



### Assessment of Hematological Parameters and Immune Cell CD Markers in Iraqi patients with Acute Myeloid Leukemia

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Article's Information	Abstract
Received: 05.07.2024 Accepted: 26.08.2024 Published: 15.03.2025	Acute Myeloid Leukemia (AML) is a heterogeneous hematologic malignancy characterized by varying genetic and immunophenotypic profiles. This study includes 120 participants classified into 40 healthy subjects, 40 newly diagnosed AML, and 40 treated AML patients analyzed demographic characteristics, hematological parameters, and immunological biomarkers in newly diagnosed and treated AML patients
<b>Keywords:</b> AML Hematological Parameters CD Markers Immunophenotyping Flowcytometry CBC	in a Baghdad teaching hospital, Baghdad, Iraq. From the period of December 2023 and March 2024. The results revealed statistically significant differences (P value <0.05). CD marker analysis revealed high positivity rates for CD33, CD13, CD117, MPO, and CD64 in AML patients, with CD33 being the most frequent marker. No statistically significant differences were found in HB, monocytes, and PLT counts across the in both groups (P Value >0.05). The study underscores the significance of regular assessment of parameters for effective diagnosis and treatment, highlighting the potential of CD33 and CD13 as therapeutic targets in AML.

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### 1. Introduction

Acute Myeloid Leukemia (AML) is a highly aggressive blood malignancy characterized by the rapid proliferation of immature myeloid precursors in the bone marrow and peripheral organs [1]. The prevalence of AML in Iraq, accounting for 19.2% of leukemia cases, highlights the urgent need for region-specific studies to improve diagnosis. treatment. and healthcare strategies 2 Hematological parameters such as white blood cell (WBC) count, red blood cell (RBC) count, and platelet count are critical for understanding disease progression and treatment response in AML patients [3, 4]. The expression of cluster of differentiation (CD) markers, such as CD33, CD13, and CD117, is essential for diagnosing and classifying AML subtypes, as well as for developing targeted therapies [5]. CD markers provide valuable insights into the immunophenotypic profile of AML, treatment which can significantly influence outcomes [6]. Recent studies have shown that newly diagnosed AML patients exhibit significantly higher

WBC counts and lower RBC counts compared to healthy controls, reflecting the aggressive nature of the disease and the impact on hematopoiesis [7]. Moreover, the presence of specific CD markers is associated with the prognosis and therapeutic targets, underscoring their importance in the management of AML [8]. Recent studies have shown that newly diagnosed AML patients exhibit significantly higher WBC counts compared to healthy controls, reflecting the aggressive nature of the disease and the impact on hematopoiesis [9]. Elevated WBC counts are often accompanied by a marked increase in blast cells, which are immature cells that proliferate uncontrollably and hinder normal blood cell formation [10]. This disruption in hematopoiesis leads to various complications, including anemia, thrombocytopenia, and increased susceptibility to infections [11]. Furthermore, the analysis of CD marker expression in AML patients provides essential prognostic information. For example, high levels of CD34, a stem cell marker, are associated with a poorer prognosis and

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[12].resistance to standard chemotherapy Conversely, patients with low CD34 expression tend to respond better to treatment. The presence of specific CD markers, such as CD33 and CD13, is also linked to the effectiveness of targeted therapies, emphasizing the importance of comprehensive immunophenotyping in AML management [13]. This study aims to evaluate the hematological parameters and Immune cells CD marker expressions in newly diagnosed and treated AML patients compared to healthy controls in a teaching Baghdad. Bv hospital in analyzing these parameters, the research seeks to provide critical clinical the biological and insights into characteristics of AML in Iraqi patients, ultimately contributing to more effective diagnosis and treatment strategies tailored to this population.

### 2. Materials and Methods

#### 2.1. Subjects

The sample includes 80 patients with AML, 40 newly diagnosed patients, and 40 those who have received treatment, as well as 40 healthy controls. The study was conducted between December 2023 and March 2024 at the College of Biotechnology, Al-Nahrain University, Baghdad, Iraq. The study was approved by the Ethics Committee 2374/3/2, date 22-10-2023 of the College of Biotechnology, Al-Nahrain University, Baghdad, Iraq.

Five ml of peripheral blood was transferred to an EDTA tube (2ml). The tubes were allowed to mix using roller mixer for 15 min. Each sample was run

using fully automated CBC differential count analyzer (Genex, China). Surface markers were added to whole blood, incubated for 15 cell minutes. followed by RBC lysis and centrifugation. The conjugated WBCs with specific monoclonal Antibody was washed twice with cell wash buffer, and then added to 500 µl of cell sheath, and read using BD Biosciences, Germany flow cytometer.

### 2.2. Statistical analysis

GRAPH Pad Prism v-8 and IBM SPSS Statistics v-27 were used to calculate the mean and standard error.

#### 3. Results and Discussion

In three distinct groups: control, newly diagnosed, and treated. In the control group, 57.5% are males (23 out of 40) and 42.5% are females (17 out of 40). For the newly diagnosed patients, males comprise 52.5% (21 out of 40), and females account for 47.5% (19 out of 40). In the treated group, males increase to 62.5% (25 out of 40), while females decrease to 37.5% (15 out of 40). Overall, each group consists of 40 individuals, totaling 120 across all groups. The statistical analysis reveals no significant difference in sex distribution among the groups, with a probability value of 0.231, indicating that sex does not significantly impact the distribution of AML patients across these groups (Table 1).

Table 1. Distribution of finite patients and control according to the sex					
Groups Control No.		Control No. (%)	Newly diagnosed No. (%)	Treated No. (%)	Р
SEX	Males	23 (57.5)	21 (52.5)	25(62.5)	0.231  NS
	Females	17 (42.5)	19 (47.5)	15 (37.5)	
	Total	40 (100)	40 (100)	40 (100)	
P; p. value.	NS; No significant di	fferences, p>0.05			

Table 1. Distribution of AML patients and control according to the sex

Hematological profile analysis

The study assessed hematological parameters in newly diagnosed and treated AML patients. Results showed that newly diagnosed AML cases had significantly higher WBC count and lower RBC count compared to the control and treated groups. Lymphocyte percentages were also low in newly diagnosed AML patients. However, no significant differences were found in HB, monocytes, and platelet counts between the two groups. Hemoglobin levels were comparable between controls, newly diagnosed AML patients, and treated AML patients. No significant difference was observed in monocyte percentages or platelet counts. The results are presented in table 2.

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Groups Controls		10^9/L			(Mean±SE)			
		WBC	RBC	HB (g/dl)	Lymph (%)	Mono (%)	PLT 10^9/L	
		7.4±0.3 A	4.7±0.3 A	11.3±1.4 A	44.2±6.3 A	6.1±0.8 A	185.9±6.8 A	
AML	Newly Diagnosed	33.3±2.9 B	2.7±1.2 B	8.2±1.3 A	12.2±3.4 B	10±3.6 A	106.5±8.6 A	
	Treated	7.2±1.4 A	4.8±0.3 A	9.8±1.7 A	27.5±5.4 B	6.2±1.3 A	165.7±5.7 A	
P. value		0.014 *	0.0001 **	0.841 NS	0.0001 **	0.291 NS	0.253 NS	

Different letter in columns represent significant differences; NS: no significant differences, p>0.05; \*: p<0.05; \*\*: p<0.001

### Distribution according to FAB subtypes

The statistical analysis of the newly diagnosed AML patients in the figure (1) recorded various subtypes of AML according to their frequency distribution. The AML-M2 is the most common subtype with a frequency of 36% while AML-M3 is the second most

common with a frequency of 24%. Also, the incidence of AML-M1 is 10. 5% and the frequency of AML-M4 is 19%. AML-M5 has a prevalence rate of 7%, while AML-M0 has the least prevalence rate of 3. 5%. The data shows that the various subtypes of AML have varying incidences.



Figure 1. Distribution of AML patients according to FAB subtypes

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# Flow cytometric immunophenotyping and CD markers in AML patient.

The expression of a variety of CD markers in patients with acute leukaemia is illustrated in the figure 2, the figure shows a series of dot plots obtained from flow cytometry analysis. The surface antigen expression of each subpopulation of WBCs is used to differentiate between the various plots, which each display a unique combination of markers. In this study, significant differences were observed in CD marker reactivity among the control group, newly diagnosed group, and treated group as shown in table (3).



Figure 2. The expression of CD markers in AML patient

all control subjects Notably, exhibited negative results for all CD markers tested. In contrast, 75% of newly diagnosed patients and 70% of treated patients were positive for CD33, with a highly significant difference indicated by a chi-square value of (56.33) and a P value of (0.001). Similarly, CD13 positivity was observed in 60% of newly diagnosed patients and 80% of treated patients, again showing a significant difference with a chi-square value of (55.71) and a P value of (0.00). For CD117, 55% of newly diagnosed patients and 52.5% of

treated patients were positive, resulting in a significant difference with a chi-square value of (33.56) and a P value of (0.00). MPO positivity was found in 55% of newly diagnosed patients and 60% of treated patients, with a chi-square value of (37.51) and a P value of (0.00), indicating significant differences. CD64 showed positivity in 55.5% of newly diagnosed patients and 44.5% of treated patients, with a chi-square value of (32.53) and a P value of 0.01, demonstrating significant differences. For CD34, 35% of newly diagnosed patients and 37.5% of

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treated patients were positive, with a chisquare value of (19.19) and a P value of (0.00), reflecting significant differences. HLADR positivity was noted in 32.5% of newly diagnosed patients and 27.5% of treated patients, with a chi-square value of (15.31) and a P value of (0.00), indicating significant differences. Lastly, CD7 showed no significant difference. The positive reactivity of a variety of CD markers in both AML patients' groups are illustrated in the figure 3. CD33 exhibits the maximum positive reactivity at 72.5%, followed by CD13 at 70%. The CD117 and MPO markers exhibit positive reactivity levels of 53.75% and 57.5%, respectively. exhibits a symmetrical positive CD64 reactivity of 50%. HLADR has a positive reactivity of 30%, whereas CD34 has a positive reactivity of 36.25%. Finally, CD7 exhibits the lowest positive reactivity at 8.75%. CD marker reactivity differences patients among AML reveal disease progression, aiding in understanding AML diagnosis, progression, and treatment efficacy.

React		Reactivity	Control	Newly	Treated No.	Chi-	P. Value
		_	No. (%)	diagnosed No.	(%)	square	
				(%)		$(\chi^2)$	
	CD33	+	0 (0)	30 (75)	28 (70)	56.33	0.001 **
		-	40 (100)	10 (25)	12 (30)	56.33	0.00 **
	CD13	+	0 (0)	24 (60)	32 (80)	55.71	0.00 **
		-	40 (100)	16 (40)	8 (20)	55.71	0.00 **
Markers	CD117	+	0 (0)	22 (55)	21 (52.5)	33.56	0.00 **
		-	40 (100)	18 (45)	19 (47.5)	33.56	0.00 **
	MPO	+	0 (0)	22 (55)	24 (60)	37.51	0.00 **
		-	40 (100)	18 (45)	16 (40)	37.51	0.00 **
	CD64	+	0 (0)	23(55.5)	18 (44.5)	32.53	0.01 **
CD		-	40 (100)	17 (44.5)	22 (55.5)	32.53	0.00 **
	CD34	+	0 (0)	14 (35)	15 (37.5)	19.19	0.00 **
		-	40 (100)	26 (65)	25 (62.5)	19.19	0.00 **
	HLADR	+	0 (0)	13 (32.5)	11 (27.5)	15.31	0.00 **
		-	40 (100)	27 (67.5)	29 (72.5)	15.31	0.00 **
	CD7	+	0 (0)	5 (12.5)	2(5)	5.76	0.06
		-	40 (100)	35 (87.5)	38 (95)	5.76	0.06
*, p	<0.01, **; r	o<0.001					

Table 3. CD marker in	control and AML patient
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### 4. Discussion

The study shows that males are the majority of acute myeloid leukemia patients, with a higher frequency (62.5%) compared to females (37.5%) [14]. This disparity is attributed to heredity, hormonal influences, and environmental impacts, with females showing better survival and prognosis [15]. The improvement in hematological parameters observed in AML patients before and after treatment provides critical insights into the disease's progression and the effectiveness of therapeutic interventions [16, 17]. Treated AML patients, a significantly decline in WBC count compared newly diagnosed patients aligns with findings that induction therapy effectively reduces leukemic cell burden, normalizing WBC counts as part of achieving remission [18]. The substantial reduction in RBC counts in newly diagnosed patients compared to treated and control groups (p=0.0001) reflects the marrow suppression characteristic of AML and its partial recovery post-treatment [19]. The study found no significant differences in Hb levels, but anemia management improved after therapy.

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Lymphocyte count decreased in newly diagnosed AML patients, indicating immune dysregulation. Platelet recovery was observed post-treatment, indicating recovery of bone marrow megakaryocytes [20].



Figure 3. The frequency of total CD markers in AML patient.

Hematological parameters like WBC, RBC, and platelets are crucial in assessing therapy response and management of AML patients and should be regularly monitored for improved patient outcomes [21]. The M2 subtype of disease activity was the most prevalent among newly diagnosed patients, this study confirming previous research that revealed M2 to be the most common subtype of AML. The asymmetric distributions of other subtypes, such as M0, M4, and M5, emphasized the heterogeneity of AML and the need for subtype categorization in treatment strategies [22]. Sex should be considered as a biological marker in AML research and management for better patient outcomes, with further understanding of molecular biology of sexual dimorphism [23]. Acute myeloid leukemia (AML) is a heterogeneous disease further classified into several subtypes that differ in CD marker profiles. The study examines sex distribution, disease subgroups. and CDactivity marker prevalence in AML patients, finding no difference in treated significant group outcomes, [22]. This aligns with previous research establishing M2 as the most common subtype of AML. The imbalance among subtypes like M0, M4, and M5 further supports the necessity for categorization to determine treatment courses. Identifying CD markers is significant in the diagnosis, prognosis, and targeted therapy of AML. CD33 was the most frequent marker in all disease stages, followed by CD13 [24]. Both indicators have potential therapeutic use in AML. CD117, MPO, and CD64 were also brightly stained. The distributions of the groupings were notably diverse. Interestingly, CD79A, CD10, and CD3 were not detected in any AML subtypes, indicating these proteins may not be significantly involved in AML development or are associated with other blood diseases [25]. The distribution of CD markers in different AML subtypes reflects disease heterogeneity. The M2 subtype had

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the highest positivity for CD33 and, along with the M3 subtype, had the highest positivity for CD13 [26]. This data indicates these markers can help identify and intervene in AML with certain subtypes. Identifying CD markers in AML patients is crucial as they serve diagnostic and therapeutic purposes. Observational studies, including our own, imply that CD33 might be a reasonable target for AML treatment. This finding aligns with the present study, where CD33 is the most common marker. We evaluated CD13 and found it expressed in all stages despite focusing mainly on early AML stages [26]. Among the studied biomarkers, CD79A, CD10, and CD3 were conspicuously absent in all patients. Notably, the control group had these antigens at approximately 11%. Consistent with other studies, the outcomes suggest that CD33 expression patterns can help differentiate APL from AML-M2. CD marker expression, particularly CD33 and CD13, suggests they can be treatment targets. The absence of CD79A, CD10, and CD3 in all AML subtypes suggests they are not significant determinants of AML or are associated with other diseases [25]. Therefore, this study supports CD markers as prognostic tools and emphasizes their role in AML diagnosis, risk stratification, and targeted treatment. Furthermore, it highlights the heterogeneity of the disorder and the need for tailored management approaches [28].

### 5. Conclusion

In this study, significant differences in hematological parameters and CD marker expressions were identified between newly and treated AML diagnosed patients compared to healthy controls. The high positivity rates of CD33, CD13, CD117, MPO, and CD64 in AML patients underscore the importance of these markers in diagnosis and of treatment. Regular assessment hematological parameters and CD markers is crucial for effective management and tailored therapeutic strategies in AML patients.

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