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Diagnostic Concordance and Superiority of Flow Cytometry Over Immunohistochemistry in Acute Leukemia Immunophenotyping: A Single-Center Study

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Background: Early diagnosis is crucial for effective treatment and management of acute leukemia. Flow cytometry (FC) is a fast and reliable immunophenotyping technique. This study aimed to compare the diagnostic agreement between FC and histopathological evaluation using shared parameters from bone marrow samples.

Material/Methods: A total of 144 patients presenting to Adult Hematology, Pediatric Hematology, and Pediatric Oncology clinics at Çukurova University Faculty of Medicine were enrolled; bone marrow samples were processed at the Central Laboratory FC Unit. The cohort included 70 patients with acute myeloid leukemia, 59 with B-cell acute lymphoblastic leukemia, and 15 with T-cell acute lymphoblastic leukemia. The chi-square test, Cohen's kappa, adjusted kappa coefficient, sensitivity, specificity, and accuracy were used to assess FC–IHC concordance.

Results: The concordance between FC and immunohistochemistry (IHC) was 82.6% for AML ($P > 0.999$, PABAK = 0.99) and 61.0% for B-ALL ($P = 0.054$, PABAK = 0.89). FC achieved 95% sensitivity for AML and 100% for B-ALL. CD33, MPO, and CD117 showed significant concordance in AML, with sensitivities of 94%, 91%, and 90%, respectively. CD34 was the top-performing biomarker for AML, with 93% sensitivity and 95% specificity. For B-ALL, TdT, CD33, and CD34 showed sensitivities of 77%, 75%, and 81%, respectively.

Conclusions: FC showed higher concordance with IHC in AML than in B-ALL and better diagnostic sensitivity for leukemic lineage classification. CD33, MPO, and CD117 were strong AML markers, while TdT, CD33, and CD34 were effective for B-ALL. To quantitatively analyze marker expression with high sensitivity, precise lineage differentiation, and a quick turnaround time, same-day reporting and rapid initiation of targeted therapy make FC essential in modern hematopathology.

Keywords: flow cytometry • acute disease • immunochemistry • hematologic diseases

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Introduction

Leukemias are generally classified into 2 main categories—lymphoid and non-lymphoid (myeloid)—based on cellular origin, clinical features, disease progression, and response to treatment. These categories are further divided into acute and chronic forms depending on disease progression, prognosis, and other clinical factors [1,2].

Flow cytometry (FC), which identifies the immunophenotypic profiles needed for diagnosing, classifying, and determining the lineages of blast cell populations in acute leukemias, and immunohistochemistry (IHC), which shows the spatial distribution of cellular markers within the architecture of tissue sections, are 2 essential diagnostic tools in modern hematopathology.

FC can analyze large numbers of cells in suspension obtained from small bone marrow aspirates. By enabling simultaneous multiparametric analysis of both cell-surface and intracellular antigens, it allows for quick and detailed characterization of leukemic blasts soon after sample collection. Additionally, FC can accurately and efficiently distinguish all maturation stages of hematopoietic cells in bone marrow samples—from early progenitors to fully mature cells—offering a comprehensive view of normal hematopoiesis and leukemic blast populations within a single platform. In contrast, IHC generally provides information about antigen expression within the tissue architecture of bone marrow biopsy sections. A key limitation of IHC is its inability to assess multiple parameters on a single cell at once. As a result, IHC can confirm the diagnosis of acute leukemia but cannot always determine the blast lineage, while FC can definitively identify lineage (myeloid or lymphoid) by evaluating multiple parameters across many individual cells simultaneously. The rapid diagnostic capability of FC thus supports quick and accurate treatment decisions in clinical practice [3,5]. Furthermore, FC easily allows the addition of extra test parameters when clinically needed.

Hematopoietic cells display specific surface molecules called Cluster of Differentiation (CD) markers in patterns that are characteristic of their lineage and maturation stage. These cells progressively acquire distinct CD marker combinations as they progress through stages of maturation; for instance, B cells express CD19 and CD20, T cells express CD3, CD4, and CD8, and myeloid cells express CD13, CD33, and CD15. Leukemic blasts are generally immature and undifferentiated, expressing progenitor-associated CD markers such as CD34, CD117, CD10, CD19, and HLA-DR. Additionally, the abnormal expression of CD markers not normally present on a given cell type further helps identify blasts and determine lineage in diagnostic practice [4].

Flow cytometry (FC) has emerged as a rapid, reliable, and pivotal diagnostic modality for the early detection and immunophenotyping of acute leukemia. Flow cytometric immunophenotyping (FCI) enables the phenotypic characterization of blasts by comprehensively evaluating surface membrane and intracellular antigens expressed by leukemic cells. This methodology allows for precise lineage assignment, assessment of potential therapeutic targets, and prediction of specific genetic lesions. Furthermore, FC's high-throughput capacity enables the evaluation of large cell numbers, allowing the detection of rare cell populations. Crucially, FC enables rapid determination of phenotypic profiles within hours of sample collection [6]. Over the last 2 decades, FCI has become a fundamental laboratory tool for diagnosing and classifying various hematologic malignancies, playing a critical role in disease monitoring and assessing therapeutic response [7]. The utility of this technology is particularly well-established and widely implemented in the evaluation of acute leukemias and lymphomas [8,9].

Comparative studies of FC and immunohistochemistry (IHC) indicate that results from these 2 modalities are rarely contradictory and are frequently concordant. While both methods are complementary, FC is generally regarded as more effective for lineage assignment and precise classification [10].

Acute leukemias (ALs) are clonal hematopoietic stem cell disorders characterized by dysregulated cellular proliferation and differentiation. Based on the expression profile of CD markers, ALs are classified into 2 principal categories: acute lymphocytic leukemia (ALL) and acute myeloid leukemia (AML). Beyond leukemia subtyping, FC plays a pivotal role in identifying and characterizing aberrant antigen expression [11].

ALL is the most common malignancy in children, with an overall cure rate exceeding 90% in most developed countries. It encompasses a group of malignant neoplasms derived from precursor B- or T-lymphoid cells, termed lymphoblasts. FCI is an essential component in the diagnostic evaluation and clinical management of patients with ALL. Additionally, it enables the detection and quantification of rare lymphoblasts that may persist following treatment [12,13].

AML is a heterogeneous blood disorder marked by an accumulation of myeloblasts in the bone marrow. These can also infiltrate peripheral blood or other tissues [14]. FC typically focuses on analyzing myeloid progenitors, which are consistently affected across disease subtypes and are more stable than maturing myelomonocytic cells. FC also determines the maturation stages of bone marrow elements, aiding AML subclassification [15,16].

In most AML cases, myeloblasts test positive for myeloid markers (CD13, CD33, and MPO) and for CD34, CD117, and HLA-DR. CD11c expression is typically dim. In acute monoblastic

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leukemia, bright positivity for CD11b, CD11c, and CD64 is typical. Aberrant expression of CD7, CD56, CD4, and, less frequently, CD19 may also be observed [16].

Lymphoblasts can be classified as B-cell or T-cell lineage based on markers expressed during FC analysis. The most common markers are CD19 or CD22 for B-cell leukemia, and CD3 and the T-cell receptor for T-cell leukemia [17]. In blasts, stem cell antigen CD34 and immature lymphoid markers HLA-DR and TdT are almost always at least partially positive. CD7 is a sensitive marker for T-ALL, but it is also found in many myeloid leukemias and is therefore not exclusive to T-ALL. Other T-lymphoblast-associated antigens in T-ALL include CD1a, CD2, CD3, and CD5. In B-ALL, with few exceptions, the blasts co-express CD10 and CD19. Other B-lymphoblast-associated antigens common in precursor B-ALL are CD9, CD22, and CD24; these markers are also found in intermediate and late stages of normal B-cell maturation [18]. In studies by Bradstock et al of B-ALL patients, the most common aberrant expression was CD33 (11%), followed by CD13 (5%), CD11b (4%), and CD7 (2%) [19,20].

The diagnosis and classification of acute leukemia require a multimodal approach combining morphology, cytochemistry, immunophenotyping, and cytogenetic (or molecular genetic) analysis. FCI plays a vital role in this process. Although no single marker allows for accurate typing, analysis using antibody panels enables the differentiation of acute leukemia into myeloid and lymphoid groups and further sub-classification into various subtypes, paralleling normal hematopoietic stages [18,21].

Technological advancements over the last decade have solidified flow cytometry (FC) as a powerful immunophenotyping tool critical for diagnosing various leukemias. It can rapidly identify abnormal cell populations, characterize phenotypes, classify lineages, diagnose, or narrow the differential diagnosis within hours of sampling. Compared to immunohistochemistry (IHC), FC is not only faster but also capable of easily and efficiently correlating multiple parameters on a single cell. The rapid turnaround time of flow cytometry testing allows for quick diagnosis or the timely selection of other appropriate ancillary tests. However, correlation with morphology, clinical data, and occasionally cytogenetic/molecular findings is always required for accurate results [22].

The present study investigated the diagnostic consistency between FC and IHC by comparing common test parameters in bone marrow aspiration samples analyzed by FC and bone marrow biopsy samples processed by IHC in patients with acute leukemia at initial presentation. The capacity of FC to reliably and rapidly perform lineage identification and assignment, combined with its high diagnostic sensitivity, is expected to offer significant prognostic advantages by enabling early diagnosis and ensuring prompt initiation of treatment. These findings are also expected to contribute to the literature on diagnostic approaches in acute leukemias.

Material and Methods

This study was conducted as a prospective cross-sectional observational study in accordance with the ethical principles outlined in the Declaration of Helsinki, and all patient data were anonymized before analysis. The study protocol was approved by the Non-Interventional Clinical Research Ethics Committee of Çukurova University (Decision No: 134, Date: June 02, 2023). The study cohort included 144 patients who presented to the Adult Hematology, Pediatric Hematology, and Pediatric Oncology Outpatient Clinics at Çukurova University Faculty of Medicine. Informed consent was obtained from all participants. Bone marrow samples were processed by the Central Laboratory Flow Cytometry Unit and the Department of Medical Pathology within the same institution. The cohort consisted of 70 patients diagnosed with AML, 59 with B-ALL, and 15 with T-ALL. Inclusion criteria specifically targeted patients diagnosed with acute leukemia at their initial presentation who were responsive to treatment. Leukemic cases were classified according to the 2022 World Health Organization (WHO) classification systems. The study compared the concordance of shared test parameters obtained from flow cytometry (bone marrow aspiration) and immunohistochemistry (bone marrow biopsy) performed on samples collected on the same day. Patient-related data, including age, sociodemographic variables, and other relevant clinical parameters, were retrieved from the hospital and laboratory information management systems (Mergentech-Enlil, version 2025).

Consecutive eligible patients who met the pre-specified inclusion criteria during the study period were enrolled in the study.

The inclusion criteria were as follows:
1. Newly diagnosed acute leukemia at the time of presentation,
2. Availability of complete clinical and laboratory data, and
3. Absence of any concomitant chronic disease (applicable to both pediatric and adult patients).

The exclusion criteria were as follows:
1. Relapsed acute leukemia at the time of presentation,
2. Presence of any concomitant chronic disease,
3. Incomplete clinical or laboratory records, and
4. Insufficient sample material for analysis (applicable to both pediatric and adult patients).

Bone marrow aspiration samples for flow cytometric analysis were collected in 5 mL EDTA tubes (Becton, Dickinson and Company, Preanalytical Systems, BD). Bone marrow biopsy

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Table 1. Flow cytometric (FC) and immunohistochemical (IHC) panels.

Acute leukemia panel (FC)	cMPO, c CD79a, cCD3, CD33, CD64, CD3, cTdT, CD20, CD34, CD7, CD117, CD14, CD11b, CD10, CD13, CD38, HLADR, CD45
AML panel (FC)	cMPO, CD10, CD19, CD34, CD13, CD64, CD14, CD11b, CD33, CD15, CD56, CD38, CD117, HLADR, CD45
B-ALL panel (FC)	cTdT, CD22, CD34, Ccd79a, CD38, CD20, CD10, CD19, HLADR, CD45
T-ALL panel (FC)	cTdT, CD10, CD19, CD34, CD2, HLADR, cCD3, CD4, CD1a, CD8, CD7, CD5, CD3, CD45
IHC panel	CD34, CD117, TdT, CD3, MPO, CD33, CD13, Pax5, CD61

specimens for immunohistochemical studies were collected in 15 mL Falcon tubes containing 10% formaldehyde (Labosel, Türkiye).

Flowcytometric analyses were performed using a 10-color, 3-laser (488 nm blue, 638 nm red, and 405 nm violet) Beckman Coulter Navios flow cytometer. Specific panels for Acute Leukemia, AML, B-ALL, and T-ALL were established for sample analysis (**Table 1**).

For the analysis of cellular subsets, samples were stained with the following conjugated monoclonal antibodies (Beckman Coulter, USA): CD19-PC5 (Phycoerythrin 5, clone J3-119), CD34-PC5 (clone 581), CD15-PC5 (clone 80H5), CD22-PC5 (clone HD239), CD1a-PC5 (clone SFCI19Thy1A8), cMPO-FITC (Fluorescein Isothiocyanate, clone CLB-MPO-1), cTdT-FITC (clone HT1+HT4+HT8+HT9), CD11b-FITC (clone Bear1), CD20-PE (Phycoerythrin, clone B9E9), CD10-PE (clone J5), CD33-Alexa Fluor 700 (APC-A700, clone 906), HLA-DR-Pacific Blue (PB, clone Immu-357), cCD79a-APC (Allophycocyanin, clone HM47), CD4-ECD (Phycoerythrin-Texas Red, clone SFCI12T4D11), CD13-PC7 (Phycoerythrin-Cyanin 7, clone Immu103.44), CD56-PC7 (clone N901), CD8-PC7 (clone SFCI21Thy2D3), CD5-APC-A700 (clone BL1a), CD117-Alexa Fluor 750 (APC-A750, clone 104D2D1), CD64-APC-A750 (clone 22), CD38-A750 (clone T16), CD3-PB (clone UCHT1), CD14-PB (clone RMO52), and CD45-Kro (Krome Orange, clone J33). Standard surface and cytoplasmic antibody staining protocols were used. Data were evaluated using dot plots that mapped 2 parameters on the X-Y axes. A gating strategy was used to isolate and visualize the cell populations of interest. Antigenic expression in bone marrow aspiration samples was defined as positive if the percentage of positive blast cells was ≥20% [23-25]. This threshold was used as the diagnostic criterion because molecular mutation data were unavailable.

Flowcytometric data were analyzed using Kaluza Analysis Software (Beckman Coulter, Miami, FL). Interpretation and reporting were performed by 2 medical biochemistry specialists with specific training and expertise in FC.

Manufacturer-calibrated instruments were used for all FC analyses. Periodic quality control procedures were performed on a daily basis in accordance with the manufacturer's recommendations, encompassing laser alignment verification, photomultiplier tube (PMT) voltage optimization, and fluorescence compensation parameter adjustment. Furthermore, the suitability of the monoclonal antibodies and reagents employed in the analyses was confirmed, and both sample preparation and data analysis procedures were conducted in strict accordance with standard laboratory protocols.

Bone marrow biopsy specimens submitted to the pathology laboratory underwent decalcification using 10% formic acid. Subsequently, the specimens were processed using an automated tissue processor, which involved dehydration in graded alcohols, clearing in xylene, and embedding in paraffin blocks. Four-micron-thick sections were cut from the paraffin blocks and stained with hematoxylin and eosin (H&E).

Following the initial morphological evaluation, immunohistochemical staining was performed to characterize blastoid cells. Sections selected for immunohistochemical analysis were mounted on positively charged slides, incubated at 60 °C for 1 hour, and deparaffinized using xylene. Following rehydration through alcohol and distilled water, a panel containing the following antibodies was applied (**Table 1**): **CD34** (NCL-L-END, liquid mouse monoclonal, Novocastra); **CD117/c-kit** (YR145, rabbit monoclonal primary antibody, 1/500, Cell Marque); **TdT** (rabbit polyclonal antibody, Cell Marque); **CD3** (NCL-L-CD3-565, mouse monoclonal antibody, 1/500, Novocastra); **MPO/Myeloperoxidase** (rabbit polyclonal antibody, 1/400, Cell Marque); **CD33** (Clone sp266, mouse monoclonal antibody, Cell Marque); **CD13** (Clone sp187, mouse monoclonal antibody, Cell Marque); **Pax5** (Clone SP34, rabbit monoclonal antibody, Cell Marque); and **CD61** (Clone Y2/51, mouse monoclonal antibody, 1/50, Dako)

Staining was performed on a Ventana BenchMark XT automated stainer using the UltraView DAB Detection Kit. Slides were coverslipped using a liquid-based mounting medium. Final diagnoses of acute leukemia were established based on

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Table 2. Mean age of the study population.

	AML mean ± SD median (min-max)	T-ALL mean ± SD median (min-max)	B-ALL mean ± SD median (min-max)
Age	41.74 ± 23.54 46.5 (2-84)	17.40 ± 12.68 13 (2-41)	15.64 ± 19.55 6 (1-77)

a synthesis of peripheral blood smear findings, immunohistochemical (IHC) and flow cytometric (FC) analyses, clinical presentation, and therapeutic response.

The study statistically compared the test parameters of monoclonal antibodies commonly used in FC and IHC analyses. The chi-square test was used to compare categorical variables between groups. To evaluate the concordance between FC and IHC results, Cohen's kappa coefficient was calculated. Additionally, the prevalence-adjusted bias-adjusted kappa (PABAK) coefficient was calculated to account for prevalence bias. To evaluate the diagnostic performance of FC, sensitivity, specificity, and accuracy values along with their corresponding confidence intervals were calculated.

Categorical variables were expressed as frequencies and percentages, while continuous variables were summarized as mean ± standard deviation or median (minimum–maximum), as appropriate. The chi-square test was used to compare categorical variables between groups. Agreement between FC and IHC results was assessed using Cohen's kappa coefficient. Given that kappa values may be susceptible to the effects of prevalence and bias, the prevalence- and bias-adjusted kappa (PABAK) coefficient was additionally computed. The diagnostic performance of FC was evaluated by calculating sensitivity, specificity, and accuracy along with their corresponding confidence intervals, using IHC as the reference gold standard. All statistical analyses were performed using IBM SPSS Statistics, version 20.0, and a 2-tailed *P* value of less than 0.05 was considered statistically significant.

Results

Demographic analysis revealed that 45% of the patients were female and 55% were male. In terms of age distribution, the cohort was stratified into adults (> 18 years, 51%) and pediatric patients (< 18 years, 49%). The overall mean age of the study population was 28.51 ± 24.60 years (**Table 2**). Additionally, an evaluation of migration status indicated that 22% of the study cohort consisted of immigrants of Syrian origin (**Table 3**).

Evaluating the diagnostic concordance between the 2 methods for AML, CD13 detected by flow cytometry (FC) had low

Table 3. Frequency distribution of sociodemographic data.

		n (%)
Gender	Female	65 (45)
Gender	Male	79 (55)
Race	Turkish	112 (78)
Race	Syrian	32 (22)
Age	< 18	71 (49)
Age	> 19	73 (51)

sensitivity (13%), indicating it failed to detect positivity in most AML cases. Despite this low sensitivity, its higher specificity (80%) suggests a better ability to correctly exclude non-AML cases. However, the low accuracy (50%) and kappa value (0.10) reflect poor agreement between these 2 methods, suggesting that CD13 is not a reliable standalone marker for AML diagnosis in this comparative context.

The CD33 biomarker demonstrated high sensitivity (94%), indicating successful detection of AML cases. Conversely, its specificity was notably low (6%), implying a high likelihood of classifying AML-negative individuals as false positives. The low accuracy (26%) and negligible kappa value (0.003) indicate minimal agreement between these 2 methods. Consequently, despite its high sensitivity, CD33 lacks the reliability required for definitive AML diagnosis due to its poor specificity and concordance metrics.

The CD117 biomarker had a sensitivity of 91% and a specificity of 27%. These rates indicate that while CD117 is highly effective at detecting AML-positive cases, it is less effective at discriminating AML-negative cases. Although it may be considered a moderately reliable biomarker with an accuracy of 75%, the kappa value of 0.21 indicates only weak agreement between the methods. Thus, despite its high sensitivity, CD117 is not considered highly reliable for AML diagnosis due to its limited specificity and concordance.

MPO demonstrated 90% sensitivity, enabling the identification of AML-positive cases with high confidence. However, a specificity of 20% results in a significant number of AML-negative

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Table 4. Comparison of the diagnostic test performance of FC and IHC in the diagnosis of acute myeloid leukemia (AML).

	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	Kappa
CD13	0.125 (0.022, 0.471)	0.800 (0.490, 0.943)	0.500 (0.290, 0.710)	-0.080
CD33	0.938 (0.717, 0.989)	0.038 (0.010, 0.128)	0.246 (0.160, 0.360)	-0.012
CD117	0.913 (0.797, 0.966)	0.267 (0.109, 0.520)	0.754 (0.633, 0.845)	0.213
MPO	0.896 (0.778, 0.955)	0.200 (0.039, 0.624)	0.830 (0.708, 0.908)	0.088
CD34	0.925 (0.801, 0974)	0.950 (0.764, 0.991)	0.933 (0.841, 0.974)	0.854*
TDT	0.286 (0.080, 0.642)	1.000 (0.918, 1.000)	0.900 (0.786, 0.956)	0.408*

Kappa values ≥0.40 were marked as significant.

Table 5. Comparison of diagnostic test performance of FC and IHC in the diagnosis of B-ALL.

	Sensitivity	Specificity	Accuracy	Kappa
CD33	0.75	0.71	0.73	0.441*
CD34	0.81	0.50	0.67	0.284*
TdT	0.77	0.50	0.76	0.093

Kappa values ≥0.40 were marked as significant.

individuals being classified as false positives. Although the accuracy was relatively high at 83%, the kappa value of 0.088 reveals poor concordance between the methods. While MPO's high sensitivity helps capture positive cases, its low specificity and poor agreement suggest that MPO results require careful clinical interpretation.

CD34 emerged as the highest-performing biomarker, with 93% sensitivity and 95% specificity. These metrics demonstrate CD34's ability to successfully discriminate between AML-positive and negative cases. The accuracy was 93%, with a kappa value of 0.854. This high kappa value signifies strong concordance between the 2 methods. The superior sensitivity, specificity, and agreement metrics establish CD34 as a reliable biomarker for AML diagnosis, playing a critical role in ensuring consistency between the 2 diagnostic modalities.

The TdT biomarker had a sensitivity of 28%, indicating that it could not identify the majority of AML-positive individuals. However, with a specificity of 100%, it accurately classifies all AML-negative individuals. While TdT demonstrated 90% accuracy, the kappa value of 0.408 indicates moderate agreement between the methods. These results suggest that despite its low sensitivity for AML, TdT prevents false-positive classifications in non-AML individuals due to its excellent specificity. Therefore, TdT is expected to be valuable in clinical decision-making, particularly for its high specificity (**Table 4**).

Kappa values ≥0.40 are marked as significant.

In the diagnosis of B-ALL, the CD33 biomarker exhibited high sensitivity (75%), demonstrating its efficacy in identifying B-ALL, particularly in cases with aberrant expression. A specificity of 71% indicates that CD33 also distinguishes non-B-ALL individuals. The 73% accuracy rate reflects a good level of overall diagnostic performance. The kappa value of 0.441 indicates moderate agreement between the 2 methods.

The CD34 biomarker, with a sensitivity of 81%, correctly identified a large proportion of B-ALL-positive individuals. However, a specificity of 50% indicates that CD34 has limited diagnostic discriminatory power in non-B-ALL individuals. The accuracy rate of 67% suggests moderate overall diagnostic performance, while the kappa value of 0.284 indicates weak-to-moderate agreement between the methods. This level of concordance limits the utility of CD34 as a strictly reliable standalone biomarker in this diagnostic context.

The TdT biomarker demonstrated a sensitivity of 77%, indicating the ability to correctly identify most B-ALL positive cases. The specificity rate of 50% implies that TdT correctly identified only half of the B-ALL-negative individuals. This low specificity suggests a potential for false-positive results in non-B-ALL individuals (eg, due to the presence of hematogones). While the 76% accuracy rate suggests good general diagnostic accuracy, the kappa value of 0.093 indicates very poor agreement between the methods (**Table 5**).

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Table 6. Comparison of FC and IHC in the diagnosis of AML and B-ALL.

		Pathology Negative n (%)	Pathology Positive n (%)	Pathology Total	p	PABAK
AML diagnosis						
Flow	Negative	0 (0.0)	3 (100.0)	3 (4.3)	> 0.999	0.99
Flow	Positive	9 (13.6)	57 (86.4)	66 (95.7)	> 0.999	0.99
Flow	Total	9	60	69	> 0.999	0.99
B-ALL diagnosis						
Flow	Negative	3 (13.0)	0 (0.0)	3 (5.1)	0.054	0.89
Flow	Positive	20 (87.0)	36 (100.0)	56 (94.9)	0.054	0.89
Flow	Total	23	36	59	0.054	0.89

Table 7. Concordance and discordance between FC and IHC in the diagnosis of AML and B-ALL.

Diagnostic	Cases	FC and IHC agreement n (%)	Diagnostic only defined IHC n (%)	Diagnostic only defined FC n (%)	Non-diagnostic by FC/IHC n (%)
AML	69	57 (86.4)	3 (5.0)	9 (13.6)	0 (0.0)
B-ALL	59	36 (64.3)	0 (0.0)	20 (35.7)	3 (13.0)

Table 8. Percentage distribution of diagnoses by FC and IHC in acute leukemias.

	Number of cases	FC	IHC	Clinical diagnosis
AML	69	66/69 (%95.7)	60/69 (%87)	69/69 (%100)
BALL	59	56/59 (%94)	36/59 (%61)	59/59 (%100)
TALL	15	13/15 (%86)	8/15 (%53)	15/15 (%100)
ALL (BALL+TALL)	74	69/74 (%93)	44/74 (%59)	74/74 (%100)
Total	144	135/144 (%93)	103/144 (%71)	144/144 (%100)

Kappa values ≥ 0.40 are marked as significant.

Due to the limited sample size of patients diagnosed with T-ALL (n = 15), a kappa concordance analysis could not be performed for this subgroup.

For the diagnosis of AML, the observed agreement between FC and IHC was 82.6% (57/69). However, due to the high prevalence of positive cases (60/69; 86.0%), the calculated kappa value was -0.070 ($P = 0.493$), indicating that chance-corrected agreement was not statistically significant. With IHC as the reference standard, the sensitivity of FC was 95% (57/60), while the specificity was 0% (0/9). When metrics less susceptible to prevalence bias were applied, the prevalence-adjusted bias-adjusted kappa (PABAK) was 0.99. These results suggest that while the method is highly successful in detecting positives, it is insufficient in distinguishing negatives. This discrepancy explains the low kappa value despite the high observed agreement (**Table 6**). (PABAK interpretation criteria: < 0.00 poor, 0.00-0.20 slight, 0.21-0.40 fair, 0.41-0.60 moderate, > 0.61 substantial/good agreement).

For the diagnosis of B-ALL, the observed agreement between FC and IHC was 61.0% (36/59). A slight but statistically significant agreement was found between the methods (kappa = 0.155, p = 0.026). Using IHC as the reference, FC sensitivity was 100% (36/36) and specificity was 13.0% (3/23) (PABAK = 0.89). While observed agreement was moderate and kappa was low but

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significant, the method excelled at detecting positives but performed poorly in excluding negatives (**Table 6**).

An evaluation of discordance between flow cytometry (FC) and immunohistochemistry (IHC) revealed that FC performed better at establishing diagnoses by accurately assigning lineages. Specifically, detection rates for both AML and B-ALL were higher with FC than with IHC. Notably, FC successfully diagnosed all AML cases with no false negatives. In the B-ALL cohort, although 3 patients were not diagnosed during the initial FC assessment, a definitive diagnosis of B-ALL was established in subsequent FC analyses (**Table 7**).

The diagnostic rates and lineage assignment capabilities of FC and IHC were evaluated in all patients with acute leukemia. FC demonstrated superior diagnostic yields compared to IHC for both AML (95.7% vs 87.0%) and B-ALL (94.0% vs 61.0%). When the combined ALL cohort was analyzed, FC achieved a significantly higher diagnostic rate than IHC (93.0% vs 59.0%). Furthermore, across the entire study population of acute leukemia patients, FC exhibited superior overall diagnostic success (93.0%) (**Table 8**).

Discussion

In the present study, FC and IHC were found to be mutually supportive and complementary diagnostic modalities in patients with acute leukemia, with no discordant results observed between the 2 methods.

In a retrospective study by Landry et al conducted in patients with hematologic malignancies, concordant results between FC and IHC were obtained in 60 of 74 acute leukemia cases (81%). In their study, one patient with acute promyelocytic leukemia (APL) was diagnosed via FC. Additionally, in a patient with myelodysplastic syndrome (MDS) progressing to AML, FC showed a higher blast percentage than IHC. Although it was noted that one method might be more advantageous than the other in certain cases, neither IHC nor FC yielded discordant or contradictory results; rather, they were observed to be complementary [10].

In the present study, the concordance rate between FC and IHC was found to be higher in AML than in B-ALL (**Table 7**). In 1 T-ALL case and 2 AML-M3 (promyelocytic leukemia) cases, diagnosis was established using FC when IHC was inconclusive; treatment was planned based on FC findings, resulting in remission. In 1 case, FC enabled the diagnosis of biphenotypic leukemia (AML and T-ALL), guiding treatment. In 3 patients, FC performed lineage assignment, but a definitive B-ALL diagnosis was only confirmed in subsequent FC analyses, which provided a clearer distinction of hematogones. In 2 cases diagnosed as chronic myelomonocytic leukemia (CMML) and chronic myeloid leukemia (CML) by IHC, FC identified transformation to AML. While IHC could diagnose "acute leukemia" by reporting blast percentages, it was unable to differentiate between lymphocytic and myelocytic lineages. FC, due to its high sensitivity and quantitative capabilities, was more effective in achieving accurate diagnosis and lineage assignment. This discrepancy is attributed to the fact that FC, by utilizing bone marrow aspirate material, enables the analysis of substantially larger cell quantities, whereas IHC relies on sectional biopsy specimens, which inherently limits the number of cells available for evaluation.

Mhawech et al, in a study of a pediatric cohort, reported that lineage classification was compatible in all cases, with concordance between the 2 methods in 80.32% of cases (80.32% overall agreement). Concordance was shown to be 79% (89/112) in ALL cases and 77.7% (7/9) in AML cases. It was stated that while IHC is necessary to define AML subtypes, FC has become a standard tool for the evaluation and management of leukemia patients, despite its limited ability to distinguish ALL subtypes [26].

Kheiri et al compared IHC and FC diagnoses in 93 cases of acute leukemia. Their study demonstrated lineage agreement in 95.8% of cases. However, when non-diagnostic and biphenotypic diagnoses established by either methodology were included, complete concordance was observed in only 77.4% of cases. They reported concordance of 89.2% for myeloid leukemias and 80% for ALL [27].

Boyd et al emphasized that in B-ALL, FC detects positive staining for CD19, CD79a, and CD34 at lower expression levels more frequently than IHC does [28].

In a study conducted by Aguilera et al on 74 cases, the sensitivity of the B-lineage markers cCD79a, CD19, CD20, and CD22 was compared. cCD79a demonstrated 100% sensitivity and 80% specificity, followed by CD22, which exhibited 97% sensitivity and 88% specificity [29].

In the present study, expression levels could not be compared because IHC assessments were reported qualitatively as positive or negative (±) rather than as quantitative CD expression percentages.

Similarly, in our study, concordance between the 2 methods was high for CD34 in AML diagnosis. Additionally, CD117, CD33, and MPO showed high sensitivity for AML. TdT negativity showed high specificity for AML. However, unlike in FC, CD33 was not a significant parameter for AML diagnosis in IHC. Particularly in pediatric patients, FC enabled rapid treatment initiation by providing results—including accurate lineage assignment and a preliminary diagnosis of B-ALL—on the same day.

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Since IHC evaluations in this study were reported qualitatively (±) without percentage ratios, quantitative expression levels could not be compared.

Aguilera et al, in a study of 74 cases comparing B-lineage markers, showed that cCD79a had 100% sensitivity and 80% specificity, followed by CD22 with 97% sensitivity and 88% specificity [26].

The analyses in this study showed that CD34 has a high sensitivity, allowing for the accurate detection of most B-ALL-positive cases. However, its low specificity indicates limited ability to distinguish individuals without B-ALL. When combined with the calculated accuracy rate, CD34 displays moderate overall diagnostic performance in B-ALL, with low to moderate agreement between methods.

In diagnosing AML, CD34 emerged as the top-performing biomarker, showing both very high sensitivity and specificity. These values suggest that CD34 can effectively distinguish between AML-positive and AML-negative cases. The high accuracy rate, along with a high kappa coefficient, indicates strong agreement between the 2 methods. This high kappa value signifies a strong agreement between the 2 methods. Overall, the high sensitivity, specificity, and kappa value of CD34 demonstrate its reliability as a biomarker in AML diagnosis. Thus, these levels of accuracy and agreement indicate that CD34 plays a critical role in ensuring consistency between the 2 diagnostic methods.

In diagnosing B-ALL, the high sensitivity of CD33 indicates abnormal binding, showing it is effective in identifying B-ALL-positive cases. Additionally, the high specificity confirms that CD33 is also good at distinguishing individuals without B-ALL. The high accuracy rate reflects strong overall diagnostic performance for CD33. The kappa value shows moderate agreement between the 2 methods.

In diagnosing AML, the high sensitivity of CD33 indicates its effectiveness in detecting AML-positive cases. However, this high sensitivity is balanced by a relatively lower specificity, leading to a significant number of false positives among AML-negative individuals. The low accuracy and kappa values reflect only slight agreement between the 2 methods. Therefore, despite its high sensitivity, CD33 is considered insufficiently reliable for AML diagnosis due to its low accuracy, specificity, and kappa values.

The high sensitivity of TdT indicates that it can accurately identify most B-ALL-positive individuals. However, regarding specificity, TdT correctly identified only about half of B-ALL-negative individuals, suggesting that this low specificity could lead to many false-positive results in individuals without B-ALL. The overall diagnostic accuracy of TdT is demonstrated by its good accuracy rate. When considering the kappa value, the agreement between methods was found to be quite low. As a biomarker for AML diagnosis, TdT showed very low sensitivity, indicating it is inadequate for identifying most AML-positive cases. Nonetheless, its very high specificity allows for accurate classification of AML-negative individuals. The combination of TdT's high accuracy rate and the calculated kappa value indicates moderate agreement between the methods. These findings suggest that, despite its low sensitivity, TdT's high specificity prevents the misclassification of non-AML cases as false positives. Therefore, TdT may be a useful marker in clinical decision-making, especially because of its high specificity.

When evaluating the diagnostic performance and agreement between the 2 methods for diagnosing AML, CD13 was found to have low sensitivity, indicating it is inadequate for detecting AML-positive cases. Despite this low sensitivity, its relatively higher specificity suggests a better rate of correct identification in AML-negative individuals. The very low accuracy rate and kappa value indicate poor inter-method agreement, while also showing that CD13 cannot be considered a sufficiently reliable marker for AML diagnosis.

CD117 showed high sensitivity but low specificity. These results suggest that CD117 is effective at identifying AML-positive cases but has limited ability to distinguish AML-negative cases. Although this marker might be seen as a moderately reliable biomarker based on its calculated accuracy, the low kappa value indicates poor agreement between methods. Therefore, despite its high sensitivity, CD117 is considered insufficiently reliable for AML diagnosis because of its low specificity and kappa values.

MPO, in turn, demonstrated high sensitivity, indicating that it can accurately identify AML-positive cases. However, its very low specificity results in a significant number of AML-negative individuals being misclassified as false positives. Although the accuracy rate is relatively high, the low kappa value signifies poor agreement between methods. While MPO's high sensitivity is useful for detecting positive cases in AML diagnosis, its low specificity and lack of consistent results across different methods suggest that MPO must be interpreted carefully in a clinical setting.

When examining the diagnostic performance of the immunophenotypic markers evaluated in this study collectively, CD34 emerged as one of the most reliable biomarkers—especially in diagnosing AML—due to its high sensitivity and specificity. However, in diagnosing B-ALL, the lower specificity of CD34 indicates limited ability to distinguish between conditions. This may be because CD34 is expressed across various immature hematopoietic cell populations in different leukemia subtypes within the bone marrow, as well as in immature B-cell

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precursors like hematogones, which can restrict its diagnostic accuracy. The strong performance of CD34 in AML may relate to its expression in early hematopoietic progenitor cells and leukemic blasts. Since CD34 is linked to immature progenitor cell populations, its consistent presence in AML blasts increases its diagnostic reliability, especially when combined with other myeloid markers.

CD33 showed moderate sensitivity and specificity in diagnosing B-ALL. However, in diagnosing AML, despite its high sensitivity, the low specificity may be due to this marker being expressed in certain lymphoid or aberrant-phenotype leukemia cells. Similarly, CD117 and MPO exhibited high sensitivity in identifying AML blasts. Nonetheless, the low specificity and kappa values of these markers imply that agreement between methods may be limited when they are used alone.

CD13, by contrast, was found to be inadequate for detecting AML-positive cases because of its low sensitivity. However, its comparatively higher specificity indicates that it can still help differentiate AML-negative individuals to some degree.

Considering the biological mechanism of TdT, its distinctive diagnostic profile likely stems from the fact that it is primarily a nuclear enzyme found in immature lymphoid precursor cells. Although it shows moderate sensitivity in diagnosing B-ALL, it has high specificity for AML. Based on these sensitivity and specificity features, the findings suggest that TdT negativity alone may not be sufficient to completely rule out lymphoid leukemia (**Tables 4-6**).

When comparing the diagnostic distribution percentages of FC and IHC, FC was found to have a higher rate of diagnostic success (**Table 8**).

There have been few detailed studies conducted in the last decade comprehensively addressing the diagnostic power of FC in acute leukemias, specifically evaluating monoclonal antibody levels in terms of sensitivity and specificity. The diagnostic power of FC has been demonstrated in our study. The increasing validity of the FC method is evident, driven by its ability to establish accurate diagnoses through correct lineage classification and to provide rapid results within the same day or even hours.

IHC is recognized as one of the gold standard reference methods in the diagnostic evaluation of acute leukemias due to its ability to preserve bone marrow architecture and provide the morphological context that may be lost during cell suspension preparation in FC. While flow cytometry offers advantages in rapid and multiparametric analysis of surface antigens, IHC allows for direct visualization of blast cells within their native bone marrow microenvironment. This enables the assessment of the spatial distribution of cells, such as paratrabecular or interstitial localization, and their correlation with stromal changes, including fibrosis and necrosis [30].

Together, these findings indicate that the diagnostic performance of immunophenotypic markers in acute leukemia varies, and no single biomarker may be sufficient for case discrimination despite its individual advantages. CD34 showed strong diagnostic value in diagnosing AML due to its high sensitivity and specificity. In contrast, the diagnostic usefulness of TdT, CD33, CD117, and MPO depended on their respective sensitivity–specificity trade-offs. Therefore, evaluating immunophenotypic markers using a multiparametric panel, alongside clinical and morphological findings, may improve diagnostic accuracy and support appropriate treatment decisions.

There are several important limitations in this study. Due to the earthquake that occurred in Türkiye on February 6, 2023, regular molecular testing could not be performed in the Medical Genetics department; consequently, patient test results could not be evaluated at the molecular level. Comparison studies were conducted based on clinical decisions and diagnosis-treatment outcomes for acute leukemia. Additionally, due to delays in procuring sufficient kits and consumables for both departments, certain markers could not be analyzed in patients and were therefore excluded from the study. This prevented a comparative analysis of more common test parameters. Furthermore, a sufficient number of patients could not be recruited to compare T-ALL cases. Finally, because CD ratios in IHC evaluations were reported qualitatively, expression levels could not be compared quantitatively.

Potential sources of bias were also evaluated. Selection bias might have resulted from including patients from only 1 clinical setting. Measurement bias was reduced by using standardized diagnostic assessments and IHC as the gold standard. Recall bias was considered minimal because the analyses mainly relied on recorded clinical and laboratory data rather than patient self-reports.

Although all eligible patients during the study period were included, the lack of a formal a priori sample size calculation and the relatively wide confidence intervals observed for certain markers suggest limited precision for some diagnostic estimates.

Conclusions

We found that individual monoclonal antibody parameters remain crucial as robust markers for confirming diagnoses in both AML and B-ALL, whether assessed via FC or IHC. The results from both methodologies were complementary and mutually supportive, with no contradictory findings.

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However, FC demonstrated superior diagnostic sensitivity and lineage assignment capabilities, particularly because it enables simultaneous evaluation of multiple monoclonal antibodies within the same cell population. Diagnostic concordance between FC and IHC was higher in AML cases than in B-ALL cases.

The ability of FC to successfully identify positive cases while simultaneously excluding negative cases in the diagnosis of AML demonstrates that its specificity performance was fully satisfactory in terms of discriminatory capacity. FC offers significant advantages over IHC, specifically regarding the ability to provide quantitative percentage data for markers and its high diagnostic sensitivity, combined with precise lineage differentiation. Furthermore, FC's rapid turnaround time enables same-day reporting and the immediate initiation of accurate, specific therapeutic regimens. Consequently, FC has established itself as an indispensable diagnostic powerhouse in the current clinical management of acute leukemias.

Comprehensive, multicenter studies with larger patient cohorts may yield further insights. Such studies should aim to expand the panel of common test parameters compared between FC and IHC and integrate the evaluation of molecular level mutations into the comparative analysis.

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