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Immunophenotyping in Acute Leukemia at a Central Laboratory Installation RSUP Dr. Mohammad Hoesin Palembang 2020 to 2022: A Descriptive Study

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ABSTRACT

Leukemia is a malignant disease of blood cells originating from the bone marrow, characterized by irregular and uncontrolled proliferation of leukocytes with the manifestation of abnormal cells in the peripheral blood. Flow cytometry is the method of choice for determining the immunophenotype characteristics of hematological malignancy in terms of diagnosis, monitoring minimal residual disease, during and after therapy. The increase in the number of uses of this method is due to its relative simplicity, high sensitivity and specificity, and short examination time. This study is a descriptive study at the Central Laboratory Installation of Dr. Mohammad Hoesin Hospital Palembang by looking at examination data from 427 patients suspected of acute leukemia who were examined for flow cytometry immunophenotyping with the Flow cytometer BD Fasc Lyrics for the period January 2020 to December 2022. From a total of 427 samples of acute leukemia suspect, 364 (85.2%) cases of acute leukemia and 63 (14.8%) cases of non-conclusive were obtained. Of the 364 cases of leukemia, 113 cases (31%) were Acute Myeloid Leukemia (AML), 210 cases (57.7%) were Acute Lymphoblastic Leukemia type B (B-ALL), 34 cases (9.3%) were Acute Lymphoblastic Leukemia type T (T-ALL), and 7 cases (2%) were Mixed Phenotype Acute Leukemia (MPAL). As a maturity determinant, CD34 most commonly appears in B-ALL (87.6%) while HLA-DR is most expressed by AML (23.9%). The most common antigens appeared in AML, B-ALL and T-ALL in the form of CD117 (30.1%), CD19 along with CD79a (100%), and CD7 (100%). Immunophenotyping is a tool that is able to diagnose and classify acute leukemia, AML, ALL, and MPAL.

Keywords: *Acute Leukemia, Immunophenotyping, flowcytometry*

INTRODUCTION

Leukemia is a malignant disease of blood cells originating from the bone marrow, characterized by irregular and uncontrolled proliferation of leukocytes with the manifestation of abnormal cells in the peripheral blood.[1] Epidemiological data show that leukemia accounted for about 2.5% of the total incidence of all types of

cancer and 3.1% of all cancer deaths globally in 2020.[2,3] According to WHO in 2019, there were 35,870 cases in the last five years in Indonesia with 11,314 deaths.[4]
The diagnosis of leukemia is established based on cytomorphology, cytochemistry, cytogenetics, and immunophenotyping examinations.[5] Immunophenotyping is a technique to identify the phenotype of a cell population based on antigen-antibody binding. Immunophenotyping in acute leukemia is aimed at detecting abnormal populations of hematopoietic cells in peripheral blood and bone marrow.[5] The application of immunophenotyping in acute leukemia includes identifying the presence of leukemia populations, identifying the hematology lineages involved, identifying multi- lineage involvement, and identifying minimal residual disease (MRD).[6,7] Flow cytometry is the method of choice for determining the immunophenotype characteristics of hematological malignancy in terms of diagnosis, monitoring minimal residual disease, during and after therapy. The use of this method is increasing due to its relatively simple use, high sensitivity and specificity, and short examination time.[8]
The purpose of this study is to find out the description of the results of hematological malignancy examination with the Flow Cytometry Immunophenotyping BD Fasc Lyrics tool at the Central Laboratory Installation of Dr. Mohammad Hoesin Hospital Palembang from 2020 to 2022.

MATERIALS & METHODS

This study is a descriptive research by looking at past flow cytometry immunophenotyping examination data. The sample was blood or bone marrow from 427 patients suspected of acute leukemia who were examined for flow cytometry immunophenotyping using the BD Fasc Lyrics flow cytometer for the period January 2020 to December 2022.
Data processing and analysis were carried out by data tabulating and data percentages based on age, gender, examination results and conclusions using descriptive statistical calculations using the SPSS application

RESULT

This study was conducted on patients with suspected acute leukemia at Dr. Mohammad Hoesin Hospital using the BD Fasc Lyrics Flow cytometer for the period January 2020 to December 2022. From a total of 427 samples of acute leukemia suspect research, 364 (85.2%) cases of acute leukemia were confirmed immunophenotyping and 63 (14.8%) cases were non-conclusive or no dominant marker of acute leukemia antigen were found. Of the 364 cases of acute leukemia, 113 cases (31%) were AML, 244 cases (67%) were ALL, and 7 cases (2%) were Mixed Phenotype Acute Leukemia (MPAL). In ALL, the results showed that 210 cases were B-ALL and 34 cases were T-ALL. The classification diagram of the type of acute leukemia can be seen in figure 1.

Figure 1. Classification of Acute Leukemia in this study

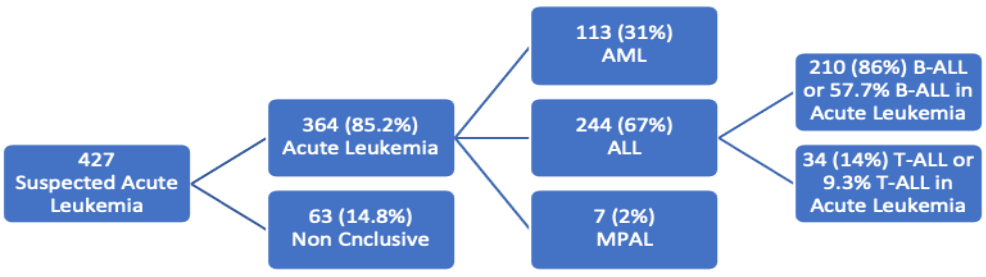

Table 1. shows an overview of the dermography characteristics of acute leukemia patients by gender and age group. Based on gender, the overall incidence ratio between males and females was 1.7:1 (63.2% *vs* 36.8%). For AML cases, the ratio of men to women is 1.5:1 (59.3% vs 40.7%), as well as for ALL ratio of 1.9:1 (65.2% vs 34.8%). So, in this study, there is a dominance of men compared to women in the incidence of acute leukemia.

Table 1. Characteristics of Acute Leukemia Based on Gender and Age

Characteristic	AML (n 113)		ALL (n 244)		MPAL (n 7)		Total	
	No.	%	No.	%	No.	%	No.	%
Gender								
Man	67	59.3	159	65.2	4	57.1	230	63.2
Woman	46	40.7	85	34.8	3	42.9	134	36.8
Age (years)								
0-10	37	32.7	156	63.9	4	57.1	197	54.1
11-20	22	19.5	51	20.9	2	28.6	75	20.6
21-30	11	9.7	11	4.5	0	0	22	6
31-40	10	8.8	10	4.1	1	14.3	21	5.7
41-50	12	10.6	6	2.5	0	0	18	4.9
51-60	9	8	3	1.2	0	0	12	3.3
61-70	8	7.1	6	2.5	0	0	14	3.8
>71	4	3.5	1	0.4	0	0	5	1.4

In this study, the age group with the most cases of leukemia was 0-10 years old (n: 197; 54.1%), followed by the age group of 11-20 years (n: 75; 20.6%) and 31-40 years (n: 21; 5.7%). The highest percentage of AML was in the age group of 0-10 years (n: 37; 32.7%), while 22 cases (19.5%) were found in the age group of 11-20 years. In ALL, the highest cases were also obtained in the age group of 0-10 years (n: 156; 63.9%). 51 cases (20.9%) were in the age group of 11-20 years and 11 cases were in the age group of 21-30 years. It is known that the percentage of acute leukemia incidence decreases after age 70 (1.4%).

Table 2. Results of reactive CD antigen in AL (*Acute Leukemia*)

Marker	AML (n 113)		B-ALL (n 210)		T-ALL (n 34)	
	No.	%	No.	%	No.	%
Markers of progenitors:						
CD34	90	79.6	184	87.6	22	64.7
HLA-DR	27	23.9	1	0.5	0	0
Myelo-monocytic markers:						
MPO	15	13.3	0	0	0	0
cyMPO	78	69	0	0	0	0
CD13	26	23.0	0	0	0	0
CD33	33	29.2	0	0	0	0
CD64	10	8.8	0	0	0	0
CD14	5	4.4	0	0	0	0
CD117	34	30.1	0	0	0	0
B lineage markers:						
CD19	18	15.9	20	98.6	7	20.6
CD20	10.9	0.9	1	0.5	0	0
CD79a	3	2.7	207	98.6	2	5.9
CD10	1	0.9	1	0.5	0	0
T lineage markers:						
CD7	45	39.8	12	5.7	34	100
CD3	5	4.4	17	8.1	23	67.6
cyCD3	0	0	0	0	23	67.6

The expression of CD34 and HLADR antigens as determinants of maturity stage can be seen in table 2. CD34 expression most often appeared sequentially in B-ALL (87.6%), AML (79.6%), and T-ALL (64.7%). Meanwhile, HLADR expression was obtained at 23.9% AML, 0.5% B-ALL, and no expression on T-ALL.

Markers of myeloid lineage evaluated include cyMPO, CD13, CD33 and CD117. cyMPO is the most common marker antigen expressed in 78 out of 113 (69%) AML cases. CD117 was expressed in 34 of 113 (30.1%) cases, and CD33 and CD13 were expressed in 33 (29.2%) and 26 (23%) cases, respectively. Other positive AML antigens were MPO 13.3%, CD64 8.8% and CD14 4.4%.

In the B-ALL lineage, 207 out of 210 cases (98.6%) expressed CD19 and CD79a, followed by CD20 and CD10 in one case. Among the T-ALL lineage, the dominant marker was CD7 (100%) followed by CD3 and cyCD3 with the same results in 23 out of 35 cases (67.6%).

In this study, CD7 was the most commonly expressed aberrant lymphoid antigen in AML (39.8%), followed by CD19 expression (15.9%). However, on the contrary, no expression of myeloid antigen was found in the case of ALL. Among B-ALL cases, CD3 appeared to be the most frequent T-cell antigen (8.1%) followed by CD7 (5.7%). Meanwhile, in T-ALL, the expression of B-cell antigens included CD19 (20.6%) and CD79a (5.9%).

Table 3. Antigen markers expressed by MPAL

Myeloid markers		**Lymphoid markers**		**Diagnosis**
Spesifik	Others	Spesifik	Others	
cyMPO	CD33, CD13, CD64, CD14	CD19	CD79a, CD10	B/Myeloid
cyMPO		CD19	CD79a	B/Myeloid
cyMPO		CD19	CD79a	B/Myeloid
cyMPO	CD33, CD13	CD19	CD79a	B/Myeloid
cyMPO	CD33, CD13, CD117	CD3, cyCD3	CD7	T/Myeloid
	CD33, CD13, CD117	CD19	CD79a	T/Myeloid
		cyCD3	CD79a, CD7	B/T

There were 7 cases of MPAL, which accounted for 2% of all acute leukemia in this study, including 4 cases of B/Myelocit MPAL, 2 cases of T/Myelocit MPAL, and one case of B/T MPAL.

DISCUSSION

Immunophenotyping in hematological malignancies is one of the most relevant applications of flow cytometry.[8] Flow cytometry's ability to detect antigen molecules or cell proteins is ideal for use in leukemia studies. In addition, this technique also has high sensitivity and specificity and short examination time.[1,9]

Based on the results of flow cytometry immunophenotyping on 427 samples, 345 patients were acute leukemia. 113 cases (26.5%) of them were AML, 244 cases (67%) were ALLs, and 7 cases (2%) were Mixed Phenotype Acute Leukemia (MPAL). These results are similar to the most previous studies in Indonesia, which reported 23% and 77% AML and ALL respectively.[10] In the Akrabi et al study in Yemen, the percentage of AML was 47.3% and ALL was 52.7%.[11] However, these results are contrary to a study in Egypt by Hamed, *et al*, which reported a greater percentage of AML than ALL (61.1% and 38.9%).[12]

Dermography data in this study showed that there was a dominance of men (63.3%) compared to women (36.8%) in the incidence of acute leukemia. These results are consistent with some previous studies.[9,13,11]

Acute leukemia affects all age groups. The highest incidence was seen in the age group of 0-10 years (n: 197; 54.1%), followed by the age group of 11-20 years (n: 75; 20.6%) and 31-40 years (n: 21; 5.7%). This result is

in contrast to the study in Yemen which reported the order of age groups from the most common incidence of acute leukemia, namely 16-30 years (29.1%), under 16 years (25.5%) and 46-60 years (20%).[11] In this study, the majority of cases in the 0-10 year age group were ALL (n: 156; 42.8%). This is in accordance with a study in Indonesia by Syahbani et al, which revealed the incidence of ALL in children at Dr. Soetomo Surabaya Hospital in 2019-2021 with the results of 39 cases (70.9%) at the age of 1-10 years and 1 case at the age of <1 year.[14] Research by Akrabi et al, Saleh et al, Hamid and Ahmad et al ALSO has the same conclusion.[11,15,16,13]

In AML, the most cases were also from the age group of 0-10 years. This is not in accordance with research by Ahmad, which found that AML cases are common at the age of 41-50 years.[13] Meanwhile, in Yemen, AML is most common in the age group of 16-30 years (30.8%).[11]

Markers of progenitor

Early signs (progenitors) were most expressed in AML cases. The earliest differentiation antigen to appear in stem cells is CD34. CD34 is expressed in many cell types, specifically in myeloblasts and low in promieloblasts. CD34 cells are found in cord blood, bone marrow, and normal peripheral blood, with amounts of about 1.5% and 0.1-0.01% of the components, respectively. The presence of CD34 antigen may describe a poor prognosis, and conversely the absence of CD34 antigen expression is associated with a high percentage of remission.[7,11,15] In this study, CD34 was positive in 79.6% of AML cases and 84% in ALL cases. Similar results were reported by Osman, et al., from Sudan where 78.7% of AML expressed CD34.[17] Meanwhile, the study by Akrabi and Hamid reported that CD34 antigen was positive for 84.6% in AML and 55.2% in ALL.[11]

HLA-DR was positive at 23.9% AML and 0.5% (one case) at B-ALL. This is different from the results obtained by Akrabi, where the percentage of positive HLADR was 38.5% in AML and 34.5% in ALL, without significant differences. In the AML subtype based on FAB classification, AML M3 has a unique immunophenotyping compared to other types of AML. The combination of HLA-DR and CD34 antigens is very helpful in differentiating M3 AML cases from other types.[11] However, this study does not classify AML based on FAB. Further research on this is highly recommended.

AML lineage markers

In AML, the antigen sequence of the most specific marker of the myeloid lineage is cyMPO (69%), then CD117 (30.1%), CD33 (29.2%), and CD13 (23%). This finding is similar to the study of Tegagen et al from Ethiopia, that cyMPO was the most abundant antigen (95%) in AML.[9] Akrabi et al also reported that the most common expression of AML markers was CD13, CD33 and *cytoplasmic* MPO.[11]

MPO (*Myeloperoxidase*) is a cytoplasmic enzyme stored in phagosomes and plays a role in the formation of hydrogen peroxidase. This enzyme is expressed at varying levels in almost all myeloid cells. MPO expression is the earliest marker and will develop into a myeloid lineage.[5] In this study, 69% and 13.3% of cyMPO and MPO antigens were expressed by AML, respectively. Akrabi et al reported that in AML as much as 42.3% of cMPO antigens were expressed. Likewise by Salem et al, who supported the findings of this study.[18]

CD117 is normally expressed by bone marrow hemopoietic precursors, and can be detected in all stages of myeloid lineage up to promielocyte maturation and in erythroid lineage up to pro-erythroblastic stages.[7] In this study, 30.1% was obtained in AML. CD117 positive for most cases in Akrabi et al, Salem DA, and Osman et al by 53.8%, 74.3%, and 83.8%. [11,18,17]

CD13 is usually expressed by hematopoietic stem cells, both mature and imaturable myeloid and monocyte lineage, as well as eosinophils and basophils. During the maturation process of myeloids, CD13 appears before CD33 on the myeloid

precursor CD34. Although it is often expressed, CD13 cannot be targeted in all cases of AML, its absence is associated with a good prognosis.[19] The study found 23% positive for CD13 antigen in AML, meanwhile, a higher percentage was found in the Akrabi et al study.[11]

CD33 is a myeloid antigen and appears during the myeloid differentiation process after CD13 in the hemopoietic process. CD33 expression is high in monocytes, and drastically decreased in basophils, neutrophils, and eosinophils.[7] In this study, CD33 was expressed lower by 29.2%, compared to Akrabi et al 76.9% and Osman 76.1%.[11,17]

B-ALL lineage markers

In the B-ALL lineage, almost all cases (98.6%) expressed CD19 and CD79a, followed by CD20 and CD10 in one case. This is in line with Salem et al, where CD19 and CD79a are expressed 100% by B-ALL, then in the Akrabi study reported 82.8% CD19 and 55.2% CD79a. [18,11]

T-ALL lineage markers

In the T-ALL lineage, CD7 is a T antigen that appears during the maturation of T lymphocytes.[20] CD7 and CD3 are common marker antigens of the T strain.[11] CD7 is not entirely specific because it has been shown to cross-react with AML cases. This study shows the dominance of CD7 in T-ALL (100%), In fact, 67.6% of T-ALL cases express cyCD3 and CD3 together. Bhattacharyya et al and Salem et al showed that CD7 and CD3 are the most commonly expressed T cell antigens.[21,18] However, lower expression was reported by Akrabi et al where there were only 2 cases (6.9.%).

Aberrant Expression

In many cases, blast cells from a lineage do not show normal markers of differentiation but express unusual markers of hematopoietic maturity. It is often found that myeloid marker antigens are expressed in lymphoblasts and lymphoid marker antigens are expressed in myeloblas. This phenomenon is called the aberrant phenotype. Based on the prognosis, the expression of aberrant antigen can affect clinical response, remission rate and survival in acute leukemia patients.[22,23]

In AML, the most frequent aberration by CD7 antigen (39%) is followed by CD19 expression (15.9%). Consistent results were obtained by CD7 and CD19 in previous studies.[11,24,25,17] Salem et al, reported CD7 prevalence (23%) but with low CD19 prevalence (1.8%).[18]

Among B-ALL cases, CD3 appears to be the most frequent T-cell antigen at 8.1% followed by CD7 (5.7%). these results contradict Salem et al and Bhattacharyya et al, where no B-ALL was found expressing T-cell antigens.[18,21] In the case of T-ALL in this study, the expression of B-cell antigens included CD19 (20.6%) and CD79a (5.9%). In the case of Momani et al, only 1 case (8%) of CD79a antigen aberration in T-ALL cases was found.[26]

Mixed Phenotyping Acute Leukemia (MPAL)

A small number of acute leukemia has a rare characteristic in which blast cells express antigens specific to more than one strain. This acute leukemia is grouped into MPAL (mixed phenotyping acute leukemia). MPAL includes leukemia with >1 population of blasts expressing specific lineage antigens, and leukemia with 1 population of blasts expressing specific antigens for >1 lineage. The WHO classification no longer distinguishes MPAL into acute biphenotypic leukemia and *bilineage*. In 2008, the WHO proposed a simpler diagnostic algorithm based on more specific strain markers. The new consensus criteria for MPAL were published in *the 4th edition of the WHO classification of tumors of hematopoietic and lymphoid tissues* and remained unchanged as of the 2016 update.[27,28]

Table 4. WHO Criteria 2008/2016 for MPAL (*mixed phenotyping acute leukemia*).

Lineage	Markers
Lineage Myeloid	MPO (Flow cytometry, immunohistochemistry, or cytochemistry) -OR- Monocytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)
Lineage sel T	Strong cytoplasmic CD3 -OR- Surface CD3
Lineage sel B	Strong expression of CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, or CD10 -OR- Weak expression of CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10

In this study, there were 7 cases of MPAL, which accounted for 2% of all acute leukemia. This is in accordance with the data published by Gupta M et al and Renu S et al that the incidence of MPAL was 2.96% (15/506 cases) and 2.7% (7/256 cases), respectively.[24,27]

Of the 7 cases of MPAL, B/Myeloid was the most dominant type (4/7 cases), this is in line with previous research.[24,27]

Limitations

There are a number of limitations in this study. (1) Patients' initial clinical were not included for analysis. (2) AML classification based on FAB was not performed. (3) Morphology, cytogenetics and molecular analysis were not performed. (4) Patients were not followed up on treatment and prognosis.

CONCLUSION

Immunophenotyping is a tool that is able to diagnose and classify acute leukemia, both AML, ALL, and MPAL. Immunophenotyping can also assess the frequency of acute leukemia aberration.

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