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RESEARCH ARTICLE

Evaluation of Nucleated Red Blood Cell Morphology Flag Screening with ADVIA® 2120

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Abstract

The evaluation of nucleated red blood cell morphology flag screening with ADVIA® 2120 on behalf of blood smear has been investigated. The accurate and timely reporting of nucleated red blood cells (NRBC) is an important function of the clinical hematology laboratory because the presence of NRBC in human peripheral blood can show a special clinical status. It has been used 40 samples to evaluate an NRBC screening for the ADVIA® 2120 Hematology System with peripheral blood smears. The positive samples have a criteria % NRBC in range 0 – 15%. (in 100 leucocytes). The sensitivity and specificity for the presence of NRBC for all samples analyzed was 90 % and 63,33 %, respectively. There is a correlation on NRBC result screening between ADVIA® 2120 and peripheral blood smears with α 0,003 of spearman correlation.

Keywords: *Nucleated red blood cell (NRBC, flag, Clinical test, ADVIA® 2120.*

Introduction

Routine hematology which is often performed in clinical laboratories includes Complete Blood Count (CBC) automatically and examination of blood cell morphology [1]. Examination of blood morphology to support the diagnosis of various diseases, for example, an examination of red blood cells to diagnose morphological disorders (spherocytosis, anisocytosis), early detection of possible ß-thalassemia or sickle cell disease; white blood cell examination for the diagnosis of leukocytosis, leukopenia, atypical lymphocytes, acute leukemia, chronic leukemia, myeloid leukemia, or lymphoid leukemia; and platelet examination as a support for the diagnosis of thrombocytosis or thrombocytopenia [2].

A hematological examination can not be separated from microscopic techniques. With microscopic techniques, accurate results according to the morphology of blood cells that appear in the microscope will be obtained [3]. However, the microscopic examination requires time, expertise, and accuracy to obtain accurate results. Experience and comprehensive library access need to be had to support the identification of blood morphology [4]. The current era of technological and information advancements demands a Hematology Analyzer tool that has accuracy, speed of results, accuracy, friendliness to the environment, and a small risk of misuse. ADVIA® 2120 is one of the sophisticated tools with innovations for hematological examination based on the flow cytometry method [2]. Morphology examination of the nucleated erythrocyte flag from ADVIA® 2120 is a relatively breakthrough hematology analyzer.

ADVIA® 2120 uses a standard reagent for blood cell type counts and leukocyte counts for nucleated erythrocyte examinations and analyzes them based on a combination of basophil and peroxidase methods [5].

Morphology reading of nucleated erythrocyte flags is very important as a marker of diagnosis of various diseases, such as thalassemia, sickle cell anemia, and myeloma fibrosis [6, 7]. Clinical laboratories are expected to be able to provide accurate patient examination results with a Fast Examination Time (WSHP) [8, 9].

The results of the hematological examination of nucleated erythrocytes from ADVIA® 2120 can help accelerate WSHP because ADVIA® 2120 can correct the number of leukocytes if nucleated erythrocytes are found in blood samples [5]. Several studies have been carried out to determine the sensitivity and specificity of nucleated erythrocyte examination of ADVIA® 2120 [5, 10-12].

However, this research has never been done in Indonesia and in fact, the morphology of the ADVIA® 2120 nucleated erythrocyte flag during routine field checks is sometimes incompatible with manual examinations. Therefore, an evaluation study of the morphology examination of the erythrocyte flag core with ADVIA® 2120 tool on the removal of peripheral blood needs to be done to determine the sensitivity, specificity, and correlation of the two tests on environmental conditions in Indonesia.

Materials and Methods

Materials

The materials used in this study were blood samples + 3 mL, 70% alcohol, 95% alcohol, tourniquet, K3EDTA + 1.8 mg / 1 mL, immersion oil (Merck), absolute methanol (Merck), Wright color streaks (methylene blue and eosin in methanol) (Merck), xylol (Merck), pH 7.2 buffered distilled water, ADVIA® TESTpoint Hematology Controls, ADVIA® SETpointTM Calibrator, and ADVIA® 2120 reagent.

Population and Sample

The sample population was taken from daily Prodia Clinical Laboratory patients, Jl. Kramat Raya No. 53 Central Jakarta, which conducts routine blood tests during the period from May to June, as well as baby patients under one year at the Prodia Clinical Laboratory of the Mother and Child Hospital of Central Jakarta. Infant patients were used in this study because they were expected to have more chance of nucleated erythrocytes in peripheral blood.

The number of patients needed was 40 patients, with the criteria of 20 positive patients with nucleated erythrocytes and 20 patients with negative nucleated erythrocytes, all of which were detected in ADVIA 2120. The sample used did not have exclusion requirements.

Research Methods

ADVIA® 2120 Calibration and Quality Control

This calibration includes physical examination and calibration of the tool's functions. The calibration was carried out by experienced technicians using ADVIA® 2120 SETpointTM Calibrator. The ADVIA® 2120 hematology system was always controlled daily regularly or after removal of an analytical component and reagent changes with different lot numbers.

The implementation of Quality Control (QC) was carried out using ADVIA® 2120 Testpoint Hematology Controls which consisted of three control samples, namely low control, normal control, and high control.

Microscope Calibration and Quality Control

Microscope calibration was carried out by experienced technicians by checking the light pattern on the microscope from the light source to the eye of the observer, the components of the microscope, and the focus and regulation of the light. Daily control of the microscope was done by cleaning the ocular lens and microscope objective using lens paper. The light source glass and microscope condenser ought also to be ensured to be free of dust and dirt by cleaning it using methanol and lens paper.

Experiment Subject Setup

The sample used was a human blood specimen. Venous blood was drawn by damaging the base of the arm with a tourniquet, then aseptic action was carried out with 70% alcohol in the area to be injected, then the aseptic zone was allowed to dry. The syringe was inserted at an angle of 10-30^{o}. When the venous had been affected, the blood was sucked (+ 3 mL) slowly to avoid hemolysis with a vacuum tube that already contained K3EDTA. Blood was mixed immediately by shaking the tube to form number 8.

Hematology Examination with ADVIA® 2120

Blood samples containing K3EDTA were then analyzed by the ADVIA® 2120 hematology system using the flow cytometry principle. The morphology flag results will appear in a computerized program and histogram. The NRBC area was on the proxies cytogram between noise and lymphocyte population, and the basophile program to the right of the polymorphonuclear area. If there were NRBCs that did not include white and differential white blood cells, then these results indicated the presence of NRBC in the sample. Examination of blood bank preparations using a microscope

- Making the peripheral blood erase preparation
- Wright Coloring
- Read the preparation for erasing under a microscope

Analysis of Results

The analysis of the results included the calculation of the sensitivity and specificity of ADVIA 2120, as well as the determination of the morphological correlation of nucleated erythrocyte flags on the ADVIA 2120 instrument against the examination of peripheral blood erase preparations. Calculation of sensitivity and specificity using the formula:

$$\text{Sensitivity} = \frac{TP}{TP + TN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN + FP} \times 100\%$$

Erythrocyte flag morphology data core in ADVIA® 2120 instruments for examination of peripheral blood erasure preparations using SPSS version 13.0 with descriptive analysis. The statistical test used was the analysis of the relationship between the examination of nucleated erythrocyte ADVIA® 2120 to the erasure of peripheral blood to see the correlation between the two tests with the Spearman Correlation approach.

Results

The study used 40 samples, 20 of which were positive nucleated erythrocytes on ADVIA® 2120 and 20 others were negative. Six samples of infants aged one year or less were used in this study to increase the likelihood of morphology of nucleated erythrocyte flags. Under normal circumstances, nucleated erythrocytes are not found in peripheral blood, except in fetuses, infants, or children with pressure on the bone marrow [9, 13]. Two baby samples showed positive nucleated erythrocytes and the rest were negative. Table 1 and Table 2 show, respectively, characteristics and relationship between erythrocyte nucleated ADVIA 2120 and microscopic examination results.

Table 1: Characteristics of the sample in the study

Characteristics	N	Value Min.	Value Max.	Means	Standard deviation
Age (years)	35	0	82	36,52	23,98
% NRBC	20	0,60	13,70	3,925	3,677
# NRBC (x 10^9/L)	20	0,20	2,68	0,517	0,665
# WBC (x 10^3/µL)	40	2,79	316,68	16,00	49,09
# RBC (10^6/ µL)	40	2,51	7,25	4,70	1,08

Table 2: Relationship between erythrocyte nucleated ADVIA 2120 and microscopic examination results

Peripheral blood smear sample	ADVIA® 2120		Results
	Morphology Flag	Quantity	
+	+	9	TP
-	-	19	TN
+	-	1	FN
-	+	11	FP

Notes: TP = True Positive, FN = False Negative
TN = True Negative, FP = False Positive

The results of the calculation of the sensitivity and specificity of ADVIA 2120 were 90% and 63.33%. The calculation results showed that the morphology examination of the ADVIA® 2120 nucleated erythrocyte flags for peripheral blood eradication at an NRBC percentage of less than 15% showed a correlation

Discussion

Red blood cells have an important role in the body's homeostatic system. The main function of red blood cells is to carry oxygen from the lungs to the tissues and cells of the body through binding with hemoglobin. All body tissues and cells need oxygen as an energy source to carry out these tissues and cell functions. Energy is obtained by cells through the mechanism of cellular respiration.

Without oxygen, the cell will die [14]. The presence of nucleated erythrocytes in peripheral blood vessels indicates that the body needs more red blood cells than normal. The body detects a lack of oxygen in cells or tissues (hypoxia) and secretes greater erythropoietin which will stimulate erythropoiesis activity. To maintain homeostasis for cells and tissues that need red blood cells to send enough oxygen, the immature erythrocytes must be removed by the body from its factory, bone marrow [15].

The presence of nucleated erythrocytes in peripheral blood vessels can show certain pathological meanings for hemolytic anemia, sickle cell anemia, thalassemia, and myelofibrosis [8, 9, 16-18]. Kratz *et.al* [19] have examined ADVIA® 2120 nucleated erythrocytes using 960 samples from Boston (USA), Paris (France) Leeds (United Kingdom), Rome (Italy), and Benevento (Italy) with a range of 0-150 nucleated erythrocytes per 100 blood cells white.

The examination showed that ADVIA 2120 had a sensitivity and specificity of 77.3% and 74.6%. They claimed errors in counting nucleated erythrocytes occur in less than 10 nucleated erythrocytes per 100 peripheral blood cells (<10%). Other data showed that at a percentage of less than 15 nucleated erythrocytes per 100 peripheral blood cells, discrepancies are often found, namely the appearance of false-positive tests (positive results on ADVIA® and negative on manual examinations [20].

Therefore, an evaluation study of the morphological examination of the nucleated erythrocyte flag on the ADVIA® 2120 device for peripheral blood erases needs to be carried out. Core nuclei erythrocytes were normally found only in peripheral blood from the fetus, newborns, or under conditions of pressure on the marrow as a child [9]. To increase the chances of nucleated erythrocyte flag morphology, in addition to using daily patients Prodia Clinical Laboratory, Jakarta Pusat, this study also used a blood sample of a one-year-old baby or less from the Prodia Clinical Laboratory of the Mother and Child Hospital.

However, only two out of six infants had cored erythrocyte morphological abnormalities in their peripheral blood. One-day-old babies were not found nucleated erythrocytes in their peripheral blood. This proves that not all newborns have nucleated red blood cells, only babies with certain conditions, such as bleeding or blood disorders. The condition of mild bleeding in infants can stimulate the release of nucleated erythrocytes because at this stage of growth blood cells and other cells are still developing very fast [9, 17].

Manual peripheral blood erase examination was used as the gold standard of examination because the microscopic examination was supported by experience, expertise, and a comprehensive library could be identified whether or not there was a real nucleated erythrocyte in the sample.

Table 1. Shows that there were still quite a lot of false positives (11 samples).From the results of the study, NRBC Gauss Fit and NRBC Residual curves in false-positive stated showed a low type of curve and tend to be flat (not so sharp). This false-positive situation with a low and flat curve showed a very sensitive tool to detect the presence of NRBC. The sensitivity of this tool is important as safety or "alarm" to immediately confirm the results of the examination with blood erasure preparations that are the gold standard [5, 9].

False-positive circumstances could be caused by several factors, namely the presence of erythrocytes that were not lysis, Erythrocytes in fetal blood samples, newborn margins, or patients with blood abnormalities (such as thalassemia) were more resistant to not lysis at the NRBC identification stage, so the

erythrocytes were detected as NRBC in the same area on the perox cytogram, the presence of large platelets.

In the case of certain diseases, peripheral blood contains large platelets which were also detected as NRBC because they occupied the same area in the peroxidase channel as NRBC, the existence of a lipemic sample. Blood samples that contain high fat interfere with the NRBC examination. This effect was observed in the perox and basophil ducts and could affect NRBC examination results [20].

The original positive state of the data from this study was found in the cored erythrocyte examination. The original positive state of the study data was found in the examination of nucleated erythrocytes with a high percentage (> 3.4%) or large amounts (> 2x109 cells / L). Also, the sample has a sharp NRuss Gauss Fit and NRBC curve that was visible between the unstained event and the alpha event curve. The existence of this sharp curve could be used as a reference for the presence of nucleated erythrocytes in peripheral blood.

The results of false-negative examinations were known to have a low but sharp NRuss Gauss Fit and NRBC residual curve. However, ADVIA® 2120 did not detect nucleated erythrocytes. ADVIA® 2120 detects the presence of nucleated erythrocytes in conditions where there were at least 200 NRBC/µL or 2% with requirements for a minimum white blood cell count of 3000/µL.

The presence of false-negative results could be caused by errors in the reading of the device so that the nucleated erythrocytes in the sample are identified as white blood cells. This was supported by a large white blood cell count of 11.23 x 103 cells/µL. Examination data for the original negative sample from ADVIA® 2120 was quite good (19 samples from 20 negative samples).

The state of the original negative sample had characteristics without the presence of NRBC Gauss Fit or Residual NRBC curves, or even if there was only a very low curve and was suppressed by an unstained event curve. Reading NRBC gauss fit and residual curves could be an alternative observation of the presence of nucleated erythrocytes in addition to the percentage and amount of NRBC data released by ADVIA® 2120.

The results of the calculation of sensitivity and specificity of ADVIA® 2120 differ from the results of the study of Kratz, *et al*., which stated that the sensitivity and specificity of ADVIA® 2120 were 77.3% and 74.6%. This was caused by differences in research conditions, such as the number of samples used by Kratz *et al*. more (960 samples) with a range of percentages of NRBC used 0-150%. Also, samples collected from various countries (US, Italy, United Kingdom, and France) allowed for differences in blood characteristics, such as total blood volume in the body or differences in the percentage of blood composition [9, 21].

ADVIA® 2120 was programmed to shoot laser beams at the right angle and time so that the volume, shape, core complexity, and refractive index of blood cells could be known precisely [20]. In this study, the nucleated erythrocyte examination technique used was to clear the viewing zones of 1-3 peripheral blood eraser preparations allowing for more accurate results in the presence or absence of nucleated erythrocytes in blood samples.

This was addressed with greater sensitivity (90%) than the sensitivity of the study of Kratz *et.al* (77.3%) who only used 100 leukocytes as reference parameters. Poor shaking when making blood erasure preparations could result in uneven blood erasure which results in negative results on manual examination [9]. The specificity of the calculated results (63.33%) was smaller than the study of Kratz *et. al* (74.6%) because the percentage of NRBC percentage used in this study was 0-15% while Kratz *et al*. using the NRBC range of 0-150%.

A large percentage of NRBCs coming out of the tool could increase the chance of NRBC in the inspection of erasure. Core examination of erythrocytes using the combined method and base on ADVIA® 2120 had been shown to have compatibility with blood smear preparations.

Using this combined method was efficient in the examination of nucleated erythrocytes because it did not use variations of other reagents which could add to the cost of examination and was effective for installing nucleated erythrocytes as evidenced by the source supported by Spearman's Correlation with α 0.003. To get the results of the examination, the core that needed the best

care ADVIA® 2120, specifically in the perox and meatball channels.

From observations during this research, the study concluded that dirty perox channels could examine nucleated erythrocytes and could produce false-positive results. But after the channel was removed and re-examined by ADVIA® 2120, the morphological flag did not reappear. ADVIA® 2120 had the core programming capability of detection of erythrocyte nuclei that could be adjusted and adjusted to the inspection conditions. To obtain accurate inspection results, the ADVIA® 2120 program could be further adjusted. From the results of the study, false-positive data was more distributed in data with a percentage and a small number of nucleated erythrocytes (<2x109 cells / L and <3.4%).

Conclusion

The results of data processing showed the sensitivity and specificity of ADVIA® 2120 were 90% and 63.33%. The results of statistical data processing using the Spearman correlation approach showed that there was a correlation between the examination of nucleated erythrocytes ADVIA® 2120 with the erasure of peripheral blood with α 0.003

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