hegglin anomaly,

microangiopathic thrombopathies, and leukemias and lymphomas. High platelet counts should be confirmed microscopically with a blood smear; falsely high counts may be the result of other particles (red-cell fragments, fragments of leukemic cells, or fungi) being counted as platelets^(8,9,10).

Examination of the blood smear is also important in patients with thrombocytosis to look for evidence of a myeloproliferative disorder, such as giant platelets, or an increase in the basophil count; the latter is not reliably detected by automated counters. A sudden, unexpected improvement in the platelet count also should be confirmed by blood-smear examination, since such an improvement may be factitious⁽⁹⁾.

Until recently, the only reference method for platelet counting was the manual phase contrast microscope chamber counts (11) in which platelets are counted manually with a haemocytometer, such as Neubauer chamber. This is laborious, time-consuming and above all, an imprecise technique. The interoperator coefficient variant of this method can be up to 25%. However, it is still most widely used reference method⁽¹²⁾.

Even if the manual platelet numeration, using a counting chamber, remains the technique of reference, it consumes more time and, to be more precise, requires a phase-contrast microscope, which is not always available in routine laboratories⁽¹³⁾. That is why the proposed method is better, since it is faster, taking only five minutes on average per patient, while demonstrating good precision.

Some authors recommend calculating the average number of platelets counted in 10 immersion fields; the adequate values are included between 8 to 20 platelets per field^(14,15). The average number of platelets is then multiplied by a factor of 20,000

for wedge preparations or 15,000 for monolayer preparations in order to obtain and estimate the platelet count, but this method is approximative and does not give the real number of platelets.

Comparing automated and manual, using red cell:platelet ratio method, platelets counting techniques showed that there was no significant difference ($P < 0.05$) between the mean, median, and range of platelet counts using these two methods.

The ICC was calculated in order to identify the reliability of the manual technique in comparison to the automated method⁽¹⁶⁾. The ICC value is measured on a scale of 0 to 1, and good reliability was generally assumed as an $ICC > 0.75$ ⁽¹⁷⁾. In this study, the ICC was equal to 0.988, which is widely greater than this limit. In addition, 93% of the differences between automated and manual counting methods were within the agreement limits ($\text{mean} \pm 2SD$).

Red blood cell: platelet ratio method requires only an accurate RBC count performed on a calibrated hematology analyzer to calculate platelet count. This method is precise, simple, and consumes less time than using a counting chamber, and therefore, potentially should supersede ordinary manual counting.

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