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Coexpression of ABCB1 and ABCG2 as prognostic marker for treatment response in acute myeloid leukemia patients

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Abstract

Treatment resistance is nowadays the main challenge in the field of AML. ABC transporters are considered one of the main mechanisms of treatment resistance in AML. Moreover, its prognostic impact on short and long-term remission rate remains of great concern. The development of targeting therapy is an urgent need for improving remission rate and prolongs survival in AML patients. This study aimed at assessing the relationship between co-expression of ABCB1 and ABCG2 transporters and their relation with the response to induction therapy as well as their prognostic significance in newly diagnosed AML patients.

This study included forty newly diagnosed AML patients and thirty healthy subjects of matched age and sex as a control group. Complete blood count, and Flowcytomeric measurement of ABCB1 (CD 243) and ABCG2 (CD 338) were done for all study participants while Bone marrow aspiration (BMA) was carried out for AML patients only. Patients were reevaluated after 28 days from receiving induction treatment in the form of (3+7) protocol for remission achievement. A statistically significant correlation between co-expression of ABCB1 and ABCG2 and treatment response was observed. This denoted that ABCB1 and ABCG2 co-expression can be used as a prognostic marker for remission achievement in AML patients. However, further studies are still needed to confirm this finding in Egyptian population.

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Introduction

Acute Myeloid Leukemia (AML) is the most common type of acute leukemia, and despite availability of chemotherapeutic drugs, mortality rates remain highly variable, ranging from 90 to 10% [1]. One of main characters of AML is having distinct cytogenetic and molecular subgroups. Despite trials trying to improve short- and long-term remission rate, fate of AML patients remains doubtful, resistance and relapse rates remains high. Many questions have been addressed to solve the problem of drug resistance [2].

Treatment resistance remains an obstacle and a challenge to hematologist. Many mechanisms of resistance have been described in AML including functional mechanism in leukemic blasts or due to host or acquired factors [3].

One of the main mechanism of resistance is ATP membrane transporter responsible for chemotherapeutic drug efflux as (ABCB1, ABCG2,..... etc). Overexpression of these transporters has a poor prognostic impact on remission rates in acute myeloid leukemia. Coexpression of multiple ABC transporters results in a worse prognosis [4].

P-glycoprotein is one of the most studied ABC transporters which has a role in drug resistance. It is encoded by the multidrug resistance gene 1 [5]. Other ABC-transporters with clinical impact on drug resistance in adult AML are the breast cancer resistance protein (BCRP), encoded by adenosine triphosphate binding cassette transporter G2 (ABCG2) [6].

The increased expression of ABC transporters causes efflux of chemotherapeutic drugs out of leukemic cells and reducing the effectiveness of these drugs resulting in increasing of the resistance and relapse rate in these patients [7-9]. Approximately 50% of AML patients express ABC transporters, with increased level of expression in elderly and relapsed patients [3]. Indeed, more than one ABC transporters is expressed in leukemic blast of patients especially relapsed patients [10].

Fortunately, clinical trials paid a great attention to overcome problem of drug resistance through developing ABC transporters inhibitors. Indeed, with the appearance of these new data about possibility of developing target therapy, we propose to reassess the role of these transporters in multidrug resistance [11-13].

The aim of this study was to investigate the expression levels of ABCB1 and ABCG2 and their effect on the achievement of hematological remission after induction therapy as well as their prognostic value in newly diagnosed AML patients.

Patients and methods

The present study was conducted on 40 newly diagnosed AML patients, received 3+7 protocol as induction therapy. Assessment of hematologic remission was carried out after 28 days. Flowcytometric measurement of CD243 and CD338 was done at diagnosis. Patients were recruited from the Hematology outpatient clinic of Alexandria Main University Hospital during the period from May 2016 to December 2017.

Thirty healthy subjects of matched age and sex were included in the study as a control group. Patients with hepatic or renal failure, concomitant chronic illness and pregnant females were excluded from the study. Written consent was taken from all study participants. This study was approved by the Alexandria Faculty of Medicine Ethics Committee.

All subjects included in the study were subjected to the following:

1. Complete history taking.
2. Thorough clinical examination.
3. Laboratory investigation including:

I. Routine Lab Investigation:

- Complete blood count.
- ALT, AST, S.urea, S.creatinine.

4. Bone marrow aspiration and examination.
5. Immunophenotyping by flowcytometry.

Immunophenotyping of the leukemic blast cells was performed on bone marrow samples using BD FACSCalibur flow cytometry analyzer equipped with BD Cell Quest Pro Software.

The following McAbs were used in combination.

The following panel applied for all cases (CD2, CD7, CD10, CD14, CD19, CD13, CD33, HLD-DR, CD34, CD45, CD 11c).

a) Secondary panel: CD11b, CD64, cyt MPO, cyt CD 22, cyt CD 3, CD 117, CD 41, CD 61, CD 235a.

b) Analysis of CD 243 (ABCB1) CD 338 (ABCG2) expression on leukemic blasts of AML by flowcytometry.

ABCB1 (CD 243) PE, Clone: 41C2, Catalog number: 12-2439-42Lot number: 13, Product of Thermo-Fischer, scientific, San Diego.

ABCG3 (CD 338), Clone: 5D3, Catalog number: 13-8888-82, Lot number: 114531, Product of Thermo-Fischer, scientific, San Diego. Labelled by APC.

Results

Regarding demographic data, there was no statistically significant difference between patients and controls as shown in table 1.

Table 1: Comparison of demographic data between patients and controls.

	Groups				t-Test p
Age (years)	**Patients**		**Control**		
Range	25.0-52.0		23.0-50.0		2.196
Mean	38.9		35.8		0.143
S.D.	8.3		8.6		N.S.
Sex	No.	%	No.	%	
Male	29	72.5	23	62.5	0.152
Female	11	27.5	7	37.5	

P is significant if ≤ 0.05

When testing conventional cytogenetics in relation to CD 338 expression and CD 243, it was noted that there is statistically significant difference between the level of both CD expression and karyotyping (p= 0.037, p= 0.001) respectively as shown in table 2 and 3.

Table 2: Relation between conventional cytogenetics in relation to CD338 expression.

Cytogenetics		CD338		Total
		-ve	+ve	
Complex	No.	5	3	8
	%	8.5%	27.3%	11.4%
Hyperdiploidy	No.	0	1	1
	%	0.0%	9.1%	1.4%
Inversion 16	No.	1	1	2
	%	1.7%	9.1%	2.9%
Normal	No.	19	1	20
	%	32.2%	9.1%	28.6%
t (8;21)	No.	4	0	4
	%	6.8%	0.0%	5.7%
failure to obtain	No.	5	0	5
	%	8.5%	0.0%	7.1%
X^2 p		12.554 0.037*		

Table 3: Relation between conventional cytogenetics in relation to CD243 expression.

Cytogenetics		CD243		Total
		-ve	+ve	
Complex	No.	7	1	8
	%	10.6%	25.0%	11.4%
Hyperdiploidy	No.	0	1	1
	%	0.0%	25.0%	1.4%
Inversion 16	No.	2	0	2
	%	3.0%	0.0%	2.9%
Normal	No.	19	1	20
	%	28.8%	25.0%	28.6%
t8:21	No.	3	1	4
	%	4.5%	25.0%	5.7%
Failure to obtain	No.	5	0	5
	%	7.6%	0.0%	7.1%
X2 p		12.554 0.037*		

Regarding Treatment, According to bone marrow aspirate results, the following table (4) shows response to standard treatment protocol (3+7) among our patients.

Table 4: Response to the planned treatment among AML cases.

	Number	Percent
Remission (CR)	26	65.0
Partial remission (PR)	14	35.0
Total	40	100.0

After induction treatment, CD 243 expression in patients who achieved complete response was negative in 100% and positive in zero %, regarding patients with partial remission was negative in 71.4% and positive in 28.6% and this was statistically significant ($p < 0.05$). As regards CD 338 expression in patients who achieved complete response after induction chemotherapy was negative in 96.2% and positive in 3.8%, regarding patients with partial remission was negative in 64.3% and positive in 35.7% and this was statistically significant ($p<0.05$) as seen in table 5.

Table 5: Relation between the CD 338 and CD 243 expression and response to treatment.

			Response		X^2 P
			Complete response	Partial response	
CD338	-ve	No.	25	9	
		%	96.2%	64.3%	7.248 0.014*
	+ve	No.	1	5	
		%	3.8%	35.7%	
CD243	-ve	No.	26	10	
		%	100.0%	71.4 %	4.13 0.035*
	+ve	No.	0	4	
		%	0.0%	28.6 %	

Figures (1-3) show different patterns of expression of CD 243 and CD 338 in three of our patients.

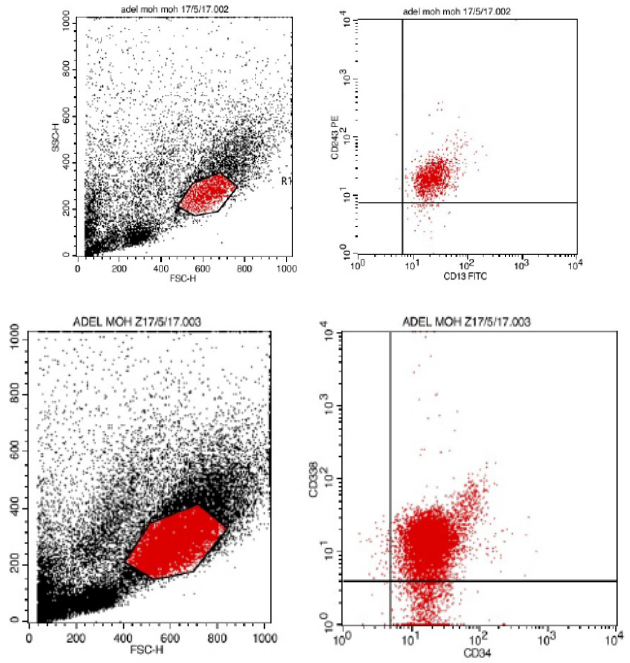

Figure 1: (a) Double expression of CD 243 and CD 13 in a patient achieved partial remission on induction chemotherapy. (b) Double expression of CD 338 and CD 34 in the same patient who achieved partial remission on induction chemotherapy.

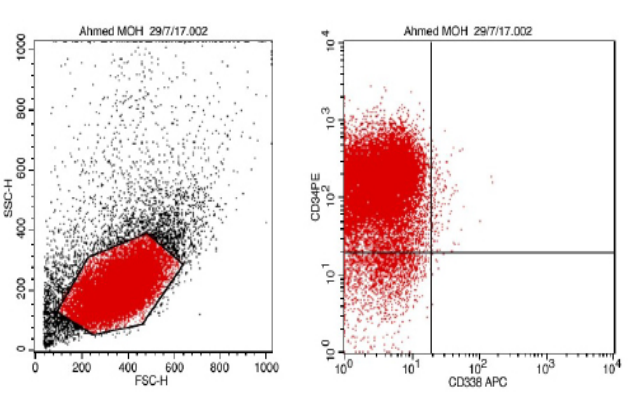

Figure 2: Negative expression of CD 338 in a patient who achieved complete remission on induction chemotherapy.

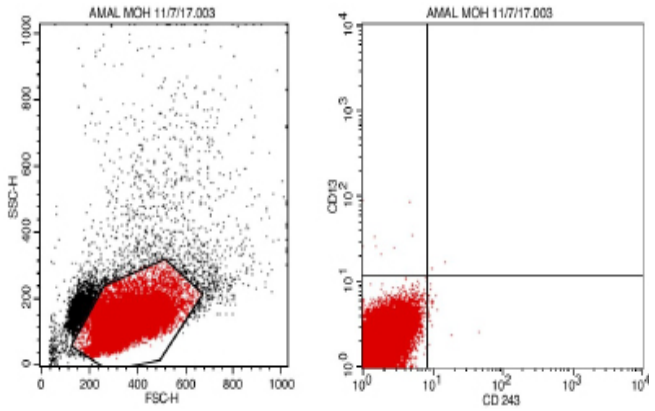

Figure 3: Negative expression of CD 243 in a patient who achieved complete remission on induction chemotherapy.

Discussion

Coexpression of multiple ABC transporters carries the worse prognosis in adulthood AML patients and contribute to treatment resistance which remains a challenge in those patients [4]. MDR is a multifactorial process including the following mechanisms as i) altered membrane transport. ii) altered target enzymes. iii) altered drug activation or degradation. iv) enhanced DNA repair. v) failure to undergo apoptosis. vi) abnormal autophagy [14].

Effectiveness of chemotherapeutic drugs depends on transport mechanism (drug efflux) through ATP transporters which remains one of the main mechanisms contributing to treatment resistance [15].

The ABC superfamily consists of 49 ABC genes identified in the human genome. Currently at least 15 ABC transporters have been implicated to confer resistance to clinically active drugs, notably P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) [16].

Resistance conferred by ABC transporters is not only to the classic chemotherapeutic agents but also against the new targeted therapy. Also many researchers discussed interactions between ABC transporters and target therapy [17-20].

Coexpression of Multiple ABC-Transporters is strongly associated with inferior outcomes in AML. Researchers' main concern nowadays is inhibiting these transporters which will improve the short term response and decrease relapse rate [21].

To date, many ABCB1 inhibitors have been developed, such as cyclosporine A. But astonishing, inhibiting ABCB1 transporter only was associated with inferior results and did not overcome MDR. THIS raised into mind the idea about co-expression of multiple ABC transporters has more great impact on treatment resistance [22-25].

We designed our study depending on this point of view, we concluded that coexpression of ABCB1 and ABCG 2 rather than one transporter is associated with poor prognosis in newly diagnosed AML patients after induction chemotherapy.

Declarations

Ethics approval and consent to participate: All patients provided written informed consent prior to their inclusion within the study.

Consent for publication: All patients provided written informed consent for the publication of their data.

Competing interests: The authors declare that they have no competing interests.

Author contributions: This study and research were designed by AM, AG, AE, AA, ME. The experiments were conducted and analyzed by AM and AE, flow cytometry by AE. Reagents were provided by AM. The manuscript was written by AM, AG, AE.

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