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Determination of Platelet Count Estimation Factor on Peripheral Blood Smear Confirmation Using Field Number 22 Microscope

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ABSTRACT

The automatic platelet count sometimes requires confirmation on the peripheral blood smear. Platelet count estimation can also be used for reporting platelet count if an automatic cell counter is not available, with an estimation factor according to the Field Number (FN) of the microscope used. This study aimed to determine the platelet count estimation factor based on peripheral blood smear confirmation using an FN 22 microscope. An observational cross-sectional study was carried out in patients who had routine hematological and peripheral blood smear examinations during September 2021 by determination of platelet count using the automatic cell counter method and an average number of platelet counts per field of view with 100x objective magnification. The estimation factor is the total ratio divided by sample size. The total ratio of 254 samples was 4.086. The platelet count estimation factor was 16, indicating that 1 platelet per field of view was equivalent to 16x103/µL. There was a very strong significant correlation between mean platelet count per field of view and platelet count using the automatic cell counter ($p<0.001$, $R>0.750$). The field number is the image diameter of the microscope eyepiece. The latest generations of microscope use FN 20 or more, which provides a wider field of view, enabling the observation of more platelets. Factor estimation was used to determine the estimated platelet count on a peripheral blood smear. A big difference between automatic cell counter and peripheral blood smear might indicate pre-analytic, analytic, and post-analytic errors. The platelet count estimation factor based on peripheral blood smear confirmation using the FN 22 microscope was 16. Each laboratory needs to determine the estimation factor according to the FN microscope used.

Keywords: Estimation factor, platelet count, FN 22

INTRODUCTION

Platelet count is one of the parameters in routine hematology tests, which can be carried out by automated and manual methods. Indirect platelet count can be performed using the Peripheral Blood smear (PBS) with a method known as the Fonio method and the Barbara Brown estimation. Currently, more platelet counts are performed using the automated method (hematology analyzer).[1]

The advantages of the automated method are the more efficient and effective work process and valid results due to a more standardized analysis process compared to the manual method. However, the hematology analyzer used in the automated method is unable to count platelets properly if there are clusters of platelets, giant thrombocytes, erythrocyte fractions, and leukocyte fractions. This is indicated by flagging the test results. To overcome this, a confirmatory test using PBS was required. All platelet count determined by automated or manual counting devices must be cross-examined on PBS to confirm an increased or decreased platelet count and to estimate any difference between the manual and automated platelet count results.[1-3]

Estimation of platelet count in PBS has so far been determined based on the Barbara Brown method using a 100x objective lens magnification of microscope in zone V, an area where the erythrocyte is evenly distributed or slightly overlaps. The average number of platelets per field of view is determined and then multiplied by the number 20,000/µL (0.02/L); this formula is valid for normal and abnormal platelet counts. However, the microscope's Field Number (FN) is not explained. Field number determines the field of view (field of view diameter), which affects the number of platelets per field of view. Factors that estimate the number of platelets will be more precise if they are determined based on the FN microscope.[1,4,5]

The field number is the diameter of the image observed through the eyepiece, which is measured in millimeters. The eyepiece of a modern microscope shows a wider field to enable observation of a higher number of platelets compared to a regular eyepiece. On this eyepiece, a certain FN is listed according to the characteristics of the manufacturer, for example, 18, 20, or 22. This FN can vary from one microscope to another. Most microscopes have an FN of 18, while the latest generation of microscopes have started to use an FN of 20 or above.[4,6]

Research on the determination of the estimation factor for the platelets count in PBS using an FN 18 microscope has been carried out by Rohmawati, Wahyuni, and Tarmizi.[4] Determination of the estimation factor of the platelets count in PBS using an FN 20 microscope has been carried out by Juharuddin.[4] The estimation factor is determined based on the total ratio between the platelet count according to the automatic cell counter to the average platelets per field of view of the number of samples. These studies suggest that the estimation of the platelet count can be used for reporting the results of the platelet count if an automatic cell counter is not available, with an estimation factor that is in accordance with the FN microscope. For laboratories that use microscopes with different FNs, it is better to use an estimation factor according to the FN of the microscope used.[4]

The purpose of this study was to determine the estimation factor for the platelet count based on PBS confirmation using an FN 22 microscope.

METHODS

This research was an observational study with a cross-sectional approach conducted at the Clinical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital, Makassar in September 2021. The research sample was patients who performed routine hematology tests and PBS at the Clinical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital. Exclusion criteria were PBS in which platelet aggregation or giant platelets were found.

Data on the results of the platelet count using the automated method were obtained using an automatic counting device. The average number of platelets in PBS was determined in 10 fields of view with 100x objective magnification and 10x ocular magnification of an FN 22 microscope. The estimation factor for the platelet count was obtained from the total ratio of the platelet count using the automated method and the average number of platelets per field of view in PBS was then divided by the sample size.

$$\text{Estimation factor} \quad \frac{\Sigma xi}{n}$$

Σxi = Total ratio of platelet count to average number of platelet
n = Sample size

Spearman correlation test was used to determine the correlation between the mean platelet count per field of view of the FN 22 microscope on PBS and the platelet count using the automatic cell counter method. The results of statistical tests with $p < 0.05$ were reported as significant.

This research was conducted after obtaining ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine, Hasanuddin University/Hasanuddin University Hospital/ Dr. Wahidin Sudirohusodo Hospital (KEPK FKUH-RSUH-RSWS) with number 625/UN4.6.4.5.31/ PP36/2021.

RESULTS AND DISCUSSIONS

There was a total of 254 subjects who met the research criteria; most of them were from the age group of 0-5 years (37.8%) and the least were those with age >65 years (5.1%). Based on gender, most of the subjects involved in this study were male as many as 133 patients (52.4%). Based on the classification of platelet count, the most subjects in this study as many as 100 patients (39.4%) had normal platelet count and in the least subjects, as many as 11 patients had grade 2 thrombocytopenia (4.4%) (Table 1).

Table 2 shows the comparison between the platelet count using the automatic cell counter method, the average number of platelets per field of view of the FN 22 microscope on PBS, and their ratio. The data were not normally distributed.

Table 3 illustrates the correlation of the mean platelet count per field of view of the FN 22 microscope on PBS with the platelet count using the automatic cell counter method. There was a significant correlation between the average number of platelets per field of view of the FN 22 microscope on PBS and the platelet count using the automatic cell counter method ($p<0.001$). Based on the correlation coefficient of 0.966 (96.6%), the strength of the correlation between both parameters was classified as a very strong category.

Table 1. General characteristics of research subjects

	Characteristics of Subjects	Total (n=254)
Gender	Male n (%)	133 (52.4%)
	Female n (%)	121 (47.6%)
Age group	0-5 years n (%)	96 (37.8%)
	5-11 years n (%)	28 (11.1%)
	12-25 years n (%)	31 (12.2%)
	26-45 years n (%)	38 (14.9%)
	46-65 years n (%)	48 (18.9%)
	>65 years n (%)	13 (5.1%)
Platelet count based on an automatic cell counter method	Grade 1 thrombocytopenia (75.000-150.000/µL) n (%)	38 (14.9%)
	Grade 2 thrombocytopenia (50.000-<75.000/µL) n (%)	11 (4.4%)
	Grade 3 thrombocytopenia (25.000-<50.000/µL) n (%)	13 (5.1%)
	Grade 4 thrombocytopenia (<25.000/µL) n (%)	32 (12.6%)
	Normal platelet count (>150.000-450.000/µL) n (%)	100 (39.4%)
	Thrombocytosis (>450.000/µL) n (%)	60 (23.6%)

Table 2. Comparison between platelet count using automatic cell counter method and average number of platelet per field of view of the FN 22 microscope on PBS (n=254)

Variable	Median (min-max)	Mean±SD	p*
Platelet count using automatic cell counter method (10^3/µL)	242.00 (2-1240)	285.51±235.87	<0.001
The average number of platelet per field of view of FN 22 Microscope on PBS (cells/field of view)	15.00 (0-82)	17.32±14.98	<0.001
Ratio	16.00 (0-94)	16.09±8.30	<0.001

*Kolmogorov-Smirnov test

Table 3. correlation between the average number of platelet counts per field of view of FN 22 microscope on PBS and platelet count using automatic cell counter method

Type	Variable	Statistic	Platelet Count Using Automatic Cell Counter Method
Spearman's rho	The average number of platelet per field of view of PBS using an FN 22 microscope	R	0.986
		P	0.000
		N	254

Abbrev: Spearman's Correlation test R=Correlation coefficient
Weak correlation R <0.250, moderate correlation R = 0.250-0.500, strong correlation R = 0.501-0.750, very strong correlation R >0.750

Based on the result of Spearman's rho analysis with a strong positive relationship, the correlation is presented in Figure 1.

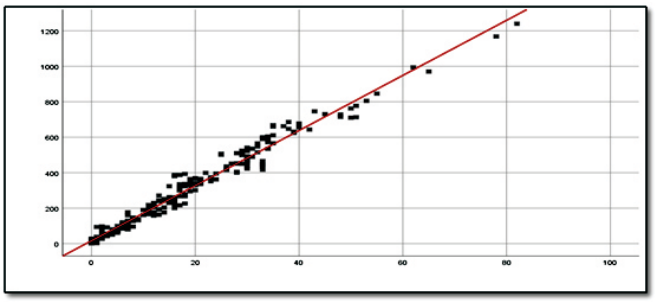

Figure 1. Scatter plot of correlation between average number of platelets per field of view of the FN 22 microscope on PBS with the platelet count using the automatic cell counter method

The total ratio of the platelet count and the average platelet count per field of view was 4.086. Therefore, the estimation factor for the platelet count based on PBS using an FN 22 microscope was 16, indicating that 1 platelet in a 100x objective field was equivalent to $16x10^3/\mu L$.

The microscope used in this study was a microscope with an FN of 22 (Figure 2), enabling observation of more platelets per field of view compared to a microscope with an FN of 18 or 20.[1,4-6]

Figure 2. Microscope with FN of 22

The field of view is the area in which the specimen can be observed. The use of a larger objective magnification will result in a smaller area with a more detailed image. The actual diameter of the field of view in millimeters can be calculated by FN divided by the objective magnification used. This calculation is important if a laboratory uses 2 microscopes with different FN.[4] An objective magnification of 100x was used in this study with an FN of 22, resulting in the diameter of the field of view of 22/100 = 0.22 mm.

Automated platelet count results sometimes require confirmatory tests using PBS to enable the estimation of platelet count. If there is a large difference between the two may indicate a possibility of errors in the platelet identification analyzer such as platelet aggregation, giant platelets, etc. Another possibility comes from pre-analytical, analytical, and post-analytic processes. Incorrect identification of samples, wrong labeling, and clots in samples are examples of pre-analytical errors. Analytical errors may occur if the PBS does not meet the requirements or if the calculator used is damaged. In addition, an error in reporting the results of the platelet count is an example of a post-analytic error. Peripheral blood smear should be made as soon as possible (<2 hours after blood is drawn) to prevent the development of morphological artifacts such as degenerative changes (cytoplasmic vacuolization of neutrophils and monocytes, lobulation or fragmentation of nuclei of nucleated cells, and apoptotic changes) or swelling of platelets. High temperatures and shocks must be avoided during blood transport to the laboratory to avoid the generation of artifacts such as erythrocyte budding and fragmentation. The characteristics of a good PBS, which meet the feasibility of the PBS technique include size, edges, and surface. The size of the blood smear covers 2/3-3/4 length of the slide, the lateral edge of the smear does not touch the edge of the slide, thin and slightly rounded edge, smooth, regular, and not perforated surface, and all drops of blood are used in the smear.[4,7]

The estimation factor is used to determine the estimated platelet count in PBS. The estimation of the platelet count can be used for reporting the results of the platelet count if an automatic cell counter is not available. Factors that estimate the number of platelets will be more precise if they are determined based on an FN microscope. The accuracy of the estimation factor also depends on the examiner's ability to identify platelets in the PBS.[1,4,5] The FN microscope will affect the field of view on the microscopy of PBS (Figure 3).

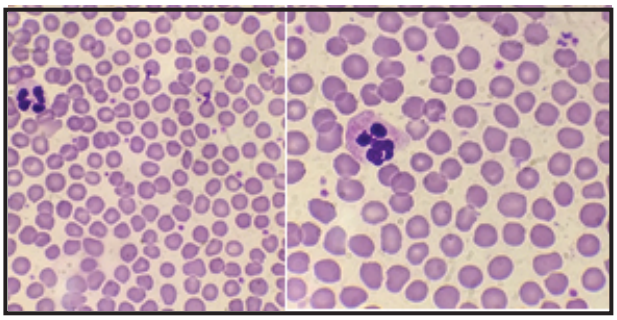

Figure 3. Microscopy of PBS

The estimation factor obtained in this study was 16, not in line with the estimation factor obtained in a study by Juharuddin, which was 18 using an FN 20 microscope and was not in line with the estimation factor obtained in a study by Rohmawati, Wahyuni, and Tarmizi, which was 22 using an FN 18 microscope. The difference in the estimation factors obtained might be due to the use FN 22 microscope in this study. The FN 22 microscope shows a wider field of view than the FN 20 and FN 18 microscopes, enabling observation of more platelets in one field of view and obtaining a smaller estimation factor for the platelet count based on PBS confirmation using an FN 22 microscope compared to that of FN 20 and FN 18 microscope. Using a larger FN microscope will

result in a wider field of view, allowing it to observe more platelets in one field of view; therefore, the estimation factor for platelet count will be smaller.

This study found a significant correlation between the mean platelet count per field of view of the FN 22 microscope on PBS and the platelet count using the automatic cell counter method with a very strong correlation coefficient of 0.966 (96.6%). Both increased or both decreased ($p<0.001$). This was appropriate because a higher platelet count using the automatic cell counter method leads to a higher number of platelets found on PBS if there is no platelet aggregation or giant platelets.

Laboratories can use an estimation factor of 16 to determine the estimated platelet count using an FN 22 microscope. Laboratories using a microscope with a different FN must use a different platelet count estimation factor.

CONCLUSIONS AND SUGGESTIONS

The estimation factor for platelet count based on PBS using an FN 22 microscope was 16, indicating that 1 platelet per field of view was equivalent to $16x10^3$/µL. The estimation of the platelet count can be used for reporting the results of the platelet count if an automatic cell counter is not available, with the estimation factor according to the FN microscope. Laboratories that use microscopes with different FNs must use different estimation factors according to the FN of the microscope used.

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