Relativity of Platelet Count with Automated Cell Counter & Manual Haemocytometer using Peripheral Blood Smear

Purnendu Sinha[1], Moattar Raza Rizvi[2], *Ranjay Kumar Choudhary[3]

ABSTRACT

BACKGROUND: Obtaining accurate, precise, and reliable platelet counts as a reference for the calibration of hematology analyzers has been a continuing problem due to varying results. We proposed the relativity of platelet count by Automated Cell Counter and Manual Haemocytometer with Peripheral smear for focusing the accuracy and & cost effectiveness, without compromising the accuracy of test results. **METHODS:** Seventy-Five blood samples in EDTA-anticoagulant vacutainer tubes were obtained and randomly divided into 3 group; Group I-normal control (n = 25), Group II-mild thrombocytopenia (n = 25) and Group III-severe thrombocytopenia (n = 25). Each blood sample had two peripheral blood smears made and stained on an automatic slide stainer. Peripheral blood smears were analyzed for platelet count by manual microscopy, an automated hematology analyzer Beckman Counter Diff 2 in all groups. The agreement between the two methodologies were assessed using the paired t-test and correlation coefficient analyses. **RESULTS:** The coefficient of variation values significantly differed in Group C and were high for Automated cell counter. High postitive correlation was also observed between Automated cell counter values and manual method ($r=0.82$, $p<0.001$) in group A and B. However, values in group C were not significantly correlated. **CONCLUSION:** Based on the results observed in severe thrombocytopenia, the automated cell counter was more reliable and significant than the manual method. However, manual platelet count using by haemocytometry and morphological study of the blood smear may be promising if platelets are very low and giant platelets.

KEY WORDS: Thrombocytopenia peripheral blood smear, platelet count, automated Cell Counter and haemocytometer

الملخص

مقدمة: تحديد قيمة عدد الصفائح الدموية بصورة دقيقة ويعتمد عليها كمرجع لمعايرة اجهزة تحليل الدم يعتبر من المعضلات المستمرة وذلك لتفاوت قيم النتائج. نقترح نسبية عد الصفائح الدموية بعداد الخلايا الاتوماتيكي والعداد اليدوي مع عمل مسحة دم طرفي وذلك للتركيز علي الدقة والتكلفة والفعالية مع عدم الاخلال بدقة نتائج الاختبار. **المنهج:** تم استخدام 57 عينة دم في أنابيب EDTA المضادة للتخثر ووزعت عشوائيا الي 3 مجموعات , المجموعة الاولي طبيعية وهي للمقارنة وعددها 25 والمجموعة الثانية تعاني من انخفاض بسيط في عدد الصفائح الدموية وعددها 25 اما المجموعة الثالثة فعددها 25 وتعاني من انخفاض شديد في عدد الصفائح الدموية. كل عينة دم صاحبها مسحتان من الدم الطرفي تم تحضيرهما وصبغتهما علي شرائح صبغية اتوماتيكية. تم تحليل المسحات في كل المجموعات بعد الصفائح الدموية يدويا بالميكروسكوب , و اوتوماتيكيا بجهاز بيكمان لتحليل الدم . تم اختبار التوافق بين الطريقتين احصائيا باستخدام اختبار t ذو الطرفين و اختبار معيار العلاقة . **النتائج:** معيار قيم المتغيرات اختلفت اختلافا ذو دلالة في المجموعة الثالثة وكانت قيمها كبيرة في النتائج المستخلصة من جهاز العد الاتوماتيكي, لوحظ ايضا علاقة ايجابية كبيرة بين النتائج المستخلصة من جهاز العد الاتوماتيكي والنتائج اليدوية ($r = 0.82$) ($p<0.001$) للمجموعتين الاولي والثانية. اما قيم المجموعة الثالثة فلم يكن لها علاقة ذات دلالة . **الإستنتاج:** اعتمادا علي النتائج التي لوحظت من العينات التي تعاني من انخفاض شديد في عدد الصفائح الدموية فان جهاز العد الأوتوماتيكي اكثر اعتمادية وموثوقية من الطريقة اليدوية . ولكن ايضا الطريقة اليدوية باستخدام عداد الخلايا الدموية مع دراسة شكل الخلايا في مسحات الدم يمكن ان تكون واعدة في حالة كانت الصفائح الدموية قليلة جدا وعملاقة.

INTRODUCTION

In many disorders, Platelet count estimation is an important element of the diagnostic and treatment process. In patients with abnormal thrombocytes where platelet transfusion is required, the reliability of the platelet count is highly desired and necessary to provide appropriate treatment. Hematology analyzers are intended to count (patient) blood

Received on: November 21, 2013 Accepted on: December 24, 2013

*Correspondence: r.choudhary@mu.edu.sa; m.rizvi@mu.edu.sa

[1]Research Scholar (Medical Lab. Technology), Singhania University, Jhunjhunu, Rajashthan, India; [2]Assistant Professor (Medical Physiology), Department of Medical Lab. Technology, College of Applied Medical Sciences, Majmaah University, Majmaah, KSA; [3]Lecturer (Hematology), Department of Medical Lab. Technology, College of Applied Medical Sciences, Majmaah University, Majmaah, KSA

samples. Many blood centers use these analyzers to perform quality control on the blood components that they produce. The platelets are counted with hematology analyzers, but varying results among different hematology analyzers are observed, making comparisons very difficult.

Measurement of platelet counts using automated hematology analyzers is usually quite precise and accurate. However, the accuracy of automated platelet counts can be compromised when measuring very low platelet counts or in the presence of interference from non-platelet particles or platelet abnormalities.

Recent studies, mainly focusing on the counts of low levels of platelets, demonstrated that automated counts were not as accurate in severely thrombocytopenic samples. Traditional microscopic methods are very intense with variable results and are highly dependent on the individual training. Recent developments in automated peripheral blood differentials using a computerized system have shown many advantages as a viable alternative. But some cases like morphology abnormality and very low platelet count in not give accurate results.

The aim of this study was to determine the reliability and accuracy of the Beckman Counter Diff2 automated system with manual microscopic estimates from the peripheral Blood smear

METHODS

Blood Samples

Blood samples were obtained from 75 patients who were randomly divided into three groups. Group I (n = 25) had the patients with normal count, Group II (n = 25) had the patients with mild thrombocytopenia and Group III (n = 25) had the patients with severe thrombocytopenia. Venous blood specimens were collected and within 20 seconds from blood sampling, blood was transferred to a tube containing ethylenediaminetetraacetic acid (EDTA), and within 4 hours a whole-blood count was performed using an automated cell counter and manual method. 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 disproportionately high concentration of EDTA would be present; these samples were excluded from the study.

Automated Analyzer Platelet Counts

After thorough mixing of each blood sample on an automated mixer for 10 min, a complete automated blood count was performed using Beckman coulter Diff 2 (Beckman, USA), which was maintained and calibrated as recommended by the manufacturer.

Manual Platelet Estimation

This study done by using improved Neubaure chamber (Paul Marienfeld GmbH & Co). Thin air-dried blood smears made after thorough mixing of each sample were stained manually with a May-Grünwald-Giemsa 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. If neither aggregates nor macrothrombocytes were found, 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, which is a laborious and time consuming method. Then all the platelets in the same field were counted. Other fields were examined in the same way until we reached a minimum number of 1000 erythrocytes. The number of platelets per 1000 erythrocytes was multiplied by the automated Red Blood Count (RBC) ($x10^6$cells/µl) to give an approximate manual count ($x10^3$cells/µl). A morphological study done by peripheral blood smear stained by Leishman's stain. After processing the samples, the cell counter values and manual method values were compared in all the groups.

STATISTICAL ANALYSIS

The data was entered into Excel spreadsheet (Microsoft, Redmond, Washington) but was exported to SPSS 20.0 for data analysis. Averages were reported for quantitative variables. Two-independent sample t-test was applied to compare quantitative values. Pearson-correlation was also applied to observe correlations. A p-value of <0.05 was considered to be statistically significant.

RESULTS

Seventy-five patients randomly divided into three

groups were compared for platelet count by automatic methods and manual cell count using hemacytometer. Group-A: Consisted of twenty-five patients with normal platelet count values ranging from 1, 50,000 to 4, 00,000/mm^3 Group-B: - consisted of twenty-five patients ranging from 30,000 to 1,50,000/mm^3 and Group-C: consisted of twenty-five patients with low platelet count ranging from 5,000 to 30,000/mm^3

In the present study, all group samples were analyzed and coefficient of variation was calculated by statistical method. Group A samples were estimated by Automated cell counter and manual method by number chamber, No significant difference was observed in the coefficient of variation between automated cell counter and manual method (33.44 vs 24.97, p=0.189). In Peripheral smear, the platelets appeared to be adequate with normal morphology. In group B, again no significant difference was observed in the coefficient of variation between cell counter result and manual method (41.86 vs 36.93 p=0.239). However, in group C, coefficient of variation values were significantly high for automated cell counter as compared to manual method values (24.50 vs 22.06, p=0.009) Figure 1. When correlations were applied, high postitive correlation was observed between Automated cell counter values and manual method (r=0.82, p<0.001) in group A and B. However, values in group C were not significantly correlated.

Table1: Distribution of Coefficient of Variation bewteen Different Groups

Groups	Methods	CV
Group I	Automated Cell Counter	33.44
	Manual	24.97
Group II	Automated Cell Counter	41.86
	Manual	36.93
Group III	Automated Cell Counter	24.5
	Manual	22.06

DISCUSSION

The comparison was done irrespective of age, sex and specific disease. The patient's sample were taken randomly and processed for study. The aim of the study was correlation of various methods of platelet counting so that we can get reliable assessment of platelet count to avoid unnecessary platelet transfusion therapy and the main objectives are the reliable platelet counting for prophylactic platelet transfusion in severe thrombocytopenic patients and the second most important object was to find out the factors responsible for unreliable platelet counting. Stibbe W et al were measured and the platelet counts compared using the various blood cell counters of the central laboratory, University Clinic, Göttingen. Using the H 6000, Hemalog 8 (both Technicon) and ELT-8 (Ortho Instruments), product-moment correlation coefficients (r) ranging from 0.88 to 0.92 were obtained between the different counters. They found the correlation coefficients of the thrombocounter (Coulter) to the other instruments were only 0.84 and 0.85. Discrepant platelet counts (difference greater than 40x10^9/L) were reinvestigated using the Neubauer chamber. From a total of 354 platelet measurements, falsely high concentrations were observed only in three cases and falsely low concentrations in seven cases [3, 4]. Our study shows group I and group II not significant

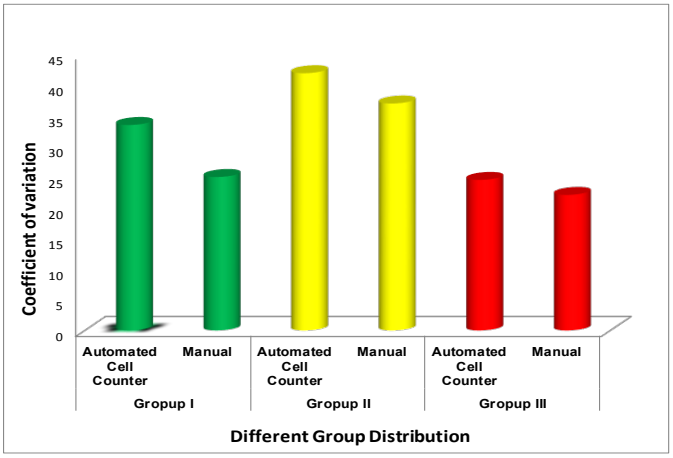


Figure 1: Comparison of platelet count reproducibility (coefficient of variation) in 3 different groups using manual and automated methods

Lawrence JB et al studied on very low platelet count about 20 × 10^9/L (20,000/µl) threshold for prophylactic platelet transfusion may be unnecessarily high. The widespread use of this threshold may reflect lack of confidence in the reliability of low platelet count [5]. They evaluated the performance of automated platelet counts and their relation to clinical bleeding. First, they prepared serial blood dilutions with "target" platelet counts from 2 to 40 × 109/L for the 48 measurements on 2 × 10^9/L "target" dilutions, values of 1 or 2 × 10^9/L were obtained with the Sysmex NE-8000 analyzer (mean 1.44 × 109/L; SD 0.31 × 109/L). Similarly, for 5 × 10^9/L "target" counts, automated

counts were 3-6 × 10^9/L (mean 4.42 × 109/L; SD 0.18 × 10^9/L) [6]. Similar results were observed with all other "target" levels, with coefficients of variation (CV) from 6.39% to 7.71% with 10-40 × 10^9/L "target" values. Secondly, they compared triplicate automated and manual platelet counts in thrombocytopenic patients with platelet counts from 4–30 × 10^9/L. The triplicate automated platelet counts differed by no more than 5 × 10^9/L among themselves, whereas the manual counts varied by as much as 30 × 10^9/L. Mean platelet counts: automated, 14.40 × 10^9/L (CV 10.12%); manual, 16.48 × 10^9/L (CV 30.39%) (P = 0.038 for counts; P < 0.001 for CV). Finally, they observed highly significant correlations between the automated platelet count and major and minor bleeding manifestations. Thus, automated platelet counts are highly reliable and accurately predict clinical bleeding.

The use of automated analyzers should facilitate improved prophylactic platelet transfusion protocols. In this condition our result also support if platelet count is very low than automated cell count is given accurate and reliable results [7,8]. Malok M estimated that the traditional method of estimating platelet counts from peripheral blood smears to evaluate automated results appears to provide adequate quality assurance. Accurate platelet counts in severe thrombocytopenia are very difficult to take decisions at prophylactic platelet-transfusion thresholds.

Until recent years, the accuracy of platelet counts has been limited by the reliance of hematology analyzers on calibration material values derived from the manual platelet counting method. The calibration of automated cell counter in thrombocytopenia and the reduction of variation between instruments have been hindered by a lack of adequate quality control materials, making the accuracy of automated methodologies in routine practice difficult to assess. This situation could now be highly improved by the use of the International Reference Method (IRM) to assign calibration materials and by further knowledge of the accuracy and limitations of the particular types of automated platelet count available to the clinician These changes will improve clinical confidence in the accuracy of a platelet count and thus inform clinical decisions at the current level of prophylactic platelet transfusions.

The clinical decision to proceed with prophylactic platelet transfusions is widely based on trigger points for platelet counts are equal to 20, 10, or even 5 x 10 [(9)] /L. But an increasing number of publications show evidence that the conventional automated platelet counting methods are unable to provide consistently accurate results in this lower thrombocytopenic range.

These measurement errors are mainly associated with the most commonly used impedance principle; optical methods seem to be more precise. The problems of counting imprecision in the low thrombocytopenic range can be avoided with direct or indirect immunological counting methods using monoclonal antibodies or by time-consuming manual procedures. But how should new counting procedures be evaluated? Which method should be used as the "gold standard" for platelet counting? A way out of this apparent dilemma is the application of a statistical procedure as proposed by Gautschi et al. This mathematical model allows a reference method independent evaluation of new methods by calculation of the limits of detection (LD) and limits of quantification (LQ) based on the imprecision profile of the investigated method. Using this evaluation procedure, it can be shown that immunological automated counting methods can provide reliable, sufficient, and prompt platelet counts, especially in the thrombocytopenic range [9,10].

Briggs C was observed the platelet count four main procedures, manual phase contrast microscopy, impedance, optical light scatter/fluorescence and flow cytometry. Earlier methods to enumerate platelets were inaccurate and irreproducible. The manual count is still recognized as the gold standard or reference method, and until very recently the calibration of platelet counts by the manufacturers of automated cell counters and quality control material was performed by this method [11]. However, it is time-consuming and results in high levels of imprecision. The introduction of automated full blood counters using impedance technology resulted in a dramatic improvement in precision. However, impedance counts still have limitations as cell size analysis cannot discriminate platelets from other similar-sized particles. More recently, light scatter or fluorescence methods have been introduced for automated platelet counting, but there are still occasional cases where an accurate platelet count

remains a challenge. Thus, there has been interest in the development of an improved reference procedure to enable optimization of automated platelet counting.

This method utilizes monoclonal antibodies to platelet cell surface antigens conjugated to a suitable fluorophore. This permits the possible implementation of a new reference method to calibrate cell counters, assign values to Calibrators, and to obtain a direct platelet count on a variety of pathological samples. In future, analysers may introduce additional platelet parameters; a reliable method to quantify immature or reticulated platelets would be useful [11,12].

There were different methods of platelet count in hematology laboratory and these methods were manual counting, automated cell counting, platelet count by peripheral blood smear, immunoplatelet counting and radioisotope labeling technique for platelet counting. Many authors had given their thoughts on the methods of platelet counting. Reliability of platelet count by comparison with manual method was studied by Lawrence J.B (1995). Koh T (1996) also concluded the discrepancy of platelet numbers between automated blood cell analysis and manual counting in patients with thrombocytopenia. In 21st century (2004), Web DI, suggested the platelet count assessment from peripheral blood smear (PBS) [13,14,15].

Mainly automated systems were two types, semi-automated and fully automated. Semi-automated required sample preparation (for example dilution) by a technician and fully automated equipment performed all steps subsequent to obtaining the sample itself. The principle of electronic counting based on Electrical Impedance or Light Scattering [16]. The methods were used for comparison of platelet counts are automated cell counter Act Diff 2 and manual platelet counting by hemacytometer with diluting fluid 1% ammonium oxalate which lyses the red cells and preserves the platelet. In automatic analyzer the processing was done automatically within few minutes. In automated counting the principle is based on Impedance and Impedance counting first described by Wallace Coulter (1956). Lindsay RM (1975) evaluated the counting of platelets by electronic methods, had become widely accepted by laboratories and method was considered to give better reproducibility than the conventional microscopic count. Mayer K (1980) gave a comparative evaluation of the latest instrumentation by automated platelet counters [17,18,19,20].

Conclusion

In this study, on the basis of results, it is recommend that a manual platelet count using haemocytometer and morphology study of the blood smear should be performed if platelets is very low and giant platelets.

Conflict of Interest

This research project was self-sponsored without any financial aid from any institution/ company. There was no financial interest of/in any company or institution that might benefit from their publication. The authors have no competing/conflict of interests associated with this publication.

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