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"STUDY ON PLATELET COUNT FROM BLOOD SMEAR ON THE BASIS OF RED BLOOD CELL: PLATELET RATIO: A HOSPITAL BASED STUDY"

Pathology

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ABSTRACT

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. Aim of the study was to estimate the platelet count indirectly by using the automated red blood cell (RBC) and calculating the platelet count on the basis of the red cell: platelet ratio in a stained blood film. In this study, we attempted to verify the reliability of this technique. The peripheral blood smear 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.

KEYWORDS

Manual Method, Automated Method and Red cell: platelet ratio.

Introduction:

Platelets play a key role in hemostasis and thrombosis. Platelet count is one of the critical parameters in patient care. 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] 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 crucial diagnostic aid.[4] However, in certain cases these analyzers produce erroneous platelet results, for example pseudothrombocytopenia[5], or pseudo thrombocytosis or at least obvious overestimation of the real number of platelets as in patients with acute leukemia. Because of their shape and size, hematology analyzers add several undefined particles to the platelet cluster. In some cases, this may even lead to the masking of a (possible life threatening) thrombocytopenia, and consequently the withholding of proper medication or other crucial supportive measures.[6] The International Council for Standardization in Hematology (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 semi-automated, single-channel apertureimpedance counter as a reference method for the enumeration of platelets.[7]

MATERIAL AND METHODS:

This present study was conducted in the Department of Pathology, Shantiram Medical College & General Hospital, Nandyal, Kurnool, Andhra Pradesh, India. Randomly selected 70 patients attending OPD in the Shantiram Medical College & General Hospital, during the period from November 2013 to February 2014 were included in the study. Ethylene Diamine Tetra Acetic acid (EDTA) anticoagulated blood samples sent to Central laboratory, Pathology Department 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. The samples were analyzed by following two methods:

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

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) (x106cells/µl) to give an approximate manual count (x103cells/µl).[8] 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 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. Data was processed using SPSS Program version18.

Results and Discussion:

The report of evaluation on all 70 individual samples with the two laboratory methods were as follows: the range was between 100-499 x103/µl, the mean platelet count was 304.25±41.3x 103/µl and by using the automated method, platelet count ranged between 95-485 x103/µl, the mean was 329.6±52.02x103/µl (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= 0.9873x-1.8631 (r=0.956). The paired t-test showed no significant difference between the two methods (p>0.05). The ICC was equal to 0.903. Accurate and reproducible platelet counts are essential for the management of thrombocytopenic patients at risk of bleeding4. Platelet count is important to evaluate the risk of occurrence of spontaneous bleeding in a patient1. 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]

Table-1: comparison of platelet count in manual and automated methods:

Parameters	Automated Platelet Count (103/µl)	Manual Platelet Count (103/µl)	P- value
Range	95-485	100-499	<0.05
Mean±S.D.	329.6±52.02	304.25±41.3	
Median	240.5	246.5	

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.[4] 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.[9,10] 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, microangiopathicthrombopathies 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.[11-13] 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.[12] Until recently, the only reference method for platelet counting was the manual phase contrast microscope chamber counts[14] in which platelets are counted manually with a haemocytometer, such as Neubauer chamber. This is laborious, timeconsuming and above all, an imprecise technique. The interoperater coefficient variant of this method can be up to 25%. However, it is still most widely used reference method.[15] 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.[16] 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.[17,18] 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 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.[19] The ICC value is measured on a scale of 0 to 1, and good reliability was generally assumed as an ICC>0.75.[20] In this study, the ICC was equal to 0.903, which is widely greater than this limit. In addition, 93% of the differences between automated and manual counting methods were within the agreement limits (Mean±2Sd). In the present study, peripheral blood smear 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

In conclusion, the 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. This is a reliable technique and can be used for microscopic validation of automated platelet counts.

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