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ORIGINAL ARTICLE

Prognostic Role of Tetraspanin CD81 in Patients withAcute Myeloid Leukemia

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ABSTRACT

Background. Acute myeloid leukemia (AML) is characterized by clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and bone marrow failure. Identification of new prognostic markers remains important; especially those refining therapeutic options. The aim of this study was to evaluate the value of Tetraspanin CD81 as a prognostic marker in patients with de novo AML.

Methods. Thirty patients with newly diagnosed AML were included in this study, and were subjected to immunophenotyping by flow cytometry. The patients were followed up for one year to evaluate overall survival (OS) and disease-free survival (DFS).

Results. CD81 was expressed in the thirteen patients with a mean percentage 31.15 ± 12.14 %. However, 17 AML patients were $CD81^-$ expression with mean percentage 9.06 ± 3.78 %. After induction therapy, complete remission achieved in 15 patients (50%). On the other hand, 13 patients (43.3%) did not achieve complete remission, and the remaining 2 patients were not evaluated. OS in AML patients ranged from (8- 12 months) with median 11.57 was 71.4%. DFS in AML patients with median 11 months was 53.6%. There was a highly statistically significant difference between studied groups, where OS and DFS were lower in patients with $CD81^+$ expression compared tothose with $CD81^-$ expression (P= 0.00).

Conclusion. $CD81^+$ expression has a potential role as a prognostic marker; where its expression is associated with M4 and M5 FAB subtypes. Moreover, patients with $CD81^+$ expression have higher incidence of relapse and decreased OS & DFS than patients with $CD81^-$ expression.

Key words: Tetraspanin CD81, AML, Overall survival, Disease free survival.

INTRODUCTION

Acute myeloid leukemia (AML) is characterized by clonal expansion of undifferentiated myeloid precursors, resulting in impaired hematopoiesis and bone marrow failure. The affected cells undergo an uncontrolled proliferation and impaired differentiation due to block at various maturation steps [1]. Over the past few years, identification of new prognostic markers remains important; especially those refining therapeutic options [2].

CD81 is a cell surface protein which belongs to the Tetraspanin family, which is a cell surface transmembrane protein. A murine monoclonal antibody was identified by its ability to induce a reversible antiproliferative effect on a human lymphoma cell line. Many of the lymphoid cell lines, in particular those derived from large cell lymphomas, were susceptible to the antiproliferative effects of the antibody. TAPA-1 may therefore play an important role in the regulation of lymphoma cell growth [3].

It regulates activation of B and T cells, and immune receptor signaling [4]. Also, it was reported to be involved as a gateway molecule in hepatocytes for HCV infectionand may have a similar role in Plasmodium infection of red blood cells in

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malaria [5, 6].

CD81 allows hematopoietic stem cells to re-enter to quiescence [7]. In hematologic malignancies, CD81 has mostly been studied in multiple myeloma where its expression on plasma cells is associated with worse progression free survival (PFS) and overall survival (OS) [8]. Lastly, CD81 may be a new prognostic marker for diagnostic risk classification in AML [9].

Aim

The aim of this study is to evaluate the value of Tetraspanin CD81 as a diagnostic and prognostic marker in patients with de novo AML.

PATIENTS AND METHODS

This study was carried out in Clinical Pathology and Internal medicine Departmentsat our university hospitals, during the period from February 2018 to February 2019. A total of 30 patients with newly diagnosed AML were included in this study after obtaining informed consent from all individual participants. The responsible ethics committees (Clinical Pathology Department committee & Institutional Review Board (IRB), Faculty of Medicine, Zagazig University) have also given their approval. This study was also conducted in compliance with Declaration of Helsinki.

Participants enrolled in the study were subjected to the following: full history taking; clinical examination; complete blood count; bone marrow aspiration and examination; immunophenotyping by flow cytometry (FCM) using Becton Dickenson FACSCalibur device to detect the following markers: MPO, CD13, CD33,HLADR, TDT, CD14, CD64, CD34, CD3, CD19, CD20,CD22 and CD81; conventional cytogenetic analysis and karyotyping.

Specific laboratory investigations were done with detection of CD81 (tetraspanin) on blast cells by FCM. The percentage of blast cells positive for the relevant studied marker was determined as a percentage from the gated blast cells population. Cut-off of MoAb percent was defined for CD81 by running samples for 10 apparently normal healthy individuals (control group) - according to the following formula; cut-off = mean + 2SD. The mean of CD81 positivity was 9.5 and the SD was 4.6. Accordingly, the cut-off was 18.7, with CD81 <20 was considered negative, and CD81 >20 was considered positive.

There were 24 males and 6 females. Their ages ranged from 17 to 75 years, with a mean value of 40.9 ± 15.44 years. They were followed up for one year. Peripheral blood and bone marrow samples were collected from all patients; at the time of presentation and before initiation of therapy.

Patients were treated by an induction regimen 3+7 regimen consisting of continuous infusion cytarabine (100 mg/m²) daily for 7 consecutive days combined with 3 days of doxorubicin (30 mg/m²). Patients with 60 years or poor performance status were treated by 2+14 protocol consisting of 2 days of doxorubicin (25 mg/m^2) combined with 14 days of subcutaneous cytarabine (10 mg/m^2/12 hours). Bone marrow aspiration was performed on day 28 after receiving induction chemotherapy to evaluate morphological remission.

Patients were followed once every 3 months with clinical examination and complete blood cell counts. Bone marrow examination was done if there was any doubt of a relapse on clinical examination or blood smear. The patients were followed up for one year to evaluate OS and DFS.

Statistical analysis

Analysis of data was performed using SPSS computer program (version 20; SPSS Inc. Chicago, Illinois, USA). $\chi 2$ -test, t-test, and Mann–Whitney test were used for statistical analysis. DFS and OS were estimated by the Kaplan–Meier method and compared using the log-rank test. P value less than 0.05 was considered statistically significant and Pearson's correlation coefficient were used as tests of significance.

RESULTS

This study included 30 adult patients with newly diagnosed de novo AML. There is a highly statistically significant increase in the age of the patients with CD81 positive expression compared to those with negative expression (P=0.003) (Table 1). There was a statistically significant difference between

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both $CD81^+$ and $CD81^-$ expression as regards FAB classification (Table 2).

According to CD81 expression on BM blast cells, 13 of the AML patients were CD81+ with mean 31.15 ± 12.14 ranging from (21 – 66) and 17 of the AML patients were CD81- with mean 9.06 ± 3.78 ranging from (3 – 19).There was15 patients (50%) of the negative CD81 expression achieved complete remission (CR), 13 patients (43%) of the positive CD81 expression did not achieve CR, while 2 patients not evaluated

Regarding survival rates in 28 AML patients, OS (within 12 months) was 71.4% (Table 5) and DFS (within 11 months) was 53.6% (Table 6) as shown in (Figure 1).

Table (1): Demographic data of AML patients and studied groups according to CD81 expression

Demographic data	AML patients (n= 30)		$CD81^+$ (n=13)		$CD81^-$ (n=17)		*P-value*
Age(years) Mean ± SD	40.9 ± 15.44 (17 – 75)		50.00 ± 14.42		33.94 ± 12.53		**0.003***
Gender	**Frequency**	**%**	**Frequency**	**%**	**Frequency**	**%**	
Male	24	80	12	92.3	12	70.6	0.2^+
Female	6	20	1	7.7	5	29.4	

**Statistically significant difference between CD 81+ & CD 81- (P< 0.05)*
+Test of significant Fisher`s exact test (not significant)

Table (2): FAB classification in the AML patients according to CD81 expression

FAB Types	AML patients (n= 30)		$CD\ 81^+$ (n=13)		$CD\ 81^-$ (n=17)		*P-value*
	Frequency	**%**	**Frequency**	**%**	**Frequency**	**%**	**0.02***
M1	2	6.7	1	7.7	1	5.9	
M2	10	33.3	2	15.4	8	47.1	
M4	12	40	4	30.8	8	47.1	
M5b	3	10	3	23.1	0	0	
M6	2	6.7	2	15.4	0	0	
M7	1	3.3	1	7.7	0	0	

**Statistically significant difference between CD 81- & CD 81+ (P< 0.05)*

Table (3) CD81 expression in the studied group

	Number	Mean	Range
CD 81+	13	31.15 ± 12.14	21-66%
CD 81-	17	9.06 ± 3.78	3-19%

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Table (4) Response to treatment in AML patients

Response to treatment	AML patients	
	Number	**Frequency**
CR	15	50
Non-CR	13	43
Missed	2	7

CR: *complete remission*

Table (5): OS in the AML patients.

OS	All patients (n=28)
12 month	71.4 %

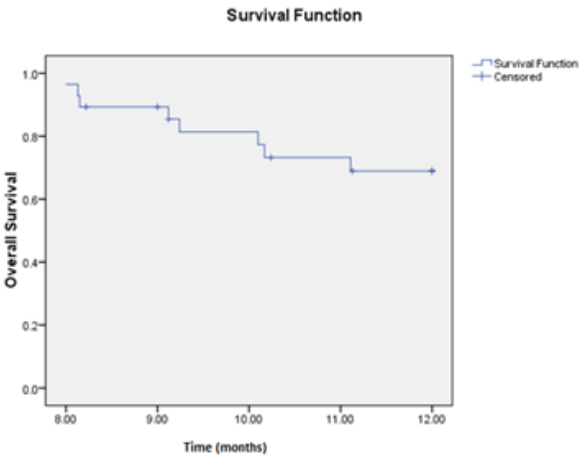
OS: *overall survival*

Table (6): DFS in the AML patients.

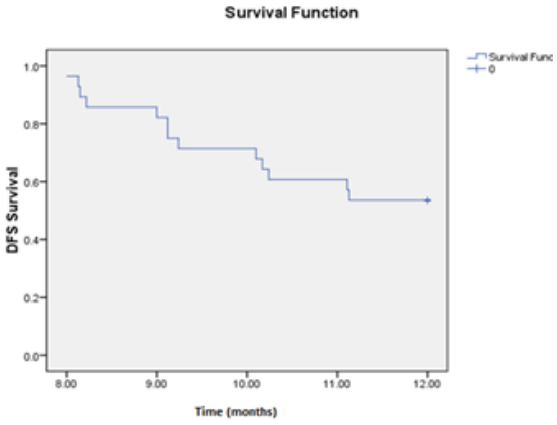
DFS	All patients (n=28)
11 month	53.6 %

DFS: *disease free survival*

Figure (1): Kaplan-Meier curves for overall survival and disease free survival in AML patients, and studied groups according to CD81 expression



A



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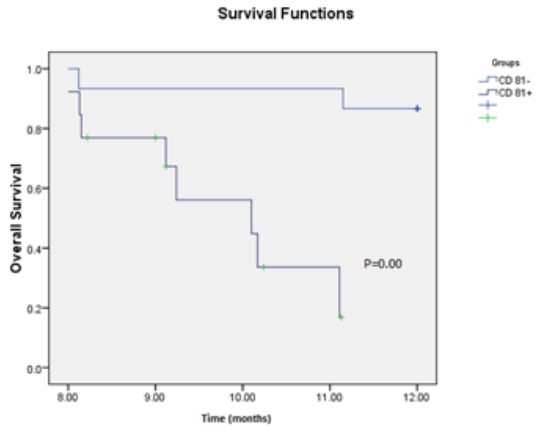

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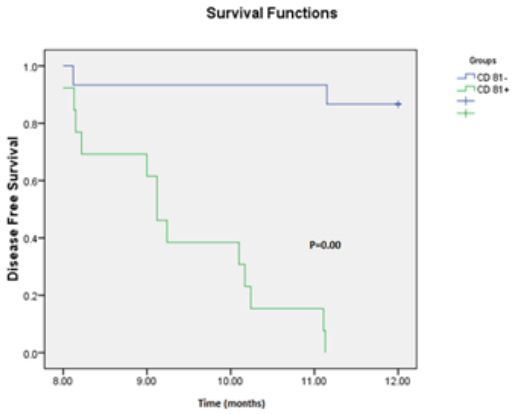

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Figure (1):
(a) Kaplan-Meier curve for overall survival (OS) in AML patients ranged from (8-12 months) with median 11.57 (OS in 28 AML patients was 71.4%). **(b)** Kaplan-Meier curve for disease free survival (DFS) in AML patients (DFS in 28 AML patients was 53.6%). **(c)** Kaplan-Meier curve shows that there was a highly statistically significant difference between both $CD81^+$ and $CD81^-$ expression as regards OS (P= 0.00).**(d)** Kaplan-Meier curve shows that there was statistically significant difference between both $CD81^+$ and $CD81^-$ expression as regards DFS (P= 0.00).

DISCUSSION

AML is a malignant disorder characterized by abnormal growth and differentiation of hematopoietic stem cells (HSCs), in which immature myeloid precursors (myeloblasts) are accumulated in the bone marrow and peripheral blood. This expansion of immature myeloid cells occurs at the expense of the normal production of their terminally differentiated counterparts, such as red blood cells (RBCs), platelets and white blood cells (WBCs) [9].

CD81 is physiologically implicated in the re-entry of hematopoietic stem cells into the quiescent state in order to control self-renewal after induced proliferation [10]. CD81 is a glycoprotein that mediates signal transduction events that is important in multiple processes as in development of the cell, its activation, motility and growth. CD81 gene is localized in the region of tumor suppressor gene and thus it is a candidate gene for malignancies [11].Various cellular functions are linked to CD81 i.e., BCR signaling in B cells [12] B-T cell interaction and cell entry for different infectious diseases [13].

$CD81^+$ expression was present in 13/30 (43%) patients of the studied group, and its level ranged from 21 to 66%, with mean value 31.15±12.14, whereas $CD81^-$expression was present in 17/30 (57%) patients of the studied group, and its level ranged from 3 to 19%, with mean value of 9.06±3.78. This was in agreement with *Boyer T et al.*who stated that the frequency of positive CD81 expression was 35% among patients with AML [10].

Overall, eight patients (26.6%) with $CD81^+$ expression died, whereas two patients (6.7%) with $CD81^-$ expression died during induction therapy, with no statistical difference between the two expressions. These results could be explained by the finding of *Gonzales F et al.*who proved that blast cells expressing CD81 were 30 to 50% more resistant to chemotherapy, and overexpression of CD81 increased AML cell adhesion, migration and blast homing and engraftment efficiency [14].

As regards response to treatment, 50% (15/30) of the negative expression patients shared in this study achieved complete remission (CR), while 43% (13/30) failed to respond to treatment. Patients with positive CD81 expression had a high statistically significant shorter DFS and shorter OS compared with patients with negative CD81 expression (P=0.00).

These results were in agreement with those reported by *Boyer T et al.* [10] and *Gonzales F et al.*[14] who stated that CD81 expression had a negative effect on patient survival.

CONCLUSION

$CD81^+$ expression has a potential role as a

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prognostic marker; its expression is associated with M4 and M5 FAB subtypes. Moreover, patients with $CD81^{+}$ expression have higher incidence of relapse and decreased OS & DFS than patients with $CD81^{-}$ expression.

Conflicts of interest: None to declare.
Financial disclosure: None to declare.

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