



The Prognostic Value of Apoptotic Marker (CD95) in Adult Acute Leukemias

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Abstract: Fas-mediated cell death is involved in drug-induced apoptosis in various cell types. Hence, failure of apoptosis could lead to chemoresistance in acute leukemia. The participants of this study were 80 adult acute leukemia patients classified as follows: 40 acute myeloid leukemia (AML) patients, 40 acute lymphoblastic leukemia (ALL) patients. In addition, 10 healthy controls were also included in the study. Fas expression was measured using flow cytometry. The mean value of Fas expression by blast cells from AML patients at diagnosis was $41.72 \pm 10.3\%$. AML patients were divided into the Fas-positive group [30 patients (72.5%)] and the Fas-negative group [10 patients (27.5%)]. The mean value of expression increased significantly in M5 ($52.91 \pm 11.3\%$) with highly significant differences ($P < 0.001$) between Fas expression levels in different FAB subtypes of AML. The mean value of Fas expression in ALL patients was $43.87 \pm 11.5\%$. 23 (57.5%) patients were positive for Fas expression, whereas 17 (42.5%) were negative. Fas expression was positive in 14/24 (63.2%) precursor B-ALL patients and in 12/16 (84.6%) T-ALL patients. The mean value of Fas expression was significantly higher ($P = 0.039$) in T-ALL ($55.15 \pm 7.8\%$) in comparison with precursor B-ALL ($34.47 \pm 5.76\%$). The mean value of Fas expression by blast cells from AML and ALL, patients at diagnosis was 41.72 ± 10.3 and 43.87 ± 11.5 . We can conclude that Fas receptor expression on blast cells from ALL and AML patients could serve as an independent prognostic factor.

Keywords: Cell Death, Fas, CD95, Blast Cells, ALL, AML, Apoptosis

1. Introduction

Apoptosis is a morphologically distinct form of programmed cell death that plays a major role during development, homeostasis [1]. It is an active death process genetically encoded to eliminate abnormal unwanted cells [2].

Fas (Apo-1/CD95) is a cell surface transmembrane molecule belonging to the tumor necrosis factor/nerve growth factor receptor superfamily that is characterized by cysteine-rich extracellular domains [3].

Fas antigen is expressed on a variety of human B and T cell lines, on various normal human tissues, and on many different tumor cells [4]. The interaction between Fas receptor (CD95) on the target cell and Fas ligand (Fas L) on cytotoxic T-lymphocytes has been shown to be an important

mechanism of apoptosis [5].

The deregulation of apoptosis may contribute to the pathogenesis of a number of human malignant disorders [6]. Some researchers demonstrated that Fas was expressed on a majority of human leukemia cells, although the intensity of expression was variable [7].

Chemotherapeutic drugs used for leukemia treatment can kill target cells by several mechanisms, including apoptosis [8]. In addition, Fas-mediated cell death is involved in drug-induced apoptosis in various cell types [9]. Hence, failure in the apoptosis machinery could lead to chemo resistance and may therefore have an impact on clinical outcome [10].

This study was conducted to evaluate the percentage of Fas receptor expression on blast cells of adult patients with acute leukemia and rule out the impact of Fas expression on

prognosis and overall survival

2. Participants and Methods

The participants of this study were 80 adult acute leukemia patients: 40 acute myeloid leukemia (AML) patients, 40 ALL patients. 10 age-matched and sex-matched healthy individuals were also included in the study as the control group. The age of AML patients ranged from 19 to 75 years and comprised 21 (52.5%) men and 19 (47.5%) women. There were 22 (56.25%) male ALL patients and 18 (43.75%) female ALL patients and their ages ranged from 16 to 62 years.

Patients were selected from Hematology/Oncology Unit, National Cancer institute, Cairo, Egypt

Patients with acute leukemia were studied at first diagnosis and were previously untreated. The diagnosis of AML, ALL, were assessed by morphological study, cytochemistry, and immunophenotyping of peripheral blood (PB) and bone marrow aspirate (BMA) according to the FAB classification of acute leukemia [11].

Immunophenotyping was carried out on PB or BM samples on EDTA using Becton Dickinson FAC Scan flow cytometry (Becton Dickinson, San Jose, California, USA) with panels of monoclonal antibodies (MoAbs) stained with either fluorescein isothiocyanate or phycoerythrin (PE) including B-lymphoid markers (CD10, CD19, CD20, and CD22), T-lymphoid markers (CD2, CD3, CD5, and CD7), myeloid and monocytic markers (CD117, CD13, CD33,

CD14, and CD64), nonlineage-specific markers (CD34, HLA-DR), and megakaryoblastic and erythroid markers (CD41, CD61, and glycophorin-A). These markers were supplied by Coulter Immunology (Coulter Corporation, Hialeah, Florida, USA).

Cytogenetic analyses were performed using pretreatment samples from all patients according to standard protocols including conventional cytogenetic analysis using the G-banding technique; chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclatures (ISCN) [12], and fluorescence in-situ hybridization analysis using a comprehensive DNA probe set allowed for the detection of most relevant AML-associated and ALL-associated genomic aberrations [13, 14].

The ALL patients, poor risk included t(9;22), t(4;11), t(1;19), t(2;8), t(8;14), t(8;22), or hypodiploidy; good risk involved hyperdiploidy or t(12;21); and intermediate risk consisted of other cytogenetic abnormalities or normal karyotypes.

The AML patients, the poor risk group consisted of -5/5q-, -7/7q-, or 11q23 abnormalities; and the favorable risk group consisted of t(8;21), t(15;17), t(16;16), or inv(16).

After diagnosis, patients received treatment according to the type of acute leukemia and they were followed up for periods ranging from 20 to 36 months, from June 2014 to June 2016, with special attention to clinical and laboratory markers of remission and relapse, taking care to estimate the date of first complete remission, date of relapse, death, or last seen alive.

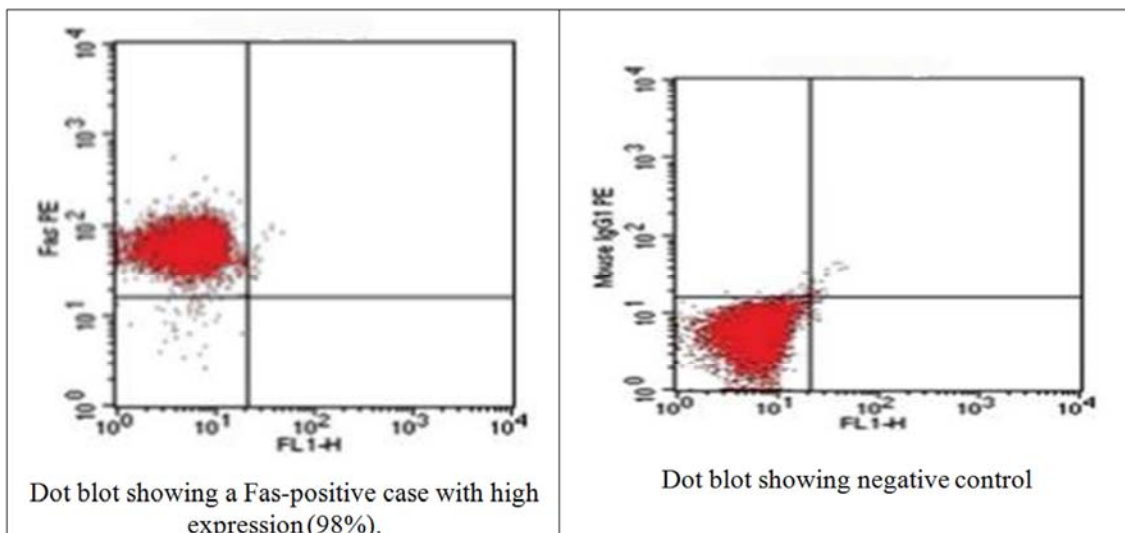


Figure 1. Fas (CD95) Expression on blast cells by Flowcytometry.

3. Analysis of Fas Expression

Expression of Fas in blast cells from BM or PB samples of the patients or in normal monocytes, granulocytes, and lymphocytes obtained from PB samples of controls and measured by flow cytometry was studied. Using the whole blood lysis method, 100 μ l of fresh EDTA PB or BM samples was prepared for flow cytometry by adding 10 μ l

of PE-conjugated anti-human CD95 MoAb (Becton Dickinson). PE- conjugated IgG1 MoAb (0.10 μ l) (Becton Dickinson) was used as isotype-matched nonspecific control antibody. Samples were incubated for 30 min at 4°C. The erythrocytes were lysed using 1 ml FACs lysis solution (Becton Dickinson) on each tube sample and were incubated for 20 min at 4°C. Cells were washed twice by adding 0.5 ml PBS (pH 7.4) and centrifuged at 3000 rpm for 5 min each. The supernatant was discarded and

resuspended in 0.3 ml PBS, and then analyzed on a fluorescence-activated cell scanner (FACs Caliber; Becton Dickinson). Data acquisition and analysis was performed using Cell Quest Procount software version 2.1 (Verity Software House, Topsham, ME, USA). A total of 10 000 events were acquired and gating was performed using forward scatter and side scatter and the percentage of positive cells was calculated [15]. Values equal to or greater than 20% were considered positive (Fas positive), whereas values less than 20% were considered negative for Fas expression (Fas negative).

We tried to correlate Fas expression to prognostic factors (age, sex, total leukocyte count, serum LDH, and cytogenetic risk categories) by studying Fas expression levels on blast cells of leukemic patients at diagnosis.

4. Results

The 80 patients in this study were divided into 40 AML patients, 40 ALL patients. The baseline characteristics of the patients are shown in Table 1.

Table 1. Demographic data of patients groups at diagnosis.

Parameters	AML (n = 40)	ALL (n = 40)
Age (range) (years)	19–75	16–62
Sex [N (%)]		
Males	21 (52.5)	18 (56.25)
Females	19 (47.5)	22 (43.75)
WBCs ($\times 10^3/\text{mm}^3$) (range)	1.7–182	2.3–210
LDH (range) (U/l)	320–5120	670–3420

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia

Table 4. Relation between Fas expression and FAB subtypes in AML patients.

	M1 (n = 7)	M2 (n = 13)	M3 (n = 3)	M4 (n = 6)	M5 (n = 11)
Positive Fas	4 (42.9)	10 (69.2)	3 (66.7)	6 (83.3)	11 (90.9)
[N (%)]	20.28 \pm	43.69 \pm	30.67 \pm	49.17 \pm	52.91 \pm
Mean \pm SD	5.3	8.63	6.47	10.77	11.3
X-test	3.15		P value		0.009*

AML, acute myeloid leukaemia. *Significant.

ALL patients were either precursor B-ALL or T-ALL as estimated by immunophenotyping. The mean value of Fas expression was significantly higher ($P = 0.039$) in T-ALL ($55.15 \pm 7.8\%$) when compared with precursor B-ALL ($34.47 \pm 5.76\%$), as shown in Table 5.

Table 5. Relation between Fas expression and immunophenotype in ALL patients.

	Precursor B-ALL (n = 24)	T-ALL (n = 16)
Positive Fas [N (%)]	14 (63.2)	12 (84.6)
Mean \pm SD	34.47 \pm 5.76	55.15 \pm 7.80
t-test	4.749	
P value		0.039*

*Significant.

Using flow cytometry in controls we found that monocytes expressed the highest level of Fas ($32.2 \pm 6.95\%$), followed by granulocytes ($23.8 \pm 7.61\%$), with lymphocytes expressing the lowest levels ($16.1 \pm 4.01\%$), with a statistically highly significant difference ($P < 0.001$) as shown in Table 2.

Table 2. Fas expression of controls on different cell types.

Type of cells	Lymphocytes (n = 10)	Granulocytes (n = 10)	Monocytes (n = 10)
Mean \pm SD	16.1 \pm 4.01	23.8 \pm 7.61	32.2 \pm 6.95
F-test	12.21		
P value	0.001*		

On comparing the three patient groups, the observed difference did not reach statistical significance ($P = 0.524$) with respect to Fas expression on different types of Leukemias as shown in Table 3.

Table 3. Fas expression in different patient groups.

	AML (n = 40)	ALL (n = 40)
Positive/negative	30/10 (72.50)	23/17 (71.88)
\times test	0.340	P value
Mean \pm SD	40.72 \pm 10.3	34.87 \pm 11.5
F-test	0.652	P value

The mean values of Fas expression in AML patients were significantly increased from M1 to M5 with the weakest expression in M1 ($20.28 \pm 5.3\%$) and the strongest in M5 ($52.91 \pm 11.3\%$) with a significant difference ($P = 0.009$) between Fas expression levels in different FAB subtypes of AML, as shown in Table 4.

5. Discussion

The two main pathways involved in apoptosis are the extrinsic pathway, which is triggered through the Fas death receptor, and the intrinsic pathway [16]. After being triggered by its natural ligand (FasL), Fas recruits the relative proteins to form the death-inducing signaling complex. A cascade of caspase activation is initiated in turn, which finally leads to apoptosis of the cell [17]. In the present work, controls showed significant variation in the mean percentage of Fas expression by different normal cells in the peripheral blood. Monocytes expressed significantly higher levels of Fas compared with granulocytes, whose expression levels were higher than that of lymphocytes. Many previous studies have found Fas expression on a variety of hematopoietic cell populations, including T and B lymphocytes, monocytes,

macrophages, and eosinophils [18].

The present study demonstrated that Fas receptors are expressed on a significant proportion of blast cells in adult AML and ALL patients. An overall 73 % of AML patients were positive expressors of Fas (with Fas being expressed by 20% or more of their leukemic cells). AML patients expressed Fas with variable intensity ranging from 3 to 98% of their blast cells. Several studies have reported variable intensities of Fas expression on AML blast cells [19], which are in agreement with our results, and may reflect the heterogeneity of AML.

Our study also showed that Fas expression varies significantly between different FAB subtypes of AML, being strongest in M4 and M5 and weakest in M1 with intermediate levels in M2 and M3. In normal haematopoiesis, immature cells do not express a significant level of Fas [20]. Fas expression becomes enhanced with maturation of the myeloid series [21].

Hence, variable expression levels of Fas in different AML subtypes may simply reflect the difference in maturation stage of leukemic cells [22].

Regarding ALL, 72 % of our patients were positive expressors of Fas, which is similar to the results in previous studies [28, 29]. We found that expression of Fas by T-ALL blast cells was significantly higher than that of precursor B-ALL cells. [30] found that T-ALL showed significantly higher percentage of Fas expression as compared with B-ALL cells. Also [23] found that leukemic cells from all patients with T-ALL strongly expressed Fas.

Another study proved that normal mature B cells are Fas-negative and stated that Fas expression status on leukemia and lymphoma B cells from different disease entities is controversial [24].

Previous studies have suggested a low expression of Fas to be associated with low complete remission rates after induction chemotherapy [25]. Impairment of Fas expression in human cancer cell lines was reported to be associated with drug resistance *in vitro* [26]. Hence, it is possible that low surface expression of Fas by leukemic cells is associated with resistance to apoptosis and antileukemic therapy in our patients [27].

Our study also showed that overall survival was prolonged in Fas-positive AML and Fas-positive ALL patients compared with Fas-negative patients. These results may suggest that Fas expression on AML and ALL cells is a possible marker suggesting sensitivity to chemotherapy or to immune-mediated apoptosis.

We can conclude that the expression of Fas receptors on blast cells from ALL and AML could serve as an independent prognostic factor, helping in monitoring the outcome of therapy. It is recommended that Fas should be included as a cell surface marker in the immunophenotyping panel of acute leukaemia. Patients with low Fas expression levels may require intense chemotherapy to achieve remission. Trials to enhance Fas expression and Fas-mediated apoptosis of blast cells may be the future hope in acute leukaemia therapy.

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