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Research Article

Association of Bcl-xL Expression with Blast Count, CD 34 and CD 7 Expression in Adult Acute Myeloid Leukemia Patients

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ABSTRACT

Background: Acute myeloid leukemia (AML) is a hematological malignancy generally marked by the unregulated proliferation of myeloid series blast cells. The condition of hematologic malignancy is often associated with increased anti-apoptotic activity. One of the Bcl-2 protein families, Bcl-xL, has an important role in controlling apoptosis / programmed cell death in hematologic malignancies. This study, determined the correlations between anti-apoptotic activity from Bcl-xL expression analysis with the number of bone marrow blasts, CD34 activity as a marker of blast cells, and CD7 as an aberration marker are often found in AML patients. **Aim:** Analysis of the correlation between blast number, and expression of Bcl-xL, CD34, and CD7 in adult Acute Myeloid Leukemia patients. **Method:** An observational cross-sectional study was performed on 30 adult patients who have recently been diagnosed with Acute Myeloid Leukemia using bone marrow aspiration for examination of the number of blasts by a microscope. Examination of the expression of Bcl-xL, CD34, and CD7 was performed by BD FACSCalibur based on the measured Mean Fluorescence Intensity (MFI). **Results:** A total of 30 AML patients had a range of blast count 20 - 82%, Bcl-xL expression with MFI 93.06 - 441.09, CD34 expression with MFI 1.06 - 1,452.48, CD7 expression with MFI 9.31 - 90.58. In this study, there was no significant correlation between Bcl-xL expression as an indicator of anti-apoptotic properties with blast count r = 0.118 (p = 0.534), CD34 expression r 0.225 (p = 0.231) and CD7 expression r = 0.148 (p = 0.435). **Conclusion:** Bcl-xL expression as an indicator of anti-apoptotic properties in adult AML patients had no correlations with the proliferation of blast cells in AML. This suggests that increased anti-apoptotic activity is not the primary mechanism in the pathogenesis of AML.
Key words: AML, Bcl-xL, blast, CD34, CD7.

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BACKGROUND

Acute Myeloid Leukemia (AML) is a hematological malignancy that is generally characterized by uncontrolled proliferation of myeloid series blast cells and diffuse infiltration of the bone marrow with heterogeneous clinical features depending on the underlying genetic disorder. Globally in 2020, leukemia accounted for approximately 2.5% of all cancer incidents with a mortality rate of 3.1%.[1] In the United States, AML accounts for 1.3% of all cancer cases, with a 2018 incidence rate of 4.3 cases per 100,000 individuals. Conversely, in Europe, the frequency of AML has been on the rise, notably in the United Kingdom, where the incidence reached 4.05 cases per 100,000 individuals in 2017. Typically affecting older individuals, AML is most commonly diagnosed in individuals aged 68 to 70 years. With the global aging population, the incidence of AML has seen a steady rise worldwide.[2-4]

Hematological malignant conditions are often associated with increased anti-apoptotic activity. Apoptosis, a fundamental process in multicellular organisms, involves a series of morphological alterations such as cell shrinkage, pyknosis, karyorrhexis, plasma membrane blebbing, and the generation of apoptotic bodies. This mechanism regulates cellular life development. However, cancer cells evade apoptosis by either diminishing pro-apoptotic proteins or enhancing anti-apoptotic proteins. Targets of the apoptotic pathway have been widely developed in the discovery and development of drugs for cancer.[5-7]

The main regulator of apoptosis is the Bcl-2 protein. One of the Bcl-2 protein families, namely Bcl-x, has an important role in controlling apoptosis/programmed cell death in hematological malignancies. The mitochondrial transmembrane protein Bcl-x is involved in regulating the intrinsic apoptotic pathway. Alternative splicing in exon 2 of Bcl-x leads to the production of two isoforms with opposing effects on cell viability: Bcl-xL, the long isoform, exhibits anti-apoptotic properties, while Bcl-xS, the short isoform, promotes apoptosis. Bcl-xL, the predominant isoform, inhibits apoptosis through various mechanisms, including the inhibition of Bax. Conversely, Bcl-xS directly binds to and inhibits anti-apoptotic Bcl-xL and Bcl-2 proteins, leading to the release of pro-apoptotic Bak. Overexpression of Bcl-xL, commonly observed in cancer, suppresses mitochondria-mediated apoptosis, thereby promoting cancer cell survival and enhancing the risk of metastasis formation.[5,8]

Acute leukemia has various expression patterns of surface antigens (CD antigens). Acute leukemia presents a range of surface antigen expression patterns, known as CD antigens. These surface molecules, expressed by blast cells, are categorized as clusters of differentiation (CD) antigens. Specific CD markers expressed by each type of leukemia aid in hematological malignancy classification and diagnosis. Flow cytometry is employed to evaluate CD marker expression, enhancing acute leukemia categorization through immunophenotyping, thus improving accuracy.[9]

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CD7, a T cell antigen, is detected in a small fraction of AML patients, with its gene localized to chromosome 17 via somatic cell hybridization. Despite its rarity, CD7 is a frequently observed abnormality marker in AML across various studies. While traditionally linked to the initial phase of myeloid differentiation, CD7 expression is not exclusive to T-lineage, as it's commonly found in AML patients. Its aberrant expression in these individuals significantly impacts clinical responses, remission rates, and overall survival outcomes.[9]

High levels of the cell surface glycoprotein CD34 are expressed by hematopoietic progenitor and stem cells, which gradually decrease as these cells mature and differentiate. The tumor burden, determined by cell proliferation and anti-apoptotic activity, is reflected by the number of blast cells and CD34 cells. This study explores the association between the percentage of blasts in the bone marrow with CD34 expression with the expression of Bcl-xL (an anti-apoptotic agent) and CD7 expression which causes a poor prognosis and determines the treatment outcome in AML patients with this mutation.[10-13]

The aim of this research is to explore the association between Bcl-xL expression and the number of blast cells, CD34, and CD7 in adult AML patients using the BD FACSCalibur® flow cytometry tool so that it can be used by clinicians as a basis for monitoring and treating patients.

METHODS

In this research, an observational analytical approach is utilized, employing a cross-sectional design. Data was taken from 30 male and female patients with a diagnosis of AML obtained from bone marrow aspirate readings based on FAB (French American British) criteria obtained from inpatient and outpatient installations at Dr. Soetomo Hospital Surabaya from September 2022 to November 2022.

The examination used a bone marrow aspirate sample stored in a 2 mL EDTA tube using the flow cytometry method using the BD FACSCalibur® device. 50 µL of homogenized sample was mixed with phosphate buffer saline (1:1), then added 2.5 µL of anti-CD45 monoclonal antibody conjugated with the chlorophyll protein peridinin (PerCP), 2.5 µL of anti-CD34 conjugated with phycoerythrin (PE) (added 1 mL lysing solution, 250 µL cytofix/cytoperm and 1 mL perm wash reagent from Becton Dickinson®) to the first tube, then 2.5 µL anti-CD45 monoclonal antibody conjugated with PerCP, 2.5 µL CD7 and 2.5 µL anti-Bcl-xL conjugated to Santa Cruz Biotechnology Inc® PE (added 1 mL lysing solution, 250 µL cytofix/cytoperm and 1 mL perm wash reagent from Becton Dickinson®) in the second tube. The cell suspension in FACS is passed through a vibration column so that charged droplets containing single cells are formed. The droplet flow will be shot with a laser beam and the transmitted light will be captured by a detector. The detector is connected to software to analyze the signals obtained so that cells can be analyzed, separated, and collected.

Visualization of data on specific marker molecules further requires the binding of specific cell antigen antibodies and the addition of markers (fluorochromes). The binding of antibodies to the target antigen must be specific so that labeling will provide accurate results. By labeling the antibodies used, the expression of the antigen (protein) can be determined. The analysis of Median Fluorescent Intensity (MFI) was conducted to evaluate the expression of CD34, CD7, and Bcl-xL in individual patient (Figure 1).

All statistical analyses used IBM SPSS 25.0 statistical software. Data normality test using Kolmogorov-Smirnov. The relationship between Bcl-xL expression and blast number, CD34 expression, and CD7 expression was tested using the Spearman correlation test with mean + standard deviation (SD). A p-value < 0.05 indicates statistical significance.

This research has received a certificate of ethical worthiness from the ethics committee of Dr. Soetomo Hospital Surabaya with ethical number 0514/KEPK/X/2022.

RESULT

Patient Characteristics

The research comprised 30 patients, with 12 males and 18 females among them. Research participants, ranging from 18 to 71 years old, had a median age of 41 years. Notably, the majority, constituting 63.3%, belonged to the age group exceeding 40 years. The AML subtype based on FAB criteria was found to be AML-M5 with a proportion of 43%. Table 1 provides an overview of the characteristics of the research subjects.

The results of data collected from 30 research subjects showed the highest number of blasts with CD34+ and the highest Bcl-xL expression in AML-M1 patients (Figure 2).

Results of analysis of the relationship between Bcl-xL expression and blast number, CD34 expression, and CD7 expression

Testing the normality of expression data Bcl-xL, number of blasts, CD34 expression, and CD7 expression. Adult AML patients as research subjects were carried out using the Kolmogorov-Smirnov test. A p-value greater than 0.05 (α = 5%) from the Kolmogorov-Smirnov test indicates that the data conforms to a normal distribution.

A total of 30 LMA patients had a presentation of blast count 20 - 82% Bcl-xL expression with an MFI range of 93.06 - 441.09, CD34 expression with MFI range of 1.06 - 1,452.48, CD7 expression with MFI range 9.31 - 90.58. Kolmogorov-Smirnov test results in data Bcl-xL expression, number of blasts, CD34 expression, and CD7 expression in adult AML patients as research subjects are as follows:

The Kolmogorov-Smirnov test on Bcl-xL expression data from adult AML patients as research subjects produced a p-value> 0.05. These results conclude that the Bcl-xL expression data of adult AML patients as research subjects are normally distributed, However, the test results of data on the number of blasts, CD34 expression, and CD7 expression of adult AML patients as research subjects were not normally distributed, so to test the relationship between Bcl-xL expression, blast number, CD34 expression, and CD7 expression in research subjects, the Spearman correlation test was used.

If the Spearman correlation test produces a p-value <0.05 (α=5%), it is evident from the results that a meaningful association exists between Bcl-xL expression, blast number, CD34 expression, and CD7 expression in adult AML patients. If the Spearman correlation test produces a p-value> 0.05, it is evident from the results that no meaningful association exists between Bcl-xL expression, blast number, CD34 expression, and CD7 expression in adult AML patients.

Figure 3 presents the results of the correlation test between Bcl-xL expression and the number of blasts in adult AML patients at RSUD Dr. Soetomo.

The correlation test between Bcl-xL expression and the number of blasts in adult AML patients as research subjects produced a value of r = 0.118 (p = 0.534), concluding that no meaningful association existed between Bcl-xL expression and the number of blasts in adult AML patients as research subjects.

Correlation test results between the expression of Bcl-xL and CD34 in adults with AML at RSUD Dr. Sutomo are shown in Figure 4.

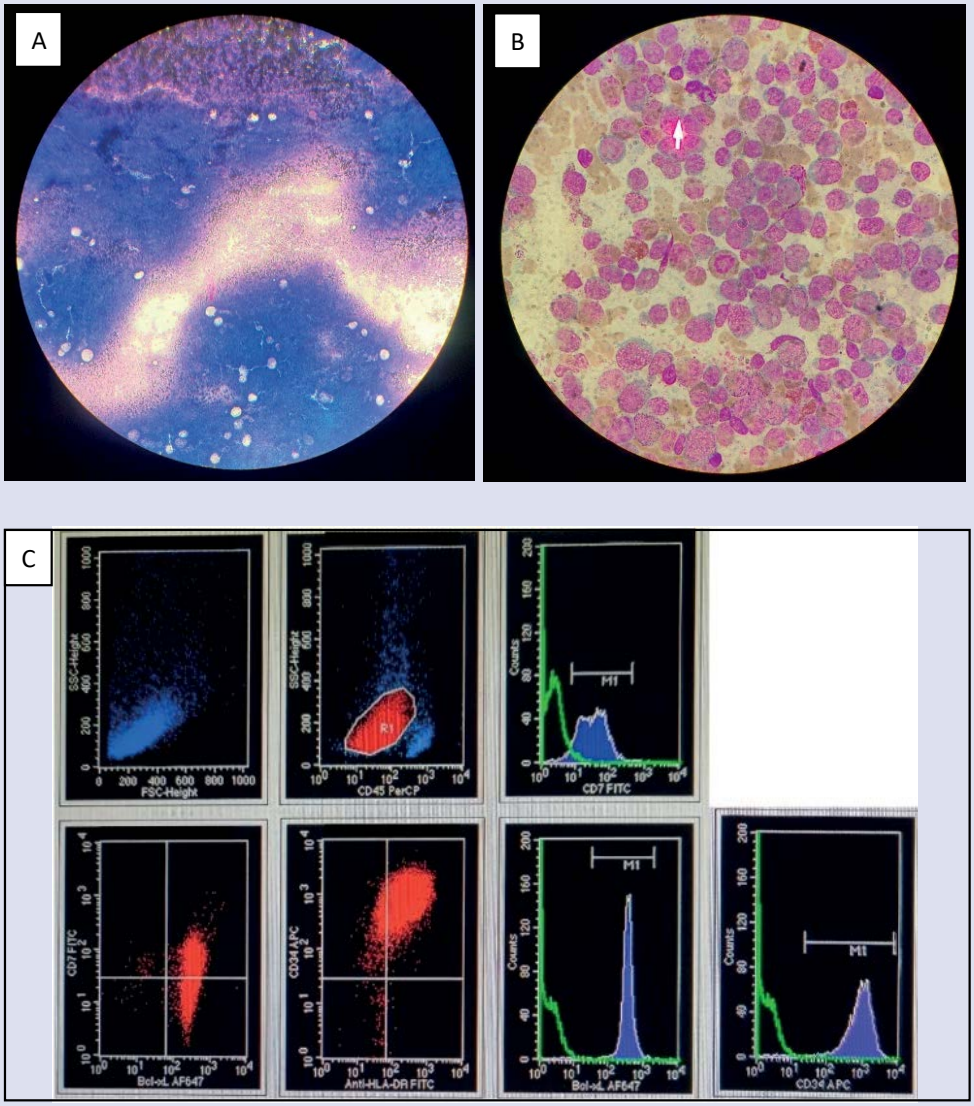

Figure 1. Cytometry analysis in 19-year-old man diagnosed as AML-M1 with 81% blasts had MFI CD34: 850.53, MFI CD7: 30.78, and MFI Bcl-xL: 321.97. Figures 1A and 1B were the microscopic imaging of the bone marrow analysis that shows the hypercellularity and dominated by blast cells. The scattergrams for CD45, CD34, CD7, Bcl-xL, and anti HLA-DR in Figure 1C were analyzed for mean fluorescence intensity (MFI) of CD34, CD7, and Bcl-xL.

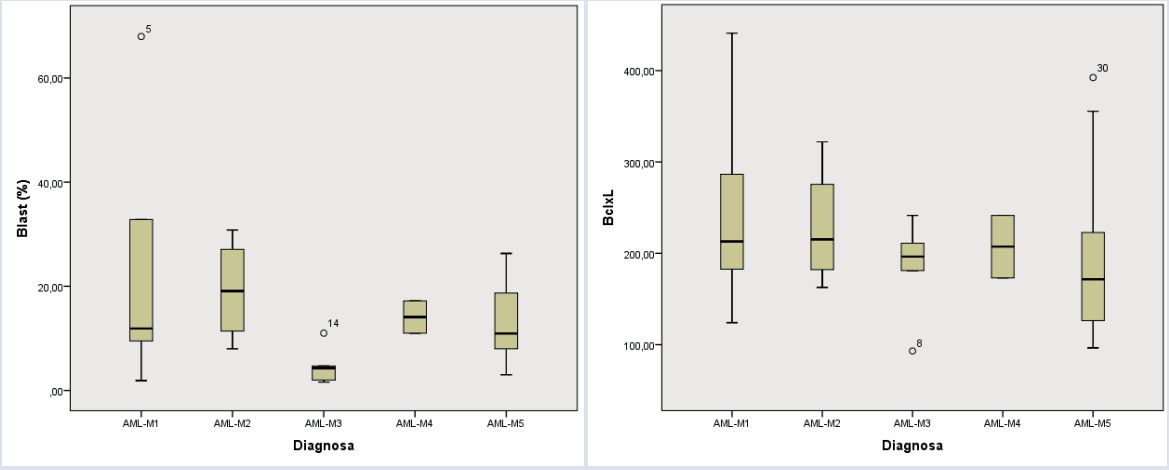

Figure 2. Boxplot of the number of blasts with CD34+ in each AML subtype (top image) and the highest Bcl-xL expression was found in the AML-M1 subtype (bottom image).

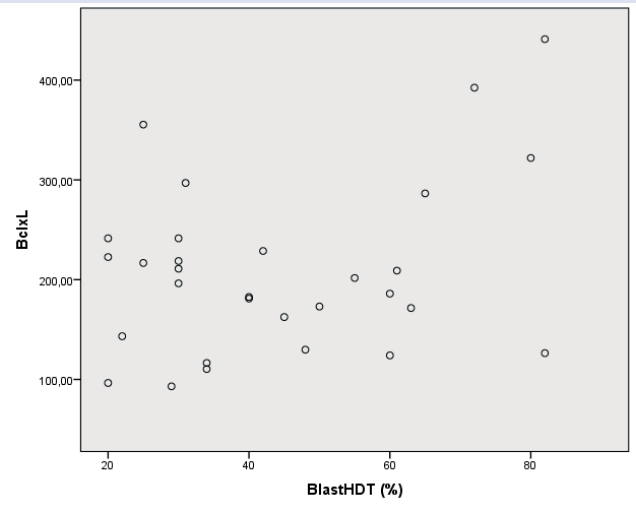

Figure 3. Relationship between Bcl-xL expression and blast number in adult AML patients.

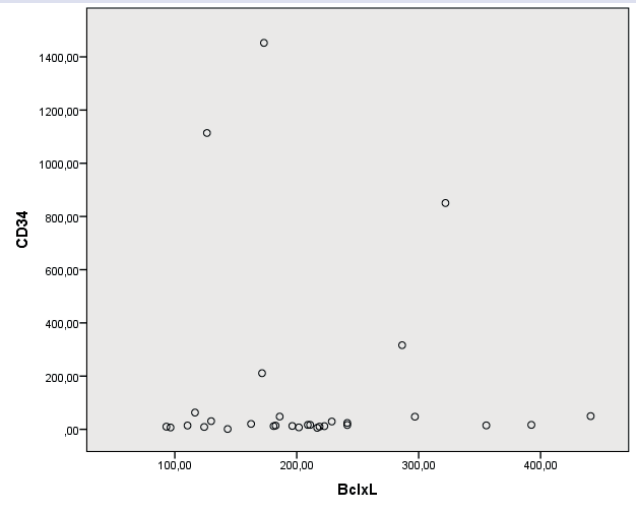

Figure 4. Relationship between the expression of Bcl-xL and CD34 in adults with AML.

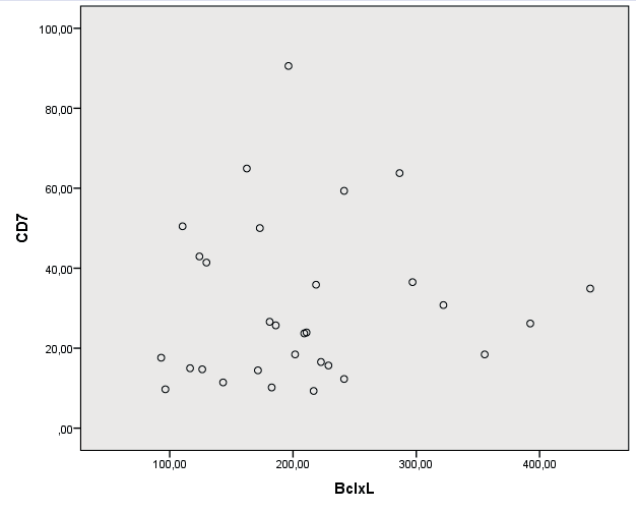

Figure 5. Association between the expression of Bcl-xL and CD7 in adults with AML.

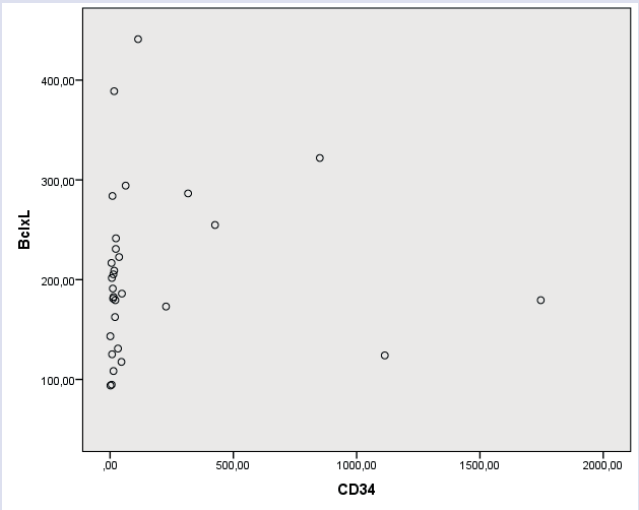

Figure 6. Relationship between Bcl-xL expression and the number of CD34+ blasts in adult AML patients.

Table 1. Demographic data on the characteristics of adult AML patients at Dr. Soetomo Hospital.

Characteristics	Amount	Percentage
Gender (n=30)		
Man	12	60%
Woman	18	40%
Age group (n=30)		
18-40 years old	11	36.7%
41-71 years old	19	63.3%
AML subtypes (n=30)		
AML-M1	6	20%
AML-M2	4	13.3%
AML-M3	5	16.7%
AML-M4	2	7%
AML-M5	13	43%

Table 2. Kolmogorov-Smirnov test data on blast number, CD34 expression, CD7 expression, and Bcl-xL expression of adult LMA patients at Dr. Sutomo Hospital.

Data	p-value
Number of blasts	p = 0.036
CD34	p = 0.000
CD7	p = 0.020
Bcl-xL	p = 0.066

The correlation test between the expression of Bcl-xL and CD34 in adults with AML as research subjects produced a value of r = 0.225 (p = 0.231), concluding that no meaningful association existed between Bcl-xL and CD34 expression in adult AML patients as research subjects.

Correlation test results between the expression of Bcl-xL and CD7 in adults with AML at RSUD Dr. Sutomo are shown in Figure 5.

The correlation test between the expression of Bcl-xL and CD7 in adults with AML as research subjects produced a value of r = 0.148 (p = 0.435), concluding that no meaningful association existed between the expression of Bcl-xL and CD7 in adults with AML as research subjects.

Figure 6 presents the correlation test results between Bcl-xL expression and the number of CD34+ blasts in adult AML patients at RSUD Dr. Sutomo.

The correlation test between Bcl-xL expression and the number of CD34+ blasts in adult AML patients as research subjects produced a

value of r = 0.132 (p = 0.488), concluding that no meaningful association existed between Bcl-xL expression and the number of CD34+ blasts in adult AML patients as research subjects.

DISCUSSION

This study analyzed 30 adult patients diagnosed with AML according to FAB criteria with a blast count of 20% from a count of 500 nucleated cells from bone marrow aspirate analyzed in less than 24 hours.

Based on O'Donnell et al 2017 in the United States patient's age range AML is 65-74 years. There is a tendency for the number of AML patients under the age of 60 to increase.[14,15]

The most common type of AML subtype in this study was AML-M5 at 43.3% followed by AML-M1 at 20%, AML-M3 at 16.7%, and AML-M2 at 13.3%, and AML-M4 at 6.7%. The average results of leukocyte, hemoglobin, and platelet examination showed the characteristics of AML with the highest number of blasts and Bcl-xL expression found in patients diagnosed with AML-M1.

Research subjects had a blast count range of 20 - 82%, Bcl-xL expression with an MFI range 93.06 - 441.09, CD34 expression with MFI range1.06 - 1,452.48, CD7 expression with MFI range of 9.31 - 90.58. This study shows that there is no relationship between Bcl-xL expression as a marker of anti-apoptotic activity with the number of blasts r = 0.118 (p = 0.534), CD34 expression r = 0.225 (p = 0.231), and CD7 expression r = 0.148 (p = 0.435).

CONCLUSION

The initiation of cancer begins with the proliferation of a cell population, which distinguishes itself from the body and can be identified as tumorigenic (tumor-initiating) cells, distinguished from non-tumorigenic cancer cells by specific cell surface markers.[16] Analysis of Bcl-xL expression on the number of blasts, CD34 expression, and CD7 expression is expected to provide additional information for clinicians in the management of adult AML patients so that they can provide maximum output in providing therapy and monitoring to patients.

The most significant role of Bcl-xL in Acute Myeloid Leukemia (AML) lies in its association with resistance to apoptosis, poor response to chemotherapy, and a poor prognosis.[17] Bcl-xL overexpression in AML patients has been linked to resistance to apoptosis, leading to challenges in treatment efficacy and patient outcomes.

Based on this study, Bcl-xL expression as a marker of anti-apoptotic activity in adult AML patients is not related to blast cell proliferation in AML. This suggests that increased anti-apoptotic activity is not the main mechanism in the pathogenesis of AML.[18]

The limitation of this research is that the subjects in this study cannot represent the AML population in Indonesia in general. Differences in characteristics of the number of female patients being greater than male patients can be caused by many factors, including population distribution in an area, and genetic or hereditary factors. Flow cytometry analysis in this study was also only carried out once when the patient was first diagnosed with AML so changes in blast cell expression after chemotherapy were not known.

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CONFLICTS OF INTEREST

None of the authors has any commercial or other conflicts of interest with this work.

ETHICAL CONSIDERATION

Ethical committee approval for this study was obtained from the ethics committee of Dr. Soetomo Hospital Surabaya with ethical number 0514/KEPK/X/2022.

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