Characterization of CD Marker Expression in Acute Myeloid Leukemia

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Abstract:
Acute Myeloid Leukemia (AML) is a heterogeneous hematological malignancy characterized by diverse subtypes and variable CD marker expressions. This study aimed to analyze the gender distribution, disease activity subtypes, and prevalence of CD markers in AML patients across different stages. A total of 160 participants were categorized into Control, Newly Diagnosed, Treated, and Relapsed groups. Blood samples were analyzed using flow cytometry for CD markers. Our findings revealed that the M2 subtype was the most prevalent among the Newly Diagnosed group. CD33 and CD13 were the most expressed markers across all stages. Notably, CD79A, CD10, and CD3 were absent in all AML subtypes. The study underscores the significance of CD markers in AML diagnosis, prognosis, and therapeutic targeting, emphasizing the disease's complexity and the need for personalized treatment approaches.
Keywords: Acute Myeloid Leukemia (AML); CD markers; Flow cytometry

توصيف تعبير المعلمات الابتدائية في تشخيص سرطان الدم النخاعي الحاد

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الخلاصة:
سرطان الدم النخاعي الحاد (AML) هو ورم خبيث دموي غير متجانس يتميز بأنواع فرعية متنوعة وتعبيرات مختلفة للمعلمات المناعية. هدف هذه الدراسة إلى تحليل تردد المرض بين الجنسين، والأنواع الفرعية لنشاط المرض، وانتشار العلامات الخلوية لدى مرضى سرطان الدم النخاعي الحاد عبر مراحل مختلفة. تم تسجيل (160) مشاركًا وتقسيمهم الى مجموعات الأشخاص الاصحاء، والمجموعات التي تم تشخيصها بمرض (AML) حديثًا، وبعد اخذ العلاج، وعودة المرض. تم تحليل عينات الدم باستخدام قياس التدفق الخلوي للمعلمات (CD). كشفت النتائج التي توصلنا إليها أن النوع الفرعي (M2) كان الأكثر انتشارًا بين المجموعة التي تم تشخيصها حديثًا. كانت (CD33) و (CD13) أكثر العلامات التي تم التعبير عنها في جميع المراحل. والجدير بالذكر أن (CD79A) و (CD10) و (CD3) كانت غائبة في جميع أنواع AML الفرعية. تؤكد الدراسة على أهمية المعلمات الحيوية في تشخيص سرطان الدم النخاعي الحاد والتشخيص والاستهداف العلاجي، مع التركيز على تعقيد المرض والحاجة إلى أساليب علاج شخصية.
الكلمات المفتاحية: سرطان الدم النخاعي الحاد (AML) ، المعلمات الخلوية، فلوسايتومتري.

Introduction

Clonal growth of undifferentiated myeloid progenitor cells in the bone marrow causes impairment of hematopoiesis and the buildup of blast cells in the bone marrow and peripheral circulation, resulting in the development of acute myeloid leukemia (AML) [1]. Patients with AML have a broad variety of clinical manifestations, prognoses, and reactions to treatment, making the illness a spectrum [2]. Depending on their morphological and cytochemical properties, AML is classified into subtypes M0 through M7 by the World Health Organization (WHO), each with different clinical and prognosis consequences. [3] M0 (Undifferentiated AML) describes this kind of illness. poor morphological distinction; difficult to diagnose. Myeloblasts make up the vast majority of M1 (acute myeloid leukemia) cells. Myeloblasts and more developed cells coexist in M2 (mature AML). When promyelocytes are present, a diagnosis of M3 (acute promyelocytic leukemia, APL) is made. Both myeloid and monocytic lineages are present in M4 (Acute

Myelomonocytic Leukemia). Leukemia of the monocytic cell type (M5), also known as Acute Monocytic Leukemia. The M6 subtype (Acute Erythroblastic leukemia) of acute leukemia affects cells of the erythroid lineage. There are megakaryoblasts in M7 (Acute Megakaryoblastic Leukemia). In order to distinguish between various subtypes of AML, immunophenotyping using cluster of differentiation (CD) markers has become a standard part of the diagnostic workup [4]. Flow cytometry-identified CD markers improve the precision of AML categorization [5] by providing information into the lineage and differentiation stage of leukemic cells. While most AML subtypes share the expression of CD13 and CD33, M7 (Acute Megakaryoblastic Leukemia) is distinguished by the presence of CD41 and CD61 [6]. CD markers have been shown to have an increasingly important role in the treatment of AML [7], not only in diagnosis but also in prognosis and therapeutic targeting, as our knowledge of the illness grows. Leukemic cells go through a series of phases of differentiation and proliferation as AML develops. To keep tabs on these shifts and provide light on how the illness develops, researchers rely heavily on CD markers, which are cell surface proteins. [8] In the earliest stages of AML, stem cell marker CD34 is often expressed, suggesting the existence of leukemic stem cells. These cells are a crucial therapeutic target because of their disease-initiating potential and resistance to standard treatments [9]. In the development of an illness, certain CD markers are expressed differently by AML cells at different stages of development. Changes in CD34 status, for instance, from CD34+ to CD34-, suggest a change from immature to more differentiated leukemic cells, which might affect prognosis and responsiveness to therapy [10]. Regarding the aggressiveness of diseases, there is a correlation between the aggressiveness of AML and some CD markers. For instance, greater CD123 expression is associated with higher blast proliferation and worse clinical outcomes [11], suggesting that it may serve as a marker for the aggressiveness of a disease. Disease Detection Down to Its Barest Essentials (MRD): The ability to identify minor remaining illnesses after therapy is critical for recurrence prediction. CD markers have a great sensitivity for detecting MRD, particularly when used in combination. Certain markers, such as CD33 and CD96, might suggest the existence of residual leukemic cells and the possibility of disease return even in low numbers [12]. Treatment Aiming: Alterations in the expression of CD markers are a common feature of AML development and provide a potential therapeutic target. For a more individualized approach to treating leukemia, agents such gemtuzumab ozogamicin have been designed to precisely target leukemic cells expressing this marker [13]. As CD markers are critical for AML diagnosis, classification, disease prognosis, treatment response evaluation, and the development of tailored therapeutic strategies, this research aims to better understand the processes and roles they play in AML progression.

Subjects

The study involved 160 participants, divided into four groups: Control, as a case control study. The AML patients were classified into Newly Diagnosed, Treated, and Relapsed. The control group had 23 males and 17 females, the newly diagnosed group had 21 males and 19 females, the treated group had 25 males and 15 females, and the relapsed group had 22 males and 18 females as inclusion criteria, while the exclusion criteria were those with CML, other cancer types, infection, and other autoimmune disorders. The samples were collected from Baghdad Teaching Hospital, Baghdad, Iraq.

Method

3ml of venous blood samples were collected in EDTA and immediately transferred to the Hematological diseases department, which related to the mentioned hospital for processing, validation and promoting using Flowcytometer, Antibodies against CD33, CD13, CD117, MPO, CD64, CD34, HLADR, CD7, IREM2, CD11B, CD35, CD79A, CD10 and CD3.

Results

The study analyzed the gender distribution and disease activity subtypes of Acute Myeloid Leukemia (AML) patients across different stages. Males dominated in the Control (57.5%), Treated (62.5%), and Relapsed (55%), while females made up 42.5% of the Control, 47.5% of the Newly Diagnosed, 37.5% of the Treated, and

45% of the Relapsed groups. The gender distribution did not show a statistically significant difference. The M2 subtype was the most prevalent in the Newly Diagnosed group at 45%, followed by the Treated group at 40% and the Relapsed group at 32.5%. Other subtypes, such as M0, M4, and M5, showed varying distributions across the groups. The data provides insights into the gender distribution and prevalence of AML subtypes across different stages of the disease. (Table1)

Table 1: Showed the demographic distribution of patients and control subjects.

Groups		Control No. (%)	Newly diagnosed No. (%)	Treated No. (%)	Relapsed No. (%)	Probability
Gender	Males	23 (57.5)	21 (52.5)	25 (62.5)	22 (55)	0.231
	Females	17 (42.5)	19 (47.5)	15 (37.5)	18 (45)	
	Total	40 (100)	40 (100)	40 (100)	40 (100)	
Diseases Activity (Subtypes)	M0	0 (0)	5 (12.5)	3 (7.5)	3 (7.5)	0.012
	M1	0 (0)	8 (20)	6 (15)	6 (15)	0.001
	M2	0 (0)	18 (45)	16 (40)	13 (32.5)	0.000
	M3	0 (0)	4 (10)	7 (17.5)	7 (17.5)	0.003
	M4	0 (0)	3 (7.5)	4 (10)	5 (12.5)	0.021
	M5	0 (0)	2 (5)	4 (10)	6 (15)	0.042

P<0.05 considered significant differences.

The data shows the distribution of CD markers among Acute Myeloid Leukemia (AML) patients. CD33 was found in 70% of Newly Diagnosed patients, 77.5% of Treated patients, and 72.5% of Relapsed patients, with a prevalence of 72.5%. CD13 was present in 62.5% of Newly Diagnosed, 75% in Treated, and 65% in Relapsed patients, with a frequency of 65%. Other markers, such as CD117, MPO, and CD64, showed varying distributions across groups. Other markers showed – CD results. (Table 2)

Table 2: CD+ markers in the AML patients

Groups		Newly diagnosed No. (%)	Treated No. (%)	Relapsed No. (%)	Total AML	Probability
CD Markers	CD33	28 (70)	31 (77.5)	29 (72.5)	88 (72.5)	0.001
	CD13	25 (62.5)	30 (75)	26 (65)	81 (65)	0.012
	CD117	21 (52.5)	23 (57.5)	21 (52.5)	65 (52.5)	0.002
	MPO	20 (50)	21 (52.5)	22 (55)	63 (55)	0.001
	CD64	21 (52.5)	20 (50)	23 (57.5)	64 (57.5)	0.003
	CD34	16 (40)	13 (32.5)	15 (37.5)	44 (37.5)	0.001
	HLADR	16 (40)	14 (35)	15 (37.5)	45 (37.5)	0.001
	CD7	7 (17.5)	4 (10)	4 (10)	15 (10)	0.001
	IREM2	2 (5)	2 (5)	3 (7.5)	7 (7.5)	0.011
	CD11B	4 (10)	2 (5)	1 (2.5)	7 (2.5)	0.002
	CD35	4 (10)	2 (5)	1 (2.5)	7 (2.5)	0.000
	CD79A	0 (0)	0 (0)	0 (0)	0 (0)	1.00
	CD10	0 (0)	0 (0)	0 (0)	0 (0)	1.00
	CD3	0 (0)	0 (0)	0 (0)	0 (0)	1.00

P<0.05 considered significant differences.

A comprehensive analysis of the distribution of various CD markers across different subtypes of Acute Myeloid Leukemia (AML), ranging from M0 to M5. The most prevalent CD marker is CD33, with 32 (26.7%) occurrences in the M2 subtype, followed by M4 at 15%. The least occurrences are seen in M0 and M5, both at 5.8%. CD13 is the most prominent CD marker in the M2 subtype, with 22 (18.3%) occurrences. CD117 is the most observed CD marker in the M2 subtype, with 16 (13.3%) occurrences,

followed by M3 at 11.7%. MPO leads with 17 (14.2%) occurrences, followed by M3 at 10.8%. CD64 is the most prevalent CD marker in the M2 subtype, with 21 (17.5%) occurrences. CD34 and HLADR have similar distribution patterns, with the M2 subtype having the highest occurrences. CD7 has the highest presence in the M2 subtype with 5 occurrences. IREM2, CD11B, and CD35 also show similar distribution patterns, with the highest occurrences in the M2 subtype at 4.3%. CD79A, CD10, and CD3 are absent across all subtypes. (Table 3), (Figure 1)

Table 3: The expression of CD markers in AML based on subtypes.

CD, Subtypes	M0	M1	M2	M3	M4	M5	P. Value
CD33	7 (5.8)	11 (9.2)	32 (26.7)	13 (10.8)	18 (15)	7 (5.8)	0.002
CD13	9 (7.5)	13 (10.8)	22 (18.3)	12 (10)	11 (9.2)	14 (11.7)	0.016
CD117	6 (5)	8 (6.7)	16 (13.3)	14 (11.7)	12 (10)	9 (7.5)	0.061
MPO	5 (4.2)	7 (5.8)	17 (14.2)	13 (10.8)	11 (9.2)	10 (8.3)	0.051
CD64	6 (5)	9 (7.5)	21 (17.5)	11 (9.2)	10 (8.3)	7 (5.8)	0.071
CD34	3 (2.5)	5 (4.2)	13 (10.8)	9 (7.5)	8 (6.7)	6 (5)	0.06
HLADR	3 (2.5)	6 (5)	13 (10.8)	9 (7.5)	8 (6.7)	6 (5)	0.08
CD7	1 (0.8)	2 (1.7)	6 (5)	2 (1.7)	3 (2.5)	1 (0.8)	0.12
IREM2	0 (0)	0 (0)	4 (3.3)	2 (1.7)	1 (0.8)	0 (0)	0.11
CD11B	0 (0)	0 (0)	4 (3.3)	2 (1.7)	1 (0.8)	0 (0)	0.23
CD35	0 (0)	0 (0)	4 (3.3)	2 (1.7)	1 (0.8)	0 (0)	0.411
CD79A	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
CD10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1
CD3	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1

P<0.05 considered significant differences.

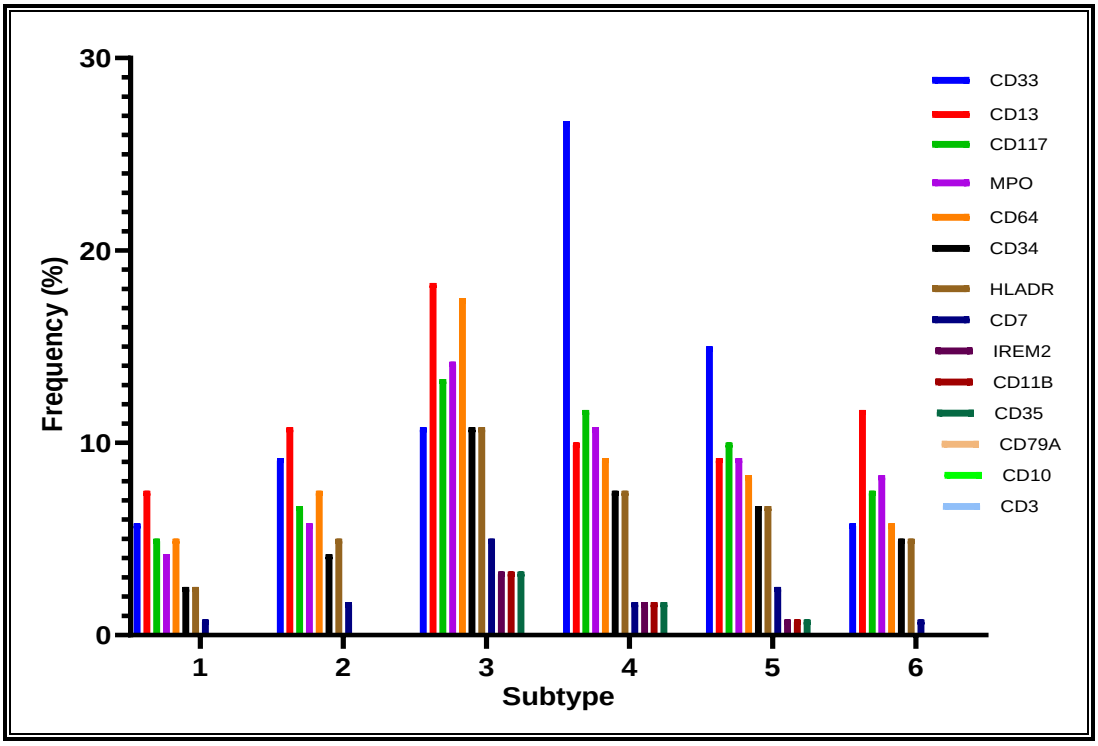

Figure 1: the CD markers profile in AML subtypes

Discussion

Multiple subtypes and variable expression of CD markers define Acute Myeloid Leukemia (AML), a heterogeneous hematological malignancy. The gender breakdown, disease activity subtypes, and CD marker prevalence in AML patients at various stages are all thoroughly analyzed in our research. This distribution, however, did not show a statistically significant difference, indicating that gender may not play a crucial role in the development or response to therapy of AML. The M2 subtype of disease activity was the most common overall and among those with a recent diagnosis. This confirms the results of prior research [15] that found M2 to be the most prevalent subtype of AML. The variability of AML and the necessity of subtype categorization for treatment methods were highlighted by the asymmetric distributions of other subtypes, such as M0, M4, and M5. CD marker expression is a key factor in AML diagnosis, prognosis, and therapeutic targeting. CD33 was the most common marker we found in all phases, with CD13 coming in a close second [16]. It has been shown that both markers are good candidates for therapeutic targeting in AML. There were also notable expressions of CD117, MPO, and CD64. However, their distributions differed considerably throughout the groups. Notably, markers including CD79A, CD10, and CD3 were not detected in any of the AML subtypes, which may indicate a minor involvement for these molecules in AML pathogenesis or a relationship with other hematological disorders [17]. CD marker distribution among AML subtypes provides more evidence of the disease's inherent complexity. The M2 subtype was found to have the greatest expression of CD33, whereas the M2 and M3 subtypes had the highest prevalence of CD13 [18]. Based on these results, these markers show promise as diagnostic and therapeutic targets, particularly for certain subtypes of acute myeloid leukemia. Because of its importance in both diagnosis and therapy, studying the expression of CD markers in AML patients is a vital field of study. The results of several studies [19], including our own, suggest that CD33 is a promising therapeutic target in AML, which is consistent with our discovery that CD33 is the most frequent marker. Although CD13 expression was most strongly linked to the early stages of AML, our data on CD13 indicated considerable expression across all stages [20]. Among the most intriguing findings of our research was the universal absence of markers CD79A, CD10, and CD3. None of our patients lacked this antigen, in contrast to roughly 11.1% of AML-M1/2 cases, and these findings were consistent with that indicated by other researchers, suggesting that the expression pattern of CD33 contributes to the differentiation of APL and AML-M2. When it comes to hematological malignancies and solid tumors, the phenotypic of the implicated cells, which is typically defined by the expression of surface CD markers, is another factor playing a significant role in the diagnosis and prognosis [20]. Our results showed that CD117 was the most often discovered myeloid antigen in 54% of ALL [21] patients, while another research indicated that CD7 was the most frequently detected lymphoid related antigen in 33% of AML cases [22], [23]. The abnormal appearance of CD 117 is crucial. Consistent with previous studies, the expression patterns of the CD markers, especially CD33 and CD13, highlight their potential as therapeutic targets. Some markers, such as CD79A, CD10, and CD3, are not present in any of the AML subtypes, suggesting they play a minor role in AML or are associated with other disorders. Additional insight into the complexities of AML is provided by the comparison with other research, such as the unique expression patterns of CD33 and the abnormal expression of CD117. The study's findings emphasize the importance of CD markers in AML diagnosis, prognosis, and therapeutic targeting while revealing the disease's heterogeneity and the necessity for individualized care.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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ETHICS COMMITTEE APPROVAL AND PATIENT CONSENT

Before performing study and collecting samples, we obtained approval from the Institutional Review Board (Ministry of Higher Education and Scientific Research, Al-Nahrain University, Scientific Research Ethics Committee, number 4924, date 31-1-2022). In order to safeguard the patient's confidentiality, the inquiry did not include any identifying information about the patient or any identifying information about healthy persons or any portion of them.

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