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ORIGINAL ARTICLE

The Difference in Blast Number Between Manual Count and Siemens Advia 2120i Automatic Hematology Analyzer

Perbedaan Jumlah *Blast* pada Hitung Manual dengan Alat Hematologi Siemens Advia 2120i

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ABSTRACT

Background

The development of sophisticated automated blood-cell analyzers caused the proportion of blood-count samples requiring a manual different count to diminish steadily. Blood smear remains a crucial diagnostic aid in determining the type of leukemia by the appearance and blast numbers in blood smear. Siemens ADVIA 2120i has a parameter of blast cell percentage. This study was undertaken to determine the difference in blast number between manual count and Siemens ADVIA 2120i automatic hematology analyzer.

Methods

This was an analytical observational study with a cross-sectional design. Thirty samples (22 peripheral blood and eight bone marrow) detected blast numbers from Siemens ADVIA 2120i were examined. Samples were collected from November 2015 to August 2016. A manual count was performed on each sample using a blood smear and bone marrow evaluation.

Results

Twenty-three cases of AML and 7 cases of ALL were found. Blast percentage from the manual count was between 0 to 95% (Mean 28.5%); from Advia 2120i was between 0.1 to 99% (Mean 16.2%). There was a significant difference in conformity results from blast number between manual count and Siemens ADVIA 2120i with $p<0.05$.

Discussion

The difference in blast numbers between manual count and Siemens ADVIA 2120i could be caused by: (1) in manual count, determining blast cells is based on cytoplasm characteristics, granules, nuclear cells, nuclear chromatin, and nucleoli. (2) in ADVIA 2120i, determining blast cell is based on complexity and resistance from BASO reagent.

Conclusion

Blast numbers were significantly different between manual count and Siemens ADVIA 2120i.

Keywords: ADVIA 2120i; Blast numbers; Blood smear evaluation; Leukemia

ABSTRAK

Latar Belakang

Kemajuan teknologi alat hematologi otomatis menyebabkan permintaan pemeriksaan hitung jenis manual menurun. Hapusan Darah Tepi (HDT) tetap memiliki peran dalam membantu menentukan jenis leukemia, dengan melihat tipe dari jenis dan jumlah/hitung blast pada hapusan darah yang semestinya tidak ditemukan pada HDT normal. Pada alat hematologi Siemens ADVIA 2120i terdapat parameter persentase jumlah sel *blast*. Penelitian ini menilai perbedaan jumlah sel *blast* yang didapat dari hitung manual dengan alat hematologi Siemens ADVIA 2120i.

Metode

Penelitian bersifat analisis observasional dengan rancangan *cross sectional*. Sampel penelitian berjumlah 30 (22 sampel darah tepi, 8 sampel sumsum tulang) yang terdeteksi adanya sel *blast* pada alat hematologi Siemens ADVIA 2120i, Sampel dikumpulkan dari bulan November 2015 s/d Agustus 2016. Masing-masing sampel dilakukan perhitungan manual jumlah sel *blast* dengan pemeriksaan hapusan.

Hasil

Dari 30 sampel didapatkan 23 kasus AML dan 7 kasus ALL. Rentang jumlah sel blast pada hitung manual 0 sampai 95% dengan mean 28.5%, rentang jumlah sel blast pada alat hematologi Siemens ADVIA 2120i 0.1 sampai 99% dengan mean 16.2%. Kesesuaian hasil perhitungan jumlah blast antara hitung manual dan alat hematologi Siemens ADVIA 2120i terdapat perbedaan bermakna dengan nilai $p < 0.05$

Pembahasan

Perbedaan hasil perhitungan jumlah sel *blast* antara hitung manual dan alat ADVIA 2120i dikarenakan pada perhitungan manual penilaian sel *blast* dari ciri sitoplasma, adanya granula, bentuk inti sel, kromatin inti, anak inti, sedangkan pada alat hematologi Siemens ADVIA 2120i hanya berdasarkan atas kompleksitas dan ketahanan terhadap reagen BASO.

Kesimpulan

Terdapat perbedaan bermakna jumlah *blast* antara hitung manual dengan alat hematologi Siemens ADVIA 2120i.

Kata Kunci: ADVIA 2120i; Jumlah blast; Hapusan darah; Leukemia

INTRODUCTION

Leukemia is a malignancy associated with the bone marrow and blood, characterized by changes in hematopoiesis progenitor cells and widespread infiltration in the bone marrow. There are four types of leukemia, namely Acute Myeloid Leukemia (AML), Chronic Myeloid Leukemia (CML), Acute Lymphoblastic Leukemia (ALL), Chronic Lymphoblastic Leukemia (CLL).[1]

Worldwide, the incidence of leukemia is estimated to be 15th (474,519 incidents) with a death rate of 11th (311,594 deaths) of all types of cancer. [2] Leukemia is also the most common type of cancer in children under five years of age and has the highest percentage of deaths which places an increased burden on individuals, families, and a country.[3]

A complete blood test is important for doctors to determine the type of leukemia. Anemia sufferers will have fewer red blood cells and platelets from a whole blood test. Examination of

peripheral blood smears (SADT) in leukemia patients revealed the presence of abnormal blast cells, where blast cells should not be found in the SADT of normal people.[4] Manual diagnosis of leukemia has several disadvantages, such as lack of trust, the subjectivity of the examiner, and requiring longer time, so Automatic inspections are becoming more popular because they are more accurate and have cheaper costs. There is no subjectivity on the part of the examiner.[4]

Immunophenotyping examination based on determining antigens found on the surface of blast cells, such as CD13 or CD33 (Cluster Designation), is the gold standard in diagnosing types of acute leukemia. Still, the equipment is expensive and not commonly available in various health facilities. In addition, Polymerase Chain Reaction (PCR) examination can be carried out to see changes in the structure or function of genes, such as FLT3 and NPMI.[5,6]

The development of automated hematology tools has decreased the number of peripheral blood smear (HDT) examinations, with a proportion of around 10-15% of the total number of examinations.[7] HDT examinations still have a role in assessing blood cell morphology to help diagnose disease and verify if there is flagging from the results. Complete blood examination using an automatic hematology tool.[8]

The Siemens ADVIA 2120i is an automatic hematology tool that measures the number and count of types of leukocytes using the principles of flow cytometry and a combination of reactions in the peroxidase and basophil methods. Cluster analysis of each method will produce cytograms and patterns, which are said to help determine hematological malignancies.[9]

Peroxidase Cytogram examination uses a peroxidase reagent to stain intracellular myeloperoxidase. As cells pass through the flow cell, the absorbed light is used to measure myeloperoxidase activity, and the luminescence is used to measure cell size and complexity. This will produce a diagram with the X axis depicting the intensity of peroxidase staining and the Y axis expressing cell size.[9]

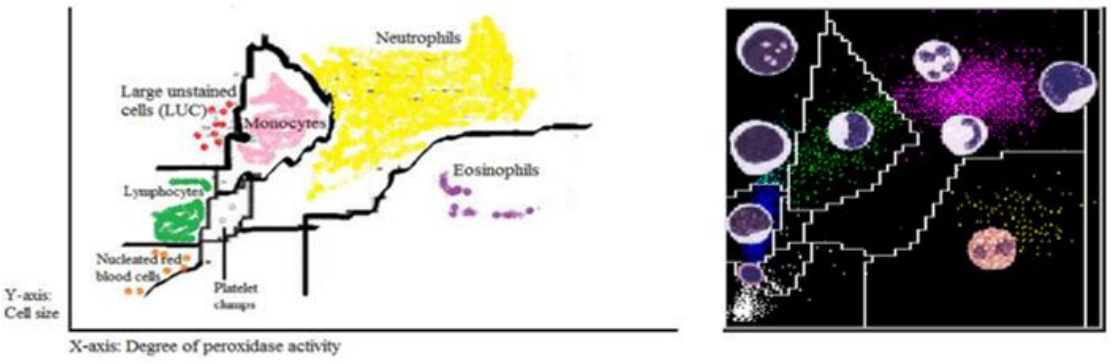

Figure 1. Peroxidase cytogram.[9, 11]

In the Basophil Cytogram examination, the Baso reagent will lyse erythrocytes, thrombosis, and the cytoplasm of all leukocyte cells except basophils. Leukocytes will pass through the laser flow cell, and the size and complexity of the cells will be detected and included in the basophil cytogram. The X-axis will describe the complexity of the cell nucleus, and the Y-axis will define the size of the cell.

Figure 2. Basophil cytogram.[9, 11]

This study aims to determine the capability of detecting the number of blasts on the Siemens ADVIA 2120i automatic hematology tool compared to manual counting on peripheral blood smears (SADT) in cases of acute leukemia.

METHODS

The type of research is observational with a cross-sectional design. The study used venous blood samples with the anticoagulant Ethylenediamine Tetra Acetic Acid (EDTA) and bone marrow samples from acute leukemia patients who were newly diagnosed or had already received therapy. The sample size was 30 samples, with details of 22 samples coming from venous blood with EDTA anticoagulant and eight samples coming from bone marrow. Diagnosing acute leukemia in research subjects was done by immunophenotyping examination using the flow cytometry method, the gold standard in diagnosing leukemia. Immunophenotyping examination uses specific markers for each series (myeloid, B, and T cells).

All samples were then analyzed with a Siemens ADVIA 2120i automatic hematology instrument to obtain the number of blasts. The leukocyte differentiation method on the Siemens ADVIA 2120i hematology device consists of the peroxidase method and the basophil/lobularity method to measure leukocyte parameters quantitatively. Flagging blasts on the Siemens ADVIA 2120i hematology device will appear if the blast % is between 1.5% - 5.0% and the LUC % ≥ 4.5% or % blast > 5.0% of total leukocytes or % BASO + % BASO suspect + % BASO Saturation ≥ 10%.9

The principle of examination of the peroxidase method is to classify leukocytes based on the characteristics of each cell when given cytochemical staining. The peroxidase enzyme is present and active in several types of leukocytes, which, when added to hydrogen peroxide and dyes, will give a dark color that is precipitated in the cells. Normal neutrophils and eosinophils have high levels of peroxidase activity, where enzyme activity is directly proportional to cell maturation.10

The examination principle of the basophil/lobularity method aims to determine the number of basophils and assess cell lobularity. This method accurately identifies basophils because basophils are more resistant to lysis caused by acids and surfactants. When the sample is mixed with BASO reagent from ADVIA, erythrocytes will lyse, and the cytoplasm of all types of leukocytes will be lost except basophils. Using a laser diode, the sample will then be analyzed based on two-angle laser light scattering detection. Leukocytes will be grouped based on three categories: basophils, mononuclear and polymophonuclear.10

SADT staining was performed with Wright-Giemsa stain. Manual counting was carried out on 100 leukocyte cells at 1000x magnification. Two clinical pathologists carried out the SADT examination separately to prevent bias.

The research was conducted at the Clinical Pathology Laboratory of Dr. Soetomo. The samples used in this research were collected from November 2015 to August 2016.

Statistical analysis used the IBM SPSS version 20 program. The data normality test used the Kolmogorov-Smirnov test. The data homogeneity test used the Shapiro-Wilk test. The difference in the number of blasts between manual counts from smears and Siemens ADVIA 2120i was tested using the Independent Samples T Test. A P value < 0.05 is considered to be a statistically significant difference.

RESULTS

In this study, 30 research samples were found that had confirmed acute leukemia based on the results of the immunophenotyping examination. The research subjects were 18 (60%) men and 12 (40%) women, with an average age of 44 years and a range between 3 and 85 years. A total of 14 samples were new cases, and 16 were patients with a history of leukemia. Table 1 explains that 15 of the 30 samples (50%) were leukemia from the myelocytic series, seven samples (23.3%) were leukemia from the monocytic series, seven samples (23.3%) was leukemia from the lymphocytic series, and 1 sample (3.3%) is leukemia of the megakaryocyte series.

Based on sample type, in peripheral blood samples, there were ten samples (33.3%) of myelocytic series leukemia, six samples (20%) of monocytic series leukemia, and six samples (20%) of lymphocytic series leukemia. In the bone marrow samples, there were five samples (16.7%) of myelocytic series leukemia, 1 sample (3.3%) of monocytic series leukemia, 1 sample (3.3%) of lymphocytic series leukemia, and 1 sample (3.3%) megakaryocytic series leukemia (Table 1).

Table 1. Characteristics of Research Subjects

Variable	n (%)
Gender	
Male	18 (60)
Female	12 (40)
Sample type	
SADT	22 (73,3)
BMA	8 (26,7)
Blast series	
Myelocytic	15 (50)
Monocytic	7 (23,3)
Lymphocytic	7 (23,3)
Megakaryocytic	1 (3,3)
Blast Series Based on Sample Type	
SADT	
- Myelocytic	10 (33,3)
- Monocytic	6 (20)
- Lymphocytic	6 (20)
BMA	
- Myelocytic	5 (16,7)
- Monocytic	1 (3,3)
- Lymphocytic	1 (3,3)
- Megakaryocytic	1 (3,3)

Table 2. Number of Blast Counting Method Manual and Siemens ADVIA 2120i

No.	SAMPLE TYPE	SERIES	BLAST NUMBER	
			MANUAL COUNT (%)	Siemens ADVIA 2120i (%)
1.	SADT	Myelocytic	10	9.5
2.	SADT	Myelocytic	28	20.6
3.	SADT	Myelocytic	14	12.5
4.	SADT	Myelocytic	7	5.5
5.	SADT	Myelocytic	41	8.8
6.	SADT	Myelocytic	10	12.7
7.	SADT	Myelocytic	12	11
8.	SADT	Myelocytic	2	2.3
9.	SADT	Myelocytic	1	99
10.	SADT	Myelocytic	80	23.7
11.	SADT	Lymphocytic	5	1.2
12.	SADT	Lymphocytic	10	9
13.	SADT	Lymphocytic	45	22
14.	SADT	Lymphocytic	8	5.2
15.	SADT	Lymphocytic	0	0.3
16.	SADT	Lymphocytic	1	1
17.	SADT	Monocytic	43	16.9
18.	SADT	Monocytic	71	25.2
19.	SADT	Monocytic	63	34.5
20.	SADT	Monocytic	95	3.3
21.	SADT	Monocytic	24	76
22.	SADT	Monocytic	19	15.1
23.	BMA	Myelocytic	65	11.9
24.	BMA	Myelocytic	60	14.3
25.	BMA	Myelocytic	3.5	4.6
26.	BMA	Myelocytic	0.5	0.2
27.	BMA	Myelocytic	6	5.3
28.	BMA	Monocytic	44	12.1
29.	BMA	Megakaryocytic	82	21.3
30.	BMA	Lymphocytic	3.6	0.1

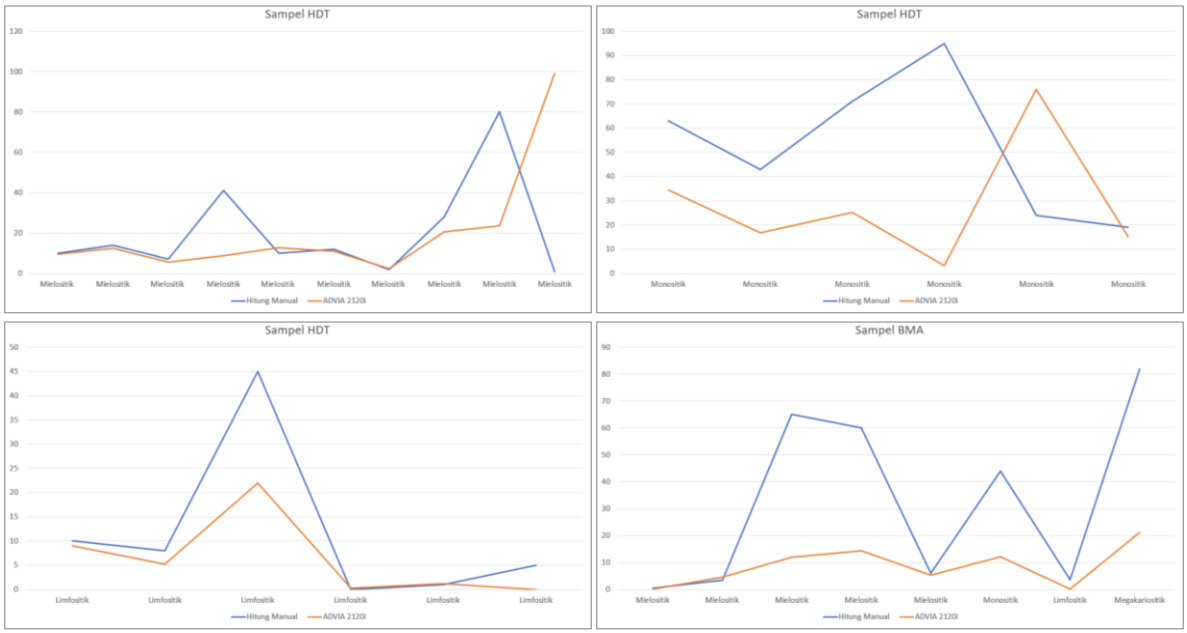

Figure 3. Comparison diagram of the number of blasts by manual counting method and Siemens ADVIA 2120i based on sample type and type of leukemia

Testing the normality of blast count data using the manual counting method and the Siemens ADVIA 2120i hematology tool with Kolmogorov-Smirnov showed that the data had a normal distribution. Testing the homogeneity of the blast count data using the manual counting method and the Siemens ADVIA 2120i hematology tool with Shapiro Wilk obtained homogeneous data. Based on the results of the Independent Samples T Test analysis, it was found that there was a significant difference in the results of the number of blasts between the manual counting method and the Siemens ADVIA 2120i hematology equipment with p=0.001.

DISCUSSION

As shown in Table 2 and Figure 3, there was a significant difference in the results of the number of blasts between manual counting and the Siemens ADVIA 2120i hematology instrument in this study. This can be caused by the method used to detect blasts on the Siemens ADVIA 2120i hematology device, which is based on the complexity of the cells and the resistance of the blast cells to the lysis reagents in the BASO chamber, while blast detection using the manual counting method is based on the characteristics of the cytoplasm, the presence of granules, the shape of the cell nucleus, nuclear chromatin, and daughter nuclei.

The largest difference in the number of blasts was found in the Monocytic series AML cases (6 out of 30 samples). This can be caused by the influence of cell shape and complexity on monoblasts, which are detected as other cells. The results of this study are in accordance with research conducted by Meintker et al., which stated that the Siemens ADVIA 2120i hematology instrument was inferior in counting the number of monocytic series, resulting in lower specificity in calculating the number of blasts.[12] Tan et al. also stated that the low number of monocytic series read on the Siemens ADVIA 2120i hematology instrument could be caused by errors in classifying small immature monocytes, causing discrepancies compared to manual counts.[13,16]

The difference in the results of the number of blasts on the Siemens ADVIA 2120i hematology device compared to manual counting on blood smears is also likely due to the presence of a Large Ungranulated Cells (LUC) parameter of more than 3%, which can cause the appearance of flagging of blast cells,11 thereby causing the results of the number of blasts on the Siemens ADVIA hematology device. 2120i goes false low; This can also be caused by the large number of blasts being counted as LUC.

The blast count for the lymphocytic series in this study was also lower on the Siemens ADVIA 2120i hematology device than manual counting. This is in accordance with research conducted by Meintker et al., which states that classifying atypical cells into the LUC population causes a low count of lymphocytes.[12] Canovi et al. also stated that the diagnostic accuracy of flagging blasts was less good when compared with other parameters (hemoglobin, platelet count, neutrophils, and monocytes) on the Siemens ADVIA 2120i hematology device in cases of acute lymphocytic leukemia.[19]

The results of this study are in accordance with research conducted by Bennaoum et al. and Meintker et al., where it was stated that there were many false positive cases with a Positive Predictive Value (PPV) below 50%. This is likely due to the low threshold set by tool manufacturers so that users remain careful in diagnosing leukemia so that cytological examination is still needed in diagnosing acute leukemia.[12,14,20] Research by Meintker et al. also states that the presence of

Immature Granulocytes (IG) can cause the calculation of the number of blasts on the Siemens ADVIA 2120i hematology instrument to be falsely high or falsely low.[12]

This study obtained the number of blasts for the myelocytic series using a manual counting method that differed from the Siemens ADVIA 2120i hematology instrument. This is in accordance with research conducted by Rocco et al. in 2018, which stated that the Siemens ADVIA 2120i hematology tool had sensitivity (86.3%) and specificity (99.83%) in diagnosing acute myelocytic leukemia.[15]

Differences in the number of blasts were also found in samples with leukopenia but had good sensitivity and specificity in samples with leukocytosis. The results of this research are in accordance with research conducted by Meintker et al. and Melet et al.[12,17] Conclusions of research conducted by Aidoudi et al. are different from what was found in this study; this is possible because the research conducted by Aidoudi et al. used normal patient subjects where the morphology of leukocytes had not undergone many changes so they could better differentiate between blast and non-blast cells. In contrast, the subjects of this study used samples that had confirmed leukemia. , whether new cases or those who have received therapy so that there are changes in leukocyte morphology.[18]

CONCLUSION

The ADVIA 2120i hematology tool has blast parameters, which help officers manually calculate the number of blasts. However, the percentage results of the number of blasts on the Siemens ADVIA 2120i hematology instrument cannot be used as a benchmark for the actual number of blasts in the sample.

In detecting the presence of blast cells with good sensitivity to prevent false negatives on the Siemens ADVIA 2120i hematology device, it is best not only to look for flagging blast cells but also to increase vigilance if flagging IG is found, and there are many abnormalities in the results of a complete blood test; because the Siemens ADVIA 2120i hematology equipment may not detect samples containing blasts.

The Siemens ADVIA 2120i hematology device is beneficial in initial screening for the presence of a hematological malignancy. PEROX and BASO cytograms are very helpful in increasing awareness of detecting blasts. Still, blast parameters on the Siemens ADVIA 2120i hematology tool cannot replace blood smear examination in calculating the number and identification of blast cell types often missed by automatic hematology tools.

This research is an initial study in looking at the difference in the number of blasts between manual counting and the Siemens ADVIA 2120i hematology instrument with a small number of samples, so further research needs to be carried out with a more significant number of samples to provide better conclusions.

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AUTHORS CONTRIBUTION

Study conception and design: M,PBN; data collection: M and PBN; analysis and interpretation of results: M and PBN; draft manuscript preparation: M.

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CONFLICT OF INTEREST

"Competing interests: No relevant disclosures".

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