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Determining Acute Leukemia Lineage Using Mie Map Red Blood Cell

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ABSTRACT

The determination of myeloid and lymphoid lineage is essential for the diagnosis and therapy of acute leukemia. Immunophenotyping is the gold standard to determine the lineage of acute leukemia, but it is still constrained and relatively expensive. Mie Map RBC in the ADVIA 2120i is a parameter that can give additional information about myeloid and lymphoid lineage but has never been studied before. It is expected that Mie Map RBC can be used to differentiate the lineage of acute myeloid and lymphoid leukemia if immunophenotyping is not present. This study aimed to analyze the diagnostic value of Mie Map RBC with ADVIA 2120i towards immunophenotyping in determining myeloid and lymphoid lineage in acute leukemia. Child and adult patients diagnosed with acute leukemia (n=30) that had peripheral blood smear and bone marrow aspiration with blasts > 20% were examined using ADVIA 2120i. The Mie Map RBC lineage results were compared to the lineage of immunophenotyping. The sensitivity and specificity of the Mie Map RBC myeloid series are respectively 60.00%, 93.33%. The sensitivity and specificity of the Mie Map RBC lymphoid series are respectively 93.33% and 60.00%. The diagnostic accuracy value of Mie Map RBC is 76.67%. The determination of acute leukemia myeloid series lineage has high specificity. If there is no population outside the matrix of Mie Map RBC, it highly suggests myeloid series. On the other hand, the determination of acute leukemia lymphoid series lineage has a relatively low specificity meaning that the population outside the matrix of Mie Map RBC does not always suggest a lymphoid lineage.

Keywords: Mie Map RBC, acute leukemia, myeloid, lymphoid

INTRODUCTION

Acute leukemia is a clonal proliferation that happens rapidly in the bone marrow.[1] Leukemia is the 9th most common malignancy in Indonesia. The number of new cases in Indonesia in 2018 reached 13,398 cases or 3.9% of total malignancies. Death rates due to leukemia reached 5.5% from all malignancies. Hence the disease management of hematological malignancies from diagnosis to therapy needs to get proper attentuin.[2]

According to FAB and WHO, acute leukemia classification is divided into Acute Myeloblastic Leukemia (AML) and Acute Lymphoblastic Leukemia (ALL).[3] Immunophenotyping examination is the gold standard in determining acute leukemia lineage, but the equipment is expensive, and there are not many in Indonesia. ADVIA 2120i uses peroxidase activity using the peroxidase channel can differentiate the myeloid from the lymphoid lineage of leukemia and has been frequently discussed in previous studies.[4,5] Further information has stated that differentiating myeloid and lymphoid lineage can be done using the Red Blood Cell (RBC) Mie Map in ADVIA 2120i.[6] Studies using the Mie Map RBC in the differentiating lineage of acute leukemia have never been done before.

METHODS

This study was an observational analytical study using a cross-sectional design. This study was conducted from February–October 2020 at the Department of Clinical Pathology Dr. Soetomo General Academic Hospital, Surabaya. The subjects were a child and adult patients diagnosed with acute leukemia (n=30) examined their peripheral blood smear morphology and bone marrow aspiration with blasts > 20%. Specimens were examined using ADVIA 2120i. The lineage from RBC Mie Map ADVIA 2120i (Siemens Healthcare Diagnostics, Deerfield, Illinois, USA) and immunophenotyping (BD FACS CALIBUR) was interpreted by a minimum of two competent hematologists. In addition, myeloid and lymphoid lineage yang obtained from the RBC Mie Map examination is compared with immunophenotyping results and analyzed statistically to find the sensitivity, specificity,

diagnostic accuracy.

Data from this study underwent a diagnostic examination using the 2x2 table to determine the sensitivity, specificity, diagnostic accuracy of Mie MAP RBC in determining myeloid and lymphoid lineage in acute leukemia compared to immunophenotyping as the gold standard. The Ethical Committee has approved this study of Health Experiments of Dr. Soetomo General Academic Hospital, Surabaya, with article Number 1825/KEPK/11/2020.

ADVIA 2120i is an automatic hematology analyzer using the flow cytometry principle, while Mie MAP RBC is a channel that calculates the erythrocyte cell population. Mass erythrocytes are converted into a spherical shape so the cells will always have the same orientation when passing the flow cell. The relationship between volume (v) and hematocrit (HC) is represented in a map known as the Mie Map RBC according to the Mie Theory in ADVIA. Mie Map RBC can sometimes be used for abnormal WBC lineage that doesn't have myeloperoxidase activity (MPO). The height position of the light scatter (5-15) is plotted as the x-axis according to the cell index (erythrocyte HC and thrombocyte granulation degree). The low light scatters (2-3) are plotted as the y-axis and depend on the cell volume. Mie Map RBC shows cell population outside of the RBC matrix (yellow circle). These cells have the same size or are slightly larger than erythrocytes but do not contain hemoglobin. Cells that are seen in this position are specifically from the lymphoid series (Figure 1).[6]

Figure 1. Mie Map RBC shows the cell population outside of the matrix (yellow circle) showing the lymphoid cell position[6]

A population found outside the Mie Map RBC matrix shows a positive lymphoid population, whereas no population outside of the matrix shows a lymphoid negative or myeloid population.[4]

RESULTS AND DISCUSSIONS

The number of samples in this study was 30 samples that fulfill the inclusion and exclusion criteria. The number of samples also met the minimum samples needed for this type of study. The subjects were mostly male patients, 18 in number and 12 female patients. The age of the patients varied from 1 year to 72 years old, with a mean of 30.1 years old. Acute leukemia diagnosis was based on the FAB criteria, and for this study, AML was as follows M0, M1, M2, M3V, and M5; whereas the lymphoid series consisted of ALL L1 and L2. Demographical characteristics and clinical diagnosis based on the morphology of 30 subjects for acute leukemia can be found in Table 1.

Table 1. Study subjects characteristics

Characteristics	n	%
Gender		
Male	18	60%
Female	12	40%
Morphology		
ALL L1	11	36.6%
ALL L2	2	6.6%
AML M0 dd ALL	4	13.3%
AML M1	1	3.3%
AML M2	4	13.3%
AML M3V (hypo-granular) dd ALL	1	3.3%
AML M3V (hyper-granular)	1	3.3%
AML M5	6	20%
Age (years old)		
Mean	30.1	
Median (min–max)	38 (1 – 72)	

The male-female ratio in AML was 1.5:1.[7] The most common morphological characteristic was ALL as many as 11 patients (36.6%). The ages were from 1 to 72 years old (mean 30.1 years old and median 38) based on the age grouping. Acute lymphoblastic leukemia usually happens during childhood or adolescents, holding 75% from childhood leukemia. Acute myeloblastic leukemia usually occurs in adulthood and increases with age but rarely happens to children.[1]

Some immunophenotyping results in acute leukemia patients from either myeloid or lymphoid lineage show an aberrant result. There were four samples from the myeloid lineage that were

aberrant, where the aberrations were in the form of CD5m CD7, CD 19, and CD20, which are lymphoid markers. Two immunophenotyping samples from the lymphoid lineage showed aberrations in the form of CD5 ad CD 33 (Table 2).

Study results showed acute leukemia patients with myeloid lineage and aberrant had no concordance with Mie Map RBC. These immunophenotyping results for myeloid lineage showed aberrant CD5, CD7, and CD10, whereas the lymphoid lineage showed aberrant CD5 (in lymphoid B lineage immunophenotyping results), CD13. 80% of ALL cases were from the B lineage (BL), whereas 15–20% are from the T lineage. Patients with B-lymphoid lineage were classified as abnormal myeloid positive if their cells also expressed CD13, CD33, or both.[8]

Myeloid lineage with aberrant immunophenotyping results was not in line with Mie Map RBC. These aberrant results showed that these cells were mostly smaller in size and resemble lymphoid cells that affect the results of Mie Map RBC because Mie Map RBC was influenced by cell volume. There were two samples from the lymphoid lineage with aberrant immunophenotyping results of CD 5 and 13. Immunophenotyping results of lymphoid lineage with aberrant CD 5 still had similarities with Mie Map RBC because CD 5 was a T lymphoid marker causing the size and morphology to not differ from B-lymphoid. Results with aberrant CD 13 showed results that were not in concordance with Mie Map RBC due to the morphology of the lymphoid cells that are relatively big, resembling myeloblast. Cells with a larger size than lymphoid usually cannot pass through the flow cell hence the lymphoid population does not show on the Mie Map RBC. The measurement of Mie Map RBC is based on cell volume and hematocrit only, while differentiating the myeloid or lymphoid lineage is also based on the presence of granules, peroxidase activity, and other factors that cannot be seen in the Mie Map RBC.

The diagnostic value of Mie Map RBC with immunophenotyping as the gold standard in determining the myeloid lineage had a sensitivity of 60% and specificity of 93.33%. However, the diagnostic value of Mie Map RBC in determining the lymphoid lineage had opposite results. Mie Map RBC in determining lineage had a 76.67% accuracy (Table 3).

Determining the myeloid series lineage of acute leukemia has high specificity if the Mie Map RBC results follow immunophenotyping results, for example, if according to the Mie Map RBC. There was no population outside the matrix (myeloid) in concordance with the immunophenotyping results, showing myeloid lineage. Thus, Mie Map RBC can be used as a predictor for myeloid series acute leukemia diagnosis even though immunophenotyping hasn't been done. Low sensitivity means that negative myeloid results cannot exclude the diagnosis of non-myeloid acute leukemia.

The determination of the lineage of lymphoid series acute leukemia has a high sensitivity if the results of Mie Map RBC show the same results of immunophenotyping, so the results of Mie Map RBC can rule out non-lymphoid acute leukemia even though immunophenotyping hasn't been done. However, specificity is relatively low, so RBC doesn't always show lymphoid lineage if a population is outside the matrix on Mie Map.

Immunophenotyping accompanied by aberrant in this study caused several results to be

Table 2. Lineage Mie Map RBC and NRBC histogram results towards immunophenotyping results with aberrant

Morphology	MIE MAP RBC	Immunophenotyping	Aberrant
ALL L1	Lymphoid – (M)	Myeloid (M)	CD7
AML M2	Lymphoid + (L)	Myeloid (M)	CD7 and CD19
AML M2	Lymphoid + (L)	Myeloid (M)	CD5
AML M2	Lymphoid + (L)	Myeloid (M)	CD5, CD20
ALL L1	Lymphoid + (L)	B-Lymphoid (L)	CD5
ALL L2	Lymphoid - (M)	Lymphoid (L)	CD33

Table 3. Diagnostic value Mie Map RBC towards immunophenotyping

	Myeloid		Lymphoid	
Sn	60.00%	CI (32.29% – 83.66%)	93.33%	CI (68.05% – 99.83%)
Sp	93.33%	CI (68.05% – 99.83%)	60.00%	CI (32.29% – 83.66%)
Accuracy	76.67%	CI (57.72% – 90.07%)	76.67%	CI (57.72% – 90.07%)

Sn: sensitivity, Sp: specificity

unsynchronized between Mie Map RBC and immunophenotyping that will affect diagnostic value in this study. Mie Map RBC cannot fully replace immunophenotyping but can be used as an additional parameter to predict the lineage of acute leukemia.

CONCLUSIONS AND SUGGESTIONS

The sensitivity, specificity, and diagnostic accuracy of Mie Map RBC towards myeloid lineage were respectively 60.00%, 93.33%, and 76.67%. The sensitivity, specificity, and diagnostic accuracy of Mie Map RBC towards lymphoid lineage were 93.33%, 60.00%, and 76.67%. The determination of myeloid series of acute leukemia lineage has a low sensitivity, which means negative myeloid results cannot rule out non-acute myeloid leukemia. High specificity means that it strongly suggests myeloid series when no population is found outside of the Mie Map RBC. Determination of acute leukemia lymphoid series has a high sensitivity, meaning results of Mie Map RBC can rule out acute non-lymphoid leukemia, relatively low specificity means that if there is a population outside of the matrix of the Mie Map RBC, it does not always mean it is from the lymphoid series.

Mie Map RBC can be used as an additional parameter in predicting the lineage of acute leukemia but must still consider the patient's clinical condition, cell morphology, and peroxidase activity. The results of immunophenotyping with aberrant can be used as a reference for further research to exclude samples of immunophenotyping aberrant, to evaluate the increase in sensitivity and specificity of Mie Map RBC.

REFERENCES

1. Keohane EM, Smith LJ, Walenga JM, Longanbach S, Miers MK. Automated blood cell analysis. In: Rodak's hematology clinical principles and application. 5th Ed., St. Louis Missouri, Elsevier Inch, 2016; 219-221, 604-618.
2. Globocan. WHO international agency for research on cancer the global cancer observatory. 2018. Available from: https://gco.iarc.fr/today/data/factsheets/populations/ 360-Indonesia-fact-sheets.pdf (accessed November 6, 2019).
3. Chauhan S, Pradhan S, Mohanty R, Saini A, Devi K, Sahu MC. Evaluation of sensitivity and specificity of bone marrow trephine biopsy tests in an Indian teaching hospital. Alexandria Journal of Medicine, 2018; (54): 161–166.
4. d'Onofrio G. PANDA–innovative classification of hematopoietic malignancies. Bloodline Reviews, 2001; (2): 3–6.
5. Gibbs G. Peroxidase Activity and Nuclear Density Analysis (PANDA) in the diagnosis of hematological malignancy. British Journal of Biomedical Science, 2005; 62(3): 137-140.
6. Gibbs G. White cells. In: Advia hematology systems a guide to cytogram interpretation. Lulu Press Inc, 2014; 5-17. 39-45.
7. Sultan S, Zaheer HA, Mohammed S, Irfan D, Ashar S. Demographic and clinical characteristics of adult acute myeloid leukemia-tertiary care experience. Asian Pacific Journal of Cancer Prevention, 2016; 17: 357-360.
8. Leach M, Drummond M, Doig A. Practical flow cytometry in hematology diagnosis. England, John Wiley & Sons Ltd., 2013; 43-93.