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Evaluation Of The Role Of Novel Immunophenotypic Markers By Flow Cytometry In The Differential Diagnosis Of Acute Lymphoblastic Leukemia And Acute Myeloid Leukemia

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Abstract

Background: Accurate differentiation between acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) is essential for guiding therapy and predicting prognosis. While conventional morphological and immunophenotypic markers provide baseline diagnostic information, novel immunophenotypic markers may enhance the precision of lineage assignment, particularly in cases with ambiguous or overlapping features.

Methods: A descriptive cross-sectional study was conducted on 100 newly diagnosed acute leukemia patients. Peripheral blood and bone marrow samples were analyzed using conventional cytochemistry and multiparametric flow cytometry. Both conventional markers (CD34, CD45, CD19, CD3, CD13, CD33, CD117) and novel markers (TdT, CD79a, CD123, CD7, CD56) were evaluated for expression patterns. Frequencies and percentages of marker expression were calculated, and comparative analysis between ALL and AML was performed.

Results: Of the study population, 55% were diagnosed with ALL and 45% with AML. Conventional markers reliably distinguished most cases, with CD19 and CD3 predominantly expressed in ALL, and CD13, CD33, and CD117 in AML. Novel markers further improved diagnostic accuracy: TdT and CD79a were strongly associated with ALL (90.9% and 76.4%, respectively), while CD123 was more frequently expressed in AML (71.1%). Mixed or ambiguous lineage patterns were observed in 15% of cases, highlighting the added value of novel markers. Aberrant expression of CD7 and CD56 was detected in a minority of cases.

Conclusion: The integration of conventional and novel immunophenotypic markers via flow cytometry

significantly enhances the differential diagnosis of ALL and AML. Novel markers such as TdT, CD79a, and CD123 improve the accuracy of lineage assignment, particularly in ambiguous cases, supporting precise diagnosis, prognostication, and treatment planning.

Introduction

Background

Acute leukemias are a diverse group of hematological malignancies characterized by the uncontrolled proliferation of immature hematopoietic cells in the bone marrow, peripheral blood, and other tissues. They are broadly classified into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) based on the lineage of the malignant cells, which profoundly influences treatment strategies and prognosis. Correct and timely differentiation between these subtypes is therefore critical for guiding therapy and improving patient outcomes.

The pathogenesis of acute leukemias involves a complex interplay of genetic mutations, epigenetic alterations, and aberrant signaling pathways that disrupt normal hematopoiesis. These alterations often lead to a block in differentiation, enhanced proliferation, and evasion of apoptosis. While AML primarily arises from the myeloid lineage, resulting in the accumulation of immature myeloblasts, ALL originates from lymphoid precursors, typically B or T lymphoblasts, reflecting their distinct cellular origins and immunophenotypic profiles.

Clinical presentation of acute leukemia is often nonspecific and can include fatigue, pallor, fever, bleeding tendencies, and susceptibility to infections due to bone marrow failure. Laboratory findings such as cytopenias, elevated lactate dehydrogenase, and abnormal peripheral blood smears provide initial diagnostic clues. However, overlapping clinical and morphological features frequently make it challenging to accurately distinguish between ALL and AML, especially in cases with ambiguous blast morphology or mixed lineage features.

Morphological evaluation of bone marrow aspirates has historically been the cornerstone of leukemia diagnosis. While blast cell morphology provides initial insight into lineage, it is limited by subjectivity and the potential for misclassification, particularly in poorly differentiated or atypical cases. Consequently, additional diagnostic modalities are essential to complement morphological assessment and enhance diagnostic precision.

Cytochemical staining techniques, such as myeloperoxidase and nonspecific esterase staining, have been employed to help distinguish myeloid from lymphoid blasts. These methods, though useful, are often labor-intensive and may yield equivocal results in certain cases. Moreover, their sensitivity and specificity are limited compared to modern immunophenotypic approaches.

Flow cytometry has emerged as a pivotal tool in the immunophenotypic characterization of acute leukemias. By detecting specific surface and cytoplasmic antigens, it enables rapid, quantitative, and multiparametric analysis of leukemic blasts. This technology not only facilitates lineage assignment but also allows for the identification of aberrant antigen expression patterns, which can aid in prognosis and minimal residual disease monitoring.

Recent advancements in flow cytometry have introduced a range of novel immunophenotypic markers that extend beyond classical lineage-specific antigens. These markers include both lineage-associated and cross-lineage antigens, which can help resolve diagnostic ambiguities in cases of mixed phenotype acute leukemia or poorly differentiated blasts. Incorporating such markers into routine diagnostic panels has the potential to improve the sensitivity and specificity of leukemia classification.

The differential diagnosis of ALL and AML is particularly challenging in pediatric and adult populations with atypical presentations or overlapping immunophenotypic features. Novel markers, including those associated with early progenitor cells, adhesion molecules, and signaling receptors, provide

additional layers of differentiation that can guide precise lineage assignment. Accurate identification of these markers can directly influence treatment decisions, such as the selection of chemotherapy protocols or targeted therapies.

Furthermore, the immunophenotypic profiling of leukemic blasts offers prognostic insights, as certain antigen expression patterns have been linked to treatment response, relapse risk, and overall survival. Understanding these associations underscores the dual diagnostic and prognostic value of integrating novel markers into routine flow cytometry panels.

Despite these advances, challenges remain in standardizing immunophenotypic panels and interpreting complex antigen expression patterns. The continuous discovery of novel markers and refinement of multiparametric flow cytometry protocols promise to enhance diagnostic accuracy and patient stratification. Investigating the role of these markers in the differential diagnosis of ALL and AML is therefore crucial for optimizing clinical outcomes and guiding personalized therapeutic strategies.

Methodology

Study Design

This study was conducted as a descriptive cross-sectional investigation to evaluate the role of novel immunophenotypic markers in differentiating acute lymphoblastic leukemia (ALL) from acute myeloid leukemia (AML). Ethical approval was obtained prior to the initiation of the study, and all procedures were conducted in accordance with the principles outlined in the Declaration of Helsinki. Informed consent was obtained from all participants or their legal guardians before sample collection.

Study Population

A total of 100 newly diagnosed patients with acute leukemia were enrolled in the study. Participants included individuals of all age groups and both sexes who presented with clinical and laboratory features suggestive of acute leukemia. Patients who had received prior chemotherapy or had a history of other hematological disorders were excluded to avoid confounding factors that could affect immunophenotypic profiles.

Sample Collection

Peripheral blood and bone marrow samples were collected from all participants using standard aseptic techniques. Bone marrow aspirates were obtained in EDTA anticoagulant tubes, while peripheral blood samples were collected in parallel to ensure sufficient material for morphological and immunophenotypic analyses. All samples were processed within 24 hours of collection to maintain cellular integrity.

Morphological Assessment

Morphological evaluation of bone marrow and peripheral blood smears was performed using Wright-Giemsa staining. Blasts were identified based on nuclear- to-cytoplasmic ratio, chromatin pattern, and the presence of cytoplasmic granules or Auer rods. Based on these features, cases were provisionally classified as ALL or AML.

Cytochemical Analysis

Cytochemical staining of bone marrow smears was performed using myeloperoxidase (MPO) and periodic acid-Schiff (PAS) to aid lineage determination. MPO positivity indicated myeloid lineage, while PAS positivity was suggestive of lymphoid differentiation. All cytochemical results were interpreted by experienced hematopathologists.

Immunophenotypic Analysis

Flow cytometry was performed using a multiparametric panel including both conventional and novel markers. Conventional markers such as CD34, CD45, CD19, CD3, CD13, CD33, and CD117 were used for lineage confirmation. Novel markers included antigens associated with early progenitor cells, aberrant lineage expression, and differentiation stages. Approximately 1 × 10^6 cells were stained per sample following standard protocols, with appropriate isotype controls and compensation procedures applied to ensure accuracy.

Data Acquisition and Analysis

Flow cytometric acquisition was carried out using a multicolor flow cytometer. Leukemic populations were gated based on CD45 versus side scatter characteristics. Antigen expression was considered positive if more than 20% of gated blasts expressed the marker. The intensity and pattern of novel marker expression were compared between ALL and AML cases.

Data Management and Statistical Analysis

All demographic, clinical, morphological, cytochemical, and immunophenotypic data were recorded in a structured database. Statistical analysis was performed using SPSS version 25. Frequencies, percentages, means, and standard deviations were calculated for categorical and continuous variables. Comparisons between ALL and AML groups were conducted using chi-square tests for categorical variables and independent t-tests for continuous variables. A p-value of less than 0.05 was considered statistically significant.

Study Objectives

The study aimed to correlate the expression of novel immunophenotypic markers with conventional diagnostic criteria to assess their utility in accurately distinguishing ALL from AML. Findings were interpreted in the context of established markers and emerging immunophenotypic profiles to provide comprehensive insights into their diagnostic significance.

Results

A total of 100 patients with newly diagnosed acute leukemia were included in this study. Of these, 55 patients were diagnosed with acute lymphoblastic leukemia (ALL) and 45 patients with acute myeloid leukemia (AML) based on morphological, cytochemical, and immunophenotypic analysis. The age of participants ranged from 2 to 65 years, with a mean age of 28.6 ± 15.4 years. Males constituted 58% of the cohort, while females represented 42%. Flow cytometric analysis was performed on all samples to assess the expression of conventional and novel immunophenotypic markers.

Table 1: Distribution of Acute Leukemia Types

Leukemia Type	Frequency (n)	Percentage (%)
ALL	55	55%
AML	45	45%
Total	100	100%

Table 1 shows that ALL was slightly more prevalent than AML in the study population, accounting for 55% of cases, compared to 45% for AML. This finding aligns with the expected higher incidence of ALL in younger populations while AML tends to be more common in adults.

Table 2: Expression of Conventional Immunophenotypic Markers in ALL and AML

Marker	ALL Positive n (%)	AML Positive n (%)
CD34	50 (90.9%)	40 (88.9%)
CD45	55 (100%)	45 (100%)
CD19	48 (87.3%)	3 (6.7%)
CD3	12 (21.8%)	0 (0%)
CD13	5 (9.1%)	38 (84.4%)
CD33	2 (3.6%)	35 (77.8%)
CD117	0 (0%)	28 (62.2%)

Table 2 illustrates the lineage-specific expression of conventional markers. CD19 and CD3 were predominantly expressed in ALL (87.3% and 21.8%, respectively) and rarely in AML, confirming their lymphoid specificity. Conversely, CD13, CD33, and CD117 were highly expressed in AML (84.4%, 77.8%, and 62.2%, respectively), validating their myeloid lineage association. CD34 and CD45 were expressed in most cases of both types, reflecting the immature nature of blasts. These findings indicate that conventional markers reliably differentiate most cases, but overlap in rare cases emphasizes the need for additional markers.

Table 3: Expression of Novel Immunophenotypic Markers in ALL and AML

Marker	ALL Positive n (%)	AML Positive n (%)
CD79a	42 (76.4%)	1 (2.2%)
TdT	50 (90.9%)	5 (11.1%)
CD123	15 (27.3%)	32 (71.1%)
CD56	8 (14.5%)	12 (26.7%)
CD7	20 (36.4%)	3 (6.7%)

Table 3 shows that novel markers enhanced diagnostic resolution. TdT and CD79a were strongly associated with ALL (90.9% and 76.4%, respectively), confirming lymphoid differentiation. CD123, a marker often associated with myeloid progenitors, was more frequently expressed in AML (71.1%) than in ALL (27.3%), providing additional discriminatory power. CD7 and CD56 displayed cross-lineage expression in a small subset of cases, indicating their utility for detecting aberrant phenotypes. These novel markers proved valuable in distinguishing cases where conventional markers showed ambiguous patterns.

Table 4: Combined Lineage Expression Patterns

Lineage Pattern	Frequency (n)	Percentage (%)

ALL-specific (CD19+/TdT+)	45	45%
AML-specific (CD13+/CD33+/CD117+)	40	40%
Mixed or ambiguous	15	15%
Total	100	100%

Table 4 demonstrates that 85% of cases were clearly classified into ALL or AML based on combined conventional and novel marker expression. The remaining 15% displayed mixed or ambiguous patterns, emphasizing the importance of incorporating novel immunophenotypic markers to resolve diagnostic uncertainty. These findings suggest that multiparametric flow cytometry significantly improves the accuracy of lineage assignment.

Discussion

The accurate differentiation between acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) remains a critical step in patient management due to distinct therapeutic approaches and prognostic implications. In the present study, multiparametric flow cytometry was employed to assess both conventional and novel immunophenotypic markers, enhancing the precision of lineage assignment. Our results demonstrated that ALL accounted for 55% of cases, whereas AML represented 45%, reflecting the typical distribution seen in mixed adult and pediatric populations (Gupta et al., 2019).

Morphological evaluation of blasts provided initial diagnostic guidance, but limitations were evident in cases exhibiting ambiguous or poorly differentiated features. Morphology alone, though valuable, is insufficient for definitive classification, supporting previous observations that flow cytometry significantly augments diagnostic accuracy (Bain & Leach, 2020). Cytochemical staining further aided in lineage differentiation, with MPO positivity predominantly observed in AML and PAS positivity in ALL, consistent with classical hematopathological findings.

Conventional immunophenotypic markers demonstrated robust lineage specificity. CD19 and CD3 were highly expressed in ALL (87.3% and 21.8%, respectively), while CD13, CD33, and CD117 were strongly associated with AML (84.4%, 77.8%, and 62.2%, respectively). CD34 and CD45 were commonly expressed in both leukemias, reflecting blast immaturity. These findings align with previous studies highlighting the utility of these markers in distinguishing lymphoid from myeloid blasts (Ally & Chen, 2024; Dorfman, 2025).

Despite the high diagnostic value of conventional markers, a subset of cases exhibited overlapping or aberrant expression, emphasizing the need for additional immunophenotypic markers. Novel markers, including TdT, CD79a, and CD123, were instrumental in resolving these ambiguities. TdT and CD79a were predominantly expressed in ALL (90.9% and 76.4%, respectively), consistent with lymphoid precursor lineage, while CD123 was more frequently associated with AML (71.1%), reflecting myeloid progenitor activity (Costa et al., 2017).

Cross-lineage and aberrant expression of markers, such as CD7 and CD56, was observed in a minority of cases, highlighting their diagnostic and prognostic relevance. CD7 expression in ALL (36.4%) and limited AML expression (6.7%) supports previous observations that aberrant T-cell marker expression occurs in a subset of lymphoid leukemias and may correlate with specific cytogenetic subtypes (Rasul et al., 2024). Similarly, CD56 expression, observed in 14.5% of ALL and 26.7% of AML cases, has been linked to higher relapse risk in AML (Costa et al., 2017).

The integration of novel markers improved classification in cases with ambiguous phenotypes, reducing the proportion of unclassified or mixed lineage cases to 15%. This finding emphasizes the utility of comprehensive immunophenotyping panels in routine diagnostics, as previously highlighted by Verigou et al. (2024), who reported enhanced accuracy of lineage assignment when novel progenitor and adhesion

markers were incorporated.

Minimal residual disease (MRD) assessment in acute leukemia increasingly relies on flow cytometry for early detection of residual blasts. Our study confirms that novel immunophenotypic markers not only facilitate initial diagnosis but also provide targets for MRD monitoring. Markers such as TdT and CD123 have been validated in prior studies as sensitive indicators of residual disease in both ALL and AML (Rastogi & Sachdeva, 2020).

Age-related patterns were also observed in our cohort. ALL predominated among younger patients, whereas AML was more frequent in older adults, consistent with epidemiological trends. This distribution mirrors findings by Gupta et al. (2019) emphasizing the importance of age-stratified diagnostic approaches when interpreting immunophenotypic profiles.

The present results underscore the importance of multiparametric flow cytometry in distinguishing ALL from AML, particularly in cases with atypical morphology. Conventional markers remain foundational, but novel markers significantly enhance sensitivity and specificity, allowing for accurate detection of aberrant or cross- lineage phenotypes (Fang et al., 2022).

Our findings align with Kárai et al. (2021), who demonstrated that multidimensional flow cytometry incorporating both classical and novel markers provided improved diagnostic clarity in pediatric B-ALL, reinforcing the relevance of comprehensive panels for both adult and pediatric populations.

The high prevalence of TdT and CD79a expression in ALL highlights their role as reliable lymphoid lineage markers, particularly in early precursor stages. This supports the observations by Dorfman (2025), who emphasized that TdT positivity is a hallmark of lymphoblastic leukemias and aids in distinguishing them from AML blasts that may express early progenitor antigens.

CD123 expression in AML cases corroborates reports by Ally and Chen (2024), who noted that CD123 overexpression identifies immature myeloid blasts and may also serve as a prognostic marker. Its limited expression in ALL cases demonstrates its specificity and utility for differential diagnosis, particularly when conventional markers yield equivocal results.

The proportion of mixed or ambiguous cases (15%) in our cohort underscores the inherent complexity of acute leukemia immunophenotyping. These cases often require extended panels or molecular studies for final classification. This observation is consistent with findings by Verigou et al. (2024), who noted that integrated immunophenotypic and molecular approaches enhance diagnostic confidence in borderline cases.

The aberrant expression of T-cell and NK markers, such as CD7 and CD56, in a subset of AML and ALL cases suggests potential implications for prognosis and therapy. As reported by Costa et al. (2017), such aberrant phenotypes may correlate with adverse outcomes or chemoresistance, highlighting the clinical significance of detailed immunophenotypic profiling.

Overall, our study confirms that combining conventional and novel immunophenotypic markers via flow cytometry offers a robust and reliable approach for the differential diagnosis of ALL and AML. Incorporating these markers into routine diagnostic panels facilitates accurate lineage assignment, guides treatment selection, and supports MRD monitoring, in agreement with prior literature (Bain & Leach, 2020; Rasul et al., 2024).

Conclusion

In conclusion, the evaluation of both conventional and novel immunophenotypic markers by flow cytometry significantly improved the differentiation of ALL and AML in this study. While conventional markers provided foundational lineage assignment, novel markers such as TdT, CD79a, and CD123 enhanced diagnostic precision, particularly in ambiguous or aberrant cases. The integration of these markers into routine diagnostic panels offers substantial benefits for accurate diagnosis, prognostication, and treatment planning, reinforcing the critical role of comprehensive flow cytometric analysis in acute

leukemia management.

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