International Journal of Medical and Pharmaceutical Research

Online ISSN-2958-3683 | Print ISSN-2958-3675
Frequency: Bi-Monthly
Available online on: https://ijmpr.in/

Original Article

Immunophenotypic Profile of Acute Leukemia- Insights From A Tertiary Care Center

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OPEN ACCESS

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Received: 15-11-2025
Accepted: 20-12-2025
Available online: 31-12-2025

ABSTRACT

Background: Acute leukemia represents a diverse category of malignancies, each characterized by distinct clinical presentations, morphologic features, immunologic profiles and molecular traits. Flow cytometric immunophenotyping analysis remains the main modality in diagnosis, subtyping and lineage determination of acute leukemia. It also helps to identify mixed phenotype acute leukemia, evaluation of minimal residual disease and prognosis assessment. ***Objectives:*** To assess the morphological and immunophenotypic profiles of acute leukemia and assess the incidence of its various subtypes. To determine the frequency of cross-lineage antigen expression in cases of acute leukemia. ***Methods:*** A retrospective study was conducted over a period of two years in a Medical College Hospital on all consecutively diagnosed cases of acute leukemia by flow cytometry. These cases were reviewed and analyzed. ***Results:*** During the study period, 33 cases were diagnosed as acute leukemia. Among 33 cases, 57.5 % (19) were acute myeloid leukemia (AML) and 42.4% (14) were acute lymphoblastic leukemia (ALL). In AML cases, 15.7% showed aberrant lymphoid expression. Majority of the cases showed CD 7 expression. Out of 14 cases of ALL, 10(71.4%) were B-ALL and 4(28.5%) were T-ALL. In ALL, CD 13 was the most predominant aberrant myeloid antigen followed by CD 33 and CD117. ***Conclusion:*** Flow cytometric immunophenotyping is a rapid and accurate method for diagnosing and subtyping acute leukemia. 57.5% of our cases were Acute myeloid leukemia with AML-M3, being the most predominant subtype. Aberrant lymphoid expression was noted in 15.7 % of our cases of AML with CD 7 expression being more predominant. B-ALL was the most common subtype of ALL in our study with CD31 expression seen as aberrant myeloid antigen in ALL.

Keywords: *Acute leukemia, Immunophenotyping, Flow cytometry, Acute lymphoblastic leukemia, Acute myeloid leukemia, Aberrant phenotype.*

INTRODUCTION

Acute leukemia (AL) is a clonal hematopoietic stem cell disorder showing an increase in immature cells (≥20%) in peripheral blood and/or bone marrow with varying clinical, morphologic, immunologic and molecular characteristics.[1,2] The initial widely recognized classification was French–American–British Cooperative Group Classification (FAB), which relied solely on morphological criteria. It was later updated in 1981 and 1985. These revisions did not include the immunophenotypic characteristics typically observed in acute leukemia (AL). In 2008, the World Health Organization (WHO) introduced a more thorough classification system for AL, which encompassed morphology, cytochemistry, immunophenotyping, fluorescence in situ hybridization (FISH) and reverse transcriptase polymerase chain reaction (RT-PCR). [2,3,4] Flow cytometric immunophenotyping is an essential method used for diagnosing, classifying, and monitoring the progression of acute leukemia. It also helps in detection of mixed phenotype acute leukemia, evaluation of minimal

residual disease and provides valuable prognostic and predictive information, helping to tailor treatment strategies.[3,4,5,6,7] Leukemic blasts with specific genetic markers exhibit distinctive immunophenotypic patterns on flow cytometry.[2,3,5] Cells of the myeloid lineage typically express antigens like CD13, CD15, CD33, and myeloperoxidase (MPO). These markers can be used to identify myeloid differentiation. [1,2,8] Few cases of AML can also show erythroid lineage differentiation. These cells express CD36, CD71, CD117, and CD235a.[1,8,9,10] AML can also present with differentiation into megakaryocytic lineage, with blasts expressing CD41 and/or CD61.These markers are typically found on the surface and cytoplasm of megakaryocytes, which are involved in platelet production.[1,10] In most cases of B-lymphoblastic leukemia, there is strong expression of various B-lineage markers, such as CD19, CD22, and CD79a.[1,10,11] CD3 is widely regarded as one of the most reliable markers for identifying T-cell differentiation, with its presence either on the cell surface or within the cytoplasm indicating this lineage. Moderate to strong expression of CD3 is typically observed in T-cells.[1,10,12] Additionally, other markers associated with T-cell lineage, such as CD1a, CD2, CD4, CD5, CD7 and CD8, are expressed to varying degrees depending on the specific T-cell subtype.[1,12,13]

The World Health Organization provides specific criteria for diagnosing Mixed Phenotype Acute Leukemia in which, myeloid lineage is identified by myeloperoxidase (MPO) staining/ the expression of two or more monocytic antigens (CD11c, CD14, CD64, nonspecific esterase, lysosome). T-cell lineage is marked by strong cytoplasmic or surface CD3 expression, while B-cell lineage is characterized by strong CD19 expression coupled with weak expression of CD10, CD22, or CD79a, or by weak CD19 expression along with strong expression of at least two of these markers. [1,2,14,15]

In acute leukemia, patterns of antigen expression on cancerous cells that differ from the normal process of hematopoietic maturation are called aberrant phenotypes.[8,16] In acute lymphoblastic leukemia (ALL), these abnormalities include cross-lineage antigen expression, such as the presence of myeloid markers in ALL, B-cell markers in T-ALL, or T-cell markers in B-ALL.[1,8,10] Immunophenotyping plays a crucial role in identifying these atypical phenotypes. Recognizing these aberrant patterns is essential for understanding their incidence, clinical significance prognostic value and aids in detecting minimal residual disease during follow-up. [1,17,18]

Distinct immunophenotypes have been linked to recurrent genetic abnormalities. In acute myeloid leukemia (AML), specific antigens are associated with certain morphological FAB subtypes and are connected to the presence of these genetic abnormalities. For example, AML with the t(8;21) translocation exhibits abnormal expression of lymphoid markers such as CD19 and cCD79a, along with CD56, which is associated with a poor prognosis.[1,17,19] CD56 expression in acute myeloid leukemia (AML) has been linked to a poor prognosis.[1,19,20] Moreover, CD7 is often abnormally expressed in AML and has been linked to poor prognosis in the majority of studies. In acute lymphoblastic leukemia (ALL), a worse prognosis has been observed in cases that are positive for myeloid antigens compared to those that are negative for these antigens.[1]

Aims and objectives: The objective of this study is to examine the morphological and immunophenotypic profiles of acute leukemia and assess the incidence of its various subtypes. Additionally, this research aims to determine the frequency of cross-lineage antigen expression in acute leukemia cases.

MATERIAL AND METHODS

A retrospective study was conducted over a two-year period in the Department of Pathology, where all consecutively diagnosed cases of acute leukemia, identified through flow cytometry, were retrieved and analyzed. The demographic details and clinical diagnosis of the patients were recorded. The diagnosis of acute leukemia was established through peripheral blood counts, peripheral smear analysis, bone marrow aspiration, cytochemical testing and immunophenotyping using flow cytometry. Peripheral blood smears and aspirates of bone marrow aspirates were stained with Giemsa stain. EDTA was the anticoagulant used for flow cytometry samples. The case distribution of subtypes of acute leukemia were analyzed. Additionally, AML cases were classified according to FAB classification and aberrant expression of markers in myeloid and lymphoid leukemias were studied.

RESULTS

Over a two-year period, 33 individuals were diagnosed with acute leukemia. Among them, 24 were adults and 9 were children, with a male to female ratio of 1.7:1 (21 males and 12 females). Of the 33 acute leukemia cases, 19 (57.5%) cases were of acute myeloid leukemia (AML), while 14 (42.5%) cases were of acute lymphoblastic leukemia (ALL).

Acute myeloid leukemia (AML) represented 57.5% (19 cases) of the acute leukemia cases in this study. Of the 19 AML cases, 10 were classified as AML-M3, 6 as AML-M5, 2 as AML-M1, and 1 as AML-M0. The most common FAB subtype of AML was AML-M3 (52.8%), followed by AML-M5 (31.5%), AML-M1 (10.5) and AML-M0 (5.2%) respectively.

Cross lineage antigen expression of lymphoid antigens in AML was observed in 3 cases (15.7%) of AML. The expressed lymphoid antigen was CD7 in two cases and CD5 in one case. In terms of genetic abnormalities, a chromosomal translocation t(8;21) and the resulting oncogenic fusion gene AML1-ETO were identified in one case(5.2%).

Of the 14 cases of acute lymphoblastic leukemia (ALL), 10 cases (71.5%) were diagnosed as B-cell acute lymphoblastic leukemia (B-ALL). CALLA positivity was observed in 9 of these B-ALL cases (90%).

In B- ALL, expression of myeloid antigens was seen in 6 cases (60%). Aberrant expression of CD13 was found in four cases, CD33 in one case and CD117 in one case respectively. Among B-ALL cases exhibiting cross-lineage antigen expression, CD13 was the most frequently aberrant marker, observed in 66.6% of cases, followed by CD33 and CD117, each detected in 16.6% of cases.

Four cases (28.5%) of ALL were classified as T-ALL, with only one case (25%) showing CALLA positivity. Aberrant antigens expressed in T-ALL included CD13 and CD117, which was seen in 2 (50%) cases. CD13 expression was observed in one case, while CD117 expression was seen in another case.

DISCUSSION

Flow cytometric immunophenotyping is a highly sensitive, specific technique that plays a crucial role in the diagnostic evaluation and sub-classification of acute leukemia (AL).It helps in identifying B-cell and T-cell lineages, assessing treatment response, detecting early responders, and identifying minimal residual disease (MRD).[1,2,3,4] Additionally, flow cytometry is valuable in recognizing megakaryocytic differentiation through the expression of CD41 and CD61, as well as in identifying pure erythroid leukemia through CD235a (glycophorin A) expression.[1,2]

The overall patient demographic profile and the frequency distribution of various acute leukemia subtypes in our study were consistent with those reported in various literature. [1,3,8,9] Acute myeloid leukemia (AML) represented 57.5% (19 cases) of the acute leukemia cases in our study and was the most common type of leukemia and ALL accounted to about 42.5% of the cases. This finding was concordant with studies done by many including Simi C M et al who observed 53% of AML and 45% of ALL cases respectively.[1,21,22,23] However in the study done by Gupta M et al showed that out 631 cases, 52.9% (n=334) were ALL , 43.9% (n=277) AML, 2.2% (n=14) MPAL, 0.5% (n=3) acute undifferentiated leukemia (AUL) and 0.5% (n=3) chronic myelogenous in blast crisis (CML-BC) respectively.[3] Koju S et al study also showed that, out of 408 cases of acute leukemia, 255 were ALL and 153 was AML.[23] AML is more commonly observed in adults, whereas ALL is more prevalent in children. Our study also reflected this age-related distribution of acute leukemia comparable with studies done by Simi C M et al and Gupta M et al.[1, 3]

In our study, among the acute myeloid leukemia (AML) cases 52.6% were classified as Acute Promyelocytic Leukemia (APML), while the remaining 47.3% were non-APML. Study by Simi C M et al, showed that out of 270 cases of AML, 5 % cases were APML while remaining 95% were non APML.[1] The most common subtype of AML in our series was AML-M3, which accounted for 52.6% which is higher than the reported frequency of 5-14% in other studies.[1,21,22] Whereas in the study done by Simi C M et al showed commonest AML subtype was AML M4 that accounted for 32%.[1] AML-M5 constituted 31.5% in our study, which is consistent with findings seen in study by Simi C M et al who found 27% of AML-M5. [1] Koju S et al. reported an incidence of 2-9% of AML-M5. [23] AML-M1 was observed in 10.5% of cases, which was consistent with results found in studies by Simi C M et al, Ghosh S et al., and Koju S et al.[1.21,23] Finally, AML-M0 was present in 5.2% of our cases, which was similar with findings seen in Simi C M et al with 4.4% and was also aligning with the incidence (<6%) reported in the literature.[1,8,9,14,15]

In our study 15.7% of our AML cases showed lymphoid antigen expression which is comparable with other studies.[1,23] CD7 was the commonly expressed lymphoid antigen followed by CD5 in our study, similar observation was also seen in studies done by Simi C M et al and Koju S et al who also showed that CD7 was the most frequently expressed lymphoid marker, followed by CD5 and CD19.[1,23]

AML with the ETO translocation t(8;21), typically associated with a poor prognosis and was observed in one case in our study, aligning with the observations made by Gert J et al.[17] AML with chromosomal translocation t(8;21) and the resulting oncogenic fusion gene AML1-ETO, exhibits aberrant expression of lymphoid markers such as CD19 and cCD79a, as well as CD56.[3,8,24,25] In AML with inv(16) or t(16;16), co-expression of CD2 is frequently observed.[3,26,27] The study by Gujral S et al. demonstrated that CD19 aberrancy was associated with the t(8;21)(q22;q22) translocation in 45% of cases.[20]

In our study the predominant immunophenotype observed in ALL was B-ALL (71.5%) whereas T-ALL comprised about 28.5%. Similar observation was also seen in study done by Gupta M et al who found 81.7% of B -cell ALL and 18.3% of T-cell ALL.[3] This finding was also consistent with the studies by Onciu M et al. and Simi C M et al., who reported B-ALL accounting for 60-80% of cases, while T-ALL made up only 15-20%. [1,28]

CD10, also known as the common acute lymphoblastic leukemia antigen (CALLA), when absent in B-ALL, especially in Pro B-ALL cases are considered a significant marker of poor prognosis. It is frequently linked to MLL rearrangements and is associated with significantly poorer overall survival (OS), event-free survival (EFS), and lower rates of complete

remission (CR).[3] In our study, 90% (9/10) of the cases showed CD10 positivity and 10% (1/10) of B-ALL cases exhibited CD10 negativity. Similar observation was also seen in studies done by Gupta M et al who showed 4.8% of B-ALL cases which exhibited CD10 negativity.[3]

Studies have reported that the expression of aberrant myeloid antigens (MyAg) in B-ALL varies with reported frequencies ranging from 4.3% to 64%.[3,27,28] No significant difference has been observed in the achievement of complete remission (CR) or overall survival (OS) between the MyAg+ and MyAg− groups.[3] In our study, myeloid antigen expression in B-ALL was observed in 6 cases (60%). Among these, CD13 was the most commonly aberrantly expressed marker, present in 66.6% of cases, followed by CD33 and CD117, each detected in 16.6% of the cases with cross-lineage antigen expression. Similar observation was also seen in study by Gupta M et al who found CD13 was the commonest aberrantly expressed marker seen in 25.6% (n=70/273) cases followed by CD33 in 17.9% (n=49/273) cases. [3]

T-lineage ALL is diagnosed based on the presence of cytoplasmic CD3 in leukemic blasts.[3] Our study showed 28.5% of T-ALL, but studies by Simi C M et al showed 15-20% and Gupta M et all showed 9.6% of T-ALL cases.[3] However incidence of T-ALL in our centre was higher than other studies.[3,8,9] In our study, 25% of the T-ALL cases showed CALLA positivity whereas study by Gupta M et al showed that CD10 was expressed in 37.7% (n=23/61) cases.[3] CD13 and CD117 were expressed aberrant myeloid antigen in T-ALL, which was seen in one case each in our study, similar findings were also observed by other studies and Simi C M et al who found CD13 was the most commonly expressed aberrant myeloid antigen, followed by CD117[1,2]. Gupta M et al study also showed that CD13 was aberrantly expressed in 32.7% (n=20/61) cases and CD117 in 19.1% (n=9/47) cases.[3]

CONCLUSION

Flow cytometric immunophenotyping is a rapid and reliable method for the accurate diagnosis and subclassification of acute leukemia. In our study we documented the spectrum of leukemia, various subtypes and the predominant immunophenotype observed. AML comprised 57.5% of all acute leukemia, with AML-M3 being the most common subtype. Aberrant lymphoid expression was observed in 15.7% of AML cases, with CD7 being the most frequently expressed marker. In ALL, B-ALL was the predominant immunophenotype. Additionally, CD13 was the most common aberrant myeloid antigen observed in ALL.

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