CD 11b and CD56 As Prognostic Markers in Acute Myeloid Leukemia

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Abstract

Background:
Acute myeloid leukemia (AML) is a common adult leukemia and is characterized by a differentiation block and aberrant clonal growth of hematopoietic blasts.
Aim:
This study aims:
1-To detect CD56 and CD11b marker expression in newly diagnosed adult AML cases.
2-To study the correlation between CD56 and CD11b expression with haematological parameters in cases of adult AML.
Materials and Methods:
The study was conducted on 26 newly diagnosed AML cases presented to the flow cytometry unit, Clinical Pathology department, Assiut University Hospital, from November 2018 to October 2019. Their ages ranged from 23 to 85 years, including 13 males and 13 females. The patients were diagnosed with Acute Myeloid leukemia after Bone marrow Aspirate and Immunophenotyping.
Results:
It was noticed that patients with relapse had the highest expression of CD11b and CD56, whereas patients who achieved remission had the lowest expression of CD11b and CD56.
Conclusion:
The present study showed that CD11b positivity and CD56 were a helpful indicator of chemoresistance and a poor prognostic marker in AML patients.
Keyword: acute myeloid leukemia- CD11b- CD56- Immunophenotyping

Introduction:

Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic stem/progenitor cells that lose the ability to differentiate normally and to react to normal regulators of proliferation and apoptosis, resulting in an accumulation of a massive amount of immature blasts with different degrees of myeloid differentiation in the bone marrow and peripheral blood. (1)

Acute myeloid leukemia (AML) is a complex, heterogeneous disease involving a metastatic expansion of neoplastic myeloid cells with variable degrees of differentiation and varying clinical, morphologic, immunologic, and molecular properties. (2)

CD11b is a protein subunit of integrin alpha-M beta-2 molecules. It is necessary for cell-cell interaction between leukemic cells and their microenvironment and then participates in regulating the biological activities of leukemic cells. (3) (4)

CD11b expression level should be considered a prognostic marker for AML patients.

CD11b positivity could predict a poor prognosis for AML patients. (5)

Cell adhesion molecule CD56 is a glycoprotein of the immunoglobulin(Ig)

superfamily expressed on the surface of various cells, which can mediate hemophilic cell adhesion and is involved in cell cytotoxicity (6).

Because of its involvement in cell–cell interactions, CD56 could affect the cell homing mechanism and probably the pattern of malignant cell dissemination. (7)

Therefore, it is conceivable that the CD56 antigen expression, an inexpensive, detectable, and reproduced index, is closely related to the prognosis of AML patients. Its overexpression is an adverse prognostic factor for AML. (5)

Materials and Methods: (IRB.no 17100347)

The study was conducted on 26 newly diagnosed AML cases presented to the flow cytometry unit, Clinical Pathology department, Assiut University Hospital, from November 2018 to October 2019. Their ages ranged from 23 to 85 years, including 13 males and 13 females**.**

All Patients were subjected to the following:

1. Full Clinical Evaluation including:
 - Comprehensive history taking, including: (age, sex, therapeutic history, and blood transfusion).
 - Clinical examination data, including: (anaemic manifestation, fever, hepatomegaly, splenomegaly, lymphadenopathy, bleeding tendency, and bone tenderness).

2. Laboratory Investigations:
 a) CBC was done using ADVIA 2120i, including hemoglobin (Hb) level, RBCs indices, total leukocytic count, platelet count, and platelet indices.

b) Examination of peripheral blood films stained with Leishman stain for differential leukocytic count and detection of Blast cells.
c) Examination of Leishman stained BM aspiration smears to assess BM cellularity, morphological BM proliferation, and cytochemical examination by Sudan Black B, Non-specific esterase, and Periodic acid Schiff.
d) Immunophenotyping was done by Becton-Dickinson fluorescence-activated cell sorter (BD FACSCalibur) using MoAbs (BD and Immunostep).
e) Follow up of patients by BM blast to predict outcome and prognosis of disease after Remission induction Therapy and consolidation therapy.

Statistical Analysis:

Data analysis was undertaken using SPSS version 26. Categorical data were presented as frequencies and percentages while mean ± SD (range). A one-way ANOVA test was used to compare mean CD11B and CD56 between more than two groups. Spearman correlation was used to determine the correlation between CD11B and CD56 with other variables. The level of significance was considered at a P value < 0.05.

Results:
Immunophenotyping of studied patients with AML (n= 26):

Immunophenotyping of studied patients with AML is summarized in Table 1. It was noticed that the mean CD11b in studied patients was (53.33 ± 29.01) with a range between 6.80 and 95.90, while the mean CD56 was (18.5 ± 27.8) with a range between (0.80-91).

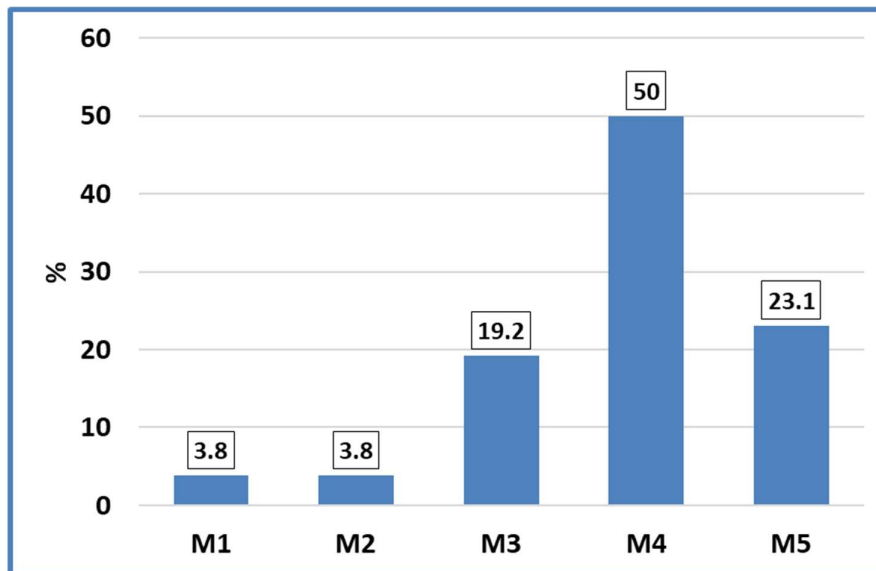**Table(1): Immunophenotyping of studied patients with AML**

Immunophenotyping	N=26
CD11b	53.33±29.01 (6.8-95.9)
CD56	18.35±5.46 (0.8-91.0)
CD34	26.80±5.66 (0.2-88.0)
CD8	4.53±0.92 (1.0-26.0)
CD4	17.24±4.99 (0.9-87.0)
CD3	6.69±1.31 (1.8-37.0)
CD19	4.07±0.55 (0.5-10.0)
CD14	23.03±3.19 (0.6-70.0)
CD10	4.78±0.66 (0.4-13.6)
CD45	71.25±17.63 (49-89)
CD13	63.74±21.77 (17.0-95.9)
CD33	66.34±30.54 (4.0-98.0)
CD117	52.44±25.96 (3.0-98.6)
CD64	59.16±23.96 (9-97)
HLA-DR	34.00±5.98 (0.8-92.0)
Cyto-MPO	59.14±29.12 (8.0-99.8)

Data expressed as mean± SD (range), **N**: number; **AML**: acute myeloid leukemia

FAB subtypes of studied patients with AML (n= 26):

FAB subtypes showed that the most frequent subtypes in the studied patients were M4 (50%), M5(23.1%), and M3 (19.2%), followed by M1(3.8%), and M2 (3.8%) patients, respectively.
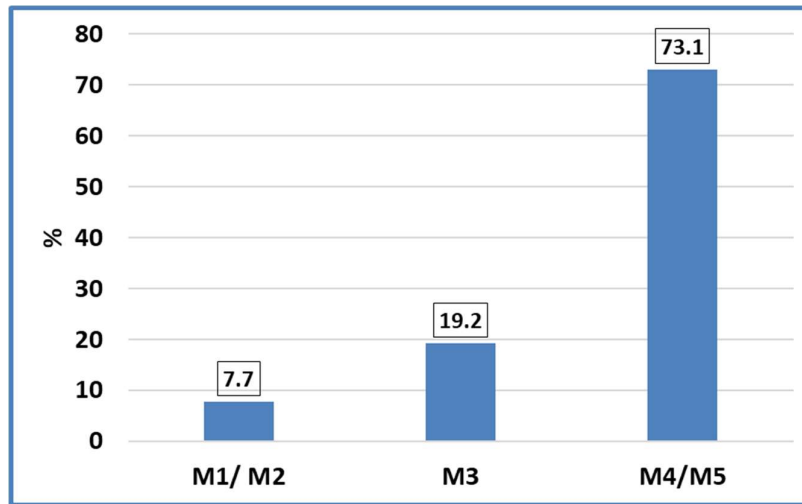
Figure (1) FAB subtypes of studied patients with AML

Immunophenotyping of studied patients with AML (n= 26):

Immunophenotyping showed that the most frequent subtypes in the studied patients were M4/M5(73%) and M3 (19.2%), followed by M1/M2 (7.7%), respectively.

Figure (2): Immunophenotyping of studied patients with AML

Outcome of studied patients with AML (n= 26):
The outcome of the patients studied with AML is shown in Figure 3. Nineteen (76.7%) patients deteriorated and died, while relapse (a recurrence of a disease after a period of improvement) occurred in 3 (11.5%) patients. It was noticed that only 4 (15.4%) patients were improved and achieved remission (disappearance of the signs and symptoms of a disease).

Figure 3: Outcome of studied patients with AML
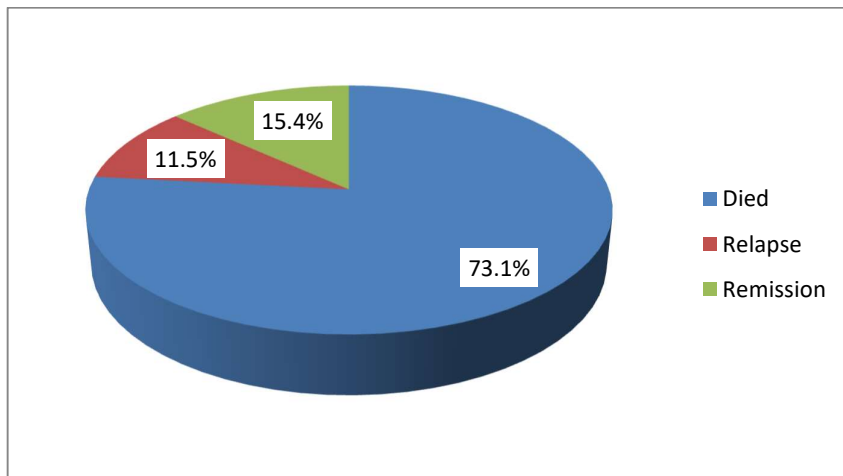
 CD11b and CD56 based on FAB subtypes:
Based on FAB subtypes, it was noticed that the highest expression of CD11b was observed with M5 (65.28±24.45), followed by M4 (61.96±24.80), while M3 had the lowest expression (24.86±13.84). It was noticed that CD56 was highly expressed in M5 (46.05±18.88) and M4 (11.58±4.02), while M1 had the lowest expression of CD56 (4.40).

Table (2): CD11b and CD56 according to FAB subtypes among patients with AML

FAB subtypes	CD11b	CD56
M1	25.40	4.40
M2	39.80	10.50
M3	24.86±13.84	6.70±3.59
M4	61.96±24.80	11.58±4.02
M5	65.28±24.45	46.05±18.88
P value*	0.071	0.081

Data expressed as mean± SD

  ***One-way ANOVA was used to compare the mean between more than two groups.

CD11b and CD56 based on Immunophenotyping:
Based on FAB subtypes, it was noticed that the highest expression of CD11b was observed with M4/M5 (63.01±24.05) and M1/M2(32.60±10.18), while M3 had the lowest expression (24.86±13.84). It was noticed that CD56 was highly expressed in M4/M5 (22.46±7.27) and M1/M2 (8.45±4.05) while M3 had the lowest expression of CD56 (6.71±1.60).

Table (3): CD11b and CD56 according to Immunophenotyping of patients with AML

| Immunophenotyping	CD11b	CD56
M1/M2	32.60±10.18	8.45±4.05
M3	24.86±13.84	6.71±1.60
M4/M5	63.01±24.05	22.46±7.27
P value*	**0.001**	0.03

  Data expressed as mean± SD,

  ***One-way ANOVA was used to compare the mean between more than two groups.
****Pairwise comparison

Figure (4): CD11b and CD56 according to Immunophenotyping of patients with AML
CD11b and CD56 based on outcome:
Based on the outcome, it was noticed that patients with relapse had the highest expression of CD11b (79.46±12.50) and CD56 (60.46±29.38), while patients who achieved remission had the lowest expression of CD11b (35.95±16.42) and CD56 (9.05±2.67).
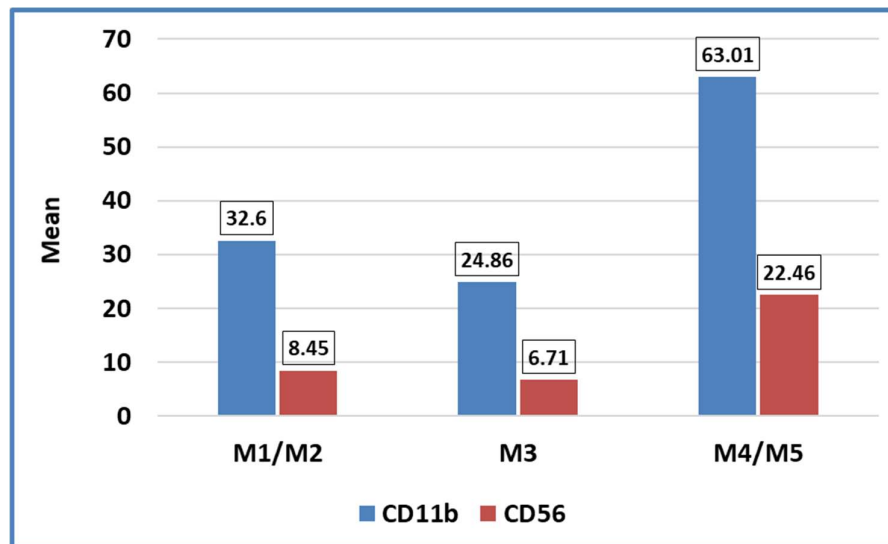
Table (4): CD11b and CD56 according to outcome :

Outcome	CD11b	CD56
Died	52.87±28.11	13.66±4.79
Relapse	79.46±12.50	60.46±29.38
Remission	35.95±16.42	9.05±2.67
P* value	0.001 <	<0.001

Data expressed as mean± SD,

*One-way ANOVA was used to compare the mean between more than two groups.
**Pairwise comparison

Correlation of CD11b and CD56 with other variables:
It was noticed that CD56 had a significant positive correlation with Blast cells (r=0.41; $P=$ 0.036), and CD11b had a significant negative correlation with CD13(r=-0.401; $p=$0.047).

Table (5): Correlation of CD11b and CD56 with other variables

Variables	CD11b		CD56	
	R	P-value*	r	P-value*
Age	0.26	0.193	0.05	0.783
Complete blood count				
Leucocytes	0.20	0.307	0.14	0.487
Platelets	0.01	0.976	0.19	0.351
Hemoglobin level	0.05	0.781	-0.01	0.975
Reticulocytes	-0.33	0.098	0.28	0.159
Blast cell	0.03	0.859	0.41	0.036
Bone marrow Blast	0.12	0.549	0.20	0.307
Immunophenotyping				
CD34	-0.05	0.774	0.16	0.409
HLA-DR	0.23	0.250	0.33	0.098
CD13	-0.401	0.047	-0.08	0.682
CD33	0.28	0.163	0.03	0.879
MPO	-0.15	0.459	-0.34	0.082

r (correlation coefficient)
*Spearman correlation is used to determine the correlation between CD11b and CD56 with other variables.

Expression of CD11b and CD56 based on age groups: Different age groups had insignificant differences regarding the expression of CD11b and CD56 (*P*=0.8, 0.385, respectively).

Table (6): CD11b and CD56 according to age groups

Outcome	CD11b	CD56
<40 years	47.16±11.31	26.72±13.15
40-60 year	58.90±26.62	12.07±7.36
>60 year	54.29±30.143	16.27±7.71
P* value	0.800	0.385

Data expressed as mean± SD
*One Way ANOVA was used to compare the mean between more than two groups

Discussion:

Acute leukemia is a heterogenous group of malignant disorders arising from hematopoietic progenitor cells. It is characterized by immature cells (blasts) in the bone marrow cells, resulting in anemia, thrombocytopenia, and an outpouring of the neoplastic blasts into the peripheral blood. They may infiltrate other parenchymatous organs such as the liver, spleen, and lymph nodes. The blasts have common characteristics, which include rapid proliferation, immaturity, and poor responsiveness to regulatory mechanisms. **(8, 9)**

AML is a common adult leukemia that has different subgroups with variable clinical features, responses to therapy, and prognoses. **(10) (11)**

A differentiation block and aberrant clonal growth of hematopoietic blasts characterize acute myeloid leukemia. It has been classified into different subtypes with respect to morphology, immunophenotype, and genetic abnormalities. **(12)**

Immunophenotyping is a convenient method for quick and reproducible diagnosis of most hematological malignancies. **(13, 14)**

Immunophenotyping data, as for any other clinical or biological characteristics of acute leukemia, cannot be used alone and must be considered together with all parameters of any patient. **(15),** (14)

CD11b is reportedly involved in restraining antitumor immunity and promoting haematological malignant cells' expansion and drug resistance. **(4)**

CD11b is critical in cellular adhesion and migration and is a surrogate marker for neutrophil activation (16) (17). Integrins, which include CD11b, are demonstrated to be involved in tumor resistance, cellular interactions, and microenvironment involvement. **(18) (19)**

CD11b is required to interact with leukemic cells in the bone marrow microenvironment. It also suppresses the immune system and is a marker for myeloid-derived suppressor cells that play a role in the progression of malignancies. Therefore, CD11b expression can be a prognostic marker in AML. **(20) (21)**

Many studies have suggested that the expression of the CD56 antigen, a neural adhesion factor, is associated with a high relapse rate and poor outcomes in acute leukemia. **(22)**

Although the actual mechanism via which CD56 reduces the sensitivity to chemotherapy is unknown, it has been suggested that CD56 is associated with the overexpression of P-glycoprotein (PGP), reducing intracellular drug concentrations. **(23)**

Our study studied the expression of CD11b and CD56 in AML patients presented to our hospital from November 2018 to October 2019.

Males and females were equally distributed in the current study and more common in the age group above 60 (42.3%). Acute myeloid leukemia (AML) is a disease of older adults with a median age at diagnosis, usually over 65 years old. **(24)**

In our study, the patients' most frequent clinical presentations were anemic manifestations at 100% and fever at 61.5%; this disagrees with **(25),** who reported that the most frequent clinical presentation was fever at 60% and anemic manifestation at 26% of cases.

Anemic manifestation may be due to bone marrow infiltration that leads to decreased production of RBCs and might be due to reduced lifespan of RBCs and autoimmune destruction. **(26)**

Thrombocytopenia was a prominent feature in most patients, and it was associated with bleeding tendency (50%). Thrombocytopenia is the result of BM failure.

The commonest FAB AML subtype was AML-M4 (50%) followed by AML-M5 (23.1%), The least AML subtype was AML-M1 and M2 (3.8%), this disagreed with (20) who reported that commonest FAB AML subtype was M2 32 cases (53.3%), followed by AML M4 12 cases(20%), AML M5 11 cases (18.3%), AML M1 3cases(5%), and this disagree with **(27)** in who reported that commonest subtype was AML-M1/M2 (40.5%), the geographical variation in different countries could explain this variation between studies.

CD11b and CD56 expression have been considered unfovbrable prognostic markers in overall survival (OS) and disease-free survival (DFS).

The expression of CD11b was associated with lower complete remission and overall survival rates. **(28)**

Our study noticed that patients with relapse had the highest expression of CD11b (79.46±12.50) and CD56 (60.46±29.38). In contrast, patients who achieved remission had the lowest expression of CD11b (35.95±16.42) and CD56 (9.05±2.67 %); this agrees with the results **(20)** and **(5)** and disagrees with (29) in which this study shows no significant differences were detected in OS or DFS or the frequency of CR according to CD11b and CD56 expression.

Our study shows a significant positive correlation between CD11b positive and CD56 positive, with non-significant correlations observed between the studied markers confirming the heterogeneous nature of AML, which agrees with this. **(20)**

The present study's findings revealed frequent high expression of CD11b, followed by CD56 expression, without significant differences in age, sex, or FAB subtype. These findings were consistent with those of many studies. **(5) (30)**

Conclusion:

It was noticed that patients with relapse had the highest expression of CD11b and CD56, whereas patients who achieved remission had the lowest expression of CD11b and CD56.

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