Original Article

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Modifications to the Manual Assessment of Platelet Counts from Peripheral Blood Smears- An Essential Correction Never Mentioned Before in Literature

Muthu Sudalaimuthu*, Koshalya Rajendran, Shivashekar Ganapathy*****

*Assistant Professor, **Postgraduate Resident, ***Professor & Head, Department of Pathology, SRM Medical College Hospital and Research Centre, Potheri, Kancheepuram District, TamilNadu

Abstract

Context: Platelet counts from automated haematologyanalysers are crosschecked in peripheral smears by counting the number of platelets in ten oil immersion fields and multiplying their average by 15000. Since different microscopes have different field diameters, the area viewed and the number of platelets counted in ten fields differs between microscopes. Hence it is inappropriate to use the same multiplication factor of 15000 in all microscopes. *Aims:* To determine whether the multiplication factor of 15000 should be modified in a microscope with field number 20. *Settings and Design:* Platelet counts were estimated by two different methods from peripheral blood smears by using a microscope with field number 20. Multiplication factor used was 12000 in method A and 15000 in method B. Results of the two methods were compared with automated platelet counts. *Methods and Material:* Automated platelet counts were obtained from Sysmex XT1800i for 200 blood samples and compared with the manual counts estimated from Leica microscope with field number 20 by the two methods mentioned above. *Statistical analysis used:* ANOVA, student's t test, correlation coefficient. *Results:* Results from method A correlated strongly with automated platelet counts (correlation coefficient of 0.978) and did not differ significantly from them (p value of 0.28). Method B results differed significantly from automated counts ($p < 0.001$). *Conclusions:* Modifications to multiplication factor are essential when microscopes of different field diameters are used for platelet count estimation. We have suggested those modifications needed for various types of microscopes in this article.

Keywords: Automated Analysers; Haematology; Microscopes; Platelet; Platelet Count.

Introduction

Accurate measurement of platelet counts is essential in the monitoring andmanagement of patients with many disorders and has got prognostic significance as well [1-5]. But unfortunately the accuracy of automated haematologyanalysers in measuring the platelet counts is low particularly in cases of thrombocytopenia [6,7]. Hence various methods have been proposed to crosscheck the platelet counts obtained from automated haematologyanalysers. One of the methods that is widely in use is by counting the average number of platelets in ten oil immersion fields in peripheral blood smear and multiplying it by 15000 [8]. But different microscope models have different field diameter and the area of slide viewed in an oil immersion field varies between microscopes. Hence this study was done to find out whether the same formula can be applied to estimate platelet count using a microscope with field number 20 or whether it needs modifications.

Corresponding Author: Muthu Sudalaimuthu, 804, New B Block, SRM Medical Staff Quarters, Potheri, kancheepuram district,TamilNadu - 603203.
E-mail: drmuthus@gmail.com

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Materials and Methods

200 blood samples that were received in our laboratory for complete blood counts were included in our study. An informed consent was obtained from the patients before doing this study. EDTA anti-coagulated blood samples were fed to the automated haematology analyser Sysmex XT-1800i within three hours of collection and platelet counts obtained. Cases of thrombocytopenia were excluded from our study as platelet counts from automated analysers can be

inaccurate in cases of thrombocytopenia. Air dried thin peripheral blood smears were prepared from the blood sample and stained with leishman stain. These smears were examined by a microscope (Leica) with Field number 20 (Field number of a microscope will be mentioned in the eyepiece of the microscopes next to the magnification). Cases with platelet clumps were excluded from the study.

An optimal area where platelets were regularly distributed at the junction of body and tail where the cells were in monolayer was selected and observed under oil immersion objective. Platelets were counted in ten fields and their average was taken. Platelet count was calculated from this value by two methods. In method A this value was multiplied by 12000 and in method Bit was multiplied by 15000. A multiplication factor of 12000 was selected in method A because the field area of a microscope with field number 20 is approximately 1.25 times the field area of a routine microscope with field number 18 (Field diameter viewed under an objective lens can be calculated by dividing the field number by magnification power of the objective, provided there is no tube lens. Field area can be calculated from this field diameter). Hence the already existing multiplication factor of 15000 was corrected to 12000 in method A. Platelet counts obtained by these two methods were compared with the automated blood counts. ANOVA, student's t test and correlation coefficient were used to analyse the results with Microsoft excel 2010.

Results

Automated platelet counts of the samples ranged from 155000 to 685000 with a mean value of 295081. Results from method A were similar with platelet counts ranging from 156000 to 720000 with a mean value of 306780. Platelet counts in this method were lower than the automated platelet count in 53 cases (26.5%) and higher in 147 cases (73.5%). In 76% of the cases (152 cases), difference from the automated count was less than 30000. Results of method B showed significant variations with platelet counts ranging from 195000 to 900000 with a mean of 383475. Platelet counts obtained by this method were higher than the automated platelet count in all the cases.Difference from the automated count was less than 30000 in only 5.5% of the cases (11 cases). ANOVA showed that results of these three methods were different from each other. Student's t test showed that results of method A and automated platelet counts were not significantly different (p value of 0.28). But the results of method B were significantly different from automated counts (p value <0.001) and from method A as well (p value <0.001). Correlation coefficient was high showing a strong correlation between method A and automated counts (r value of 0.978). Scatterplot showing the results of method A plotted against automated counts is given as Figure 1.

Table 1: Suggested modifications to the multiplication factor when using microscopes with different field numbers for assessing platelet count*

Field Number of the Microscope used	Suggested Multiplication Factor
18	15000
20	12000
22	10000
25	8000

*Assuming there is no additional tube lens in the microscope used

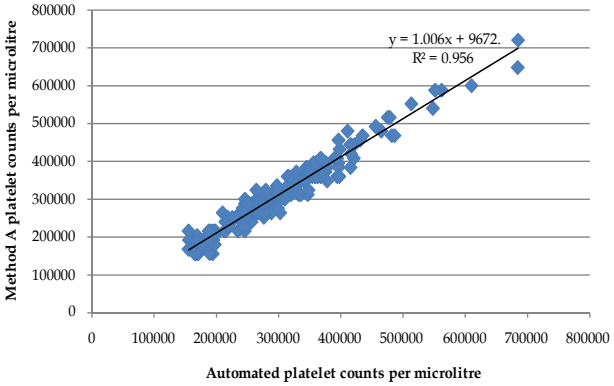

Fig. 1: Scatterplot showing minimal dispersion and a strong correlation between automated platelet counts and results of Method A.

Discussion

Accurate measurement of the platelet counts is essential in monitoring patients with various disorders and to plan further management. Besides these it has got prognostic significance as well [1-5]. But this accurate measurement of platelet counts is a challenge to the pathologist because the routinely used automated analysers are known to be less accurate in estimating platelet counts particularly in patients with thrombocytopenia [6,9]. Although the International Council for Standardization in Haematology (ICSH) and the International Society of Laboratory Haematology (ISLH) have recommended a method based on platelet/Red Blood Cells (RBC) ratio and fluorescent labeled platelets as the reference method for platelet count estimations, its high cost prevents its routine use [10].

Few more methods have been suggested for the estimation of platelet count in the recent past [11-13], particularly the method based on platelet/RBC ratio

in peripheral smear seems promising. Butthese methods are dependent on some automated analyser values. Of all the existing methods, the only reliable method that can be used even in the absence of automated analyser is the traditional method where the average number of platelets per oil immersion field is multiplied by 15000. Although it has certain disadvantages, this method is popular among pathologists as it is simple and can be used even in an emergency as well as in rural areaswhen none of the automated analysers are available.

One of the major drawbacks of this long existing method is inter-observer variability and sometimes variability even when the same person reviews the slide again. This was observed by Nosanchuket al [14] also in their study, the study which first established the validity of this method. Such variability has been noted by Gao et al [15] as well in their study. This variability is due to the differences in the field selected and field to field variations in the number of platelets. Such variations can be minimized by examining an area where the platelets are regularly distributed and where the cells are in monolayer at the junction of body and tail.

Our study results show that the routine multiplication factor of 15000 cannot be used in microscopes with field number 20 as it overestimates the platelet count significantly. This variation was more marked in patients with thrombocytosis. A multiplication factor of 12000 gave platelet count results within acceptable range.Nosanchuket al[14] had suggested that eight fields can be counted instead of ten fields in microscopes with wider field of view to compensate for the wide area measured. We feel counting for eight fields is not the ideal solution for this issue because so many varieties of microscopes are available today with a wider field and having varying field numbers (20, 22, 25 etc). Counting for ten fieldsmakes the calculations a little easier and also has the advantage of viewing more area so that the field to field variation in the distribution of platelets can be covered up.

Another dilemma in this method is some authors suggest a multiplication factor of 20000. Many studies are being published on this with some studies supporting a multiplication factor of 15000 [8,16,17] and some others supporting 20000 as multiplication factor [13-15,18]. A clear conclusion cannot be arrived at about the multiplication factor after reading these articles, more so because these articles have not mentioned the field number of the microscopes they have used in their study. Our study results are in a favor of 12000 as multiplication factor for microscopes with field number 20 which indirectly means that a multiplication factor of 15000 is optimal for microscopes with field number 18. Using the same concept, we suggest that the multiplication factors given in Table 1 can be used for microscopes with other field numbers. For people who want to persist with 20000 multiplication factor in microscopes with field number 18, ideal multiplication factors will be 16000, 13000and 10000 for microscopes with field numbers 20, 22 and 25 respectively. One must also remember that if their microscopes have an additional tube lens, corrections must be made for that as well by dividing the values given in these tables with magnification of the tube lens. However these suggestions need to be confirmed by further large scale studies in future.

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Conflicts of Interest

Declared none.

References

1. Makroo RN, Raina V, Kumar P, Kanth RK. Role of platelet transfusion in the management of dengue patients in a tertiary care hospital. Asian J Transfus Sci. 2007; 1(1):4–7.
2. Damani Z. Platelet count in women with pregnancy induced hypertension in university hospital center of mother and child healthcare 'Koço Gliozheni', Tirana, Albania. Mater Sociomed. 2016 Jul 24; 28(4):268–70.
3. Ten Berg MJ, van den Bemt PMLA, Huisman A, Schobben AFAM, Egberts TCG, van Solinge WW. Compliance with platelet count monitoring recommendations and management of possible heparin-induced thrombocytopenia in hospitalized patients receiving low-molecular-weight heparin. Ann Pharmacother. 2009 Sep; 43(9):1405–12.
4. Eggemann H, Ehricke J, Ignatov T, Fettke F, Semczuk A, Costa SD, et al. Platelet Count After Chemotherapy is a Predictor for Outcome for Ovarian Cancer Patients. Cancer Invest. 2015 May; 33(5):193–6.
5. Puertas M, Zayas-Castro JL, Fabri PJ. Statistical and prognostic analysis of dynamic changes of platelet count in ICU patients. Physiol Meas. 2015 May; 36(5):939–53.
6. De la Salle BJ, McTaggart PN, Briggs C, Harrison P, Doré CJ, Longair I, et al. The accuracy of platelet

counting in thrombocytopenic blood samples distributed by the UK National External Quality Assessment Scheme for General Haematology. Am J Clin Pathol. 2012 Jan; 137(1):65–74.

7. Segal HC, Briggs C, Kunka S, Casbard A, Harrison P, Machin SJ, et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. Br J Haematol. 2005 Feb; 128(4):520–5.
8. Webb DI, Parker L, Webb K. Platelet count assessment from peripheral blood smear (PBS). Alaska Med. 2004 Dec; 46(4):92–5.
9. Oliveira RAG, Takadachi MM, Nonoyama K, Barretto OC de O. Is automated platelet counting still a problem in thrombocytopenic blood? Sao Paulo Med J. 2003 Jan 2; 121(1):19–23.
10. International Council for Standardization in Haematology Expert Panel on Cytometry, International Society of Laboratory Hematology Task Force on Platelet Counting. Platelet counting by the RBC/platelet ratio method. A reference method. Am J Clin Pathol. 2001 Mar; 115(3):460–4.
11. Brahimi M, Osmani S, Arabi A, Enta-Soltane B, Taghezout Z, Elkahili BS, et al. The estimation of platelet count from a blood smear on the basis of the red cell: platelet ratio. Turk J Haematol. 2009 Mar 5; 26(1):21–4.
12. Sutor AH, Grohmann A, Kaufmehl K, Wündisch T. Problems with platelet counting in thrombocytopenia. A rapid manual method to measure low platelet counts. Semin Thromb Hemost. 2001 Jun; 27(3):237–43.
13. Malok M, Titchener EH, Bridgers C, Lee BY, Bamberg R. Comparison of two platelet count estimation methodologies for peripheral blood smears. Clin Lab Sci. 2007; 20(3):154–60.
14. Nosanchuk JS, Chang J, Bennett JM. The analytic basis for the use of platelet estimates from peripheral blood smears. Laboratory and clinical applications. Am J Clin Pathol. 1978 Apr; 69(4):383–7.
15. Gao Y, Mansoor A, Wood B, Nelson H, Higa D, Naugler C. Platelet count estimation using the CellaVision DM96 system. J Pathol Inform. 2013; 4:16.
16. Bajpai R, Rajak C, Poonia M. Platelet estimation by peripheral smear: Reliable, rapid, cost effective method to assess degree of thrombocytopenia. International Journal of Medical Science Research and Practice 2015; 2(2):90-93.
17. Hoffman R, Benz JR, Silberstein LE, Heslop H, Weitz J, Anastasi J. Hematology: Basic Principles and Practice. 6th edition. Philadelphia, PA: Elsevier Health - US; 2012.
18. Al-Hosni ZS, Al-Khabori M, Al-Mamari S, Al-Qasabi J, Davis H, Al-Lawati H, Al-Riyami AZ. Reproducibility of manual platelet estimation following automated low platelet counts. Oman Medical Journal. 2016 Nov; 31(6):409-13.