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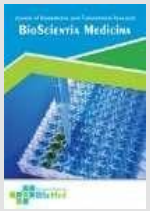

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Immunophenotyping vs. Bone Marrow Aspiration in Pediatric Acute Leukemia: A Comparative Analysis

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A B S T R A C T

Background: Acute leukemia is a significant global health concern, with increasing prevalence worldwide and in Indonesia. Accurate diagnosis and classification of acute leukemia subtypes, primarily acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML), are crucial for effective treatment. Immunophenotyping and bone marrow aspiration (BMA) are commonly used diagnostic methods, each with its strengths and limitations. This study aimed to analyze the concordance between immunophenotyping and BMA in diagnosing and classifying acute leukemia in children. **Methods:** A retrospective cross-sectional study was conducted on 46 children diagnosed with acute leukemia at Dr. M. Djamil General Hospital Padang from January 2022 to July 2023. Data were collected from medical records, including patient demographics, immunophenotyping results, and BMA findings. Concordance between the two diagnostic methods was analyzed using Fisher's exact test. **Results:** The study population consisted of 30 (65.2%) males and 16 (34.8%) females, with a median age of 4 years. Immunophenotyping identified 24 (52.2%) cases as ALL and 22 (47.8%) as AML. BMA classified 26 (56.5%) cases as ALL and 20 (43.5%) as AML. There was a high concordance between the two methods, with only 2 (4.3%) cases showing discordant results. These two cases were classified as AML by immunophenotyping but as ALL by BMA. **Conclusion:** Immunophenotyping and BMA demonstrate a high level of concordance in diagnosing and classifying acute leukemia in children. The few discordant cases highlight the importance of considering both methods, especially in challenging cases, to ensure accurate diagnosis and appropriate treatment.

1. Introduction

Acute leukemia, characterized by the uncontrolled proliferation of leukocytes, presents a formidable challenge to health systems worldwide. This malignancy disrupts the normal production of blood cells, leading to a range of clinical manifestations that can be life-threatening. The global impact of leukemia is substantial. In 2020, there were 474,519 new cases of leukemia reported globally, constituting 4.45% of all cancer diagnoses. Leukemia also accounted for a significant proportion of cancer-related deaths, with 311,594 fatalities recorded in the same year, representing 3.1% of total cancer mortality. The burden of leukemia is not evenly distributed across populations. Notably, the incidence of leukemia is higher in males compared to females. Specifically, in 2020, there were 269,503 new cases among males and 133,776 new cases among females. Regional disparities also exist. In Southeast Asia, leukemia was responsible for 37,025 new cases and 28,088 deaths in 2020. Within this region, Indonesia faces a significant burden, with leukemia ranking among the top 10 most common cancers. In 2020, Indonesia reported 14,979 new cases of leukemia and 11,530

deaths attributed to the disease. The diagnosis of leukemia is a complex process that requires a multi-faceted approach. The World Health Organization (WHO) recommends a combination of methods to accurately diagnose and classify leukemia. These methods include morphology, cytochemistry, immunophenotyping, cytogenetics, and molecular genetic analysis. This comprehensive approach is essential for distinguishing between the various subtypes of leukemia and for guiding appropriate treatment strategies.[1-4]

Acute leukemia is broadly classified into two main categories: acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML). These categories are further divided into subcategories based on the specific lineage and maturity of the leukemic cells. The accurate determination of the leukemic cell lineage is of paramount importance as it directly influences treatment decisions and prognosis. Bone marrow aspiration (BMA) is a fundamental procedure in the diagnosis, classification, and monitoring of hematologic disorders, including leukemia. This technique involves the extraction and examination of bone marrow tissue, providing valuable insights into the cellular composition of the bone marrow. Through BMA, clinicians can assess the morphology, cytochemistry, and immunophenotype of leukemic cells, which are crucial for accurate diagnosis and classification. Immunophenotyping is another essential diagnostic tool used in the evaluation of leukemia. This technique employs monoclonal antibodies to detect specific antigens expressed on the surface, cytoplasm, or nucleus of leukemic cells. By identifying these antigens, immunophenotyping enables precise characterization of leukemic cells, aiding in diagnosis, prognosis, and the monitoring of minimal residual disease (MRD).[5-7]

Both BMA and immunophenotyping play critical roles in the diagnostic workup of leukemia. However, it is important to acknowledge that discrepancies can arise between the results obtained from these two methods. These discrepancies may be attributed to a variety of factors, including technical limitations inherent to each technique, variations in sample quality, and the intrinsic heterogeneity of leukemic cells. Given the potential for discrepancies and the critical importance of accurate diagnosis in initiating timely and effective treatment, there is a need for studies that compare and evaluate the concordance between different diagnostic methods.[8-10] This study aims to contribute to this body of knowledge by investigating the concordance between immunophenotyping and BMA in the diagnosis and classification of acute leukemia in children.

2. Methods

This study employed a retrospective cross-sectional design. The study was conducted at Dr. M. Djamil General Hospital Padang. This hospital is a tertiary care center located in Padang, Indonesia. The study period spanned from January 2022 to July 2023. The study population consisted of all children diagnosed with acute leukemia at Dr. M. Djamil General Hospital Padang. during the specified study period. For the purposes of this study, children were defined as individuals under the age of 18 years. The study sample was derived from the medical records of children with acute leukemia who met specific inclusion and exclusion criteria. The use of medical records allowed for the collection of relevant data, including patient demographics, immunophenotyping results, and BMA findings.

To ensure the selection of appropriate cases for the study, the following inclusion criteria were applied; Age <18 years: Only patients under the age of 18 were included in the study. This criterion focused the study on pediatric acute leukemia cases; Complete immunophenotyping and BMA results: Cases were included only if they had complete results for both immunophenotyping and BMA. This criterion ensured that a comprehensive comparison between the two diagnostic methods could be conducted; No prior treatment for leukemia: Patients who had received prior treatment for leukemia were excluded. This criterion aimed to minimize the potential confounding effects of prior therapy on the diagnostic accuracy of

immunophenotyping and BMA. The following exclusion criteria were used to further refine the sample and ensure the quality of the data; BMA sample contaminated with peripheral blood: Cases were excluded if the BMA sample was contaminated with peripheral blood. Contamination can affect the accuracy of BMA results, making these cases unsuitable for inclusion; Immunophenotyping or BMA results indicating aberrant or mixed-phenotype acute leukemia: Cases with immunophenotyping or BMA results indicative of aberrant or mixed-phenotype acute leukemia were excluded. These subtypes of leukemia can present diagnostic challenges and were excluded to maintain the focus of the study on the major subtypes of acute leukemia (ALL and AML).

Data were extracted from the medical records of eligible patients using a standardized data collection form. The use of a standardized form ensured consistency and completeness in the data collection process. The following information was collected; Patient demographics: This included age and gender. These demographic characteristics were collected to describe the study population and to explore potential associations with the study findings; Immunophenotyping results: This included the lineage and subtype of leukemia as determined by immunophenotyping. Immunophenotyping results are crucial for classifying acute leukemia and were a primary focus of the study; BMA findings: This included the lineage and subtype of leukemia as determined by BMA. BMA findings are also essential for the diagnosis and classification of acute leukemia and were the other primary focus of the study.

Immunophenotyping is a technique that utilizes monoclonal antibodies to identify specific antigens expressed by cells. These antigens can be present on the cell surface, in the cytoplasm, or in the nucleus. In the context of leukemia diagnosis, immunophenotyping plays a crucial role in characterizing leukemic cells and classifying them according to their lineage and stage of differentiation. The general procedure for immunophenotyping involves the following steps; Sample Preparation: The bone marrow aspirate is processed to obtain a single-cell suspension. This may involve the use of enzymatic digestion or mechanical disruption to disaggregate cell clumps and ensure that individual cells can be analyzed; Antibody Staining: The cell suspension is incubated with a panel of monoclonal antibodies. Each antibody is specific for a particular antigen. These antibodies are typically conjugated to a fluorescent dye, allowing for their detection using flow cytometry. The selection of antibodies is critical and depends on the specific diagnostic question being addressed. In the diagnosis of acute leukemia, the antibody panel typically includes markers for lymphoid and myeloid lineages, as well as markers for different stages of cell differentiation; Flow Cytometry Analysis: The stained cell suspension is analyzed using a flow cytometer. This instrument measures the fluorescence intensity of individual cells as they pass through a laser beam. The data collected by the flow cytometer are used to identify and quantify cells expressing specific antigens. Flow cytometry allows for the analysis of a large number of cells in a short period of time, providing a detailed immunophenotypic profile of the sample; Data Analysis and Interpretation: The data generated by flow cytometry are analyzed using specialized software. This analysis involves gating strategies to identify specific cell populations based on their antigen expression. The results are interpreted in the context of other clinical and laboratory findings to arrive at a final diagnosis. Specific details of the immunophenotyping procedures used at Dr. M. Djamil General Hospital Padang during the study period would typically include; Antibody Panel: The specific monoclonal antibodies used for immunophenotyping. This would include details on the manufacturer, clone, and fluorochrome conjugate for each antibody; Flow Cytometer: The make and model of the flow cytometer used for data acquisition; Gating Strategy: The specific gating strategy used to identify and classify leukemic cells. This would include details on the markers used to define different cell populations (e.g., lymphoid vs. myeloid, blasts vs. mature cells); Quality Control: Procedures used to ensure the quality and accuracy of

immunophenotyping results, such as the use of control samples and calibration procedures.

Bone marrow aspiration (BMA) is a procedure used to collect a sample of bone marrow for examination. This procedure is a critical component in the diagnosis and management of various hematologic disorders, including leukemia. BMA provides valuable information about the cellular composition of the bone marrow, including the morphology, cytochemistry, and immunophenotype of cells. The general procedure for BMA involves the following steps; Patient Preparation: The patient is positioned appropriately, and the site for bone marrow aspiration is selected. Common sites include the posterior superior iliac spine or the anterior iliac crest. The skin overlying the site is cleaned and sterilized. Local anesthesia is administered to numb the area. In some cases, sedation may be used, particularly in pediatric patients; Needle Insertion: A specialized bone marrow aspiration needle is inserted through the skin and into the bone marrow cavity. The needle is advanced until it penetrates the bone cortex and enters the marrow space; Aspiration: Once the needle is in the correct position, a syringe is attached to the needle, and suction is applied to aspirate a sample of bone marrow fluid. The sample is typically small, usually around 1-2 mL; Smear Preparation: The aspirated bone marrow fluid is used to prepare smears on glass slides. These smears are then stained with various stains, such as Wright-Giemsa stain, for microscopic examination; Sample Analysis: The bone marrow smears are examined under a microscope by a trained pathologist or hematologist. The cells are evaluated for their morphology, including size, shape, and nuclear characteristics. Cytochemical stains may be applied to further characterize the cells. Specific details of the BMA procedures used at Dr. M. Djamil General Hospital Padang during the study period would typically include; Aspiration Site: The specific site used for bone marrow aspiration (e.g., posterior superior iliac spine, anterior iliac crest); Needle Type: The type and gauge of the bone marrow aspiration needle used; Anesthesia: The type of anesthesia used (e.g., local, sedation); Staining Procedures: The specific staining procedures used for the bone marrow smears (e.g., Wright-Giemsa stain, other cytochemical stains); Microscopic Examination: Details on the microscopic examination of the bone marrow smears, including the criteria used for cell identification and classification; Quality Control: Procedures used to ensure the quality and adequacy of the bone marrow aspirate, such as assessment of the sample for the presence of marrow particles and the absence of peripheral blood contamination.

Statistical analysis was performed using SPSS software, version 28. Descriptive statistics were used to summarize the characteristics of the study population. Descriptive statistics provide a clear and concise overview of the data, including measures of central tendency (e.g., mean, median) and measures of dispersion (e.g., standard deviation, range). In this study, descriptive statistics were used to describe patient demographics, immunophenotyping results, and BMA findings. Concordance between immunophenotyping and BMA in diagnosing and classifying acute leukemia was assessed using Fisher's exact test. A p-value of <0.05 was considered to indicate statistical significance.

3. Results

Table 1 presents a concise summary of the key characteristics of the patient population included in the study. It provides a breakdown of the distribution of patients based on age, gender, and the results of two diagnostic methods: immunophenotyping and bone marrow aspiration (BMA). Additionally, it shows the overall concordance between these two methods. The table reveals that the largest proportion of patients (39.1%) falls within the youngest age group, ≤4 years, with 18 patients. This is followed by the 10-14 years age group, comprising 13 patients (28.3%). The 5-9 years group includes 10 patients (21.7%), and the smallest group is the 15-<18 years category, with only 5 patients (10.9%). This distribution indicates that acute leukemia is more prevalent in younger children within this study population. The data indicates a

clear gender disparity in the occurrence of acute leukemia. The majority of the patients are male, with 30 male patients representing 65.2% of the total. In contrast, there are 16 female patients, accounting for 34.8%. This finding aligns with the general trend of leukemia being more prevalent in males compared to females. The table also presents the classification of leukemia based on immunophenotyping and BMA, the two diagnostic methods under comparison. Immunophenotyping identified a slightly higher number of ALL cases (24 patients, 52.2%) compared to AML cases (22 patients, 47.8%). BMA classified a slightly higher number of cases as ALL (26 patients, 56.5%) compared to AML (20 patients, 43.5%). These results suggest a relatively balanced distribution of ALL and AML cases within the study population, with a slight predominance of ALL. The table highlights a high level of agreement between immunophenotyping and BMA. Among the 46 patients, 44 cases (95.7%) showed concordant results, meaning both methods classified the leukemia in the same way. However, there were 2 cases (4.3%) where the results were discordant, indicating disagreement between the two diagnostic methods.

Table 1. Patient characteristics.

Characteristic	Category	Number of patients	Percentage
Age			
	≤ 4 years	18	39.1%
	5-9 years	10	21.7%
	10-14 years	13	28.3%
	15-<18 years	5	10.9%
Gender			
	Male	30	65.2%
	Female	16	34.8%
Immunophenotyping			
	ALL	24	52.2%
	AML	22	47.8%
Bone marrow aspiration			
	ALL	26	56.5%
	AML	20	43.5%
Concordance			
	Concordant	44	95.7%
	Discordant	2	4.3%

Table 2 presents a detailed analysis of the concordance between immunophenotyping and bone marrow aspiration (BMA) in diagnosing acute leukemia. It categorizes the results based on the findings of each diagnostic method and provides a statistical measure of their agreement. The table clearly demonstrates a high level of agreement between immunophenotyping and BMA in classifying acute leukemia cases. When immunophenotyping identified a case as acute lymphoblastic leukemia (ALL), BMA also classified it as ALL in 24 out of 46 patients. This represents 52.2% of the total cases and is labeled as "Concordant" in the table. Similarly, when immunophenotyping indicated acute myeloblastic

leukemia (AML), BMA agreed with this classification in 20 cases, accounting for 43.5% of the total. These cases are also designated as "Concordant." Despite the high concordance, the table also highlights instances where the two diagnostic methods yielded different results. There were 2 cases (4.3%) where immunophenotyping classified the leukemia as AML, but BMA classified it as ALL. These cases are specifically labeled as "Discordant," indicating a disagreement between the two diagnostic methods. The table includes a p-value of <0.001 for the ALL concordance. This p-value is a measure of statistical significance, indicating that the observed agreement between immunophenotyping and BMA in diagnosing ALL is highly unlikely to have occurred by chance. The very low p-value strongly supports the reliability of both methods in diagnosing ALL and their high level of agreement.

Table 2. Concordance between immunophenotyping and bone marrow aspiration in diagnosing acute leukemia.

Immunophenotyping result	**Bone marrow aspiration result**	**Number of patients**	**Percentage**	**Concordance**	**P-value**
ALL	ALL	24	52.2%	Concordant	<0.001
AML	AML	20	43.5%	Concordant	
AML	ALL	2	4.3%	Discordant	
Total		**46**	**100%**		

4. Discussion

The demographic characteristics of patients with acute leukemia, specifically their age and gender distribution, provide critical insights into the epidemiology of the disease and can have important implications for clinical practice. In this study, a distinct pattern emerged in the age and gender distribution of the pediatric acute leukemia patients, warranting a more detailed examination. The study findings revealed that the highest proportion of patients (39.1%) belonged to the youngest age group, ≤4 years. This observation is a significant one, as it aligns with the established understanding that certain subtypes of acute leukemia, most notably acute lymphoblastic leukemia (ALL), exhibit a higher prevalence in early childhood. ALL is the most common type of childhood cancer, and its peak incidence is typically observed between the ages of 2 and 5 years. The reasons for this age-specific predilection of ALL are complex and involve a combination of factors. The development of the immune system plays a crucial role. The lymphoid cells, which are the precursors of the leukemic cells in ALL, undergo significant developmental changes during early childhood. These developmental processes may render these cells more susceptible to genetic alterations or other events that can trigger leukemogenesis. Genetic factors also play a significant role. Certain genetic abnormalities are more commonly associated with childhood ALL, and these abnormalities may arise or become clinically apparent during early childhood. These genetic alterations can affect various cellular processes, including cell proliferation, differentiation, and apoptosis, ultimately leading to the uncontrolled growth of leukemic cells. Environmental factors may also contribute to the increased incidence of ALL in young children. Exposure to certain infections or other environmental triggers during early childhood may play a role in the development of leukemia in susceptible individuals. However, the exact nature and extent of these environmental influences are still under investigation. In contrast to the high prevalence of leukemia in the youngest age group, the study observed a gradual decrease in the proportion of patients with increasing age. The 5-9 years age group comprised 21.7% of the patients, the 10-14 years group constituted 28.3%, and the 15-<18 years group represented only 10.9% of the study population. This declining trend with age is consistent with the general pattern observed for ALL,

where the incidence decreases after the peak in early childhood. It is important to note that while ALL is more common in young children, other subtypes of leukemia, such as acute myeloid leukemia (AML), can occur at any age, including childhood. The age distribution of AML may differ from that of ALL, with a more uniform distribution across different age groups or a higher incidence in older children or adolescents in some studies. The age distribution pattern observed in this study has important implications for clinical practice. It underscores the importance of maintaining a high level of clinical vigilance for acute leukemia in young children presenting with signs and symptoms suggestive of the disease. These signs and symptoms can be nonspecific and may mimic common childhood illnesses, making early recognition challenging. Clinicians evaluating young children with unexplained fever, fatigue, pallor, bruising, bone pain, or lymphadenopathy should consider the possibility of acute leukemia. Prompt evaluation, including a thorough physical examination, complete blood count, and other appropriate diagnostic tests, is crucial for timely diagnosis and initiation of treatment. Furthermore, the age distribution pattern can inform public health initiatives aimed at early detection and prevention of childhood leukemia. Strategies to raise awareness among parents and healthcare providers about the signs and symptoms of leukemia in young children can contribute to earlier diagnosis and improved outcomes. In addition to the age-related patterns, the study demonstrated a clear gender disparity in the occurrence of acute leukemia. The majority of the patients in this study were male, with 30 male patients representing 65.2% of the total study population. In contrast, there were 16 female patients, accounting for 34.8% of the patients. This finding is consistent with established epidemiological trends that consistently indicate a higher incidence of leukemia in males compared to females across various age groups and populations. This male predominance has been observed in numerous studies examining the epidemiology of both ALL and AML in children and adults. The reasons for this gender difference are complex and multifactorial. While the precise mechanisms remain to be fully elucidated, several potential contributing factors have been proposed and are the subject of ongoing research. These factors can be broadly categorized as biological, genetic, hormonal, and environmental. Biological differences between males and females may play a role in the observed gender disparity in leukemia incidence. These differences can include variations in immune system function, cellular metabolism, and other physiological processes that may influence the susceptibility to leukemogenesis. The immune system plays a critical role in recognizing and eliminating abnormal cells, including pre-leukemic cells. Differences in immune responses between males and females may affect the efficiency of this process. Some studies have suggested that females may have a more robust immune response, which could provide some protection against the development of leukemia. Cellular metabolism also differs between males and females, influenced by genetic and hormonal factors. These metabolic differences could affect how cells respond to DNA damage, oxidative stress, and other factors that can contribute to leukemogenesis. Genetic factors are likely to play a significant role in the gender disparity observed in leukemia. Differences in the genetic makeup of males and females, particularly those related to gender chromosomes, may contribute to variations in leukemia risk. The gender chromosomes, X and Y, carry genes that are involved in various cellular processes, including cell growth, differentiation, and apoptosis. Alterations in these genes, or differences in their expression between males and females, could influence the likelihood of developing leukemia. For example, some genes located on the X chromosome are involved in immune regulation and tumor suppression. Females have two copies of the X chromosome, while males have only one. This difference in gene dosage could affect the expression of these genes and contribute to the observed gender disparity in leukemia. Furthermore, genetic mutations that increase the risk of leukemia may occur more frequently or have more severe

consequences in males compared to females. Differences in DNA repair mechanisms or other cellular processes could also contribute to variations in the impact of genetic mutations. Hormonal differences between males and females are another potential contributing factor to the gender disparity in leukemia. Gender hormones, such as androgens and estrogens, can influence various cellular processes, including cell proliferation, differentiation, and apoptosis. These hormones can also interact with the immune system and affect its function. Variations in hormone levels or hormone receptor expression between males and females could affect the susceptibility to leukemogenesis. Some studies have suggested that androgens may promote the development of leukemia, while estrogens may have a protective effect. However, the precise role of gender hormones in leukemia development is complex and requires further investigation. Environmental factors may also contribute to the gender disparity in leukemia, although their role is less clear. Exposure to certain environmental toxins, infections, or other triggers may differ between males and females, potentially influencing their risk of developing leukemia. Occupational exposures, lifestyle factors, and other environmental influences could also play a role. However, identifying specific environmental factors that contribute to the gender disparity in leukemia is challenging, and more research is needed in this area. The demographic findings of this study, specifically the age and gender distribution patterns, contribute to a more comprehensive understanding of the epidemiological characteristics of acute leukemia in the studied population. Recognizing these patterns has several important implications. Firstly, the age distribution pattern, with the highest prevalence in young children, reinforces the need for heightened clinical vigilance in this age group. Early recognition of leukemia in young children is crucial for timely diagnosis and initiation of treatment, which can significantly improve outcomes. Secondly, the gender disparity, with a higher prevalence in males, highlights the importance of considering gender as a potential risk factor for leukemia. Clinicians should be aware of the increased risk in males and consider this factor when evaluating patients with suspected leukemia. Thirdly, these demographic findings can aid clinicians in risk stratification. By recognizing the age and gender distribution patterns, clinicians can better assess an individual patient's risk of leukemia and tailor their diagnostic and management strategies accordingly. Fourthly, the findings can inform early detection efforts. Public health initiatives aimed at raising awareness about leukemia should consider the age and gender distribution patterns to target specific populations for education and screening. Fifthly, the observed disparities in age and gender distribution underscore the need for further research to elucidate the underlying biological mechanisms. Understanding the reasons why leukemia is more prevalent in young children and males could lead to the development of targeted prevention strategies and more effective treatments.[11-15]

The accurate diagnosis and classification of acute leukemia are of paramount importance for effective treatment planning and predicting patient outcomes. This process relies on a combination of diagnostic techniques, among which immunophenotyping and bone marrow aspiration (BMA) hold crucial roles. These methods provide complementary information about the leukemic cells and contribute to a comprehensive understanding of the disease. Immunophenotyping is a sophisticated technique that plays a pivotal role in the characterization of leukemic cells. It involves the detection and analysis of specific antigens expressed by these cells. These antigens, which are proteins or carbohydrates, are present on the cell surface, in the cytoplasm, or within the nucleus. The principle behind immunophenotyping lies in the use of monoclonal antibodies. These antibodies are highly specific, meaning each antibody is designed to bind to a particular antigen. In the laboratory, leukemic cells from a patient's sample are exposed to a panel of these antibodies. Each antibody is typically labeled with a fluorescent dye. If a cell expresses the antigen that a particular antibody is

designed to target, the antibody will bind to the cell, and the fluorescent dye will be detectable. The process of immunophenotyping often employs flow cytometry, a technology that allows for the rapid analysis of a large number of individual cells. In flow cytometry, cells stained with fluorescently labeled antibodies are passed through a laser beam. The instrument measures the fluorescence emitted by each cell, providing quantitative data on the presence and abundance of specific antigens. Immunophenotyping is instrumental in determining the lineage of leukemic cells. In acute leukemia, the leukemic cells are immature blood cells that have failed to differentiate normally. Immunophenotyping helps to identify the type of blood cell from which the leukemic cells originated. This is crucial because acute leukemia is broadly classified into two main types, acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). In ALL, the leukemic cells are lymphoid precursors, which are cells that normally develop into lymphocytes (a type of white blood cell involved in the immune response). Immunophenotyping in ALL typically involves the detection of antigens such as CD19, CD10, and TdT, which are commonly expressed by lymphoid cells at various stages of development. In AML, the leukemic cells are myeloid precursors, which are cells that normally develop into various types of myeloid cells, including granulocytes, monocytes, and red blood cells. Immunophenotyping in AML typically involves the detection of antigens such as CD33, CD13, and myeloperoxidase, which are commonly expressed by myeloid cells. Beyond simply distinguishing between ALL and AML, immunophenotyping also aids in further subclassification of leukemia into more specific subtypes. Within ALL, for example, there are different subtypes based on the stage of lymphoid cell development at which the leukemic cells are arrested. Similarly, AML has various subtypes based on the specific myeloid lineage involved and the presence of certain genetic abnormalities. Immunophenotyping plays a crucial role in identifying aberrant antigen expression. In some cases, leukemic cells may express antigens that are not normally expected for their lineage or stage of differentiation. This aberrant expression can have important implications for diagnosis, prognosis, and treatment. Immunophenotyping is also essential for the detection of minimal residual disease (MRD). MRD refers to the small number of leukemic cells that may remain in the body after treatment. Immunophenotyping can be used to detect these residual cells, even at very low levels, which is important for monitoring treatment response and predicting the risk of relapse. Bone marrow aspiration (BMA) is a fundamental procedure in the diagnosis and evaluation of hematologic disorders, including acute leukemia. It involves the collection of a small sample of bone marrow, the soft, spongy tissue found inside bones where blood cells are produced. The procedure for BMA typically involves inserting a needle into the bone marrow cavity, usually in the hip bone or sternum, and aspirating a small amount of marrow fluid. This fluid contains cells and tissue that can be examined in the laboratory. The aspirated bone marrow fluid is used to prepare smears, which are thin layers of cells spread on glass slides. These smears are stained with special dyes, such as Wright-Giemsa stain, which highlight the different components of the cells. A trained pathologist or hematologist then examines the stained smears under a microscope to assess the morphology of the cells. In acute leukemia, the morphological examination typically reveals an increased number of blast cells, which are immature, undifferentiated blood cells. The blasts crowd out the normal blood-forming cells in the bone marrow, leading to a decrease in the production of red blood cells, white blood cells, and platelets. The specific morphological features of the blast cells can provide clues to the type of leukemia. For example, in ALL, the blasts may appear smaller and have less cytoplasm compared to the blasts in AML. In addition to morphological examination, cytochemical stains may be applied to the bone marrow smears. These stains react with specific enzymes or other substances within the cells, providing further information about their lineage and

characteristics. Cytochemical stains can be particularly useful in distinguishing between ALL and AML. For example, myeloperoxidase is an enzyme found in myeloid cells but not in lymphoid cells. A positive reaction for myeloperoxidase is indicative of AML, while a negative reaction supports the diagnosis of ALL. Other cytochemical stains, such as Sudan black B and periodic acid-Schiff (PAS) stain, can also be used to aid in leukemia classification. BMA allows for the evaluation of bone marrow cellularity, which refers to the proportion of the bone marrow space occupied by blood-forming cells. In acute leukemia, the bone marrow is typically hypercellular, meaning it is packed with an excessive number of cells, primarily leukemic blasts. This hypercellularity disrupts the normal bone marrow architecture and function. BMA is crucial for assessing the overall bone marrow environment. It provides information about the relative proportions of different cell types, the presence of any abnormal cells, and the degree of bone marrow involvement by the leukemic process. Immunophenotyping and BMA are not mutually exclusive, rather, they play complementary roles in the diagnosis and classification of acute leukemia. Each method provides unique and valuable information, and their combined use enhances the accuracy and comprehensiveness of the diagnostic evaluation. Immunophenotyping provides detailed information about the immunophenotype of leukemic cells, allowing for precise lineage determination and subclassification. It is highly sensitive and specific for identifying leukemic cell populations and detecting minimal residual disease. BMA, on the other hand, provides a comprehensive assessment of the bone marrow environment, including cellular morphology, cytochemistry, and cellularity. It allows for the visualization and characterization of leukemic cells, as well as the evaluation of the overall impact of the disease on bone marrow function. The integration of immunophenotyping and BMA results is essential for a definitive diagnosis and accurate classification of acute leukemia. In most cases, the results of these two methods are concordant, meaning they agree with each other. However, in some instances, discrepancies may arise, highlighting the importance of considering both methods in the diagnostic process. In this study, both immunophenotyping and BMA demonstrated a high degree of accuracy in classifying acute leukemia. Immunophenotyping identified 52.2% of cases as ALL and 47.8% as AML. BMA classified 56.5% of cases as ALL and 43.5% as AML. These results indicate that both methods were effective in distinguishing between the two major types of acute leukemia. The proportions of ALL and AML cases identified by each method were quite similar, suggesting a high level of agreement between the two techniques. The slight differences observed in the proportions of ALL and AML cases identified by each method may be attributed to several factors. Immunophenotyping and BMA are different techniques that rely on different principles. Immunophenotyping detects specific antigens, while BMA assesses cellular morphology and cytochemistry. These inherent differences can lead to slight variations in the results, particularly in cases where leukemic cells exhibit overlapping features or atypical characteristics. In some cases, leukemic cells may express antigens or exhibit morphological features that are characteristic of both ALL and AML. This can create diagnostic challenges and lead to discrepancies between immunophenotyping and BMA results. The interpretation of BMA results, particularly morphological assessment, can be subjective and influenced by inter-observer variability. Different pathologists or hematologists may have slightly different interpretations of the same sample, which can contribute to variations in the results. Despite these slight differences, the overall agreement between immunophenotyping and BMA in this study was high, indicating that both methods are valuable tools for leukemia classification.[16-20]

5. Conclusion

This study demonstrates a high level of concordance between immunophenotyping and bone marrow aspiration (BMA) in the diagnosis and classification of acute leukemia in children. Both

methods effectively distinguished between acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML), with a strong agreement observed in the majority of cases. The study also highlights the importance of considering age and gender distribution in the epidemiology of acute leukemia. The highest proportion of cases was observed in children aged ≤4 years, aligning with the peak incidence of ALL in early childhood. Additionally, a clear male predominance was evident, consistent with established epidemiological trends. These demographic patterns underscore the need for heightened clinical vigilance in young children, particularly males, presenting with suggestive signs and symptoms. While both immunophenotyping and BMA proved to be reliable diagnostic tools, the study acknowledges the potential for discrepancies. In this study, a small number of cases showed discordant results, emphasizing the importance of integrating data from both methods for accurate diagnosis and classification. Such an approach is crucial for optimizing treatment strategies and improving outcomes in children with acute leukemia.

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