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Comparison of Bone Marrow Aspiration Interpretation with Immunophenotyping in Children's Leukemia Diagnosis

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Abstract

BACKGROUND: The prevalence of cancer in children is increasing. The morphological diagnosis of acute leukemia is divided into acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) and is sometimes incompatible with the diagnosis of flow cytometry, which shows the characteristics of malignant cells.

AIM: This study aims to compare the results of the interpretation of bone marrow aspiration with immunophenotyping in diagnosing childhood leukemia.

METHODS: Data from medical records were used in a retrospective cohort study of children with leukemia (ages 1–18 years) admitted to Dr. Wahidin Sudirohusodo Hospital Makassar from 2013 to 2017. All patients with a working diagnosis of leukemia were examined for bone marrow aspiration, followed by immunophenotyping, and each group was analyzed.

RESULTS: Of a total of 90 study subjects, the final diagnosis based on immunophenotyping was 60 ALL and 30 AML patients with a mean age of 8 years and 3 months. The male-to-female ratio was 1.7:1 (p = 0.353). The mean age of the ALL group was 7 years and 10 months, and AML, 9 years (p = 0.409). The suitability of morphology and flow cytometry to ALL and AML was 92.3% and 50%, respectively. Most markers were in the ALL CD 10 groups and CD 20 groups with a sensitivity of 100% each, and AML CD 117 with a sensitivity of 92%.

CONCLUSION: There was a correlation between the interpretation of bone marrow aspiration and immunophenotyping in diagnosing ALL.

Introduction

The American Cancer Society, in 2014, found 15,780 newly diagnosed cases of cancer and a total of 1,960 cancer deaths that occurred in children and adolescents from birth to 19 years of age. The incidence of cancer in children each year is estimated at 186.6/1 million children from birth to 19 years of age. Based on 2013 basic health research data, the national prevalence of cancer in the population of all ages in Indonesia is 1.4% or around 347,792 people [1]. Meanwhile, based on age, the prevalence of cancer in the Indonesian population is 0.3% at age under 1 year, 0.1% aged 1–4 years, 0.1% aged 1–4 years, and 0.9% aged 15–24 years. The most common cancers in children include acute lymphoblastic leukemia (ALL) (26%), brain and central nervous system tumors (21%), neuroblastoma (7%), and non-Hodgkin's lymphoma (6%) [2], [3].

Leukemia is a blood cell malignancy originating from the bone marrow. Leukocytes in the blood proliferate irregularly and uncontrollably, and their function becomes abnormal, marked by the proliferation of white blood cells with the manifestation of abnormal cells in the peripheral blood. The most widely accepted and applied classification of leukemia is the one based on morphology and cytochemistry, proposed by French American British (FAB). In general, leukemia is divided into two categories, namely, acute leukemia and chronic leukemia. Acute leukemia is divided into two major groups, namely, acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) [4], [5].

However, some facts showed better or even worse results, indicating the continued search for cellular and molecular characteristics of leukemia cells. At present, immunophenotyping for cases of acute leukemia is very important in determining the leukemia lineage and tends to be a common subject for analysis. Thus, the diagnosis and classification of leukemia are expected to better determine prognosis, assist in therapeutic choices, because leukemia cells display a characteristic pattern of surface antigen expression (CD antigen) [6]. Prompt and precise diagnosis of leukemia are essential so that appropriate treatment can be started without delay. This study was conducted to compare the immunophenotyping profile in blood samples of children diagnosed with leukemia with bone marrow aspiration results in favor of leukemia. Hence, an early diagnosis can be determined with accuracy.

This research has never been conducted in South Sulawesi.

Methods

Sample selection

This study is a retrospective cohort study, conducted at Dr. Wahidin Sudirohusodo hospital, Makassar using data from the medical records of leukemia patients who were registered at the Pediatric Hematology-Oncology, Department of Child Health, in 2013–2017. This study was approved by the Faculty of Medicine Universitas Hasanuddin Ethical Committee, Indonesia. All patients with a working diagnosis of leukemia were examined for bone marrow aspiration. Interpretations were conducted by attending physicians, followed by an immunophenotyping examination. Samples were taken from peripheral blood, and each group was analyzed. A total of 90 children aged 1–17 years and 11 months were enrolled as subjects in this study. Data were collected from medical records at Wahidin Sudirohusodo Hospital. The exclusion criteria are met if the medical record is incomplete and a bone marrow aspiration procedure failed to obtain an adequate sample for this study.

Data analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 26.0. The Chi-square test was used to test relationships between categorical variables. An independent t-test was performed to compare mean values between the two groups. A normality test was performed using Kolmogoro–Smirnov. $p < 0.05$ was considered statistically significant.

Results

This study consisted of 90 cases of suspected acute leukemia registered at Dr. Wahidin Sudirohusodo hospital, Department of Child Health in 2013–2017. This study consisted of 57 male and 33 female patients, with a ratio of 1.7: 1. Consisting of 60 patients in the ALL group and 30 patients in the AML group ($p = 0.353$). The patient ages ranged from 1 year to 17 years and 11 months, with a mean age of 7 years and 10 months in the ALL group and 9 years in the AML group ($p = 0.409$). There was no significant difference in Hb, WBC, Plt, and lymphocyte count between ALL and AML with $p > 0.05$. Initially, patients with suspected acute leukemia were examined for bone marrow puncture. The final diagnosis was given after immunophenotyping, into the ALL and AML groups (Table 1).

Table 1: Characteristics of patients based on bone marrow aspiration and immunophenotyping

Serial number	Characteristics	ALL (n = 60)	AML (n = 30)	p
1	Sex, n (%)			
	Male	40 (66.7)	17 (56.7)	0.353*
	Female	20 (33.3)	13 (43.3)	
2	Age (years)			
	Mean	7.90	9.05	0.409**
	Median	6.50	8.04	
	Minimum–maximum	1.25–17.92	1.00–17.33	
3	Peripheral blood, median (range)			
	Hb (g/dL)	6.3 (2.5–14.7)	7.2 (4.4–9.4)	0.59*
	WBC ($\times 10^3$/L)	16,500 (900–423,930)	17,400 (5300–96,070)	0.103**
	Plt ($\times 10^3$/L)	20,000 (2000–369,000)	12,000 (2000–43,000)	0.061**
	Lymphocytes (%)	78.9 (19.8–96.4)	41.4 (22.6–56)	0.204**

*Chi-square test, **Mann–Whitney U-test. ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, WBC: White blood cell, Hb: Hemoglobin, Plt: Platelets

Based on the results of bone marrow aspiration examination, the number of leukemia cases was 81 (90%), consisting of ALL 65 cases, AML 15 cases, and one mixed-lineage case. Non-leukemia based on bone marrow aspiration, nine cases (10%) consisted of five cases of myelodysplasia syndrome and four cases of aplastic anemia. Nine cases based on bone marrow aspiration were not leukemia, but nine cases were detected as leukemia based on immunophenotyping (Table 2).

Table 2: Results of immunophenotyping of leukemia and non-leukemia patients based on bone marrow aspiration

Classification (BMP)	Immunofenotyping		Total
	ALL	AML	
Leukemia			
ALL	54 (83.1)	11 (16.9)	65 (100)
AML	0	15 (100)	15 (100)
Mixed lineage	1 (100)	0	1 (100)
Non-leukemia			
MDS	2 (40)	3 (60)	5 (100)
Aplastic anemia	3 (75)	1 (25)	4 (100)
Total	60 (66.7)	30 (33.3)	90 (100)

ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, MDS: Myelodisplasia syndrome, BMP: Bone Marrow Aspiration

Based on the results of the bone marrow aspiration examination, there were 65 samples detected as ALL, but based on the immunophenotyping, there were only 60 samples, so the concordance was 92.3%. Based on bone marrow aspiration results, 15 samples were obtained for AML samples. However, there were 30 samples detected by AML based on immunophenotyping. Hence, the concordance was 50.0%, the other 15 samples that detected AML came from the results of bone marrow aspiration: ALL (11), MDS (3), and Aplastic Anemia (1) (Table 3).

In ALL, the most common markers were found on CD 10 (100%), CD 5 (100%), CD 3 (100%), and CD 19 (85%). In AML, the most common markers were found on CD 117 (92%) and CD 33 (81.8%) (Table 4).

Discussion

In this study, it was found that the frequency of incidence of leukemia in boys was greater than

that in girls, with a male-to-female ratio of 1.7: 1. In Pernambuco state, this ratio is 1.7: 1.20, while in Ribeirao Preto (a city in the state of São Paulo), it is 1.8: 1. Noronha *et al*. discovered a higher proportion of male height (3.1: 1) [7]. There was no significant difference in Hb, WBC, Plt, and lymphocyte count between ALL and AML with $p > 0.05$. It is consistent with a study conducted by Malik [8].

Based on the type of leukemia, the frequency of AML was lower (33%) than ALL (67%). Noronha *et al*. found a much lower frequency of AML (37.1%) than ALL (60%) and that there was biphenotypic acute leukemia 2 (2.9%). Similar results are described by Rego *et al*., who found that in the state of Piauí, the frequency of AML is half that of ALL [7].

Khalil *et al*. (1995) reported a concordance between immunophenotyping with morphology and cytochemistry of 97% at the King Faisal specialist hospital. Other studies have concluded that immunophenotyping by flow cytometry is a useful and reliable method for the classification of acute leukemia [9]. Supriyadi *et al*. also evaluated the suitability of morphological and immunophenotyping methods and found a good kappa score of 0.82 for the new method [4]. In this study, we found a concordance of morphological methods with immunophenotyping in ALL cases (92.3%). However, 30 cases of AML were based on immunophenotyping, and 15 cases were according to morphology so that the concordance was 50%. It was found that there was a discrepancy between the morphological diagnosis and flow cytometry. This is in accordance with a study conducted by Muliadi, who obtained the results of 14 patients with morphologically diagnosed ALL, of whom one was diagnosed as AML by immunophenotyping. As for patients with AML, all were declared AML (100%) using immunophenotyping, with a fairly good morphological concordance with ALL (92.8%). A clear difference was seen when the results of bone marrow aspiration MDS and aplastic anemia results from immunophenotyping did not match MDS: 2 (40%) ALL and 3 (60%) AML, and aplastic anemia: 3 (75%) ALL and 1 (25%) AML [10].

Table 3: The concordance between morphological diagnosis and immunophenotyping of leukemia patients

Type of leukemia	Morphology	Immunofenotyping	Diagnosis	Concordance (%)
ALL	65	60	60	92.3
AML	15	30	30	50.0

ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, MDS: Myelodisplasia syndrome.

A total of nine cases are based on the results of bone marrow aspiration examination: Five cases of MDS, four cases of aplastic anemia, and can be detected using immunophenotyping, namely, five cases as ALL and four cases as AML. Meanwhile, Murmu *et al*. found that a comparison of morphological diagnosis with flow cytometry contained complex concordances in 81% of cases, partial co-conditions in 3% of cases, and non-co-conditions in 13% of cases between the two modalities [11]. Flow cytometry is particularly useful when morphology fails to provide a diagnosis, especially when clinical evidence supports leukemia, but bone marrow aspiration results do not so that immunophenotyping can aid in accurate and timely diagnosis and treatment.

In this study, HLA-DR markers were frequently found in both patient groups. Next, CD 34 and CD 19 are the most common markers in ALL and AML patients. Philip Lanzkowsky has shown that the most common marker is HLA-DR, and CD19 is the second most important. However, the highest positive predictive value in the case of ALL is shown by the CD 20 marker with successive values (100%), CD 10 (100%), CD 19 (85%), CD5 (100%), and CD3 (100%). Whereas in the AML group, the highest positive predictive value was shown by CD 117 (92%). This result is in line with the results found by Mirbehbahani *et al*. (2011) that the dominant markers found in ALL are CD 19 (90.2%) and CD 10 (84.36%) [5]. Other studies report CD34 expression. CD34 was seen in blasts in 66.66% (50/75) of the cases. In contrast, HLA DR proved to be more useful for defining immaturity as it was expressed by blasts in 92% (69/75) of the cases. Expression of B lineage markers: CD19 was expressed in all the 75 cases. The next most common B lineage antigen was cytoplasmic CD79a, which was expressed in 96% followed by CD10 at 94.6% [12].

Kermani and Tabriz (2002) showed that the most frequent markers in ALL patients were CD7 (11–28%), CD2 (5–21%), and CD19 (3–14%). CD10 (1–5%) and CD20 (9%) rank second and third, respectively [13]. In this study, there was no specific age range. Similarly, Tong *et al*. (2010) studied 113 patients with ALL in China [5]. In this study, the most common markers in AML patients were CD34, followed by CD 13, CD 33, and HLA-DR. In this study, the common markers found were CD 34, CD 13, CD 33, HLA-DR, and CD 117.

Tong *et al*. investigated the immunophenotypic subtype profile of 192 patients with AML. The results showed that CD33, CD13, myeloperoxidase (MPO), and CD117 were the antigens most frequently expressed in AML. CD117 was represented in 84.6% of AML-M3 cases [5] Similarly, Shen *et al*. observed that the most common antigens in AML were CD7 (12.8%), CD19 (6.4%), and CD2 (5.1%) [12]. Marker covariance was observed in the different AML subgroups. Meanwhile, Shresta *et al*. (2013) found CD 13 and CD 33 to be the most important markers for AML [13].

Table 4: Distribution of leukemia markers and types

Tipe leukemia	CD34	HLA-DR	CD10	CD19	CD20	CD117	CD13	CD33	CD7	CD5	CD3
ALL (%)	40 (58)	50 (66.7)	44 (100)	51 (85)	34 (100)	2 (8)	12 (30.8)	6 (18.2)	7 (43.8)	7 (100)	8 (100)
AML (%)	29 (42)	25 (33.3)	0	9 (15)	0	23 (92)	27 (69.2)	27 (81.8)	9 (56.3)	0	0
Total	69	75	44	60	34	25	39	33	16	7	8

CD: Cluster of *differentiation*, ALL: Acute lymphoblastic leukemia, AML: Acute myeloblastic leukemia, *HLA-DR: HLA-DR: Human leucocyte antigen DR.*

In our study, the prevalence of several markers in ALL and AML patients was different from other studies, and some of them were the same. These results can be used for the differential diagnosis of AML from ALL. The current investigation is being conducted on a small population of children, and further studies with larger sample sizes will be needed to reach clear conclusions and other limitation; we did not correlate the results of the study with comorbidities of the patients.

Conclusion

Immunophenotyping is very useful in the identification and diagnosis of acute myeloid or lymphoblastic leukemia and its subtypes, so it is very important to carry out these two tests. We found the correspondence between the results of the interpretation of bone marrow aspiration with immunophenotyping in diagnosing ALL 92.3% and AML 50%. Using a combination of FAB morphology and immunophenotyping, we diagnosed and classified all patients with acute leukemia in this study.

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