

Original Research Article

Contribution of Flow Cytometric Immunophenotyping to the Diagnosis of Acute

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Article History

Received: 29.08.2026

Accepted: 26.10.2026

Published: 30.10.2026

Journal homepage:

<https://www.easpublisher.com>

Quick Response Code



Abstract: **Introduction:** The diagnosis of acute leukemia relies on the integration of morphological and immunophenotypic findings. Flow cytometry is of particular value in morphologically ambiguous cases or in myeloperoxidase (MPO)-negative cases. **Objective:** To evaluate the contribution of flow cytometric immunophenotyping to the diagnosis and classification of acute leukemias and to assess its concordance with cytological findings. **Methods:** A retrospective study was conducted in 62 patients with acute leukemia. Demographic, clinical, hematological, and cytological data were collected. Flow cytometric immunophenotyping was performed to determine lineage and characterize antigen expression by blast cells. Concordance between cytological and immunophenotypic classifications was assessed. **Results:** The study population comprised 43 men (69.35%) and 19 women (30.65%), with a median age of 62 years. Anemia and thrombocytopenia were observed in 91.94% and 88.71% of patients, respectively. Cytology identified 33 cases of acute myeloid leukemia (AML) and 21 MPO-negative acute leukemias. Immunophenotyping classified 41 cases as AML and 14 as acute lymphoblastic leukemia (ALL). Overall concordance between the two approaches was 83.87%, whereas discordance was observed in 16.13% of cases. Among AML cases, CD33 (80.49%), MPO (78.05%), CD13 (75.61%), and CD117 (75.61%) were the most frequently expressed markers. Aberrant CD7 expression was observed in 29.27% of cases. **Conclusion:** Flow cytometric immunophenotyping provides an important complementary diagnostic contribution, particularly in MPO-negative acute leukemias and discordant cases. Its combination with cytological examination improves lineage assignment and classification of acute leukemias. **Keywords:** Acute Leukemia, Flow Cytometry, Immunophenotyping, Acute Myeloid Leukemia, Acute Lymphoblastic Leukemia, MPO, Aberrant Antigen Expression.

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INTRODUCTION

Acute leukemias constitute a heterogeneous group of malignant hematological disorders characterized by the clonal proliferation of immature cells of either myeloid or lymphoid lineage. Their diagnosis and classification currently rely on an integrated approach combining clinical, morphological, immunophenotypic, cytogenetic, and molecular findings. Recent international classifications, particularly the 5th edition of the World Health Organization (WHO) Classification and the International Consensus Classification (ICC), emphasize the integration of these different data to improve diagnostic accuracy and biological characterization of acute leukemias [1, 2].

Peripheral blood and bone marrow cytology constitute fundamental steps in the initial diagnostic assessment. They allow the identification of blast cells and evaluation of their morphological and cytological characteristics. However, some situations remain difficult to interpret, particularly when blast cells exhibit nonspecific morphological features or when myeloperoxidase (MPO) is negative. In such situations, morphological analysis alone may be insufficient to establish the cellular lineage with certainty, thereby justifying the use of immunophenotyping [1, 2].

Multiparametric flow cytometric immunophenotyping is therefore an essential tool in the diagnosis of acute leukemias. It enables the simultaneous analysis of multiple membrane and cytoplasmic antigens, contributing to the determination of lineage,

differentiation stage, and aberrant antigenic profiles. This approach is particularly important in complex diagnostic situations and in acute leukemias of ambiguous lineage. It also allows the establishment of a baseline immunophenotypic profile that can subsequently be used for measurable residual disease monitoring [3-5].

In acute myeloid leukemia (AML), assessment of markers such as MPO, CD13, CD33, and CD117, together with the immaturity markers CD34 and HLA-DR, contributes to the identification of myeloid differentiation. Current classifications and recommendations nevertheless emphasize the importance of interpreting these markers as a whole rather than relying on the isolated expression of a single antigen [1-6]. Identification of aberrant antigenic profiles may also provide diagnostic information and contribute to patient monitoring, particularly in the context of measurable residual disease [3-6].

In Morocco, several studies have demonstrated the value of immunophenotyping in the characterization of acute leukemias. A study conducted at Ibn Rochd University Hospital Center in Casablanca involving 440 patients with acute lymphoblastic leukemia (ALL) reported a predominance of B-ALL (82.5%) over T-ALL (17.5%) [7]. In addition, a Moroccan study investigating aberrant phenotypes in AML involving 54 patients reported lymphoid antigen expression in 51.8% of cases, with CD7 being the most frequently expressed aberrant antigen [8]. These findings highlight the importance of immunophenotyping in the classification of acute leukemias in the Moroccan population.

In this context, the present study aimed to evaluate the contribution of flow cytometric immunophenotyping to the diagnosis of acute leukemias and to investigate its concordance with cytological findings in patients managed in the Department of Hematology and Immunohematology at Mohammed V Military Teaching Hospital in Rabat.

MATERIALS AND METHODS

1. Study Design, Period, and Setting

This was a retrospective study conducted over a two-year period, from January 2022 to December 2023, in the Department of Hematology and Immunohematology of Mohammed V Military Teaching Hospital, Rabat, Morocco.

2. Study Population

The study included 62 patients with acute leukemia who underwent both cytological examination and flow cytometric immunophenotyping.

Demographic, clinical, and biological data were collected from patient medical records. Demographic variables included age and sex. Clinical variables included, in particular, deterioration of general

condition, tumor syndrome, fever, and hemorrhagic syndrome. Hematological data included the main complete blood count parameters, particularly hemoglobin, white blood cell count, and platelet count.

3. Cytological Examination

Cytological examination was performed as part of the diagnostic work-up for acute leukemia.

Morphological analysis of blast cells provided an initial diagnostic orientation toward acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), MPO-negative acute myeloid leukemia, or an inconclusive profile.

Myeloperoxidase (MPO) activity, when available, was taken into account in lineage assignment.

4. Flow Cytometric Immunophenotyping

Immunophenotyping was performed by flow cytometry to determine the lineage of blast cells and characterize their antigenic profile.

The analysis was based on a panel of markers designed to identify the major hematopoietic lineages, as well as the degree of differentiation and cellular immaturity of blast cells. The markers analyzed included the myeloid antigens MPO, CD13, CD33, and CD117; the immaturity markers CD34 and HLA-DR; and various lymphoid markers.

Interpretation of immunophenotypic profiles was based on the combined analysis of the different markers rather than on the isolated expression of a single antigen, in accordance with the principles of multiparametric immunophenotyping of acute leukemias [1-3].

5. Immunophenotypic Classification

Patients were classified according to their immunophenotypic profile as AML, B- or T-lineage ALL, or other categories when the profile did not allow definitive lineage assignment.

Particular attention was paid to MPO-negative acute leukemias, atypical immunophenotypic profiles, and aberrant expression of lymphoid antigens in AML.

6. Cytology–Immunophenotyping Concordance Analysis

The results of cytological classification were compared with those obtained by immunophenotyping.

Concordance was defined as cases in which both methods resulted in the same diagnostic classification. Cases showing a difference between the two classifications or an inconclusive result by either method were considered discordant.

Discordant cases were analyzed to determine the contribution of immunophenotyping to the final

classification, particularly in cases of MPO-negative acute leukemia, inconclusive cytological diagnosis, or modification of the initially suspected lineage.

7. Statistical Analysis

Qualitative variables were expressed as frequencies and percentages. Quantitative variables were described using the mean, median, and range.

Associations between qualitative variables were assessed using Pearson's chi-square (χ^2) test. When the assumptions for the chi-square test were not met, Fisher's exact test was used. Statistical significance was defined as $p < 0.05$.

RESULTS

1. Demographic Characteristics

The study included 62 patients with acute leukemia managed between January 2022 and December 2023. A male predominance was observed, with 43 men (69.35%) and 19 women (30.65%), corresponding to a male-to-female ratio of 2.26.

The mean age was 52.95 years, with a median age of 62 years and a range of 18–96 years. The 65–75-year age group was the most represented, comprising 16 patients (25.81%).

Table 1: Demographic characteristics of the patients

Characteristic	n	%
Men	43	69.35
Women	19	30.65
Total	62	100
Mean age, years	52.95	—
Median age, years	62	—
Range, years	18–96	—
Age 65–75 years	16	25.81

2. Clinical and Hematological Findings

Deterioration of general condition was the most common clinical manifestation, observed in 28 patients (45.16%), followed by tumor syndrome in 27 patients (43.55%). Fever was reported in 17 patients (27.42%), while hemorrhagic syndrome was observed in 13 patients (20.97%).

From a hematological perspective, anemia was observed in 57 patients (91.94%) and thrombocytopenia in 55 patients (88.71%). Leukocytosis was observed in 34 cases (54.84%), whereas leukopenia was present in 16 patients (25.81%). Pancytopenia was observed in 14 cases (22.58%).

Table 2: Main clinical and hematological findings

Parameter	n	%
Clinical manifestations		
Deterioration of general condition	28	45.16
Tumor syndrome	27	43.55
Fever	17	27.42
Hemorrhagic syndrome	13	20.97
Hematological abnormalities		
Anemia	57	91.94
Thrombocytopenia	55	88.71
Leukocytosis	34	54.84
Leukopenia	16	25.81
Pancytopenia	14	22.58

The different abnormalities could coexist in the same patient.

3. Comparison between Cytology and Immunophenotyping

Cytology provided an initial diagnosis of acute myeloid leukemia (AML) in 33 cases (53.23%). Twenty-one cases (33.87%) were classified as MPO-negative acute leukemias. Two cases (3.23%) were considered acute lymphoblastic leukemia (ALL), two cases (3.23%) were classified as B-lineage acute leukemia, and four cases (6.45%) were inconclusive.

Following flow cytometric immunophenotyping, 41 patients (66.13%) were classified as AML and 14 (22.58%) as ALL. Two patients (3.23%) had B-lineage acute leukemia, whereas five cases (8.06%) remained inconclusive.

Thus, immunophenotyping enabled clarification of the classification in several cases that were initially difficult to characterize by cytology, particularly among MPO-negative acute leukemias.

Table 3: Comparison of diagnostic classification according to cytology and immunophenotyping

Diagnostic classification	Cytology n (%)	Immunophenotyping n (%)
AML	33 (53.23)	41 (66.13)
ALL	2 (3.23)	14 (22.58)
B-lineage acute leukemia	2 (3.23)	2 (3.23)
MPO-negative acute leukemia	21 (33.87)	—
Inconclusive	4 (6.45)	5 (8.06)
Total	62 (100)	62 (100)

Overall statistical comparison between the two methods was not associated with a p-value in this table because the data were aggregated rather than presented as a paired contingency table.

Among the 14 patients classified as ALL by immunophenotyping, 8 (57.14%) had a T-cell phenotype and 6 (42.86%) had a B-cell phenotype.

4. Immunophenotypic Profile of Acute Myeloid Leukemia

Among the 41 patients classified as AML by immunophenotyping, the most frequently expressed markers were CD33, detected in 33 cases (80.49%), followed by MPO in 32 cases (78.05%), and CD13 and CD117, each expressed in 31 cases (75.61%).

HLA-DR and CD34 were expressed in 73.17% and 65.85% of cases, respectively. Aberrant expression of the lymphoid marker CD7 was observed in 12 patients (29.27%).

Table 4: Immunophenotypic profile of AML

Marker	n	%
CD33	33	80.49
MPO	32	78.05
CD13	31	75.61
CD117	31	75.61
HLA-DR	30	73.17
CD34	27	65.85
cCD11	14	34.15
CD7	12	29.27
CD45	11	26.83
CD14	8	19.51
CD36	5	12.20
CD56	2	4.88
CD4	2	4.88
CD123	2	4.88
CD3	1	2.44
CD79a	1	2.44

Percentages were calculated among the 41 patients classified as AML by immunophenotyping.

5. Concordance between Cytology and Immunophenotyping

Comparison of the two diagnostic approaches showed concordance in 52 cases (83.87%) and discordance in 10 cases (16.13%).

Among the 10 discordant cases, three cases that were initially inconclusive by cytology could be

classified using immunophenotyping. Four cases initially interpreted cytologically as MPO-negative AML remained inconclusive after immunophenotypic analysis. The remaining three cases showed a change in diagnostic lineage. Among these, two cases initially considered biphenotypic by cytology were ultimately classified by immunophenotyping as T-ALL and AML, respectively.

Table 5: Concordance between cytology and immunophenotyping

Comparison result	n	%
Concordance	52	83.87
Discordance	10	16.13
Total	62	100

Table 6: Nature of diagnostic discrepancies

Discordant situation	n	% of discordant cases
Inconclusive cytology → classification by immunophenotyping	3	30.0
Cytological AML/MPO-negative → inconclusive immunophenotyping	4	40.0
Change in diagnostic lineage	3	30.0
Total	10	100

Table 7: Association between age and sex and leukemia type

Analysis	Test	p-value	Conclusion
Age × leukemia type	χ^2	0.562	Not significant
Sex × leukemia type	Fisher's exact test	0.438	Not significant

No statistically significant association was found between age and leukemia type ($\chi^2 = 39.94$; $df = 42$; $p = 0.562$). Similarly, no significant association was observed between sex and leukemia type (Fisher's exact test, $p = 0.438$).

These findings indicate that, in our series, leukemia type was not significantly associated with age or sex.

DISCUSSION

The diagnosis of acute leukemias currently relies on a multidimensional approach integrating clinical, morphological, immunophenotypic, cytogenetic, and molecular data. The WHO-HAEM5 and ICC classifications have reinforced this integrated approach, particularly in situations where morphological findings alone do not allow the lineage or diagnostic entity to be established with certainty [1, 2]. In this context, flow cytometry represents a major complementary tool to cytology, enabling rapid multiparametric characterization of blast cells [3, 4].

In our series of 62 patients, a male predominance was observed, with 43 men (69.35%) compared with 19 women (30.65%), corresponding to a male-to-female ratio of 2.26. The mean age was 52.95 years, whereas the median age was 62 years, with a range of 18–96 years. The 65–75-year age group represented the largest group (25.81%). This distribution reflects a predominantly adult and relatively elderly population. However, this distribution should be interpreted in light of the composition of our study population, which included several types of acute leukemia.

Clinically, deterioration of general condition was the most frequent manifestation (45.16%), followed by tumor syndrome (43.55%), fever (27.42%), and hemorrhagic syndrome (20.97%). Biologically, anemia was observed in 91.94% of patients and thrombocytopenia in 88.71%, whereas leukocytosis was present in 54.84% of cases. These findings are similar to those reported in a large Moroccan series of acute lymphoblastic leukemia, in which anemia and thrombocytopenia were observed in 86.5% and 89.4% of patients, respectively [7]. These hematological abnormalities are consistent with the bone marrow

failure frequently observed at the time of acute leukemia diagnosis.

The comparison between cytology and immunophenotyping represents one of the main findings of our study. Cytology initially suggested AML in 33 cases (53.23%), whereas 21 cases (33.87%) were classified as MPO-negative acute leukemias. In contrast, immunophenotyping identified AML in 41 cases (66.13%) and ALL in 14 cases (22.58%). This difference illustrates the added value of immunophenotyping when morphological or cytological features do not allow clear lineage assignment.

Flow cytometry enables the simultaneous assessment of multiple differentiation and immaturity markers and the establishment of an overall antigenic profile. European LeukemiaNet recommendations emphasize the importance of appropriately designed panels capable of identifying myeloid, B-cell, or T-cell lineage as well as aberrant profiles [3]. When interpreted together with morphological findings, multiparametric flow cytometry can therefore refine the diagnosis and reduce the differential diagnosis [4].

In our series, concordance between cytology and immunophenotyping was 83.87%, whereas 16.13% of cases were discordant. This concordance rate indicates that cytology remains an important diagnostic orientation method, while immunophenotyping provides complementary information in difficult cases. Published data similarly emphasize the complementary nature of morphology and flow cytometry in the diagnosis and classification of acute leukemias [3, 4].

Detailed analysis of discordant cases is particularly informative. Three cases initially considered inconclusive by cytology could be classified using immunophenotyping. Conversely, four cases initially classified as MPO-negative AML by cytology remained inconclusive after immunophenotypic analysis. Finally, three cases showed a change in the initially suspected lineage, including two cases considered biphenotypic by cytology that were ultimately classified as T-ALL and AML, respectively, by immunophenotyping. These observations illustrate the importance of multiparametric interpretation of antigen expression and the need to avoid

classifications based solely on the isolated expression of a single marker [1-3].

The diagnostic difficulty associated with acute leukemias of ambiguous lineage is precisely one of the situations in which flow cytometry is particularly valuable. The WHO-HAEM5 and ICC classifications rely on specific immunophenotypic criteria to assign lineage to blast cells and distinguish the different entities of acute leukemia of ambiguous lineage [1, 2]. The combined use of multiple markers therefore helps reduce the risk of classification based exclusively on morphological criteria or isolated antigen expression.

Among patients with AML in our study, the most frequently expressed antigens were CD33 (80.49%), MPO (78.05%), and CD13 and CD117 (75.61% each). The immaturity markers CD34 and HLA-DR were expressed in 65.85% and 73.17% of cases, respectively. This profile is consistent with the typical expression of myeloid and immaturity markers in AML. However, recent data emphasize that interpretation of these markers should be integrated with morphological, cytogenetic, and molecular findings to achieve an accurate classification [1-6].

Aberrant expression of lymphoid antigens represents another important finding of our study. CD7 was aberrantly expressed in 29.27% of AML cases. This observation is particularly relevant in light of Moroccan data. In a study conducted at Ibn Rochd University Hospital Center in Casablanca involving 54 patients with AML, aberrant lymphoid antigens were observed in 51.8% of cases, with CD7 being the most frequently expressed lymphoid antigen [8]. The authors also reported asynchronous antigen expression in 57% of cases. These findings confirm that aberrant phenotypes are relatively frequent in AML and may provide useful information for characterization and immunophenotypic monitoring.

In our population, immunophenotyping also identified 14 cases of ALL, including 8 T-ALL cases (57.14%) and 6 B-ALL cases (42.86%). This distribution differs from that reported in the large Moroccan series from Ibn Rochd University Hospital Center, which found 82.5% B-ALL and 17.5% T-ALL [7]. This difference should nevertheless be interpreted cautiously because of the small number of ALL cases in our series, as well as differences in age, study population, and recruitment setting.

Moroccan data concerning T-ALL also demonstrate substantial immunophenotypic heterogeneity. In a study involving 40 Moroccan adults with T-ALL, 45% had an immature pro-T or pre-T phenotype, 30% had a cortical CD1a-positive phenotype, and 25% had a medullary phenotype. CD34 expression was observed in 57.5% of cases, while expression of the myeloid antigens CD13 and/or CD33 was observed in

55% of cases [9]. These findings highlight the value of multiparametric analysis for accurate determination of lineage and differentiation stage.

Taken together, these results confirm that cytology and flow cytometry should be considered complementary rather than competing approaches. Cytology remains essential for initial diagnostic orientation and morphological assessment of blast cells, whereas immunophenotyping provides more precise characterization of their lineage, maturation stage, and aberrant antigenic profiles [3, 4]. This complementarity is particularly important in MPO-negative acute leukemias, acute leukemias of ambiguous lineage, and situations in which morphology is insufficiently specific.

Immunophenotyping is also valuable for patient monitoring. Establishment of an initial immunophenotypic profile may provide a baseline for subsequent assessment of measurable residual disease. In AML, European LeukemiaNet recommendations emphasize the importance of measurable residual disease in evaluating treatment response and prognostic stratification [5]. This further supports the importance of rigorous immunophenotypic characterization at diagnosis.

Study Limitations

This study has several limitations that should be considered when interpreting the results. It was a retrospective, single-center study involving a relatively limited sample of 62 patients, which may limit the generalizability of our findings to the Moroccan population as a whole. The small number of ALL cases also limits the statistical power of comparisons between different lymphoblastic subtypes.

Furthermore, cytogenetic and molecular data were not systematically incorporated into the analysis, although they currently play an important role in the classification and prognostic stratification of acute leukemias [1, 2]. Finally, the absence of complete longitudinal follow-up prevented assessment of the impact of aberrant immunophenotypic profiles on clinical outcomes and patient survival.

Prospective multicenter studies involving larger cohorts and incorporating cytogenetic, molecular, and measurable residual disease data are needed to confirm and further characterize these findings.

CONCLUSION

This study highlights the importance of flow cytometric immunophenotyping in the diagnosis and classification of acute leukemias. Although cytology remains an essential step in the initial diagnostic assessment, the 83.87% concordance observed in our series and the 16.13% of discordant cases demonstrate the added value of immunophenotyping, particularly in

MPO-negative acute leukemias and morphologically inconclusive cases.

The identification of immunophenotypic profiles, particularly the frequent expression of the myeloid markers CD33, MPO, CD13, and CD117, as well as aberrant CD7 expression in 29.27% of AML cases, highlights the value of a multiparametric approach. Combining cytology and flow cytometry can therefore improve lineage assignment and reduce classification errors.

These findings support the systematic integration of immunophenotyping into the diagnostic work-up of acute leukemias whenever this technique is available. Integration with cytogenetic and molecular data, in accordance with current international classifications, would further improve diagnostic accuracy and biological characterization of these hematological malignancies.

Acknowledgments

The authors would like to thank all staff members of the Department of Hematology and Immunohematology at Mohammed V Military Teaching Hospital, Rabat, for their contribution to patient care, laboratory analyses, and data collection, which made this study possible.

Conflict of Interest: The authors declare that they have no conflict of interest related to this study.

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Cite This Article: Jerroundi, Z., Khazraji, A., & Zahid, H. (2026). Contribution of Flow Cytometric Immunophenotyping to the Diagnosis of Acute. *East African Scholars J Med Sci*, 9(10), 421-427. <https://doi.org/10.36349/easms.2026.v09i10.002>