

Diagnosis of acute myeloid leukemia (AML) using flow cytometry

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ABSTRACT

The diagnosis of acute myeloid leukemia (AML) is frequently suspected and occasionally confirmed using peripheral blood examination. The current study aimed to evaluate the diagnostic methods used for AML diagnosis. This study included 360 newly diagnosed patients attending the Hematology Clinic at Baghdad Teaching Hospital, Medical City. The cohort included 147 men and 213 women, with a mean age of 45.8 years and an age range of 17–76 years. Diagnosis and classification were performed using peripheral blood smear examination, bone marrow aspiration, and flow cytometry. Flow cytometric immunophenotyping of bone marrow samples showed a distinct blast population on the CD45/side-scatter (SSC) dot plot, accounting for $70.0 \pm 3.5\%$ of gated events. The lowest blast percentage was 36%, observed in AML-M2 and AML-M6, whereas the highest blast percentage was 90%, observed in AML-M3. The most common FAB subtype in the AML group was AML-M0, accounting for 32% of cases, followed by AML-M3 at 26%. AML-M1 accounted for 7%, AML-M2 for 4%, AML-M4 for 12%, AML-M5 for 8%, AML-M6 for 5%, and AML-M7 for 7% of AML cases. A clear understanding of the pathological information required for AML diagnosis has become increasingly important for guiding treatment decisions and improving patient outcomes.

Keywords: AML, Diagnosis, Flow Cytometer

1 INTRODUCTION

Acute myeloid leukemia (AML) is a malignancy characterized by impaired differentiation of hematopoietic stem and progenitor cells [1]. AML is the second most common cause of childhood leukemia and the most common type of acute leukemia in adults [2]. Patient survival depends on several factors, including age and the presence of cytogenetic or molecular genetic abnormalities [3]. Most AML subtypes are treated primarily with chemotherapy, which may result in complete remission in some cases; however, many patients eventually relapse and die from the disease. Therefore, novel therapeutic strategies are needed [1,3].

Several classification systems for AML have been developed based on etiology, morphology, immunophenotype, and genetic features. The older French–American–British (FAB) classification and the

more recent World Health Organization (WHO) classification are two major systems used to categorize AML. The FAB classification, developed in the 1970s, defined eight major AML subtypes, from M0 to M7, primarily based on morphology, immunophenotype, and cytochemical criteria [4]. The WHO classification has largely replaced the older FAB classification and is currently considered the standard approach for AML classification. AML classification has traditionally been based on blast cell count, lineage commitment, and the degree of differentiation of neoplastic cells.

Since the 1970s, when cytomorphology and cytochemistry were the main diagnostic tools available, the diagnosis of acute leukemia has undergone major advances. Acute leukemia can now be identified and subclassified more accurately through the development and integration of modern diagnostic technologies using a multimodal

approach [5].

Immunophenotyping using multiparameter flow cytometry (MFC) is used to identify leukemia and lymphoma subtypes [6]. By measuring the binding of fluorescently labeled antibodies using MFC, the expression of several antigens or cluster of differentiation (CD) markers on the cell membrane can be semi-quantified. Antibody binding produces an immunophenotypic profile that is specific to a particular cellular lineage, developmental stage, or disease state [7]. To ensure accurate lineage assignment and determine the degree of differentiation, at least 8-color MFC is recommended [8]. A typical cutoff for considering leukemic cells positive for a specific marker is approximately 20%. However, lower cutoffs, such as 10%, have been used for some CD markers, including CD34, cytoplasmic CD3, and CD117 [8]. CD marker profiles can be assessed using several body fluids or specimens, including peripheral blood (PB), bone marrow (BM) aspirate, and lymph node cell suspensions. Thousands of cells from a sample can be examined within minutes.

Flow cytometry is one of the most effective analytical tools used in biological laboratories. This method is widely used to identify cellular phenotypes and investigate mechanisms in cell-based assays. The ability to rapidly and thoroughly analyze complex cell populations, together with the qualitative and quantitative nature of the obtained data, makes flow cytometry an efficient analytical technique [9]. The basic principle of this method is to measure the optical and fluorescence characteristics of specifically labeled particles as they pass in a single-file stream through a fluid system. Physical characteristics, such as cell size and internal complexity, can be determined by measuring light scatter. Fluorescent markers can also be used to distinguish various cell-surface and cytoplasmic antigens, measure DNA or RNA content, and detect several membrane-bound and intracellular proteins [9].

2 MATERIALS AND METHODS

2.1 Samples

This study included 360 patients with acute myeloid leukemia who attended the Hematology Center at Baghdad Teaching Hospital, Medical City. AML diagnosis was based on specialist physical examination, morphological evaluation of peripheral blood films, bone marrow aspirate smear analysis, and flow cytometric immunophenotyping. The patients' ages ranged from 17 to 76

years, with a median age of 46.5 years. Diagnosis was performed using standard morphological examination of peripheral blood smears according to the criteria of the FAB Cooperative Study Group and was confirmed by immunophenotyping of bone marrow aspirates according to the standards established by the British Committee for Standards in Hematology (BCSH) and the European Group for the Immunological Classification of Leukemias [9].

2.1.1 Blood samples

Peripheral blood samples of 5 mL were collected from patients and transferred into vials containing ethylenediaminetetraacetic acid (EDTA).

2.1.2 Bone marrow samples

Bone marrow aspiration was performed by a physician after cleaning the skin around the hip and injecting local anesthesia to numb the area and bone surface. A small hollow needle was then inserted into the bone, and a small amount of bone marrow fluid was withdrawn using a syringe. In the diagnosis of hematological diseases, bone marrow aspirates and biopsies provide valuable information and, in some cases, may be essential for establishing a proper diagnosis. Marrow can be sampled using a trephine or aspiration needle. Patients were positioned appropriately on the examination table before bone marrow aspiration.

2.2 Morphological diagnoses (blood film)

2.2.1 Preparation of the giemsa stain stock solution

Approximately 3.8 g of Giemsa powder (IndiaMART, India) was added to 250 mL of methanol and dissolved. The solution was heated to 60 °C. Then, 250 mL of glycerin (IndiaMART, India) was added slowly to the solution. The solution was filtered and left to stand for approximately 1–2 months before use.

2.2.2 Preparation of the working solution

The working solution was prepared by adding 10 mL of stock solution to 80 mL of distilled water and 10 mL of methanol.

2.2.3 Blood film preparation

A blood smear was prepared by placing a drop of blood near the end of a glass slide and spreading it to separate the cells. This allowed sufficient spacing for distinguishing and counting cells. The slide was left to

dry. The blood film was then fixed by briefly dipping the slide in methanol, followed by leaving the slide for 5 min to allow methanol evaporation. Fixation is essential for obtaining good staining quality and improving cellular visualization. The slide was covered with the prepared stain and left for 30 min. The slides were then washed with water, left to dry, and examined under a light microscope by a hematologist.

2.3 Flow cytometric immunophenotyping

AML diagnosis was performed using bone marrow samples and flow cytometric immunophenotyping of bone marrow aspirates. A standard red blood cell lysis protocol was used for specimen processing. Single-cell suspensions were stained using different fluorochrome-conjugated antibody combinations and compared with isotype-matched control antibodies. Samples were stained with monoclonal antibodies against the following antigens: HLA-DR, CD117, CD103, CD56, CD45, CD38, CD36, CD34, CD33, CD25, CD22, CD20, CD19, CD16, CD15, CD14, CD13, CD11c, CD11b, CD10, CD8, CD7, CD5, CD4, CD3, cytoplasmic myeloperoxidase (cMPO), IREM2, TdT, CD71, and CD2. Data were acquired using a FACSCalibur double-laser cytometer (Becton Dickinson Biosciences) and analyzed using CellQuest software (Becton Dickinson Biosciences) [10].

2.4 Blast cells immune checkpoints flow cytometry staining principle

In this study, 8-color flow cytometry analysis was used to identify the immunophenotype of blast cells. The staining protocol was applied to all collected blood samples in EDTA according to the manufacturer's instructions. CD45 was used in the initial gating strategy in combination with side scatter (SSC), allowing identification of the most important cell populations on a single plot and differentiation from residual granulocytes, lymphocytes, and monocytes. A panel of fluorochrome-conjugated anti-human antibodies (Elabscience, USA) was used. The panel included extracellular staining with anti-CD45 conjugated to fluorescein isothiocyanate (FITC).

2.5 Gating

Flow cytometry was introduced into the routine investigation of whole bone marrow samples after red blood cell lysis, based on a primary CD45/SSC gating procedure. Gating was performed under the supervision of a hematopathology advisor. A forward scatter height

(FSC-H) versus forward scatter area (FSC-A) density plot was used, followed by an SSC versus CD45 gate to identify CD45-positive leukocytes. Thereafter, gating based on size and granularity was used to identify different cell populations. Smaller and less complex cells were defined as lymphocytes and appeared in the lower part of the dot plot. Monocytes, which showed intermediate size and complexity, were located in the central part of the graph. Granulocytes, which had high internal complexity, appeared in the upper part of the graph. CD45 expression was used together with scatter characteristics to differentiate CD45-positive blast cells.

2.6 Data analysis

The relevant cell populations in each investigated group were calculated using data from the flow cytometer hierarchy tables. Analysis was performed using a BD FACSCanto flow cytometer with BD FACSDiva software [11]. Values were expressed as percentages.

2.7 Statistical analysis

IBM SPSS Statistics version 23 for Microsoft Windows (SPSS Inc.) was used for statistical analysis. Minimum and maximum values, mean, median, and standard deviation (SD) were calculated. A p value ≤ 0.05 was considered statistically significant.

3 RESULTS

3.1 Baseline characteristics of included patients

A total of 360 patients with leukemia were included in this study at the Hematology Center, Baghdad Teaching Hospital, Medical City. Sample collection was conducted between September 2022 and June 2025. The cohort included 147 men and 213 women, with ages ranging from 17 to 76 years.

3.2 Morphological diagnoses [blood film] and immunophenotyping

In this study, blast cells accounted for approximately 70% of cells in bone marrow aspirates. These cells were medium to large in size and showed immature chromatin, one or two prominent nucleoli, nuclear herniation with protrusion of chromatin material in the form of micronuclei, and abundant basophilic cytoplasm with numerous azurophilic granules and cytoplasmic fragmentation. Dysplastic changes were visible in neutrophils.

A stepwise hematological diagnostic approach began with cytological examination of peripheral blood and

bone marrow, along with quantification of blast cells. Differences in chromatin pattern and nuclear shape were observed among the various blast cell subtypes. A representative blast cell image showing irregular nuclear chromatin and an indistinct nuclear–cytoplasmic margin is presented in Figure 1.

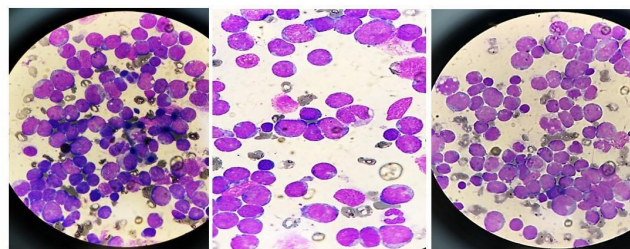


Fig. 1 Bone marrow film of a patient with AML; most cells are myeloid blasts, with moderate cytoplasm and large hyperchromatic nuclei with some nuclear indentations [Giemsa, 40X].

3.3 Immunophenotyping

Flow cytometry results showed that all patients previously diagnosed with AML by peripheral blood smear, based on the presence of blast cells, were confirmed to have AML. All cases showed more than 20% blast cells and expressed several immunophenotypic markers on the cell surface, allowing identification of AML subtypes. Leukemic blast cells were characterized using flow cytometry. The proportion of cells expressing a specific marker, with fluorescence intensity exceeding the background threshold, was used as a measure of marker expression. Thirty markers were used to describe each patient. The traditional approach for analyzing these data depends on determining whether marker expression is positive or negative. Based on this approach, patients were classified into subgroups according to the positive or negative expression of specific markers.

Newly diagnosed acute leukemia cases during the study period ($n = 360$) were classified as AML. Flow cytometric immunophenotypic analysis of bone marrow samples revealed a distinct cell cluster in the blast region on the CD45/SSC dot plot, comprising $70\% \pm 3.457$ of all gated events. The most common FAB subtype in the AML group was AML-M0, accounting for 32% of cases, followed by AML-M3 at 26%. AML-M1 accounted for 7%, AML-M2 for 4%, AML-M4 for 12%, AML-M5 for 8%, AML-M6 for 5%, and AML-M7 for 7% of cases (Table 1).

Flow cytometry results showed that all patients ($n = 360$), who had been previously diagnosed with AML by peripheral blood smear, had more than 20% primitive cells, or blast cells. The lowest blast percentage was 36%, observed in AML-M2 and AML-M6, whereas the highest blast percentage was 90%, observed in AML-M3.

In this study, approximately 32% of patients were diagnosed with AML-M0. These patients had $95\% \pm 19.4018806$ blast cells ($p \leq 0.05$), which showed the lowest degree of differentiation. These cells expressed several CD markers on their surfaces, including MPO in 40%, CD117 in 100%, CD33 in 100%, CD34 in 88%, HLA-DR in 63%, and CD13 in 88% of cases.

The results also showed that 7% of patients had AML-M1, with $48\% \pm 21.7940850$ blast cells ($p \leq 0.05$). These cells had an aspherical nucleus and slightly granulated cytoplasm with Auer rods. CD marker expression in blast cells was as follows: CD13, 61%; CD33, 92%; CD38, 75%; CD71, 54%; and CD117, 90%.

Only 4% of patients had the AML-M2 subtype, in which blast cells accounted for $56\% \pm 17.2586532$ ($p \leq 0.05$). Auer rods were observed in some cases, and some partially differentiated myeloid cells were also present. CD marker expression levels were CD34, 87%; CD117, 70%; CD13, 55%; CD33, 95%; HLA-DR, 44%; and CD56, 34%.

Approximately 26% of patients had AML-M3, in which blast cells accounted for $87\% \pm 18.3340010$ ($p \leq 0.05$). These cells were promyelocytic cells with round nuclei, strongly granulated cytoplasm, and Auer rods, which were observed in most cases.

Another AML subtype was AML-M4, which accounted for approximately 12% of cases, with blast cells accounting for $74\% \pm 14.3449565$ ($p \leq 0.05$). These cells showed both myeloid and monocytoid features. CD marker expression was as follows: cMPO, 76%; CD34, 40%; CD31, 96%; CD33, 79%; CD64, 45%; CD35, 33%; CD117, 28%; CD7, 26%; HLA-DR, 69%; CD11b, 47%; CD14, 45%; and IREM2, 43%.

In addition, 8% of patients had AML-M5, with $88\% \pm 23.9603923$ blast cells ($p \leq 0.05$). These cells had prominent nuclei, multiple nucleoli in some cases, and lightly granulated cytoplasm. They expressed CD markers at varying percentages: CD4, 40%; CD34, 40%; CD13, 90%; CD33, 90%; CD64, 45%; CD117, 60%; CD11c, 25%; and CD14, 30%.

The results also showed that approximately 5% of patients had AML-M6, with $44\% \pm 7.7053727$ blast

cells ($p \leq 0.05$). These patients were diagnosed based on the presence of early-stage cells showing abnormal proliferation of erythroid cells, leukocytes, and bizarre megakaryocytes. Their CD marker expression was CD7, 59%; CD15, 66%; CD56, 76%; CD38, 99%; CD64, 78%; CD33, 100%; CD117, 91%; and CD13, 90%.

The final AML subtype identified in this study was

AML-M7, which accounted for 7% of cases. These patients had $71\% \pm 22.1237244$ blast cells ($p \leq 0.05$). The cells were morphologically undifferentiated, with multinucleation and budding cytoplasm. CD marker expression was CD34, 100%; CD33, 100%; and CD117, 70% (Table 1; Figures 2, and 3).

Table 1 Percentage of AML patients according to FAB type

FAB type	Percentage	Blast cells (%) Mean $\pm$ SD	Morphology of blast cells	Positive CD (%)
M0	32%	$95\% \pm 19.4018806$	Blast cells were in the lowest degree of differentiation	MPO 40% CD117 100% CD33 100% CD34 88% HLA-DR 63% CD13 88%
M1	7%	$48\% \pm 21.7940850$	Blast cells had aspherical nucleus and slightly granulated cytoplasm with Auer rods in some cases	CD13 61% CD33 92% CD38 75% CD71 54% CD117 90%
M2	4%	$56\% \pm 17.2586532$	Partially differentiated myeloid cells with Auer rods in some cases	CD34 87% CD117 70% CD13 55% HLA-DR 44% CD33 95% CD56 34%
M3	26%	$87\% \pm 18.3340010$	Progranulocytic cells had round nuclei with strongly granulated cytoplasm and Auer rods seen in most cases	cMPO 15% CD13 50% CD117 50% CD7 30% CD33 90%
M4	12%	$74\% \pm 14.3449565$	Blast cells with both myeloid and monocytoid appearance	cMPO 76% CD34 40% CD31 96% CD33 79% CD64 45% CD35 33% CD117 28% CD7 26% HLA-DR 69% CD11b 47% CD14 45% IREM2 43%
M5	8%	$88\% \pm 23.9603923$	Blast cells had remarkable nuclei with so many nucleoli in some cases and lightly granulated cytoplasm	CD4 40% CD34 40% CD13 90% CD33 90% CD64 45% CD117 60% CD11c 25% CD14 30%

Continued on next page

Table 1 (continued)

FAB type	Percentage	Blast cells (%) Mean $\pm$ SD	Morphology of blast cells	Positive CD (%)
M6	5%	44% $\pm$ 7.7053727	Early-stage cells of abnormal proliferation of multicellular erythrocytes, leukocytes, and bizarre megakaryocytes	CD7 59% CD15 66% CD56 76% CD38 99% CD64 78% CD33 100% CD117 91% CD13 90%
M7	7%	71% $\pm$ 22.1237244	Undifferentiated morphologically with multinucleated and buddle cytoplasm	CD34 100% CD33 100% CD117 70%

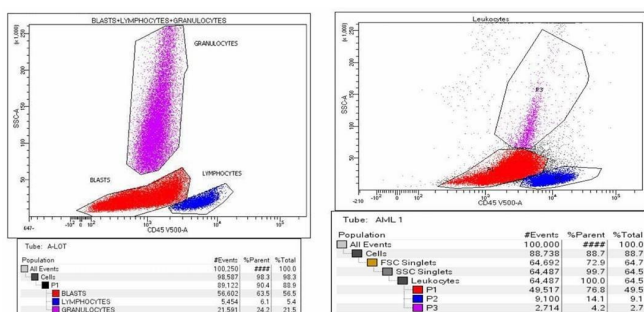


Fig. 2 Flow cytometric AML immunophenotypic analysis of the bone marrow blood samples revealed the presence of distinct cell cluster in lower granulocyte region on the CD45/SSC dot plot comprising of all gated events with moderate CD45, low to intermediate SSC signal and having a FSC with the majority of cells falling into the small size cell range.

4 DISCUSSION

The diagnosis of AML relies on an increased number of progenitor cells in the bone marrow and peripheral blood, usually exceeding 20%. In addition, the presence of specific immunological markers on the surface of progenitor cells was used to diagnose AML subtypes.

In this study, flow cytometry was used for AML diagnosis by detecting the binding of different fluorescein-labeled monoclonal antibodies to immunological markers on cell surfaces. Therefore, flow cytometry is important for identifying AML subtypes according to FAB classification.

Flow cytometry plays a crucial role in cell identification through immunophenotyping. This technique can distinguish malignant cells from normal cells because blast cells are characterized by multiple

immunophenotypic patterns that differ from those of normal cells. Furthermore, flow cytometry can identify different cell types at various maturation stages because cell-surface antigen expression changes during cellular development.

Several clinical studies on AML have been conducted in Iraq; however, most have lacked a precise diagnostic mechanism and did not determine the distribution of AML subtypes according to FAB classification. Therefore, investigating techniques that help identify different immunological marker patterns is important for keeping pace with modern classification systems, including WHO and FAB-based classifications.

In a previous Iraqi study, 34.78% of cases were identified as AML-M0 and 65.22% as T-ALL using flow cytometry; however, the other FAB subtypes were not mentioned [12].

In the current study, as the first diagnostic step, blood smears were prepared from patients and examined to observe cellular morphology. This step plays an important role in identifying disease subtypes before flow cytometry is performed. It enabled assessment of cell shape, nuclear morphology, cytoplasmic features, and the nature of cytoplasmic granules, helping to determine the maturation stage of cells in each subtype. This is consistent with previous studies in which morphology alone was used for the diagnosis and classification of leukemia. The French–American–British (FAB) classification, developed in 1976 by seven hematologists, divided acute myeloid leukemia (AML) into stages M1 through M6 according to the differentiation stage at

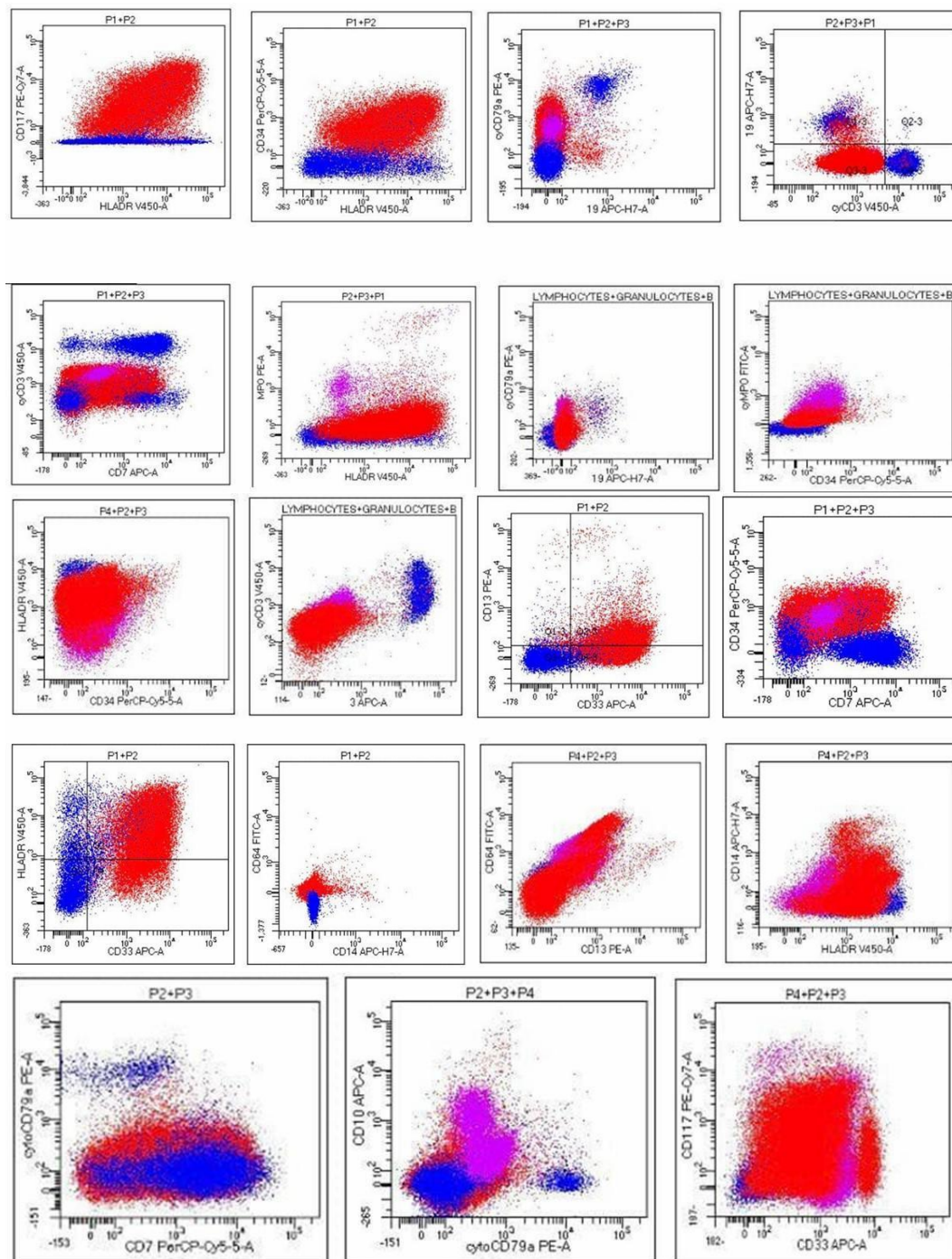


Fig. 3 Flow cytometry immunophenotyping of AML case. Immune phenotypic features of AML, bone marrow aspirate. Side scatter [SS] is plotted against antigen expression. Red Blast Cells, blue lymphocytes; purple, granulocytes. The blast cells express CD33, CD13, CD11b, HLA-DR, CD34 and CD14, but lack MPO and CD10.

which most leukemic cells were arrested [4]. Later, M0 and M7 were added to include minimally differentiated AML and acute megakaryoblastic leukemia, respectively.

In the current study, we relied on the phenotype of progenitor cells and the presence of immunological markers on their surfaces using flow cytometry to confirm subtype diagnosis and support treatment planning. This approach is consistent with the World Health Organization classification, in which AML is classified using a combination of clinical features, morphology, immunophenotype, and genetics [13]. Immunophenotyping is used to identify cells based on the types of antigens or markers present on the cell surface. Leukemia cells can be differentiated using appropriate antigen panels [14]. Flow cytometers are advanced medical tools used for immunophenotyping. However, flow cytometric techniques are expensive and require specialized personnel. Technical support and quality assurance programs for flow cytometry are often not easily accessible in countries with limited resources [15]. Nevertheless, the current standard of care requires immunophenotyping for proper classification of all acute leukemia cases.

In this study, clear differences were observed in the morphology of primitive cells in terms of cell size, nuclear shape, number of nucleoli, and the number, shape, and nature of cytoplasmic granules. This is consistent with previous studies that relied on white blood cell characteristics, such as cell size, shape, and nuclear chromatin structure, which can be directly observed through microscopic morphological examination by hematologists to distinguish normal white blood cells from blast cells [16]. These morphological characteristics can also be used to categorize blast cells as ALL or AML. Microscopic analysis of bone marrow smears is inexpensive and represents an effective screening tool in both adults and children. It provides rapid and reliable information on several hematological malignancies and reflects the functional state of the bone marrow.

In addition, because AML is highly cellularly heterogeneous, multiple markers must be used for

accurate diagnosis, as each subtype is characterized by simultaneous positive expression of several markers on cell surfaces. Numerous previous studies have highlighted the importance and effectiveness of flow cytometry in AML diagnosis [5, 12].

In clinical laboratories, immunophenotypic analysis of acute leukemia by flow cytometry has become widely used as a powerful tool for accurately identifying myeloid or lymphoid lineage. Immunophenotyping remains necessary for accurate determination of myeloid lineage in minimally differentiated acute myeloid leukemia (AML-M0) and for distinguishing B-cell or T-cell lineage in acute lymphoblastic leukemia (ALL), although many acute leukemias can be correctly identified morphologically with or without enzyme cytochemical analysis [5].

In this study, all cases expressed CD45, with a mean positivity of $70\% \pm 3.457$. HLA-DR was expressed in $65.5\% \pm 2.97$ of all AML cases, with the strongest positivity of 95% and the weakest positivity of 30%. CD34 showed a mean positivity of $63.1\% \pm 3.27$ among all AML subtypes, with the strongest expression of 100% and the weakest expression of 20%. CD117 was studied among the blast population, with a mean positivity of 50.72%, ranging from 20% to 100%. CD13 showed a mean positivity of 74.72% among all AML subtypes, with the strongest expression of 100% and the weakest expression of 42%. CD33 showed a mean positivity of $92.72\% \pm 1.42$, with the strongest expression of 100% and the weakest expression of 60%. CD7 was positive in 27% of cases, while CD10 was positive in approximately 13% of cases.

These results are consistent with other studies showing that CD13 and CD33 are traditionally expressed in AML, along with developmental markers such as CD34 and CD117. Myeloperoxidase (MPO) is detected in the cytoplasm. A proportion of blasts that mature toward the neutrophil lineage may express CD15 and/or CD65 but not CD11b. In many cases, weak or aberrant expression of the B-lineage antigen CD19 may also be observed [17]. Another study noted that blast cells frequently express CD56 and that this expression is associated with a worse

prognosis [18]. Immature granulocytes in these patients may also express CD56 [19].

Typical myeloid markers, such as CD13, CD33, and frequently CD117, are expressed by blast cells. However, CD34 expression may be absent in some AML subtypes. Therefore, testing an essential marker panel is important for strengthening the diagnosis. Flow cytometry also shows strong MPO expression. Leukemic cells in some AML subtypes may not express the classically tested neutrophil antigens CD15 and CD16. The absence of HLA-DR, which is frequently expressed on myeloid blasts in several AML types, is another important characteristic, particularly in acute promyelocytic leukemia [20].

Myeloid blasts may also express monocytic markers, such as CD4, CD36, or CD38 [21]. Neutrophil-associated markers, such as CD15 and/or CD65, may be expressed by part of the blast population. The tendency of these blasts to differentiate toward neutrophilic and monocytic lineages may also result in clear expression of CD11b, an antigen typically expressed more strongly by monocytes than by neutrophils. CD2 may also be positive in some cases.

Before fluorescence in situ hybridization (FISH) and/or conventional cytogenetic analysis confirm inversion or translocation, evaluation of cytological features, especially in bone marrow, together with immunophenotypic features, may suggest the diagnosis. In some cases, the immunophenotype demonstrates the presence of monocytic markers without CD19 expression. Some cases may show CD4 positivity. CD34 is frequently absent, and asynchronous expression between CD33 positivity and CD13 negativity may be observed. In addition, monocytic differentiation may be inferred from weak co-expression of CD15 and/or CD65, usually in the absence of CD14 [22].

In some AML subtypes, strong morphological or immunophenotypic indicators may be absent. Oyarzo et al. [23] described the immunophenotype of AML with this abnormality as immature myeloid progenitors co-expressing CD34, CD117, CD33, and CD13 in most cases, along with CD9 and CD38.

Alsabeh et al. [24] reported more variable CD34 expression in a previous series. In the absence of increased basophils, there may be no strong morphological or immunophenotypic indicators; however, this diagnosis may still be suspected in certain cases. Immature myeloid cells may co-express CD34, CD117, CD13, and CD33 and are frequently MPO-negative [24]. Although CD7, CD11c, CD11b, and CD123 are frequently expressed, CD56 positivity is less common [25].

Acute promyelocytic leukemia (APL/AML-M3) and AML-M1/M2 can be distinguished from each other by the expression patterns of CD34 and HLA-DR. Although the immunophenotypic patterns of acute leukemia are well known, the diagnostic value and specificity of immunophenotypic markers for different acute leukemia subtypes are less clearly established [26]. In flow cytometric immunophenotyping of acute leukemia, surface CD34 and HLA-DR are considered immaturity-associated antigens related to hematopoietic stem and precursor cells. CD34 is a transmembrane marker commonly expressed on hematopoietic stem cells and early thymic T-cell precursors. It is one of the most widely used markers of hematopoietic stem cells and is involved in stem cell differentiation, expansion, signaling, and adhesion regulation [27]. Kermani demonstrated that HLA-DR was present in most acute leukemias, except T-ALL [28]. Callea et al. identified a significant association with AML-M4/M5 subtypes, whereas Hamed and Fakher El-Deen demonstrated a strong association between HLA-DR and CD34 positivity and AML-M0/M1 subtypes [29, 30].

A previous study found that immaturity-associated antigens, including CD34, CD117, and HLA-DR, were expressed at high levels, along with strong MPO expression and asynchronous expression of CD15 and CD65. In addition, CD7 and CD56 were frequently expressed. Interestingly, CD64 was expressed by both neutrophils and blasts. Flow cytometry also showed features of erythroid dysplasia that could be observed morphologically, including immature erythroblasts and positivity for CD117 and CD105, together with low levels of CD36 and

CD71 [31].

Blasts from bone marrow or peripheral blood can be used to diagnose AML. Excess blast cells in bone marrow and often in peripheral blood are characteristic features of acute leukemia. For further prognostic evaluation, reliable differentiation into distinct subclasses is important. Morphology, immunophenotype, and cytochemistry are primarily used for this purpose, together with cytogenetic and molecular genetic analysis [32]. It should be noted that the blast cell phenotype in different FAB variants of AML may show unusual features shared by both children and adults [33].

The number of blast cells in bone marrow may remain constant in some patients diagnosed with “preleukemia” or “smoldering acute leukemia” for months or even years. A precise explanation for this phenomenon is not fully established. Possible explanations include the short lifespan of newly formed blast cells before intramedullary cell death, creating a steady state between blast cell production and death. In addition, some blast cells may differentiate into cells involved in normal hematopoiesis [34]. It is therefore reasonable to infer that morphologically undifferentiated cells, or blast cells, represent precursors of hematopoiesis or of a hematopoietic subset.

5 CONCLUSION

In conclusion, this study demonstrates that flow cytometry plays an important role in the classification and diagnosis of AML. AML subtypes were identified according to the FAB classification. The findings also indicate a strong relationship between the morphology of primitive cells under the microscope and immunophenotyping results obtained by flow cytometry. Identification of AML subtypes according to FAB classification depends on the presence of specific CD markers on blast cells. A clear understanding of the pathological information required for AML diagnosis has become increasingly important for guiding treatment decisions and improving patient outcomes.

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Data availability

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DECLARATIONS

Conflict of interest

The authors declare no competing interests

Consent to publish

N/A

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