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RESEARCH

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## **CORRELATION OF BLAST PERCENTAGE TO CD34 OF BONE MARROW IN ALL PEDIATRIC PATIENTS**

*(Kenasaban Persentase Blas dengan CD34 di Sumsum Tulang di Pasien LLA Anak)*

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### **ABSTRAK**

*Leukemia Limfoblastik Akut (LLA) adalah penyakit keganasan sel progenitor limfoid yang berasal dari sumsum tulang. Tanda khas dari diagnosis leukemia akut adalah sel blas. Pemeriksaan mikroskopis dilakukan untuk menentukan persentase sel blas pada diagnosis leukemia akut. Immunophenotyping merupakan metode diagnostik yang dapat membantu menegakkan diagnosis pada keganasan hematologi. CD34 merupakan antigen yang sering digunakan untuk identifikasi sel induk hemopoietik atau blas. Penelitian ini bertujuan untuk mengetahui kenasaban antara persentase blas dengan ekspresi CD34 di sumsum tulang di pasien leukemia limfoblastik akut anak sebelum dan sesudah pengobatan kemoterapi fase induksi.*

**Kata kunci:** Blas, CD34, leukemia limfoblastik akut anak

### **ABSTRACT**

Acute Lymphoblastic Leukemia (ALL) is a malignant disease of lymphoid progenitor cells from the bone marrow. Typical signs of acute leukemia diagnosis are blast cells. Microscopic examination is done to determine the percentage of blast cells in acute leukemia diagnosis. Immunophenotyping is a diagnostic method that can help establish the diagnosis of hematological malignancies. CD34 is an antigen that is often used to identify stem cells or hemopoietic blasts. The aim of this study was to determine the correlation between the percentage of blasts in bone marrow CD34 expression in children with acute lymphoblastic leukemia before and after induction phase of chemotherapy.

**Key words:** Blast, CD34, pediatric acute lymphoblastic leukemia

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### **INTRODUCTION**

Acute Lymphoblastic Leukemia (ALL) is a malignant lymphoid progenitor cell disease originating from the bone marrow, characterized by leukocyte proliferation and abnormal cell manifestations in peripheral blood. Acute lymphoblastic leukemia can affect both children and adults, 75% of whom are younger than 15 years. The incidence of acute lymphoblastic leukemia reaches its peak at the age of three to five years old. Acute lymphoblastic leukemia is more common in males than in females.<sup>1</sup>

In Asia, the incidence of leukemia in children, moreover, is higher than in white children. A research in Japan even mentions that every year there are 1000

new ALL cases in children less than 15 years. In the Dr. Soetomo Hospital, Surabaya, there were many new cases involving 82 children in 2006, in which the percentage of remission was 48.5%, 14.7% for no remission and 36.8% for death as the outcomes of the induction phase of the treatment.<sup>2</sup>

Leukemia, furthermore, can be diagnosed based on clinical symptoms and complete blood tests. However, the diagnosis of leukemia must also be confirmed with bone marrow aspiration examination supported with chest radiographic examination, cerebrospinal fluid examination and some other examinations.<sup>3</sup> Bone marrow aspiration is performed for diagnosis, evaluation of disease progression, or treatment progress according to a prescribed schedule.<sup>4</sup>

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The typical sign of the diagnosis of acute leukemia is blast cells, undifferentiated cells with diffused chromatin cell distribution and by one or more basophilic nuclei and cytoplasm. Unfortunately, in dense bone marrow with large blast cells, bone marrow aspiration is often difficult to perform.<sup>5</sup>

Microscopic examination can also be performed to determine the blast percentage in diagnosing acute leukemia. Nevertheless, examiners should recognize peripheral blood cells as well as normal and pathological bone marrow to assess the amount of nuclei in bone marrow smear preparations.<sup>6</sup>

Methods of preparation, procession and reporting of bone marrow smear preparations vary widely. The variety of methods of making bone marrow smear preparations often leads to inconsistencies in diagnosing and classifying the disease. The presence of inconsistencies in the diagnosis then will affect treatment and treatment outcomes.<sup>7</sup>

In general, treatment in ALL consists of induction phase, consolidation (intensification), direct therapy of central nervous system and maintenance therapy. The induction phase of chemotherapy lasts five to six weeks, which aims to eradicate 99% of leukemia cells and restore a normal hematopoiesis system.<sup>8</sup> Patients then can be stated to have complete remission if results of the bone marrow aspiration after the induction phase show the number of nucleated blast cells is less than 5%. Otherwise, they can be stated to have no complete remission if the number of nucleated blast cells is about 5–20%.<sup>9</sup>

In addition, relapse occurs in at least 20% of children with acute lymphoblastic leukemia treated with chemotherapy programs. A number of examination methods then can be used to estimate the recurrence in these patients, but none are truly reliable. Examination of peripheral or bone marrow cell morphology, for instance, is an approach to identify residue of residual disease. However, it is highly subjective and limited in sensitivity. Patients who are clinically declared in complete remission may in fact still contain 1010 leukemia cells.<sup>10</sup>

Immunophenotyping is a diagnostic method that can establish a diagnosis of hematological malignancy. This examination detects the phenotype expression of a cell. Immunophenotyping classifies normal or abnormal blood cells based on their surface antigen. Immunophenotyping using current cytometry method can also detect an abnormal cell in 104 or more normal cells.<sup>10</sup> A cell from a particular lineage typically expresses a specific molecule of cluster of differentiation (CD).<sup>11</sup>

Cluster of Differentiation (CD) 34 is an antigen often used for the identification of hemopoietic stem cells or blast cells. CD34 is expressed by lymphoblasts and myeloblasts.<sup>12</sup> CD34 is also considered as an antigen of a specific stage in human hematopoietic differentiation, expressed at the onset of hematopoietic differentiation.<sup>8</sup> Normal bone marrow contains less than 3% of cells expressing CD34 to be a good marker for evaluating blast cell population.<sup>13</sup>

Immunophenotyping of abnormal hematology cells is a rapid and effective way to diagnose, classify, evaluate prognosis and detect residual diseases in patients with hematological malignancies. The advantages of immunophenotyping examination using flowcytometry method are; Small sample requirement; The ability to identify different cell markers on the same cell; The ability to analyze various specimens in the form of blood cell suspension, bone marrow, body fluids and so on.<sup>14</sup>

## METHODS

This research was a non-comparable longitudinal cohort study with a population of new Acute Lymphoblastic Leukemia (ALL) pediatric cases before and after the induction phase of chemotherapy. This research was conducted in June 2015 involving outpatients and inpatients of the Hematology of Pediatric Oncology as well as the Laboratory of Clinical Pathology, Faculty of Medicine, Airlangga University/ Dr. Soetomo Hospital, Surabaya. A preliminary study was conducted to determine the sample size and to obtain at least 15 samples per group, first. Patients with acute lymphoblastic leukemia aged 1 month to 18 years who were newly diagnosed and would undergo the regular induction phase of chemotherapy, as well as who were approved with the parent/guardian consents to participate in the research were included as subjects of this research. Meanwhile, acute lymphoblastic leukemia patients who had complex congenital abnormalities with multi-organ abnormalities or did not complete the induction phase chemotherapy were excluded from this research. Those new cases of acute lymphoblastic leukemia then were diagnosed based on history, physical examination, radiological examination, laboratory examination, and bone marrow aspiration examination. The diagnosis was definitive since they were based on bone marrow aspiration examination, indicating more than 25% nucleated cells as young cells or lymphoblasts.

Bone marrow aspiration examination was performed by a pediatrician consultant from the

Division of Hematology and Oncology/Department of Pediatric. The samples then were smeared on an object glass for microscopic examination and put into a 0.25 mL heparin tube for immunophenotyping. Afterwards, blast examination was performed by using Wright staining on bone marrow smears. The blast percentage then was counted among 200 nucleated cells. Meanwhile, CD34 expressions were examined with flowcytometry method using BD FACS Calibur instrument, CD45 reagent per CP, CD19 FITC, CD3 FITC and CD34 PE. Next, a statistical test, Pearson Correlation test (normal distribution) or Spearman (abnormal distribution), was performed to compare blast percentage to CD34 expression.

## RESULTS AND DISCUSSION

There were actually 29 new patients with acute lymphoblastic leukemia in this research, but 9 of them died and 4 of them did not continue therapy. Thus, only 16 of them, consisting of 12 male children (75%) and 4 female children (25%). In other words, the ratio of males to females was 3:1. Similarly, some literatures also stated that males are more likely to suffer from ALL than females, with a ratio of 3:1. This ratio was higher than the ratio resulted in a research conducted by Widiaskara<sup>2</sup> which was, about 1.15 : 1. This difference may be due to the number of research subjects, time and type of leukemia studied.

In this research, age was categorized into four groups, namely the age group of less than 1 year, the age group of between 1 to less than 5 years, the age group of between 5 to less than 10 years and the age group of more than 10 years. The highest number of the subjects was in the age group of between 1 to less than 5 years, in line with the research conducted by Widiaskara in 2005.<sup>2</sup> The detailed characteristics of those research subjects were presented in Table 1. Next, the first examination was performed on those patients undergoing the induction phase of chemotherapy. The second examination then was carried out after the patients had completed the induction phase of chemotherapy.

The classification of ALL types based on FAB criteria in this research showed that the number of the patients with ALL type L1 was 12 or 75%, higher than those with ALL type L2. Meanwhile, the classification of those ALL patients based on cell type in this research indicates that the number of the patients with B lineage was 8 or 50%, equal to those with T lineage. Unlike this research, a research conducted by Tehuteru<sup>15</sup> depicted that the number of ALL patients

**Table 1.** Characteristics of the research subjects

Characteristics of samples	f (%)
<b>Sex</b>	
Males	12 (75%)
Females	4 (25%)
<b>Mean ± SD of age</b>	5.56 ± 3.7tahun
< 2 years	1 (6.25%)
2- < 5 years	7 (43.75%)
5- < 10 years	5 (31.25%)
≥10 years	3 (18.75%)
<b>Criteria of FAB</b>	
L1	12 (75%)
L2	4 (25%)
<b>Types of cells</b>	
B lineage	8 (50%)
T lineage	8 (50%)

with B lineage was higher than those with T lineage. This difference may be due to the number of research subjects, time and type of leukemia studied.

Moreover, there were changes in hemoglobin (Hb) level and leukocyte counts before and after the induction phase of chemotherapy. However, there was no statistically significant difference, with  $p=0.230$  and  $0.234$ . This indicated that there was an increase or decrease in Hb level and leukocyte count before and after the induction phase of chemotherapy, but the change was not statistically significant.

This condition is thought to be due to leukemia cells that accumulated in the bone marrow, leading to disruption of blood cell formation. In addition to its illness, this may be due to the induction phase of chemotherapy used to suppress stem cell growth and induce young hematopoietic cell apoptosis.<sup>16</sup> The induction phase of chemotherapy lasted 5 to 6 weeks or 35 to 42 days and it took longer to evaluate the changes in hemoglobin since turn over erythrocytes take about 120 days.

Moreover, blast percentage in the bone marrow can be used to establish diagnosis, evaluation and monitoring of therapy in patients with acute lymphoblastic leukemia. The diagnosis of acute lymphoblastic leukemia can be generated when the smears of bone marrow aspiration show that the percentage of cell blast is more than 25%. The patients then can be stated to have complete remission if the percentage of cell blast derived from the smears of the bone marrow aspiration after the induction phase indicates nucleated cells of less than 5%, or incomplete if it shows cell blast of 5–20%.

In this research, the blast percentage at the time of diagnosis or before the induction phase of chemotherapy was 73.63% with a standard deviation of 13.61%. Similarly, a research conducted by

**Table 2.** Results of the examinations in those ALL pediatric patients before and after the induction phase of chemotherapy

Parameters	Before the induction phase of chemotherapy	After the induction phase of chemotherapy	p
	range Mean ± SD	range Mean ± SD	
Hemoglobin levels (g/dL)	4.2 – 14.3 9.35 ± 2.65	5.8 – 14.14 10.3 ± 2.1	0.230
Leukocyte count (x10 <sup>6</sup> /L)	1,300 – 221,200 42,288 ± 70,138	3,500 – 40,100 8,614 ± 8,478	0.234
Blast percentage (%)	40 – 95 73.63 ± 13.61	2 – 55 9 ± 14	0.001
CD34 (%)	3.88 – 90.33 31.65 ± 29.15	6.59 – 57.18 23.05 ± 13.57	0.400

Kamazani<sup>17</sup> showed that the blast percentage at the time of diagnosis was 75 + 22%. Blast enhancement in the bone marrow can be considered as an indicator of acute lymphoblastic leukemia since ALL is a malignancy caused by lymphoid cell proliferation in the early stages of differentiation.<sup>8</sup>

In addition, the mean blast percentage after the induction phase of chemotherapy in this research was 8.75% with a standard deviation of 12.83%. It meant that there was a significant difference in the blast percentage before and after the induction phase of chemotherapy. This was because the induction phase of chemotherapy aimed to eradicate 99% of leukemia cells restore the normal hematopoiesis system.<sup>8</sup>

CD34 expression, on the other hand, at the time of diagnosis or before the induction phase of chemotherapy was 31.65% with a standard deviation of 29.15%. Similarly, a research conducted by Kamazani<sup>16</sup> showed that CD34 expression was 34.6 + 31.2%. Meanwhile, CD34 expression after the induction phase of chemotherapy was 23.05% with a standard deviation of 13.57%. It indicated that there was no significant difference in CD34 expression before and after the induction phase of chemotherapy. In other words, there was an increase or decrease in CD34 expression before and after the induction phase of chemotherapy, but this change was not statistically significant.

Furthermore, many of those ALL patients experienced increased CD34 expression, especially in those ALL patients with type T lineage. Nevertheless, further researches should evaluate future patient progress, recurrence possibility, or treatment success after treatment completion.

This research used only CD19 for B-cell marker and CD3 for T-cell marker. There are actually several other CD markers that can be used to distinguish different B cell subtypes, such as CD10, CD20, CD22 and CD79a. On the other hand, several CD markers can also be used to distinguish T cell subtypes, such as CD2,

CD5 and CD7. CD34 can be expressed by pro B ALL subtype, whereas the more mature B lineage ALL type does not express CD34. In T lineage ALL type, CD34 is expressed by immature T ALL subtype.<sup>18,19</sup> In this research, ALL is not differentiated based on subtypes, thus, the variation in CD34 expressions may be due to the ALL subtype difference.

In general, the results of this research showed that there was no correlation between blast percentage and CD34 in bone marrow in pediatric patients with acute lymphoblastic leukemia before the induction phase of chemotherapy. It may be due to aberrant in expression on CD34 in lymphoblast or a more mature ALL subtype, which was not examined further in this research. Moreover, unlike this research, a research conducted by Supriyadi<sup>20</sup> showed that the percentage of CD34 expressed in 211 ALL patients was 53%.

In addition, there were six patients with the blast percentage outcomes not equal to CD34 expression before and after the induction phase of chemotherapy. In these patients, the blast percentage decreased, but CD34 expression increased after the induction phase of chemotherapy. These six patients were identified as ALL patients with T lineage type. Two of them even had non remission outcomes. These six patients might experience overexpression of CD34 by remaining cell blast after the induction phase of chemotherapy as an attempt to maintain cell survival since T lineage ALL type had a poor prognosis. The CD34 marker is also known to be associated with a glycoprotein P as a transport protein, which presence precisely leads to the efflux of the drug out of the cells.<sup>21</sup>

The increase of CD34 expression after the induction phase of chemotherapy that did not coincide with the decrease of the blast percentage in this research was mostly found in T lineage ALL type. Consequently, further researches need to be conducted to a longer time until the maintenance phase of the therapy to evaluate whether the patient is getting improved based on blast percentage, or experienced recurrence

in certain phases. If the patient had a relapse, then this was in accordance with the results of CD34 expression.

Another possibility was that blasts in these patients have aberrant CD34, which should be high at the beginning of the diagnosis due to blast percentage. But, some patients with underexpression of CD34 have low CD34 expression. After the induction phase of chemotherapy, CD34 expression then will become normal, so is considered to be high. This was found in 3 patients with extreme CD34 expression, i.e low prior to the induction phase chemotherapy, but increased after the induction phase of chemotherapy. And this expression was not aligned with blast percentage. Similarly, a research conducted by Mazher<sup>22</sup> showed aberrant CD marker on ALL, i.e CD13 (20%), CD33 (15%) and MPO (14%).

A research conducted by Supriyadi<sup>20</sup> showed that CD34 in B lineage ALL type was associated with a standard risk group, but had no significant effect on prognosis. Survival rates of ALL patients expressing CD34 were not much different from those of ALL patients who did not express CD34. On the other hand, CD34 in T lineage ALL type was associated with poor disease free survival. A research conducted by van Grotel<sup>21</sup>, for instance, suggested that CD34 correlated with a patient aged more than 10 years due to central nervous system involvement at the time of diagnosis and high leukocyte count. These were actually considered as poor prognostic factors in ALL patients.

Finally, the results of this research showed that there was no correlation between blast percentage and CD34 in the bone marrow of those pediatric patients with acute lymphoblastic leukemia before and after the induction phase of chemotherapy. Unfortunately, supporting researches correlating the blast percentage to CD34 in the bone marrow of pediatric patients with acute lymphoblastic leukemia before and after the induction phase of chemotherapy were difficult to obtain.

## CONCLUSION AND SUGGESTION

In conclusion, there was no significant difference in blast percentage in the bone marrow of pediatric patients with acute lymphoblastic leukemia before and after the induction phase of chemotherapy. There was also no significant difference in CD34 expressions in the bone marrow of pediatric patients with acute lymphoblastic leukemia before and after the induction phase of chemotherapy. In addition, there was no correlation of blast percentage to CD34 expressions

in the bone marrow of pediatric patients with acute lymphoblastic leukemia before the induction phase of chemotherapy. Similarly, there was no correlation of blast percentage to CD34 expressions in the bone marrow of pediatric patients with acute lymphoblastic leukemia after the induction phase of chemotherapy.

As a result, the blast percentage in the bone marrow could still be used as a means of diagnosis and treatment evaluation for pediatric patients with acute lymphoblastic leukemia. On the other hand, CD34 expressions can only be used as an aids for diagnosing pediatric patients with acute lymphoblastic leukemia. Thus, the examination of CD34 expressions could not replace the examination of blast percentage in the bone marrow for diagnosing or evaluating treatment to determine the outcomes of the treatment in pediatric patients with acute lymphoblastic leukemia.

Based on this study are result it is suggested to do further research with a larger sample to observe the correlation between blast percentage and the CD34 in the bone marrow of ALL patients before and after induction phase of chemotherapy; A more complete cluster differentiation in order to study various ALL subtypes; A longer study to observe the possibility to relapse; A study concerning differentiation of CD34 before and after therapy in T lineage ALL.

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